# Research Activities

2019

The Jikei University School of Medicine

**Research Activities 2019**
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 10 students abroad and received 69 overseas students in the 2019 academic year. We have partnerships with 14 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 global society.

We owe much to the efforts of Professors Susumu Minamisawa and Masao Okazaki, and the editorial staff of the Academic Information Center in editing this report.

Senya Matsufuji
President
The Jikei University School of Medicine

November 1, 2020

# 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 Jikei Centre for International Affairs (JCIA), 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, 2020, the number of books and journals held is 262,000 by the Shimbashi Main Library and 92,646 by the Kokuryo Branch Library.
With the prevalence of academic e-resources, the libraries have replaced print journals with e-journals. In 2019, 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 (51 participants in 2019). Elsevier's abstract and citation database, *Scopus*, was introduced in August 2019. To encourage the use of *Scopus* as a tool for analyzing individual and institutional research performance, the libraries held lectures in the main hospital and 3 branch hospitals in cooperation with Elsevier's instructors.
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 (331 newly registered remote-access users in 2019).
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 these 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. Homare Eda (Chiba University/Pfizer Inc., May 14), Prof. Masako Nishikawa (Clinical Research Support Center, May 16), Prof. Masao Okazaki (Academic Information Center, May 28), and Prof. Osamu Ohara and Assistant Prof. John Surya (Medical English Division, Department of English, May 30).

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 and to give a short lecture for postgraduates, concerning *Turnitin*. The libraries also prepared *Turnitin*'s user registration form on their website and registered 14 new personal users in 2019.

The libraries maintain an institutional repository, the Academic Repository of The Jikei University School of Medicine, to preserve the academic output of this university and to make it available on the Internet. In 2019, 326 articles, including the university's publications and doctoral theses, were registered in the Academic Repository.

To address the spread of open access journals, the libraries need to provide researchers with information on the reputation and reliability of the journals to which they submit their manuscripts.

*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, 2020) 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 2019, the Medical Museum renovated 53 specimens by removing stains and renewing the explanations.

From January 20 to 31, the Medical Museum held a poster exhibition entitled *Sogo Tenji* at the Shimbashi Campus, featuring research activities of a retiring professor, Prof. Akihiro Ikai (Department of Dentistry).

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 recording videos of research topics. In addition, the Photography Room modifies photographic and video image files for manuscripts or presentation forms. Large-format and other types of color printers are available in the Photography Room.

In 2019, the Photography Room dealt with 5,341 photographs and 11,693 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 2019, the Historical Collection Room received 59 visitors and 70 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.

## The Jikei Centre for International Affairs

Ruri Ashida, *Professor and Director*

### General Summary

The 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. The activities of the JCIA include: (1) English education of medical students, nursing students, physicians, nurses, and other members of the hospital staff; (2) selecting and sending students to partnership (and nonpartnership) 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. This year, research was focused on exploring cultural issues related to taking care of foreign patients in Japan.

### Research Activities

1. Professor Ashida held simulation sessions with English-speaking simulated patients for medical and nursing students, physicians, and nurses at The Jikei University and other universities (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. To enhance healthcare professionals' ability to communicate with foreign patients in English and other languages with cultural humility, Prof. Ashida continued her research

on cultural aspects involved in caring for foreign patients, and held educational seminars and simulations to search for ways to enhance healthcare professionals' communication skills in English. Her research was presented at the 5th International Conference on Faculty Development in the Health Professions in Ottawa.
3. Professor Ashida continued her work on the use of UNESCO Bioethics Core Curriculum (Section 2: Study material) in ethics education.
4. Partnerships with the University of Hong Kong (June 2019), University of Queensland (July 2019), Mayo Clinic College of Medicine and Science (November 2019), and Medical University of Vienna (February 2020) were established to promote research and education. The number of overseas partner universities has increased to 14.
5. A seminar to enhance overseas training and study-abroad programs was held on October 4th, and Dr. Minami Taro, Brown University School of Medicine, gave a talk on clinical practice and research activities in the United States.
6. Professor Ashida was invited by Seoul National University for "SNU Medicine Exchange Partner Day" to exchange ideas on enhancing partnership in education and research. Other universities that participated included National Taiwan University, National University of Singapore, and Peking University Health Science Center.

# Continuing Medical Education Center
# The Continuing Medical Education Committee

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

## General Summary

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

## Research Activities

1. Registered members: 165 (as of April 1, 2020)
Members using the Center: 316/year
2. The 40th summer seminar Tokyo Jikei Medical University ― University Hospital Medical Cooperation Forum is held on August 1, 2019. A total of 239 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*
Hideaki Suzuki, *Assistant Professor*

## General Summary

The Office of Educational Development was founded in 1999. Staff members were recruited from the School of Medicine. Its main interests were (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 (OSCEs), and community-based medical education programs in the undergraduate curriculum. However, many improvements have been required in our undergraduate medical and nursing education, postgraduate clinical training programs, and continuing professional development for healthcare workers. In 2005, the office of Educational Development was reorganized as the Center for Medical Education. Furthermore, the secretariat was set up in the Center in 2006. The bylaws for the Center were revised in 2011, 2013, and 2015.
The Center now consists of Branches for Physician Professional Development Support, Nursing Professional Development Support, Simulation Education, Community-based Medical Education and Research, Educational Institutional Research, and 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 affiliated hospitals; and the management of an e-learning system and simulation training centers for students, faculty, and staff in affiliated 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 course director of Medicine in General I and II; the educational unit director of Introduction to Health Care Practice (year 1 of medical and nurs-

ing students), Early Clinical Exposure (year 1) and Medicine in General Practice II (year 2) and 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). Associate 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). Assistant Professor Suzuki was the educational director of Medicine in General Practice I (year 1). Regarding graduate school education, Professors Fukushima, Nakamura, and Onoue and Associate Professors Ishibashi and Okazaki were in charge of medical education in the common graduate school curriculum.

2. The Branch for Nursing Professional Development Support (Branch leader: Director Takahashi) organized seminars for education nurses, exchange workshops between basic nursing faculty and nurses working in the ward, basic nursing educational program seminars according to the End-of-Life Nursing Education Consortium-Japan, and a first-level seminar for certified nursing manager. Shinobu Hazama, RN (Master of Nursing) was in charge of Disaster Nursing and Emergency Nursing at the School of Nursing and of Consultation in Nursing and Critical Care in Nursing at the Graduate School of Nursing.

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. Faculty development and staff development activities on campus: Associate Professor Ishibashi participated as a facilitator or member of the chief task force in faculty development for clinical clerkship teachers, for clinical supervisors for clinical training in postgraduate years (PGY) 1 and 2, educational training courses for PGY 3 to 5, and for OSCEs evaluator training at years 4 and 6. Associate Professor Okazaki participated as a facilitator or member of the chief task force in faculty development for teachers of medical interviewing and evidence-based medicine sessions, clinical supervisors in postgraduate (PGY 1 and 2) clinical training, educational training courses for PGY 3 to 5, and for OSCEs evaluator training at years 4 and 6.

6. Postgraduate clinical training at affiliated hospitals: Associate Professor Ishibashi was the chair of evaluation of technical recognition in laparoscopic surgery. Associate Professor Okazaki was the tutor in "significant event analysis" session in the seminar for postgraduate clinical trainees.

7. Nursing staff development in affiliated hospitals: Hazama, RN participated in staff development of immediate cardiac life support, basic life support, endotracheal intubation, Rapid Response System as a facilitator, and carried out the training programs of intravenous injection at levels 3 and 4, and teamwork for sudden changes in the ward for nurses working in all affiliated hospitals and was in charge in consultation sessions in the certified nurse program at Kashiwa Hospital.

8. 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).

Professor Fukushima participated in third-party evaluation activities of vocational education in practical schools supported by MEXT.
As a participant in the activities of the Japan Accreditation Council for Medical Education, Professor Fukushima and Professor Nakamura served as evaluators in external evaluation teams at several schools of medicine. Professor Nakamura was a lecturer in the evaluator training workshop held by the Japan Accreditation Council for Medical Education.
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 Juntendo University and Kumamoto University. Associate Professor Ishibashi was a member of several CBT committees and was sent as a CBT monitor to Tokyo Medical University and Toho University. Associate Professor Okazaki was a member of several OSCE committees and was sent as an OSCE monitor to Fukui University, Toyama University, Miyazaki University, Iwate Medical University, and Osaka University.
As a participant in the activities of the Japan Council for Evaluation of Postgraduate Clinical Training, Associate Professor Ishibashi served as an evaluator at Kanto Rosai Hospital.
9. Contribution to other institutions of higher education (faculty development lectures and workshops): National Defense Medical College; Yamanashi University; IMS Group Patient Safety; Teacher training for occupational therapy, physical therapy, and speech therapy held by the MHLW; Showa University; Kurume University; Teikyo University; Hyogo Medical School; Hyogo Medical School Hospital; Teacher training for judo therapists held by the MHLW; Gifu University; Kansai Medical University; Yamanashi University; Kochi University; Oita University; Certified Nursing Manager course at Showa University Hospital; Certified Nursing Manager course held by Yamagata Nursing Association; Hana Gakuen Practical School; Emergency nursing training course at Tokyu Hospital; Disaster triage course held by Minato Medical Association; Shakai-igaku-gijutsu gakuin; Nihon Rehabilitation Practical School; and Fukui University.

# Clinical Research Support Center

Keigo Shikishima, *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. In April 2019, the directorship changed from Prof. Kageyama to Prof. Shikishima. We started consulting for clinical research in September 2014 and had 81 protocols of consultation, including 40 new protocols, from April 2019 through March 2020. The number of protocols the center newly consulted on was as follows: research planning, 23; protocol planning and statistics, 19; protocol for randomization/allocation/concealment of emergency key, 4; consultation of statistical analyses, 20; conducting statistical analysis, 1; preparation of articles, 1; application for competitive research fund, 10; and specified clinical research, 5. The number of protocols the center continuously consulted on was as follows: protocol planning and statistics, 2; protocol for randomization/allocation/concealment of emergency key, 1; consultation of statistical analyses, 7; conducting statistical analysis, 7; preparation of articles, 7; application for competitive research fund, 2; and specified clinical research, 2. As a result, 4 articles were published and 2 studies were approved for competitive research funding.

In cooperation with the Division of Clinical Pharmacology and Therapeutics we held a "Clinical Trial Seminar" to improve literacy about clinical trials among researchers. Since April 2019, the "Clinical Trial Seminar" was held by our center. The themes were "Basic knowledge on clinical study" (December 2019) and "Approval review and consultation regarding pharmaceuticals in the Pharmaceuticals and Medical Devices Agency" (January 2020). We stopped holding a "Biostatistics Seminar for Tomorrow," consisting of 2 basic courses and 1 advanced course, which had been held since 2015 to promote appropriate trial designs and the application of biostatistical methods. Starting this, we have instead held "Methodology for Clinical Trial" as a course for graduate students which is open to all staff members of The Jikei University School of Medicine.

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, and a certified review board was established at The Jikei University in November 2018. To meet these requirements, we prepared common forms of protocol, an informed consent form, a standard operating procedure for monitoring, and other documents. This year, we upgraded the ethics application system to address the requirements of the Clinical Trials Act. In addition, clinical research coordinators appointed by the director of The Jikei University Hospital monitored the specified clinical trials ongoing in our hospital to check whether researchers conduct them properly.

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. 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 the monthly achievement rates of hemoglobin A1c, blood pressure, and level of low-density lipoprotein (LDL) cholesterol and of all 3 variables have certain circannual rhythms in type 2 diabetes mellitus of 4,678 patients nationwide. As part of the Japan Diabetes Clinical Data Management Study Group, we used those patient records whose hemoglobin A1c, blood pressure, and LDL cholesterol were measured 12 or more times during a 24-month period from January 2013 through December 2014. We explored related factors to lowering achievement rates in summer and winter separately. Insulin use and sulfonylurea use were independently associated with the decreased achievement rates of all 3 variables in both summer and winter.

In cooperation with the Division of Nephrology and Hypertension, Department of Internal Medicine, we analyzed retrospective cohort of 1,065 Japanese patients with IgA nephropathy diagnosed between 2002 and 2004. This study was funded by the Agency for Medical Research and Development (AMED). We showed that the matched patients who underwent tonsillectomy within 1 year of the initial diagnosis of IgA nephropathy had a lower risk of renal events than those who did not undergo the procedure, and that tonsillectomy may improve renal survival rates in patients with IgA nephropathy independent of conventional therapy using renin-angiotensin system inhibitors and corticosteroids.

In cooperation with the Departments of Endoscopy, we made a study protocol of randomized controlled trial to examine the accuracy of diagnostic support system using artificial intelligence for colonoscopy. Under the support of the AMED, we started the study with the approval of the certified review board and finished the planed enrollment. We undertook to develop the analysis program.

### Publications

***Sakamoto M, Matsutani D, Minato S, Tsujimoto Y, Kayama Y, Takeda N, Ichikawa S, Horiuchi R, Utsunomiya K, Nishikawa M.*** Seasonal Variations in the Achievement of Guideline Targets for HbA(1c), Blood Pressure, and Cholesterol Among Patients With Type 2 Diabetes: A Nationwide Population-Based Study (ABC Study: JDDM49). *Diabetes Care.* 2019 May; **42**(5): 816-823. doi: 10.2337/dc18-1953. Epub 2019 Feb 10. PubMed PMID: 30739885.

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***Koshizaka M, Ishikawa K, Ishibashi R, Maezawa Y, Sakamoto K, Uchida D, Nakamura S, Yamaga M, Yokoh H, Kobayashi A, Onishi S, Kobayashi K, Ogino J, Hashimoto N, Tokuyama H, Shimada F, Ohara E, Ishikawa T, Shoji M, Ide S, Ide K, Baba Y, Hattori A, Kitamoto T, Horikoshi T, Shimofusa R, Takahashi S, Nagashima K, Sato Y, Takemoto M, Newby LK, Yokote K; PRIME-V Study Group.*** Comparing the effects of ipragliflozin versus metformin on visceral fat reduction and metabolic dysfunction in Japanese patients with type 2 diabetes treated with sitagliptin: A prospective, multicentre, open-label, blinded-endpoint, randomized controlled study (PRIME-V study). *Diabetes Obes Metab.* 2019 Aug; **21**(8): 1990–1995. doi: 10.1111/dom.13750. Epub 2019 May 8. PMID: 30993861; PMCID: PMC6767075.

***Fujita M, Nagashima K, Takahashi S, Suzuki K, Fujisawa T, Hata A.*** Handheld flow meter improves COPD detectability regardless of using a conventional questionnaire: A split-sample validation study. *Respirology.* 2020 Feb; **25**(2): 191–197. doi: 10.1111/resp.13602. Epub 2019 Jun 12. PMID: 31188538.

***Suichi T, Misawa S, Beppu M, Takahashi S, Sekiguchi Y, Shibuya K, Amino H, Tsuneyama A, Suzuki YI, Nakamura K, Sato Y, Kuwabara S.*** Prevalence, clinical profiles, and prognosis of POEMS syndrome in Japanese nationwide survey. *Neurology.* 2019 Sep 3; **93**(10): e975–e983. doi: 10.1212/WNL.0000000000008062. Epub 2019 Aug 1. PMID: 31371568.

***Sasaki T, Tsuboi N, Okabayashi Y, Haruhara K, Kanzaki G, Koike K, Kobayashi A, Yamamoto I, Takahashi S, Ninomiya T, Shimizu A, Rule AD, Bertram JF, Yokoo T.*** Estimation of nephron number in living humans by combining unenhanced computed tomography with biopsy-based stereology. *Sci Rep.* 2019 Oct 7; **9**(1): 14400. doi: 10.1038/s41598-019-50529-x. PMID: 31591408; PMCID: PMC6779756.

# Department of Anatomy (Gross Anatomy and Neuroanatomy)

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

## General Summary

Our department research activities have focused on neuroanatomy and gross anatomy. In neuroanatomical research, organizations of neuronal networks and the development are investigated to elucidate brain function and diseases using morphological and electrophysiological methods. Our primary interest is focused on quantitative architecture and dynamics of neural circuits and their relationship. In gross anatomical researches, functional importance is explored on variations of organ systems using 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.

*Spatiotemporal structure and dynamics of spontaneous oscillatory synchrony in the vagal complex*

Fundamental structure and dynamics of spontaneous neuronal activities without apparent peripheral inputs were analyzed in the vagal complex (VC), whose activities had been generally thought to be produced almost passively to peripheral cues. The analysis included the caudal nucleus of the tractus solitarius — a main gateway for viscerosensory peripheral afferents and dynamically and critically involved in cardiorespiratory brainstem networks. In the present study, a possibility of self-organized brain activity was addressed in the VC. While VC neurons exhibited sparse firing in anesthetized rats and in in vitro preparations, we identified peculiar features of the emergent electrical population activity: (1) Spontaneous neuronal activity, in most cases, comprised both respiration and cardiac cycle components. (2) Population potentials of polyphasic high amplitudes reaching several millivolts emerged in synchrony with the inspiratory phase of respiratory cycles and exhibited several other characteristic temporal dynamics. (3) The spatiotemporal dynamics of local field potentials, recorded simultaneously over multiple sites, were characterized by a stochastic emergence of high-amplitude synchrony. By adjusting amplitude and frequency (phase) over both space and time, the traveling synchrony exhibited varied degrees of coherence and power with a fluctuating balance between mutual oscillators of respiratory and cardiac frequency ranges. Full-fledged large-scale oscillatory synchrony over a wide region of the VC emerged after achieving a maximal

stable balance between the two oscillators. Distinct somatic (respiratory; ～1 Hz) and visceral (autonomic; ～5 Hz) oscillators seemed to exist and communicate co-operatively in the brainstem network. Fluctuating oscillatory coupling may reflect varied degrees of synchrony influenced by the varied amplitude and frequency of neuronal activity in the VC. Intranuclear micro-, intrabulbar meso-, and wide-ranging macro-circuits involving the VC are likely to form nested networks and strategically interact to maintain a malleable whole-body homeostasis.

These two brainstem oscillators could orchestrate neuronal activities of the VC, and other neuronal groups, through a phase-phase coupling mechanism to perform specific physiological functions.

## Publications

***Kawai Y.*** Cooperative Phase Adaptation and Amplitude Amplification of Neuronal Activity in the Vagal Complex: An Interplay Between Microcircuits and Macrocircuits. *Front Syst Neurosci.* 2019 Dec 3; **13**: 72. doi: 10.3389/fnsys.2019.00072. eCollection 2019. PubMed PMID: 31849619; PubMed Central PMCID: PMC 6901686.

# Department of Anatomy (Histology and Embryology)

Masataka Okabe, *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 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

*Mucosal vascular networks in the mouse distal colon*

We have previously demonstrated that leakage of plasma protein and bleeding in the lamina propria in the distal colon occur as primary signs in dextran sulfate sodium (DSS)-induced colitis and have suggested that disturbances and disruptions of colonic circulation, including tissue microcirculation, were involved in the pathogenesis of colitis. However, vascular networks in colonic mucosa have not been studied in detail.

In this study, we have attempted to investigate vascular networks in the mouse colonic mucosa by injecting fluorochrome-labelled gelatin into blood vessels and observing 3-dimensionally the whole mount specimen of the distal colon with a confocal laser scanning microscope. The arterial plexus was observed in the submucosa. Arterioles branched off from the plexus pierced the muscularis mucosa and entered the lamina propria. In the deep part of the lamina propria, the arterioles ramified and anastomosed each other to form a vascular plexus around the base of crypts. Some branches from the plexus run around the crypt or upwards among crypts to enter the subepithelial capillary networks. The subepithelial capillary networks were formed by interconnections of hexagonal capillary rings. Venules emerged from the capillary networks run downward and horizontally at the deep part of the lamina propria, where they received venules from the surroundings, and then pierced the muscularis mucosa to pour into the venous plexus in the submucosa.

There were no reports concerning the arterial plexus and venules in the deep part of the lamina propria and no attention has been paid to them. However, the previous findings that mucosal bleeding in DSS colitis originated at blood vessels running in the deep of the lamina propria and that disturbances in microcirculation in the mucosa occurred prior to the disorganization of the mucosal tissue architecture indicate that vasculatures in the deep part of the lamina propria play a pivotal role in maintaining colonic mucosa. A detailed investigation of these vasculatures may contribute to prevent the DSS induced colitis, a model of the inflammatory bowel disease.

*Regeneration of epidermis basal lamina during posterior lateral line development in* Polypterus

The genus *Polypterus* is the most basal extant actinopterygian fish in molecular phylogeny because of scales covered with 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* to investigate an origin of diversity of the lateral line in bony fishes and found that epidermis basal lamina is regenerated 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, as shown with periodic acid-methenamine silver staining and scanning electron microscopy, did not exist just underneath the neuromast, and neurites from the neuron bundle stained with neuron-specific antibodies innervated the neuromast cells within the epidermis. The neuron bundle away from the neuromast was clearly seen underneath the basal lamina, and continuity with the cranial ganglion, suggested that it was the lateral line nerve. Therefore it suggested that the lateral nerve bundles extending from the cranial nerve was innervated to the neuromast cells outside the basal lamina, and that the basal lamina just below the neuromast was regenerated outside the nerve bundles during posterior lateral line development.

*Functional analysis of mouse Glial cell missing 1 gene in kidney*

The glial cell missing (GCM) is a transcription factor conserved from invertebrates to vertebrates and is known to be important for placenta formation in mammals. Deficiency of the glial cell missing 1 (*Gcm1*) in mice causes placental hypoplasia, which is lethal at embryonic day 10. Although *Gcm1* has been reported to be expressed in the kidney, its function remains unclear. We constructed a flox mouse, in which the DNA binding sequence of *Gcm1* was sandwiched between loxP, and crossed it with a mouse in which Wilms tumor 1 (*WT1*)-*Cre* is specifically expressed in the kidney to analyze the kidney of the *Gcm1* conditional knockout mouse. We revealed that *Gcm1* deficiency did not affect renal development and that renal size and function did not differ even after maturation. However, we clarified that fibrosis was significantly reduced in *Gcm1*-deficient kidneys compared with control kidneys when ischemic injury was performed. In addition, we found that the expression of *Tgf-β1*, which is reported to be involved in fibrosis, is decreased in the *Gcm1*-deficient kidney, we finding that suggests that Gcm1 controls the expression of *Tgf-β1* directly or indirectly. We also revealed that cell proliferation was reduced in *Gcm1*-deficient kidney. The analysis of cultured cells showed that *Gcm1* increased the expression of *Tgf-β1*, which might promote cell proliferation. With these experiments, we revealed that *Gcm1* is involved in cell proliferation and fibrosis in renal ischemic injury. This result suggests that controlling *Gcm1* might prevent fibrosis in chronic kidney disease, leading to important results applicable to the treatment of future renal diseases.

*Functional analysis of tenascin C in the induction of DSS enteritis*
Ulcerative colitis (UC) is a diffuse nonspecific inflammation of the large intestine, and abnormalities in intestinal mucosal barrier function are thought to be involved in the disease. Mucosal epithelial cells maintain homeostasis by interacting with stromal cells and the extracellular matrix. We believed that to elucidate the intestinal mucosal barrier mechanism, the extracellular matrix supporting mucosal epithelial and interstitial cells should be analyzed. Therefore, we focus on the extracellular matrix glycoprotein tenascin C (TNC), analyze its relationship with mucosal epithelial damage when intestinal inflammation is induced, and attempt to verify its involvement in the intestinal mucosal barrier mechanism. In this study, we used the DSS-induced colitis mouse, which is a frequently used mouse model of UC, to observe TNC expression during the induction of colitis by immunohistochemical staining. We found that in the normal large intestinal mucosa, TNC is expressed around microvessels in the lamina propria just below the mucosal epithelium and that the distribution of TNC expression changes from the superficial to the deep lamina as inflammation progresses. These findings suggest that TNC functions to suppress inflammation. Currently, the distribution of TNC expression in human UC samples is being verified by immunohistochemical staining. Based on these data, we have clarified the relationships of epithelial cells, stromal tissues, and extracellular matrix and elucidated the homeostatic maintenance mechanism of colonic mucosa.

*Organ size regulation in the lives of zebrafish*
The caudal fin of zebrafish develops in a fan-like shape, grows in a different (bi-lobed) shape during juvenile stages, and becomes larger throughout adult stages. To investigate the mechanisms of fin shape regulation, we measured bone lengths of caudal fins and standard body lengths (from the tip of the mouth to tail vertebrae) at several growing periods. We found the growth-changing point: there was positive-allometric growth until an early juvenile stage (approximately 7.0 mm standard length), and isometric growth occurred after the 7.0 mm standard length stage. To analyze messenger RNAs and microRNAs expression we collected fin tissues around the point of time when growth patterns changed and found that the muscle segment homeobox gene (*msxb*) and 2 microRNAs were highly expressed molecules at an allometric growth stage and that the TTK family protein kinase gene (*mps1*) and 2 other microRNAs were highly expressed molecules at an isometric growth stage.

*The role of chorion-specific transcription factor* GCM1*in* Polypterus
Glial cells missing 1 (GCM1) is a transcription factor that is required for development of the trophoblast cells of chorion in mammals. GCM1 is a remarkable trigger for placental evolution, but the functions and spatial expression patterns of *Gcm1* in other vertebrae is unknown. We recently found that glial cells missing 1 gene (*Gcm1*) is conserved in the genome of the extant actinopterygian fish, *Polypterus*. This finding suggests that the origin of cells expressing *Gcm1* go back from the early branched group of ray-finned fishes to mammals. Therefore, we investigated the gene expression of *Polypterus* by whole-mount *in situ* hybridization with a *Gcm1* RNA probe. We found that *Gcm1* is expressed in scattered cells in the skin of external gills and in the yolk sac membrane. We also revealed

with transmission electron microscopy that these cells contain characteristic large vacuoles in the cytoplasm. These new findings suggest that cells expressing *Gcm1* might be ionocytes, which are present in most fishes to maintain body fluid ionic and osmotic homeostasis. Further analyses, such as mass spectrometry, will reveal the function of *Gcm1*-expressing cells in *Polypterus*.

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# Department of Molecular Physiology

Shigeru Takemori, *Professor*
Maki Yamaguchi, *Associate Professor*
Toshiko Yamazawa, *Associate Professor*

## General Summary

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

## Research Activities

*Functional water in skeletal muscle evaluated with differential scanning calorimetry*

Magnetic resonance images reflect not only water content but also water states in physiological tissue. By taking advantage of well-organized skeletal muscle, we have previously clarified that magnetic resonance can be used to distinguish localized water clusters of 5 states. Although water state in the tissue are not clarified yet in detail, interaction between water and macromolecules is generally considered to restrict motional freedom of the water molecules similarly to the freezing of water. As is the case with melting of ice, differential scanning calorimetry can represent the restriction as extra heat absorption at a certain temperature.

Differential scanning calorimetry measurement with skinned fibers of the sartorius muscle from bull frogs at a rigor condition showed extra heat absorption at −24, −21, 0, 46, and 65 degrees centigrade. The peaks at 46˚C and 65˚C would represent denaturation of myosin and actin filaments, respectively, because selective removal of myosin or actin filaments diminished the corresponding peaks and because the temperature values are close to those reported for the denaturation temperature of corresponding proteins from rabbit psoas muscle. The heat 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 and actin filaments. Integrated heat capacity in the range from −80˚C to +20˚C was decreased by denaturation of actin. The integrated heat capacity per protein mass, after the solution mass was subtracted, was 150% of the control muscle with myosin removal and 25% of the control muscle with actin removal. These results suggest that actin and myosin independently and cooperatively restrict surrounding water and that extra integrated heat capacity mainly depends on actin filaments.

*Insights into channel modulation mechanism of skeletal muscle type ryanodine receptor mutants using $Ca^{2+}$ imaging and molecular dynamics*

Type 1 ryanodine receptor (RyR1) is a $Ca^{2+}$ release channel in the sarcoplasmic reticulum in skeletal muscle and plays an important role in excitation-contraction coupling. 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. So

far, more than 300 mutations in *RyR1* have been reported in patients with malignant hyperthermia. However, due to a lack of comprehensive analysis of the structure-function relationship of mutant RyR1, the mechanism remains largely unknown. Thus, we combined functional studies and molecular dynamics simulations 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 simulations of wildtype and mutant RyR1s were performed using crystal structure of the N-terminal domain (NTD) monomer, consisting of A, B, and C domains. We found that the mutations located around interdomain regions differentially affected hydrogen bonds/salt bridges. Particularly, mutations at R402, which increase the open probability of the channel, cause clockwise rotation of the B and C domains with respect to the A domain by altering the interdomain interactions. Similar results were obtained with artificial mutations that mimic alteration of the interactions. Our results reveal the importance of interdomain interactions within the NTD in the regulation of the RyR1 channel and gain insights into the mechanism of malignant hyperthermia caused by mutations at the NTD.

*Effect of homogenate extract of adult skeletal muscle on expression pattern of myosin heavy chain*

In adult skeletal muscle, satellite cells are maintained in a state of quiescence until tissue damage or other stimulus causes these cells to become activated, to proliferate, and, subsequently, to differentiate into mature myofibers. Meanwhile, myosin heavy chains (MyHCs) play a critical role in contractility and have various fiber types, such as slow (I), fast (IIa, IIb, and IIx), and extraocular specific types. Previous studies are unclear as to whether activated satellite cells from adult muscle are predisposed organogenetically to express the phenotype of their founder muscle or they differentiate to a standardized post-mitotic stage dependent on extrinsic signals from the host muscle for determination of mature phenotype. To address this issue, satellite cells from mouse extraocular muscles, the diaphragm, and various hindlimb muscles were isolated, expanded, and differentiated under laminin i211-coated culture dishes. We harvested cells during 3 distinct time courses, which were: without differentiation, 3 days after differentiation, and 8 days from differentiation. Proliferating myoblasts and differentiated myofiber cultures were analyzed via sodium dodecylsulfate-polyacrylamide gel electrophoresis and mass spectrographs for expression of MyHCs. The MyHC profile of differentiated primary satellite cells was equivalent across all cultures with embryonic type MyHC and MyHC cardiac beta (MYH7B). Interestingly, slow type MyHC was expressed in satellite cells from the diaphragm but was in satellite cells from extraocular muscle or hindlimb muscles. Mass spectrographs showed expression of nonmuscle MyHC 2A (NM2A) across all cultures. These results suggest that differentiation of myofibers from satellite cells is similar to that of myogenesis and that the determination of phenotypes is due to intrinsic factors or extrinsic factors or both. The expression of NM2A indicates that NM2A plays a major role in myotube fusion.

*Effect of homogenate extract of adult skeletal muscle on proliferation and differentiation of myoblasts*

Residing next to mature skeletal muscle fibers, satellite cells are known to serve as the progenitor of myoblasts when triggered by various stimuli, including damage to the muscle fibers *in vivo*. The homogenate extract of various types of adult chicken skeletal muscles was reported to induce differentiation of primary culture of myoblasts prepared from chicken embryo to express MyHC isoforms of the source skeletal muscle of the extract. Inspired by this report, we examined the effect of homogenate extract from adult mouse skeletal muscle on primary cultures of satellite cells prepared from young mice. Unexpectedly, the extract seemed to enhance proliferation of the satellite cells without inducing obvious differentiation in the expression pattern of MyHCs. However, the extract's effect on satellite cell proliferation was unclear because of cellular contaminants, such as fibroblasts and adipogenetic cells. Therefore, we examined the effect of muscle extract on C2C12 myoblasts. The proliferation of C2C12 myoblasts was evaluated with the cytotoxic assay method. The extract was found to dose dependently enhance C2C12 myoblast proliferation confirming the stimulating effect of the extract on the growth of the myogenic precursor cells.

## Publications

***Yamazawa T, Ogawa H, Murayama T, Yamaguchi M, Oyamada H, Suzuki J, Kurebayashi N, Kanemaru K, Oguchi K, Sakurai T, Iino M.*** Insights into channel modulation mechanism of RYR1 mutants using $Ca^{2+}$ imaging and molecular dynamics. *J Gen Physiol.* 2020 Jan 6; **152**(1): e201812235. doi: 10.1085/jgp.201812235. PMID: 31841587; PMCID: PMC7034096.

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# Department of Cell Physiology

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Masato Shibuya, *Visiting Professor*
Yoichiro Kusakari, *Associate Professor*
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Norio Fukuda, *Associate 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, metabolomic changes in the diseased heart, 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 vein. In addition, we developed a high-precision analyzing system to measure the temperatures of cells.

## Research Activities

*Analysis of characteristics of pulmonary veins*
Pulmonary veins must have a character that differs from that of systemic veins due to containing a high concentration of oxygen in blood. However, the characteristics of pulmonary veins remain a mystery. We have generated the atrium-specific overexpression of paired-like homeodomain transcription factor 2 (Pitx2c), a transcription factor that is specifically expressed in the pulmonary veins and the left atrium. We found that the transgenic mice exhibited sinus node dysfunction using a telemetric electrocardiographic analysis system.

*Molecular mechanism of closure of the DA*
The DA is an essential artery that connects the main pulmonary artery and the descending aorta in a fetus. The DA closes immediately after birth in accordance with its smooth muscle contraction and vascular remodeling. We found that a new chemical compound EP4 antagonist, RQ-15986 (renamed from CJ-042794), selectively contracted the DA when we measured the isometric tension of rat DA in the presence of RQ-15986. In addition, we investigated the effect of gentamicin on DA closure. We found that standard-dose gentamicin did not increase the risk of DA patency in rat neonates. Furthermore, we examined the transcriptional profiles of chick DA, which anatomically differs from mammal DA. A DNA microarray analysis revealed newly identified genes in the chicken pulmonary artery-sided DA. Subsequent pathway analysis with the Database for Annotation, Visualization and Integrated Discovery (DAVID) Bioinformatics Resources revealed that the pulmonary artery-sided DA showed enhanced expression of the genes involved in melanogenesis and tyrosine metabolism, suggesting that tyrosinase and the related genes play an important role in the proper differentiation of neural crest-derived cells during vascular remodeling in the chick DA. These projects are a collaborative work with Waseda University.

*Regulation of cardiac metabolism*
Cardiac metabolism plays an essential role in maintaining cardiac function. Fatty acid oxidation is impaired and glycolysis is promoted in the damaged heart. We used 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 one of the earliest events 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 an isometrically contracting muscle preparation from rat right ventricular papillary muscles. We found that acute severe overstretch of an isolated rat papillary muscle caused inner mitochondrial collapsing with preserved sarcomere structure. We are now checking these phenomena in in-vivo experiments using a pulmonary banding model.

*Mechanism of sarcomere contraction in cardiac muscle*
Sarcomeres are activated via thin filament structural changes (i.e., from the "off" state to the "on" state) in response to a release of $Ca^{2+}$ from the sarcoplasmic reticulum. This process involves chemical reactions that are highly dependent on ambient temperature. We investigated the effects of rapid heating by focused infrared laser irradiation on the sliding of thin filaments reconstituted with human α-tropomyosin and bovine ventricular troponin in an in-vitro motility assay. We performed high-precision analyses measuring temperature by the fluorescence intensity of rhodamine-phalloidin-labeled F-actin coupled with a fluorescent thermosensor sheet containing the temperature-sensitive dye europium (III) thenoyltrifluoroacetonate trihydrate. This approach enabled a shift in temperature from 25°C to∼46°C within 0.2 second. We found that in the absence of $Ca^{2+}$ and the presence of ATP, infrared laser irradiation elicits sliding movements of reconstituted thin filaments with a sliding velocity that increases as a function of temperature. The heating-induced acceleration of thin filament sliding likewise occurs in the presence of $Ca^{2+}$ and ATP; however, the temperature dependence is more than twofold less pronounced. These findings might indicate that in the mammalian heart, the "on-off" equilibrium of the cardiac thin filament state is partially shifted toward the "on" state in diastole at physiological body temperature, enabling rapid and efficient myocardial dynamics in systole.

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# Department of Biochemistry

Kiyotsugu Yoshida, *Professor*

## 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 between 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

*Carbonic anhydrase 13 suppresses bone metastasis of breast cancer cells*
Metastatic progression is the leading cause of mortality in breast cancer. However, the molecular mechanisms that govern this process remains unclear. A line of breast cancer stem cells—induced cancer stem cell-like 10A (iCSCL-10A) cells—was established by introducing reprogramming factors (octamer-binding transcription factor 4 [OCT4], sex determining region Y-box 2 [SOX2], Kruppel-like factor 4 [Klf4], and c-Myc) into MCF-10A nontumorigenic mammary epithelial cells. The iCSCL-10A cells display a malignant phenotype and form tumors when injected into immunodeficient mice and possess the hallmarks of cancer stem cells. However, the metastatic ability of iCSCL-10A cells and a potential metastatic model for breast cancer has not yet been reported. Here, we found that carbonic anhydrase 13 (CA13) has the potential to suppress bone metastasis of iCSCL-10A breast cancer stem cells. The iCSCL-10A cells possess the hallmarks of cancer stem cells and indeed exerted the ability of bone metastasis in the hind limbs of mice in 5 weeks after injection, whereas no metastasis was observed in those of mice injected with control MCF-10A cells. Transcriptome analysis indicate that the expression of several genes involved in cell adhesion, signaling, and metabolism was reduced in bone metastatic iCSCL-10A cells. In-vitro and in-vivo analysis determined that overexpression of CA13 in iCSCL-10A cells suppressed migration, invasion, and bone metastasis. Furthermore, we found that breast cancer patients with low CA13 expression had a significantly shorter overall survival. These findings indicate that CA13 might act as a novel prognostic biomarker and therapeutic candidate for the prevention of bone metastasis of breast cancer.

*DYRK2-null mouse recapitulates VATER/VACTERL association with lung hypoplasia*
Congenital malformations are a major issue in pediatric healthcare and the leading cause

of infant mortality in the United States. Most rare congenital malformations, such as VATER (vertebral anomalies, anal atresia, tracheoesophageal fistula and/or esophageal atresia, and radial dysplasia)/VACTERL (VATER plus cardiac defects and limb defects) association, Alagille syndrome, and CHARGE (coloboma, heart defect, atresia of the choanae, retardation, and genital and ear abnormalities) syndrome, have multiple component features, and some are known to involve genetic mutations. The genetic knockout of causal genes in mice often reproduces congenital malformations, providing extremely valuable models for the study of rare pediatric diseases. However, there is a lack of adequate animal models recapitulating rare congenital diseases. We have previously shown that dual-specificity tyrosine-phosphorylation-regulated kinase 2 (DYRK2) exerts antitumor effects in various cancer cells. However, the effect of DYRK2 gene ablation during embryogenesis has not been previously investigated. In this study, we report the generation of DYRK2-deficient mice using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) nickase system. We found that the phenotypes of DYRK2-deficient mice recapitulated those of VATER/VACTERL human congenital malformations. Transcriptome analysis indicated close similarities between the molecular phenotypes of VATER/VACTERL association and DYRK2-deficient mice, particularly with respect to *Foxf1* reduction. Mutant pups died soon after birth owing to respiratory failure, a feature secondary to VATER/VACTERL components. Detailed analyses of primordial lungs during early development demonstrated that DYRK2 deficiency leads to altered airway branching and insufficient alveolar development. Furthermore, the Foxf1 expression gradient in mutant lung mesenchyme was disrupted, reducing Foxf1 target genes, which are necessary for airway and alveolar development. Taken together, our results confirm the establishment of a novel DYRK2-deficient mouse model that recapitulates the pathological and molecular phenotypes of VATER/VACTERL association with lung hypoplasia. Collectively, these findings provide new insights into VATER/VACTERL association.

*Molecular functions of DYRK2 during mammalian tissue development*

In this study, we aimed to elucidate the function of dual-specificity tyrosine-regulated kinase 2 gene (*Dyrk2*), which is a key regulator of p53 in response to DNA damage. In 2019, we researched the following issues: (1) the functions of DYRK2 during mammalian tissue development, (2) the functions of DYRK2 in tissue/cancer stem cells, and (3) the antitumor effect of DYRK2 in colorectal cancer.

1. The 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. However, little is known regarding the molecular functions of DYRK2 during mammalian tissue development. In this study, to identify molecular functions of DYRK2 in tissue development, we analyzed *Dyrk2* knockout (*Dyrk2*$^{-/-}$) mice. In 2019, we identified a candidate signaling by analyzing *Dyrk2* $^{-/-}$ mice and mouse embryonic fibroblasts. We would like to analyze the molecular functions of DYRK2 in the identified signaling.

2. The functions of DYRK2 in tissue/cancer stem cells:
In the process of tumorigenesis, tissue stem cells are known to transform into cancer-initiating cells. We have demonstrated DYRK2 localized in tissue stem cells in several tissues. In 2019, to analyze the functions of DYRK2 in cancer-initiating cells, we developed conditional *Dyrk2*-knockout mice specifically in leucine rich repeat containing G protein coupled receptor 5 gene (*Lgr5*)-expressing cells (*Dyrk2*$^{flox}$; *Lgr5*-CreERT2-IRES-EGFP mice). We would like to analyze these conditional *Dyrk2*-knockout mice in the process of tumorigenesis.
3. The antitumor effect of DYRK2 in colorectal cancer:
In colorectal cancer cell lines, we recently reported that knockdown of the dual-specificity tyrosine-phosphorylation-regulated kinase 2 gene (*DYRK2*) induces proliferation in vitro. In this study, we aimed to examine whether a forced expression of DYRK2 has a potential for novel gene therapy against cancer. We developed a xenograft model of colorectal cancer cell lines and are analyzing the effects of DYRK2-overexpression by adenovirus-infection in proliferation and apoptosis.

*Subcellular localization of Ser/Thr kinases*
Liver cancer has a high mortality rate. Although surgical resection has been recognized as the only curative treatment when liver cancer is at an early stage, this cancer is diagnosed in a majority of patients at an advanced stage, when present therapies are ineffective. Until now, few tumor markers can be used to discriminate liver cancer with high sensitivity and specificity. Therefore, novel biomarkers should be developed to predict initiation or progression of liver cancer. Recently, we found that novel localization machinery by which several nuclear trafficking proteins were translocated into the extracellular space. We established a proteomics approach to detect nuclear trafficking proteins from extracellular fluid of liver cancer and identified protein kinase C delta (PKCδ), which is known to be involved in various signaling events. We detected PKCδ in the culture media of several liver cancer cell lines and the sera of tumor model mice. Furthermore, serum levels of PKCδ were significantly higher in patients with liver cancers than in either healthy donors or high-risk group patients for liver cancers (chronic hepatitis and hepatic cirrhosis). We have also found that the extracellular secretion of PKCδ was induced by distinct secretion mechanism from that of other liver cancer markers (alpha-fetoprotein and proteins induced by vitamin k antagonism or absence [PIVKA] II) in living liver cancer cells. Furthermore, we revealed that extracellular PKCδ augmented phosphorylation of the growth signaling, such as extracellular signal-regulated protein kinase (ERK) 1/2 and signal transducer and activator of transcription (STAT) 3, which results in increased tumor growth. These results demonstrate a novel function of PKCδ as a secretory growth factor and suggest that serum PKCδ might be an active biomarker for liver cancer.

*Gene silencing with CRISPR/Cas9 method*
Gene silencing is the useful method for elucidating the function of a particular gene. Conventional methods using RNA interference are insufficient as experimental systems because (1) complete repression of expression is not possible and (2) recovery of expression is observed in many cases. Recently, genome editing methods have been used to sup-

press the expression of specific genes, and these methods are now being used in various fields. Therefore, we have also used the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) gene-editing system in an attempt to disrupt specific genes in cancer cell lines. Although several methods can be used for gene disruption with the CRISPR/Cas9 method, we estimated the efficiency of gene disruption in lentiviral vector systems and plasmid vector systems. First, the lentiCRISPR v2 plasmid was transfected into 293T cells along with the packaging vectors to obtain particles of the lentiviral vector. Next, these particles were infected with various cancer cell lines at a multiplicity of 1, and puromycin-resistant cells were cloned. Whereas these clones were analyzed, many of them did not undergo genome editing and were unable to disrupt their genes. In contrast, when the lentiCRISPR v2 plasmid was transfected into cells by lipofection and subjected to strong puromycin selection, genome editing occurred in a majority of surviving cells. These differences in the efficiency of genome editing might be caused by differences in the amount of Cas9 protein expressed in the cell.

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# Department of Molecular Biology

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## General Summary

The aims of our research are to clarify the mechanism and biological significance of polyamines and their elaborate regulatory system and to develop polyamine-related medical applications. Polyamines are ubiquitous biogenic amines and are essential for cell proliferation. They are related to various phenomena, such as differentiation, development, cancer, and autophagy. The latest studies have shown that polyamines have effects on longevity, memory, and arteriosclerosis. Three major polyamines—putrescine, spermidine, and spermine—are present in mammalian cells. Ornithine decarboxylase (ODC) is a key enzyme of polyamine biosynthesis in mammalian cells. The role of ODC is to convert ornithine to putrescine, which in turn leads to spermidine and spermine. To be degraded, ODC interacts with antizyme (AZ). Three AZ isoforms (AZ1-3) are present in mammals. The AZs are expressed by translational frameshifting that is induced by polyamines and negatively regulate cellular polyamines. Cellular polyamine contents are maintained by the feedback mechanism involving AZ. The AZs are further regulated by proteins termed antizyme inhibitors (AZINs).

## Research Activities

*The role of antizyme 2 in neuroblastoma tumor growth*
We have previously found that polyamine regulating protein AZ2 accelerates the ubiquitin-independent degradation of protooncogene MYCN which is a factor for the poor prognosis of patients with neuroblastoma. Last year, we revealed, with a colony formation assay in soft agar and xenograft mouse model experiment, that knockdown of AZ2 facilitates neuroblastoma tumor growth. This year, comprehensive analysis was performed with RNA sequencing of gene expression in an AZ2 knockdown neuroblastoma cell line. In AZ2 knockdown cells, mRNA expression of the Fos and Jun families, which are strongly related to cell growth, differentiation, and survival, were increased, whereas expression of effector and initiator caspases, which are deeply involved in apoptosis, were significantly decreased. These results suggest that AZ2 knockdown enhances cell growth in a neuroblastoma cell line by upregulating cell growth-related genes and downregulating apoptosis-related genes.

*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, which is used for lipid anabolism or acetylation of cellular components by the screening for AZ-binding proteins. We have recently reported that AZ1 and AZ2 bind to and activate ACLY in cancer cells. However, the significance of ACLY activity on

polyamine metabolism was unclear, despite AZ being a negative regulator of cellular polyamines. We are continuing the study for the crosstalk between polyamines metabolism and ACLY through the function of AZ. The 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 extracellular acetyl-polyamines of ACLY-overexpressed cells were measured. Contrary to expectations, both intracellular and extracellular acetyl-polyamines were significantly increased. These results indicate that a factor other than ACLY is needed for the export of acetylated polyamines.

*Translation efficiency affects the sequence-independent +1 ribosomal frameshifting by polyamines*

Synthesizing the functional AZ protein requires transition of the reading frame at the termination codon. We have reported that spermidine has the potential to shift the reading frame in the +1 direction in any sequence using a human cell-free translation system. The probability of this promiscuous +1 frameshifting by spermidine has an inverse correlation with the efficiency of translation. This sequence-independent +1 frameshifting can also be induced by putrescine and spermine, although the dose required for +1 frameshifting was quite different from that required by spermidine. These results suggest that polyamines potentially induce the sequence-independent +1 frameshifting. The polyamine-dependent +1 frameshifting was also detected in translation with *in vitro* transcribed RNA templates in place of DNA templates, supporting that +1 frameshifting in this *in vitro* protein expression system occurred during translation.

*Polyamines involved in respiratory function*

The extracellular polyamine concentration is about 0.1% to 1% of the intracellular polyamine concentration. Because polyamines are present in all organisms and in all cells, most polyamine research has focused on the intracellular function of polyamines. We found that polyamines are present in alveoli. To investigate the effects of polyamines on respiratory function, the effects of polyamines were examined with a lavage model established as a rat model of acute respiratory distress syndrome. We found that polyamines administered to the alveoli improve lung compliance, arterial blood oxygenation, and lung aeration. Many patients with acute respiratory distress syndrome die in the absence of effective treatment. Now we are continuing the verification toward the practical application to patients.

## Publications

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# Department of Pharmacology

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## General Summary

The research interests of the Department of Pharmacology include:
1. Synaptic transmission and its modulation in the basal ganglia and basal forebrain (Toshihiko Momiyama)
2. Peripheral benzodiazepine receptors on adrenal cells (Yuji Ohno)
3. Mast cells and homeostasis: involvement in melatonin synthesis (Haruhisa Nishi)
4. Analysis of the cerebro-cerebellar interaction using optogenetics (Taro Ishikawa and Misa Shimuta)
5. Mild hypothermia-mediated neuroprotection for experimental ischemia through adenosine receptors (Masahito Kawamura)
6. Theoretical characterization of chemical probes used in the study of synaptic transmission (Yukihiro Nakamura)
7. 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, its modulation, and developmental changes in the nigrostriatal or mesolimbic dopaminergic system and in the cholinergic system of the basal forebrain. These systems are involved in various psychological functions and disorders, including Parkinson's disease and Alzheimer's disease. Furthermore, optogenetic activation techniques for neurons in these areas of the brain have been introduced to analyze neuron type-specific synaptic transmission and its modulation by dopamine, serotonin, and muscarinic acetylcholine receptors. These basic analyses can lead to the identification of mechanisms underlying the related disorders mentioned above and to the development of novel therapeutic tools.

*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.

*Mast cells and homeostasis: involvement in melatonin synthesis*

Some studies suggest that mast cells release melatonin, which might play key roles in the prevention of viral and bacterial infections and the development of tumors. The present study focused on 2 enzymes for melatonin synthesis in mast cells because of their roles in the immune response. Messenger (m) RNA expression from LAD2, a human mast cell-derived cell line, was examined for key enzymes in melatonin synthesis. The LAD2 cells were positive for mRNA expression of both enzymes. The mRNA levels were enhanced by cyclic adenosine monophosphate elevation without LAD2 activation. In contrast, an increase in calcium did not enhance mRNA levels but did activate LAD2. These results suggest that melatonin release from mast cells is involved in maintaining homeostasis and is not involved in allergic responses.

*Analysis with optogenetics of the cerebrocerebellar interaction*

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 in transgenic mice whose cerebral cortex can be suppressed by light illumination. We found that direct signals from the trigeminal nucleus and indirect signals via the primary somatosensory cortex are integrated in both Purkinje cells and granule cells in the cerebellar cortex. We also found that spontaneous signals are transmitted from the somatosensory cortex to the cerebellum.

*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 has not been determined. 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 suggests that adenosine is involved in the therapeutic hypothermia (usually 32°C to 33°C) for acute stroke.

*Theoretical characterization of chemical probes used in the study of synaptic transmission*

Although chemical probes are essential in cellular biology, they are often used without a correct understanding of their characteristics and mechanisms of action. Using numerical simulations, we examined the properties of the two chemical probes used in the study of synaptic transmission.

The Ca chelator ethyleneglycol bis-(β-aminoethylether) N,N,N',N'-tetraacetic acid (EGTA) is widely used to probe the coupling distance between voltage-gated Ca channels and vesicles in the active zone. Our simulation showed that the effectiveness of EGTA also depends on factors other than the distance. We made the calibration curves for EGTA.

Due to slow dissociation rate constant, fluorescence signal from glutamate probe EOS

does not directly show the time course of the glutamate concentration transient. Deconvolution of EOS fluorescence is being developed to make more precise measurement of glutamate release.

*Cholinergic modulation of central synaptic transmission*

Acetylcholine is a neurotransmitter involved in learning and memory. In the central nervous system, several studies have shown that acetylcholine modulates the synaptic transmission and the firing property of neurons. We used an electrophysiological technique to elucidate the cholinergic modulation in adult mice striatum. We have found that GABA release from striatal medium spiny neurons onto cholinergic interneurons is inhibited by carbachol application.

## Publications

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# Department of Pathology

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Hiroyuki Takahashi, *Professor*
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Tohru Harada, *Assistant Professor*
Yasuhiko Endo, *Assistant Professor*
Takako Kiyokawa, *Professor*
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Shigeharu Hamatani, *Associate Professor*
Tomoe Lu, *Assistant Professor*
Kazumasa Komine, *Assistant Professor*

## General Summary

The aim of our study in the Department of Pathology is to examine the causes of disease on the basis of morphology and morphological changes. We studied autopsied, surgically resected, or biopsied human body materials. These materials were examined by using several techniques, including optical microscopy, electronic microscopy, morphometry, immunohistochemical staining, and molecular pathological measurements.

## Research Activities

*Research on the gastrointestinal tract*

1. Examination of predictive factors for lymph-node metastasis in patients with early colorectal cancer

The incidence of lymph-node metastasis (LNM) in patients with colorectal cancer invading the submucosa (pT1 colorectal cancer) is 10% to 15%. We examined the association between histopathological factors and lymph-node metastasis in 339 consecutive patients with pT1colorectal cancer who were treated in Shizuoka Cancer Center. Lesions were classified as polypoid growth type and non-polypoid growth type to clarify problems associated with submucosal invasion depth (SID) as defined by treatment guidelines for colorectal cancer. We prepared an algorithm excluding SID to predict LNM; LNM was found in 37 (10.9%) of 339 lesions. In our algorithm, LNM was found in 36 (16%) of 249 lesions with at least 1 of 3 factors, consisting of non-polypoid growth type, lymphatic invasion, and 2/3rds of sprouting. Of the 90 lesions with no factors, LNM was found in only 1 lesion (1%). These results showed that the use of our algorithm excluding SID allowed lesions to be classified into a high-risk group or a low-risk group.

2. Examination of appropriate handling of surgically resected specimens of colorectal cancer

How surgical materials of colorectal cancer should be handled is defined in detail by the Japanese Classification of Colorectal, Appendiceal, and Anal Carcinoma (JCCRC). In the diagnosis of advanced pT4a stage colorectal cancer, however, some lesions cannot be detected with a method for sampling of colorectal cancer as defined in the JCCRC. In the JCCRC, pT4a is defined as tumors infiltration of serosa and extra-serosa (SE). We carefully examined the sites of SE tumors in 44 patients with a diagnosis of pT4a colorectal cancer (patients who had undergone surgery at the Shizuoka Cancer Center). The sites of the occurrence of SE tumors were classified as the mesenteric or antimesenteric side of the colon. In 44 patients, the SE tumor was identified in 80 sections: 30 sections (38%) in

the serosa of the mesentery and 50 sections (62%) of the antimesenteric side. We carefully examined the distribution of the antimesenteric side and found SE tumors around the antimesenteric side in 25 sections (50%), indicating that SE tumors more frequently occurred at such sites. Grade PT4a colorectal cancers were difficult to assess accurately with a method for splitting the long axis of the large intestine in parallel, as recommended by the JCCRC. A method for vertically splitting the long axis of the large intestine which emphasizes the positional relation between lesions and the mesentery was considered useful.

*Research on the urogenital organs*

1. Continuing from the previous year, we compared the prognostic ability of clinicopathological factors before surgery in patients with prostate cancer. In 2019, we compared the prognostic ability of postoperative pathological findings as clinicopathological factors in 224 patients with intermediate-risk prostate cancer whose preoperative Gleason score was 3 + 4 = 7. Consequently, the percentage of Gleason pattern 4 on biopsy was an independent predictive factor for prostate cancer with a poor postoperative prognosis.

*Research on female genital organs*

1. About cervical adenocarcinoma

1) We demonstrated that immunohistochemical staining of trefoil factor 2 is useful for the histological diagnosis of gastric type adenocarcinoma, a representative type of uterine cancer unrelated to human papilloma virus.

2) The new classification we proposed at the meeting in the International Endocervical Adenocarcinoma Criteria and Classification, was reflected in the World Health Organization classification, which was revised and reissued in 2020. We clarified the relation of outcomes to a microcystic, elongated, and fragmented pattern of invasion in each histologic type of cervical adenocarcinoma.

3) We summarize morphological characteristics of invasive stratified mucin-producing carcinoma, a histological type that was newly advocated by International Endocervical Adenocarcinoma Criteria and Classification as described above.

2. Regarding outcomes of clear-cell carcinoma of the ovary, the following results were obtained, and each was published in a journal

1) In patients with deletion of AT-rich interactive domain-containing protein 1A, treatment with gemcitabine might improve outcomes (Gynecol Oncol 2019, 155: 489-498).

2) The expression of hepatocyte nuclear factor 1 homeobox B may be related to outcomes after chemotherapy (Clin Cancer Res 2019, 25: 3962-3973).

*Research on the respiratory system*

The deletion of regions, including 3p24, 3p12, 3p22.2, 3p25.3, and 3p14.2, in the developmental process of small-cell lung cancer and its significance

1. The background and objectives were as follows: A known culprit tumor suppressor gene in the short arm of the chromosome 3 (3q) region is inactivated at an early stage, and other chromosomal changes and gene alterations occur at an advanced stage. This is the most powerful theory about the development of smoking-associated lung cancers, such as squamous-cell carcinoma and small-cell cancer. However, culprit genes associ-

ated with the development and progression of lung cancer in the 3p region remain unknown. Microsatellite instability (MSI) analysis has been known as the most powerful tool for examining the localization of target genes using microsatellite markers that exist in or near DNA sequences on a chromosome. Last year, we used this analytical technique and found that tumor suppressor genes associated with the development of lung squamous epithelium might be located in the 3p22 region. This year, we performed a study to clarify the relation between the developmental process of small-cell cancer, another smoking-associated lung cancer, and 3p chromosomal changes.
Materials and methods were as follows: ***
Cancerous tissue and noncancerous tissue were collected from unstained formalin-fixed paraffin-embedded sections using a microdissection method in 21 surgically resected specimens of small-cell lung cancer. From these tissues, DNA was extracted. In Japanese or other Asians, 18 heterozygous microsatellite markers existing in all 3p regions were selected, and an MSI analysis was performed. Furthermore, regarded as a control group were 21 patients with pulmonary large-cell neuroendocrine carcinomas that were histopathologically classified as the same category as neuroendocrine tumors. The same analysis was performed in the control group. In the control group, the mean incidence of MSI was 27% in 18 markers for large-cell neuroendocrine cancer and 54% in 18 markers for small-cell neuroendocrine cancer. Regions in which chromosome depletion frequently occurred in the developmental process of small-cell cancer were 3p24, 3p12, 3p22.2, 3p25.3, and 3p14.2. This result suggested that tumor suppressor genes associated with the development of small-cell lung cancer might be present in several 3p regions.

*Research of the liver, the gallbladder, and the pancreas*
1. In general, the degree of fibrosis in the portal region (such as the presence or absence of bridging) can be used as an indicator of the basic structure of liver tissue. However, information about the biliary system is not reflected. Therefore, we observed the liver to examine how the connection from the hepatic cell cord and the normal liver lobules to the narrow bile duct and interlobular bile duct in the portal region are impaired. As materials, 166 liver biopsy specimens were obtained from patients with various liver diseases, including chronic hepatitis. CD10 staining was performed to visualize the bile canaliculi, and CK7 staining was performed to visualize the narrow bile duct and the interlobular bile duct. We observed the maintenance status of the biliary system and found that the state of the bile canaliculus and the proliferation of the narrow bile duct are influenced by various illnesses and underlying diseases, leading to structural changes. At present, accumulated data are being analyzed. Finally, we will submit a paper.
2. About tumor immunity in patients with pancreatic cancer
Cases of cancer with a histologically confirmed tertiary lymphoid follicles (lymphoid follicles around cancer, LFC) are considered to have good outcomes. Pancreatic cancer generally has a poor outcome. However, LFC formation is a good prognostic factor for pancreatic cancer, although LFC where the tumor is located has been confirmed in several patients. Such LFCs are considered to control immune reactions, similar to a secondary lymphoid follicles. However, the formation and maintenance mechanism remain unclear. In the present study, we approached the mechanism of LFC formation in patients with

pancreatic cancer from the aspects of immunology and of pathology on the basis of morphological observations. We performed immunohistochemical staining with paraffin-embedded sections obtained from patients who had pancreatic cancer with histologically confirmed LFC formation. We found that CXC motif ligand 13 (CXCL13), a chemokine that induces lymphoid tissue derived cells, frequently accumulated around the blood vessels in the stroma of the lymphatic device. Recently, the role of heparan sulfate on the accumulation of chemokines has being elucidated. Particularly, heparan sulfate arrays with high affinity for CXCL13 have been reported. In patients with pancreatic cancer, specific heparan sulfate arrays are considered to be expressed around blood vessels as a background factor for CXCL13 accumulation required for LFC formation. The goal of our study was to investigate this phenomenon by examining specimens from patients with pancreatic cancer. The following 2 approaches were used to prove that specific heparan sulfate arrays are expressed in LFC: (1) a method for visualizing the expression of specific heparan sulfate arrays on tissue sections and (2) a method proving that the production of these heparan sulfate arrays are promoted at the cellular level.

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# Department of Virology

Kazuhiro Kondo, *Professor* Nobuyuki Kobayashi, *Assistant Professor*

## General Summary

Human herpesvirus (HHV) is capable of establishing a lifelong latent infection of their host, is reactivated frequently. We are studying the molecular mechanism of latency and pathogenesis of human cytomegalovirus (HCMV) and HHV-6, and find a novel latent protein of HHV-6 which associate with and mood disorders. We are also trying to apply HHV-6 and HHV-7 to the tools for studying the mechanism of fatigue. Salivary amounts of HHV-6 and HHV-7 DNA increased with training and decreased with rest, suggesting the usefulness of viral DNA as biomarkers of physiological fatigue and cancer-related fatigue.

## Research Activities

*Posttranscriptional regulation of HHV-6 immediate-early 1 and 2 genes by immediate-early 2 and splicing factor squamous-cell carcinoma antigen recognized by T cells 3*

Background: Herpesviruses operate a gene regulatory cascade conventionally divided into immediate-early (IE), early, and late phases. However, this cascade is not so simple in β-herpesviruses, such as HCMV, HHV-6A, and HHV-6B, and posttranscriptional regulation has been suggested to play an important role in the replication of these viruses.

Methods: To determine the effects of IE1B/IE2B (derived from HHV-6B [strain HST]) on the alternative splicing of ie1A/ie2A messenger (m) RNA expressed from a cosmid (Cosmid SalI derived from HHV-6A [strain U1102]), ie1A/ie2A expression was examined with the reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR), using primer pairs that can discriminate HHV-6A from HHV-6B.

In addition, to reveal the mechanism underlying HHV-6B IE2B posttranscriptional regulation, an HHV-6B (strain HST)-infected peripheral blood mononuclear cell complementary DNA library was screened by the yeast two-hybrid method, with IE2B as the bait. Furthermore, the correlation between the expression levels of sart3 and ie1B/ie2B mRNA in phytohemagglutinin-stimulated peripheral blood mononuclear cells or cord blood mononuclear cells infected with HHV-6B (strain HST) was examined by RT-qPCR 24 hours after infection.

Results and conclusion: In this study, we demonstrated that the alternative splicing of ie2 mRNA is enhanced by IE2 itself through an interaction with the protein squamous-cell carcinoma antigen recognized by T cells 3 (SART3), which is known to be involved in pre-mRNA processing. In addition, we showed that sart3 mRNA expression correlates with ie1/ie2 mRNA expression in peripheral blood mononuclear cells and umbilical cord blood mononuclear cells infected with HHV-6B. These results suggest that the interaction between IE2 and SART3 plays a significant role in the posttranscriptional regulation of ie1/ie2 and that SART3 is a key cellular factor that determines viral replication and prolif-

eration during postentry stages.

*Attenuation of HHV-6B reactivation by aging*

Objective: There has been little research on HHV-6B in healthy adults, and prevalences in different age groups have been unclear. Therefore, this study evaluated seroprevalence to HHV-6 antibodies in ordinary working people and examined the effect of aging on seroprevalence. Because HHV-6B is reactivated in saliva, this study also investigated an association between age and HHV-6B reactivation based on measured salivary HHV-6 DNA levels.

Methods: The subjects were 77 ordinary office workers who underwent a health checkup. In this population, we measured anti-HHV-6 antibody titers using enzyme-linked immunosorbent assay and 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 were not significantly correlated with other factors.

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

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

A member of the β herpesvirus subfamily, HHV-6 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. We detected higher levels of interleukin (IL)-1β and basic fibroblast growth factor (bFGF) in the cerebrospinal fluid of patients with HHV-6B encephalitis when compared to those in patients with non-HHV-6B-induced febrile seizures. *In vitro*, IL-1β and bFGF enhanced HHV-6B gene expression in infected U373 astrocytes during the initial and maintenance phases of infection, respectively. These findings indicate that IL-1β and bFGF contribute to HHV-6B growth and the onset of encephalitis.

## Publications

***Kobayashi N, Nishiyama T, Yamauchi T, Shimada K, Suka M, Kondo K, Yanagisawa H.*** Attenuation of human herpesvirus 6B reactivation by aging. *J Med Virol.* 2019 Jul; **91**(7): 1335-1341. doi: 10.1002/jmv.25434. Epub 2019 Feb 27. PubMed PMID: 30788852.

# Department of Bacteriology

Yuki Kinjo, *Professor*
Akiko Tajima, *Assistant Professor*
Shinya Sugimoto, *Associate Professor*
Ken-ichi Okuda, *Assistant Professor*

## General Summary

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

## Research Activities

*Analysis of protective effect of a novel pneumococcal vaccine against pneumococcal infection*

*Streptococcus pneumoniae* is a major cause of community-acquired pneumonia and occasionally causes invasive pneumococcal diseases (IPDs), such as bacteremia and sepsis, especially in young children and older adults. *Streptococcus pneumoniae* has about 100 serotypes based on the difference in the components of capsular polysaccharides. Current pneumococcal vaccines contain polysaccharides of a limited number of serotypes as antigens. The introduction of the current pneumococcal vaccine for young children as a routine immunization has decreased the incidence of IPD. However, patients who have IPD with nonvaccine serotypes are gradually increasing. Thus, development of a novel vaccine that covers most serotypes is desired. Our laboratory generated a novel protein-based vaccine with a glycolipid adjuvant that has potent immune-stimulatory activities. This novel vaccine induced long-term protection against various serotypes, including the nonvaccine serotypes.

*Development of a universal pneumococcal vaccine*

Current polysaccharide-based pneumococcal vaccines are effective for preventing IPD with vaccine serotypes. However, patients who have IPD with nonvaccine serotypes are gradually increasing (serotype replacement). Thus, development of a novel vaccine that covers most serotypes is desired. In collaboration with other academic institutions and a vaccine company, we have worked on the development of a universal pneumococcal vaccine. A new vaccine that we have developed contains pneumococcal surface protein A antigens that cover a majority of pneumococcal strains. Our results suggest that the new vaccine provides protective effects against infection with various pneumococcal strains. The new vaccine that we have developed would be useful as a universal pneumococcal vaccine.

*Basic research against biofilm formation of S. aureus*
We found new insights regarding *S. aureus* biofilms and discovered the potential for new treatments. First, we demonstrated that RNA is a new component in biofilms. RNA localized in the biofilm by binding to the polysaccharide and wall teichoic acids which is ubiquitous in the cell wall of Gram‐positive bacteria. In addition, RNA extracted from human blood promoted biofilm formation in a catheter‐related flow model. Second, we demonstrated that *S. aureus* surface protein G promotes biofilm formation by a mechanism different from those previously reported. Third, we found *Bacillus subtilis natto* has an inhibitory effect on *S. aureus*. Transcriptome analysis revealed that sporulation and motility might be important for the expression of the effect. Fourth, we discovered a derivative of glucose inhibited biofilm formation of methicillin‐resistant *S. aureus* (MRSA) and improved the susceptibility of some antibiotics.

*Effect of transglycosylase gene deletion on antimicrobial susceptibility of MRSA*
The functions of lytic transglycosylases (LTs) in relation to cell division, biofilm formation, and antibiotic‐resistance have been determined for several bacteria. The only known *S. aureus* LTs are immunodominant staphylococcal antigen A (IsaA) and *Staphylococcus epidermidis* D protein (SceD), both of which have been shown to possess cell wall hydrolytic activity. In this study, we aimed to characterize the roles of LTs in MRSA by investigating their effects on antibiotic susceptibility. In immunodominant staphylococcal antigen A gene (*isaA)*‐deleted strains, β‐lactam resistance was significantly decreased compared with that of wild‐type strains. Plasmid‐based expression of penicillin binding protein 2 prime gene (*mecA*), a major determinant of β‐lactam resistance in MRSA, in an *isaA*‐deleted strain did not restore β‐lactam resistance, demonstrating that the β‐lactam susceptibility phenotype is exhibited by *isaA* mutant regardless of the expression level of *mecA*. Overall, our results suggest that IsaA is a potential therapeutic target for MRSA infections.

*Periplasmic oxidative burst‐mediated cell death in dormant bacteria*
Stress is known to induce bacterial dormancy, which is an unculturable state, but the details, including underlying mechanisms, are poorly understood. We found that stressed Gram‐negative bacteria, including enterohemorrhagic *Escherichia coli* O157, entered a dormant state depending on the activity of the sigma factor σs. These stressed bacteria exhibited outer membrane disintegrity and periplasmic redox imbalance which led to the periplasmic oxidative burst and cell death. On the basis of these findings, we developed a culture method that isolates dormant *E. coli* O157 from contaminated food sources. This study provides evidence of the novel stress response and cell death pathway in dormant Gram‐negative bacteria, including food‐borne pathogens, which are related to public health and food safety.

*Distinct stage of biofilm dispersed bacteria from planktonic lifestyles*
Bacteria have 2 growth modes, switching between planktonic and biofilm lifestyles. Biofilm dispersals release free‐living bacteria that can lead to bacterial spread in a new location. We discovered that *S. aureus* causes biofilm dispersal by nuclease and dispersed

bacteria evaded polymorphonuclear neutrophil phagocytosis. In a mouse model of infection, dispersed bacteria showed greater survival in the blood than did planktonic bacteria and caused a lethal infection within 24 hours. Dispersed bacteria showed decreased sensitivity against bactericidal agents, such as hydrogen peroxide and aminoglycoside antibiotics. These results indicate that biofilm-dispersed bacteria differ from planktonic bacteria and have greater virulence.

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# Department of Tropical Medicine

Hirotaka Kanuka, *Professor*

Kenji Ishiwata, *Professor*

## General Summary

Novel parasite control strategies must be developed because of the failures of current eradication approaches and the logistical difficulties to implement them. One 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 response in intermediate host, (3) immune responses to helminth infection, and (4) vector epidemiology.

## Research Activities

*Introduction of* Sparganum proliferum *and its infectivity in laboratory mice*

*Sparganum proliferum* is a larval form of cestode for which the adult stage has not been identified and, thus, is called an orphan. The sparganum multiply in the human body in the form of branching buds, like the roots of wasabi. The first case in patients was reported by Professor Ijima at the University of Tokyo in 1905. Since then, only 21 cases have been reported worldwide, and many cases had lethal outcomes. Seven cases were from Japan; the estimated infection areas are Tokyo, 3 cases; Kyoto, 2 cases; and Kumamoto, 2 cases. The most recent case was reported from Toranomon Hospital (Aoshima M et al., Nihon Kyoubu–Shikkan–Gakkai Azsshi, 1989). Although the lifecycle and infection route are completely unclear, the pathophysiology of proliferative spargaonosis is summarized as follows. In the parenchyma organs, including bones, the sparganum randomly grow and divide by branching or budding and sometimes metastasize. Current antihelmintic drugs are ineffective. From the National Museum of Nature and Science, Japan, we received the sparganum, which was the only one in the world that has been maintained and isolated from patients. We confirmed the following points in sharing the maintenance of the precious species. (1) Approximately 2 months after transplantation into the abdominal cavity of a mouse, the number of individuals increased by proliferating and dividing in the free state in the abdominal cavity. (2) Occasional lesions were observed in the peritoneum, diaphragm, liver, and lungs. (3) Histologically, no worms were observed in the lesion, but cell infiltration, consisting mainly of round cells, was found around the substance considered to be the sparganum component. (4) The sparganum did not grow or divide even, if it had been maintained in the culture medium for about 3 months, but the number of individuals increased if transplanted into the abdominal cavity of mice. (5) When a sparganum was divided into 2 pieces and the fragment was transplanted into the abdominal cavity of the mouse, it grew. Employing with both culture and animal experiment systems, we are planning to elucidate the mechanism of growth and division of this sparganum and develop therapeutic agents.

*Development of a novel method for xenomonitoring to detect virus-derived DNA in mosquitoes by loop-mediated isothermal amplification*

Increasing incidence of mosquito-borne diseases, including Dengue fever and Zika virus disease, accelerates the demand for real-time and accurate surveillance data on pathogen-infected mosquitoes. Surveillance of pathogen-infected mosquitoes, namely xenomonitoring, peculiarly achieved success in the program to eliminate lymphatic filariasis. Meanwhile, xenomonitoring for virus-infected mosquitoes has been stagnated. This stagnation is due to most of the viruses inducing mosquito-borne diseases being RNA viruses, which makes infected mosquitoes difficult to store in endemic areas. In 2016, the novel finding that virus-derived DNA (vDNA) is generated in cultured cells and mosquitoes infected with Dengue virus or Chikungunya virus was reported (Goic et al., Nat Commun, 2016). In this study, taking advantages of DNA being stable in dried mosquito samples, we aimed to develop a new xenomonitoring method to detect vDNA instead of virus genomic RNA by loop-mediated isothermal amplification (LAMP), which was named vDNA-LAMP. With an optimized primer set, LAMP was used to detect vDNA in mosquito-derived culture cells, C6/36 cells, and a mosquito host, *Aedes aegypti*, infected with either Dengue or Zika virus. After the LAMP reaction, the target sequence of vDNA was successfully amplified and detected. Moreover, vDNA-LAMP was applied to the field trial of xenomonitoring with wild mosquito samples collected in Burkina Faso, where Dengue fever is endemic. Mosquitoes collected in each household was pooled, and of which DNA was purified and provided for vDNA-LAMP targeting Dengue virus 2. As a result, vDNA of Dengue virus 2 was detected in mosquitoes collected in 4.8% of households. Together, application of vDNA-LAMP to identify virus-infected mosquitoes provided a potential as a new procedure for xenomonitoring.

*Loop-mediated isothermal amplification applied to severe fever with thrombocytopenia syndrome virus vDNA detection in ticks*

Severe fever with thrombocytopenia syndrome virus (SFTSV) is a newly identified phlebovirus causing acute hemorrhagic fever in East Asia, China, Korea, and Japan. The SFTSV has infected more than 8,000 people and has an infection fatality rate of 6.4% to 20.9%. The virus lifecycle and natural mechanisms of sustained transmission remain to be fully clarified, but transmission via ticks is considered the most plausible route. Although SFTSV infections cause human disease, infections of arthropod vectors are basically nonpathogenic and persist throughout the life of ticks. Recent studies have revealed that DNA forms of arboviral RNA genomes play a significant role in viral persistence in *Drosophila* and *Aedes* species. It is possible that SFTSV DNA forms are also generated in ticks. We conducted a study to detect the appearance of SFTSV DNA forms following infection of wild ticks.

For epidemiological analysis, we collected ticks from 20 locations on Kyushu, Japan, in 2018 and 2019. These sites were selected for the survey on the basis of the number of human cases of SFTS identified in these regions. Ticks were collected by flagging vegetation (using a white flannel cloth of 170 × 70 cm). Of the 379 tick pools subjected to vDNA-LAMP to detect SFTSV vDNA, 11 were positive. Because vDNA is more stable than RNA, this stability might also lead to new methods to study the epidemiology of

SFTSV. These results provided evidence of SFTSV vDNA synthesis in wild ticks, which might have implications for the effective management of tick-borne diseases by xeno-monitoring using vDNA.

*Dissection of blood sucking behavior of mosquitoes*

Exploring the molecular mechanism of blood sucking behavior of female mosquitoes is a critical step to fight against vector-borne diseases, such as dengue and malaria, because pathogens are transmitted when mosquitoes are gorging on blood. The ATP in erythrocytes of host blood enhances the blood sucking of mosquitoes. Furthermore, mosquitoes reportedly do not exhibit blood sucking when only plasma is presented. From our experiment, we discovered that the ratio of full-engorged mosquitoes was significantly reduced when a mixture of ATP and plasma was presented to mosquitoes compared to when only ATP was presented, suggesting an inhibitory factor for blood sucking in plasma of host blood. This inhibitory effect was observed when supernatant of boiled plasma was used, indicating that the inhibitory factor might be a nonprotein component. We also fractioned this supernatant of boiled plasma with reverse-phase high-performance liquid chromatography and examined the inhibitory effect by presenting each fraction to mosquitoes. Only one hydrophilic fraction exhibited the inhibitory activity. Although many components are still included in this hydrophilic fraction and further investigation is required to identify the factor, we revealed the existence of a negative factor for blood sucking in host blood. Mosquitoes seem to perceive both positive and negative factors when they suck blood and decide whether to continue or to stop blood sucking in a context-dependent manner.

**Publications**

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# Department of Public Health and Environmental Medicine

Hiroyuki Yanagisawa, *Professor*
Takashi Yamauchi, *Assistant Professor*
Wataru Yoshioka, *Assistant Professor*
Machi Suka, *Professor*
Shingo Yogosawa, *Assistant Professor*

## General Summary

One of our major research projects in the 2019 academic year was involved in (1) a new incident of lung diseases in workers handling acrylic acid polymers. Other major research projects focused on (2) the effects of nanomaterials on chromosomal abnormality and extracellular vesicles, (3) the effects of zinc-deficiency on the thymus, (4) the role of phospholipase $A_2$ enzymes in the adverse effects induced by aromatic hydrocarbon receptor activation, (5) decompression stress in the hyperbaric work, (6) association between work/life-related factors and injuries among workers, (7) social independence of patients with neurofibromatosis type 2, (8) the effects of polaprezinc on pica and binge eating, (9) the legacy effect in patients with type 2 diabetes, and (10) the effect of postprandial hyperglycemia on the incidence of retinopathy in patients with type 2 diabetes.

## Research Activities

*Epidemiological and experimental studies on a new incident of lung diseases in Japanese workers handling cross-linked water-soluble acrylic acid polymer powders*

Lung diseases developed in many workers in a Japanese company who handled cross-linked water-soluble acrylic acid polymer powders. Because of a lack of supporting evidence for such pulmonary toxicity, we conducted epidemiological and experimental studies to verify whether occupational inhalation exposure to cross-linked water-soluble acrylic acid polymer powders causes lung diseases.

*Experimental medicine*

1. Analyses of the biological effects of nanomaterials

To elucidate the effects of nanomaterials at the molecular level, we examined application of transmission electron microscope, scanning electron microscope, and energy dispersive X-ray spectroscopy on Chinese hamster lung cells exposed to poly(amidoamine) dendrimer ethylene diamine core, $Al_2O_3$, $CeO_2$, or ZnO nanoparticles. Encapsulated elements were detected in cells exposed to $Al_2O_3$ or $CeO_2$ but not in cells exposed to ZnO. Thus, subcellular localization may be a mechanism of ZnO cytotoxicity, such as oxidative stress. We have also investigated extracellular vesicles (EVs) secreted from human keratinocyte A549 cells exposed to ZnO nanoparticles (ZnONPs). We found several types of keratin derived from differentiated keratinocytes in EVs from cells treated with ZnONPs. We have developed a working hypothesis that ZnONPs may induce cell differentiation against the human keratinocytes and are testing this possibility.

2. Inhibitory effects of EVs derived from human colorectal cancer HT29 cells on migration of human keratinocyte HaCaT cells
EVs are thought to be involved in cell-to-cell signal transduction by changing the composition of EVs in response to environmental changes. We found the suppression of migration of human keratinocytes HaCaT cells treated with EVs released assay by human colorectal cancer HT29 cells. This effect was not seen in EVs derived from HT29 cells treated with etoposide. Furthermore, a comparative analysis of the components of each EV revealed that the clathrin heavy chain was increased in the EVs released from the etoposide-treated cells.
3. Mechanism of thymus atrophy in zinc deficiency
We investigated the potential association of immune dysfunctions and thymus atrophy induced by zinc deficiency. To this end, we examined interleukin (IL)-7, a T cell maturation factor, and peroxisomal proliferator-activated receptor (PPAR)-γ in the thymus of rats fed a zinc-deficient diet. The number of PPAR-γ-positive cells, PPAR-γ messenger RNA expression, and thymic fat in zinc-deficient rats were increased, suggesting the positive association of PPAR-γ to thymic fat. The expression of IL-7 in the thymus of zinc-deficient rats was decreased. The decrease in IL-7 messenger RNA expression may be due to thymic fat.
4. Phospholipases as potential effectors of aryl hydrocarbon receptor activation
We had previously found the major role of cytosolic phospholipase A2α (cPLA$_2$α) in the adverse effects of aryl hydrocarbon receptor activation. This year, we found that *patatin-like phospholipase domain-containing* (*Pnpla*) *7*, *Pnpla2,* and *phospholipase A2, group VII* (*Pla2g7*) have the same expression property as cPLA2α and have the potential to mediate the effects of aryl hydrocarbon receptor activation. On the other hand, no phospholipase A2 exhibited a potential to compensate the action of cPLA2α.
5. Decompression stress in hyperbaric work
Exposure to a hyperbaric environment and the subsequent decompression to the surface may cause the decompression stress. The level of decompression stress would be related to the risk of decompression sickness. However, there are no biomarkers for decompression sickness. Bubbles in the body after decompression and the number of human herpesvirus 6 cells in saliva may permit use of such biomarkers for the decompression stress.

*Epidemiological studies*
1. Longitudinal association between work/life-related factors and injuries among workers
We examined the longitudinal association between work/life-related variables and injuries by industry using 1-year follow-up data obtained from a nationally representative sample of Japanese workers. Whereas long working hours, near-misses, and depressive symptoms at baseline significantly predicted the occurrence of injuries during the follow-up period, these associations differed by industry.
2. Social independence of patients with neurofibromatosis type 2
We examined the state of social independence and its contributing factors in patients with neurofibromatosis type 2 using data from a national registry in Japan. Approximately 80% of patients were socially independent. Socially dependent participants had more neurological features than did those who were socially independent.

3. Effects of polaprezinc on pica and binge eating
Polaprezinc is a complex of zinc and L-carnosine, both of which participate in the regulation of feeding behavior. Zinc deficiency has been known to be associated with pica, although the role of zinc supplementation in the treatment of pica has not been well investigated. We performed an open-label trial to evaluate the effects of polaprezinc on pica. In addition, we performed an open-label trial to evaluate the effects of polaprezinc on binge eating and its related psychopathology in patients with binge-eating disorder or bulimia nervosa.
4. Analysis of the duration and extent of the legacy effect in patients with type 2 diabetes
The legacy effect may continue for 14 to 19 years, with a greater effect for 10 years or less. The end of the legacy effect could be at 15 to 20 years. This effect may be the greatest for diabetic retinopathy, followed by diabetic kidney disease, and the smallest for cardiovascular disease.
5. Effect of postprandial hyperglycemia at clinic visits on the incidence of retinopathy in patients with type 2 diabetes
An analysis using real-world long-term follow-up data showed that postprandial hyperglycemia at clinic visits may predict the incidence of diabetic retinopathy, independent of HbA1c. The effect of postprandial hyperglycemia on diabetic retinopathy is obvious in patients with well-controlled HbA1c and in younger patients.

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# Department of Forensic Medicine

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## General Summary

Our main research projects in 2019, in previous years, have focused on forensic pathology, DNA analysis, and forensic toxicology. Much of the research was based on forensic practice. The details of our research are described below.

## Research Activities

*Forensic Pathology*

1. Utility of rapid detection test for heart-type fatty acid-binding protein in postmortem blood

Although the rapid detection tests for heart-type fatty acid-binding protein (H-FABP) are frequently used in clinical practice, their utility for assessing postmortem blood have not yet been reported. Such rapid detection tests of H-FABP (range of detection; 6.2-2,000 ng/ml) were performed to measure serum concentrations in cardiac blood from 72 forensic autopsy cases. In the H-FABP negative group, as detected with the rapid detection test, the H-FABP concentration was significantly higher, and false negative results were shown with H-FABP concentrations greater than 5,200 ng/ml. We conclude that the H-FABP rapid detection test should not be used to assess postmortem blood because the concentrations it found were high regardless of the cause of death and because of the risk of false negative results if the H-FABP concentration is too high.

2. Utility of postmortem measurement of urine N-terminal pro-brain natriuretic peptide

The N-terminal pro-brain natriuretic peptide (NT-proBNP) is reported to be excreted in the urine. However, the utility of urine measurement in the forensic field is still unclear. We evaluated the diagnostic efficacy of NT-proBNP concentration in urine obtained postmortem in a series of forensic autopsy cases. This study suggests the diagnostic efficacy for acute myocardial infarction, congestive heart failure, and sepsis-related fatality in cases in which the postmortem interval was within 72 hours.

*DNA analysis*

1. Identification of war-dead remains with DNA analysis

We performed identification of war-dead remains that recovered and repatriated from the former Soviet Union and southern area 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 of nuclear DNA.

2. The detection and analysis of X chromosome short tandem repeats locus

The analysis of short tandem repeats (STRs) located on the X chromosome (X-STRs) is

known to be useful in kinship testing. In the present study we performed a detection and population genetic study of a novel tetranucleotide 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 Databases. We are going to investigate relevance with other X-STRs by linkage analysis.
3. Human height prediction by forensic DNA phenotyping
We examined the prediction of human height with forensic DNA phenotyping. As a result of having analyzed human height and single nucleotide polymorphisms (SNPs), they were weak correlation.
To predict human height, an accurate prediction with the number of the smallest SNP must be performed. We are going to investigate a first-line combination of SNPs for human height prediction.

*Forensic toxicology*
1. Medicines and poisonous substances (abuse 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. The fluoride was quantitatively analyzed with the standard addition method.
2. We have constructed methods for drug screening using liquid chromatography/tandem mass spectrometry. The target drugs were added, and approximately 290 types of drugs are targeted. Furthermore, we have considered adding target drugs.
3. Qualitative and quantitative analysis of fluoride was conducted with gas chromatography/mass spectrometry on a forensic autopsy of person who was suspected to have consumed hydrofluoric acid. Analyzed were liquid samples (femoral vein blood and stomach contents) and homogenized organs (muscle, fat, brain, heart, kidney, lung, liver, pancreas, spleen, and stomach). Fluoride beyond the lethal range was detected in the femoral vein blood. Furthermore, high concentrations of fluoride were detected in the stomach contents and such organs as the stomach, spleen, and pancreas. Fluoride was not detected in the fat or brain. Therefore, we concluded that the person who died had drunk hydrofluoric acid.
4. We performed a forensic anatomy of a person suspected of having consumed an alkaline solution of unknown composition. We attempted liquid chromatography/quadrupole time-of-flight mass spectrometry analysis combined with Kendrick mass defect analysis to estimate the components contained in the sample (blood and stomach contents). As a result, a polyethylene glycol compound was detected in each sample.

*Radiocarbon analysis*
1. Establishing date of birth
We studied the estimation of date of birth from carbon-14 level isolated from tooth enamel or dentin or both. This method was applied to a 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 practice, we have examined the minimum amounts of enamel and dentin required for analysis.

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# Department of Internal Medicine
# Division of Gastroenterology and Hepatology

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## General Summary

In 2016, our department reorganized into laboratories on the basis of organs and established new groups: the Gastrointestinal tract group, the Liver group, Biliary-pancreatic group, and Tumor group. The Gastrointestinal tract group aims to elucidate the pathophysiology of inflammatory bowel diseases, represented by ulcerative colitis (UC) and Crohn's disease, and to establish biomarkers and therapeutic methods. The Liver group copes with viral and alcoholic liver damage and carcinogenesis and works to elucidate the pathophysiology of autoimmune hepatitis and primary cholangitis caused by autoimmune disorder. The Biliary-pancreatic group attempts to establish highly specialized diagnostic methods and treatment techniques for the early diagnosis of pancreatic cancer and to clarify the mechanism of pancreatic cancer development. The Tumor group considers the safe use of chemotherapy and tries to elucidate the mechanism of unexpected side effects mediated by the immune mechanism.

## Research Activities

*Alimentary Tract*

1. Prostaglandin E-major urinary metabolite: a reliable marker for endoscopic remission in patients with UC

We analyzed 92 patients and observed a significant difference in prostaglandin E-major urinary metabolite (PGE-MUM) between the groups that achieved endoscopic remission and pathological remission and those who did not. There was no difference in the area under the curve between PGE-MUM, fecal calprotectin, and immunological fecal occult blood in determining the achievement of each evaluation item, indicating usefulness comparable to existing biomarkers.

2. Examination of characteristics of gastrointestinal primary malignant lymphoma

We analyzed 182 patients and found them to have malt lymphoma (49.4%), diffuse large B-cell lymphoma (28.9%), follicular lymphoma (14.5%), mantle cell lymphoma (3.0%), Burkitt lymphoma (1.8%), and T-cell lymphoma (2.4%).

3. Examination of biological malignancy analyzed from positive rate of vascular invasion in patients with resected gastrointestinal neuroendocrine tumor

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

We evaluated 352 cases and classified the posteradication changes into “the easy group” (49.7%), “the unchanged group” (24.7%), and “the difficult group” (25.6%) based on the ease of endoscopic observation. “The difficult group” included about ¼ of cases, indicating that gastric cancer might be difficult to detect.
5. Molecular imaging of cancer and development of therapeutic method using imaging as a guide
6. Randomized trial of vitamin D supplementation to prevent seasonal influenza and upper respiratory infection in patients with inflammatory bowel disease
Although vitamin D supplementation did not prevent influenza, upper respiratory inflammation was significantly prevented (P = 0.042). However, the disease activity of symptomatic UC was significantly worsened (P = 0.02).
7. Randomized trial of the prevention of colorectal tumor development by whey protein
8. Investigation of the effects of genetic polymorphisms of thiopurine S-methyltransferase (*TPMT*), inosine triphosphatase (*ITPA*), and nudix hydrolase 15 (*NUDT15*) on the pharmacokinetics of azathioprine: Measurement of intermediate metabolite thioinosine nucleotide.
9. Examination of the significance of pretreatment analysis of the genes *NUDT15*, *TPMT*, *ITPA* to prevent side effects with thiopurine preparation
10. Study of the relationship between insoluble excretion of pH-dependent 5-aminosalicylic acid formulation and UC relapse
11. Randomized trial of the efficacy of indigo naturalis (*qing-dai*) in UC

*Liver*
1. The development of targeting therapy for cancer stem cells in liver cancers: We have reported that dual-specificity tyrosine-regulated kinase 2 (DYRK2) knockdown enhances the tumor growth of liver cancer cells. Conversely, adenovirus-mediated overexpression of DYRK2 inhibits cell proliferation and tumor growth and induces apoptosis both *in vitro* and *in vivo*. Furthermore, we found that patients with liver cancer and low DYRK2 expression had a significantly shorter overall survival. The findings that DYRK2 regulates proliferation and apoptosis of cancer cells suggests that DYRK2 expression is a promising predictive marker of the prognosis and that stabilized or forced expression of DYRK2 is a potential target when treating liver cancer.
2. Clinical analysis of the long-term outcomes of combined ursodeoxycholic acid and bezafibrate therapy in ursodeoxycholic acid-refractory primary biliary cholangitis patients and identify prognostic factors
Combination therapy significantly improved transaminase, biliary enzymes, and serum immunoglobulin M; reduced liver-related death and liver transplantation; and contributed to long-term prognosis.
3. Study on long-term prognosis of autoimmune hepatitis
4. Immunohistochemical study of hepatic infiltrating lymphocytes of autoimmune hepatitis: Comparison between typical cases and centrilobular zonal necrosis cases
5. Current status and treatment of liver disease patients in super-aging society (multicenter study)
6. Study of frailty in a super-aging society

*Gall bladder and Pancreas*

1. Construction of a surveillance strategy using endoscopic ultrasonography by enclosing patients with intraductal papillary mucinous neoplasma for early diagnosis of pancreatic cancer
2. Clinical study of long-term management of autoimmune pancreatitis
3. Study of the relationship between intestinal flora and oxidative stress in pancreatic cancer
4. Qualitative evaluation of non-alcoholic fatty pancreas by endoscopic ultrasonography
5. Examination of localized pancreatic atrophy findings as predictors of pancreatic cancer onset
6. Current status and treatment of patients with pancreatic diseases in a super-aging society
7. Combining WT1 dendritic cell vaccine and standard chemotherapy for advanced pancreatic cancer (Phase I clinical trial)

*Chemotherapy*

1. Systemic chemotherapy for colorectal cancer

We investigated the therapeutic effects and side effects of drug changes on 3 available kinds of fluorinated pyrimidine preparations.

2. Systemic chemotherapy for elderly patients
3. Management of thrombosis and systemic chemotherapy for patients with cancer

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# Department of Internal Medicine
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## General Summary

Our clinical and basic research in 2019 consisted of the following topics: (1) stroke, (2) Parkinson's disease (PD), (3) neuromuscular immune-related adverse evets, and (4) amyotrophic lateral sclerosis.

## Research Activities

*Stroke*

1. We analyzed the diagnostic ability of right-to-left shunt by transcranial color flow imaging among examiners. Because the diagnostic ability may depend on the skill of the examiner, the examination maneuvers must be standardized.
2. We investigates the difference of clinical features undergoing endovascular treatment between patients who had had an in-hospital stroke and those who had had a community-onset stroke via ambulance. Even though feasible work-flow time intervals are achieved in patients who have had an in-hospital stroke, puncture to recanalization time should be improved.
3. The NAG (initial of 3 factors, NIHSS, Anticoagulants, and Glucose) scale is a simple predictive scale for early hematoma expansion in patients with acute intracerebral hemorrhage. This study aimed to validate the usefulness of this scale at multiple institutions. In our multi-institutional validation, the NAG scale showed good discrimination.
4. We aimed to determine risk factors for lacunar infarctions and giant lacunar infarctions and to clarify who they differed in pathophysiological mechanisms. These types of infarction differed in risk factors for infarct expansion. Therefore, the underlying pathophysiological mechanisms for infarct expansion should be recognized in each type of infarction.
5. We investigated the incidence and clinical characteristics of convexity subarachnoid hemorrhage with hyperacute ischemic stroke. Hemorrhage was observed in 0.5% of patients within 4.5 hours of stroke onset and in 0.5% of patients 6 days after stroke onset.

*Neurodegenerative Disease*

1. Parkinson's disease

1) This study aimed to clarify whether cognitive dysfunction in PD and dementia with Lewy bodies (DLB) can be attributed to an abnormality of blood pressure. The significant association between nocturnal blood pressure dysregulation and cognitive or executive

decline in PD might be due to impaired microvascular circulation or invasion of α-synuclein in the central nervous system. The lack of a correlation of an insufficiency of blood pressure with cognitive impairment in DLB suggests initial involvement of Lewy body pathology in the neocortex, regardless of Lewy body invasion of the autonomic nervous system.

2) In this retrospective longitudinal observational study, we examined a nationwide registry data of 20,936 patients from 2008 through 2016 for patterns and trends in anti-PD drug prescriptions. These results highlight that the state of PD treatment in Japan adheres to most of the recommendations in the 2011 national guidelines but also precedes the 2018 guidelines.

3) We compared correlations of depression and anhedonia with cardiovascular sympathetic function in patients with drug-naïve PD. Depression and anhedonia might have different pathophysiological backgrounds in patients with drug-naïve PD.

4) Chronic low back pain (LBP) is a troublesome nonmotor complications of PD. This pain was present in 40.6% of patients with PD. The severity of LBP and disability in activities of daily living were associated with the severity of postural abnormality. Stooped posture was the most common cause of LBP, but 25.6% of patients had LBP that was not associated with postural abnormality.

5) This study investigated whether characteristics of white and red blood cells are associated with clinical symptoms in patients with de novo PD. Patients with normosmia, tremor-dominant or mixed type, and patients without a low body mass index have low peripheral inflammatory indices. Relative mild peripheral inflammation might play a major role in developing a mild disease phenotype in these patients.

6) We investigated the association between sympathetic nervous denervation and hemoglobin levels in patients with PD. Hemoglobin levels in these patients appear to be closely related to noradrenergic nervous activity and nigrostriatal dopaminergic degeneration.

7) We investigated the association of striatal dopaminergic depletion accompanied by sympathetic cardiovascular failure with conditions, such as orthostatic hypotension and cardiac sympathetic denervation, in patients with early PD. The presence of orthostatic hypotension and cardiac sympathetic denervation were independently associated with uptake in the putamen of $^{123}$I-N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) nortropane ($^{123}$I-FP-CIT).

8) A questionnaire survey was conducted of patients with PD regarding their illness, nutritional status, and dysphagia. Malnutrition in patients with PD is considered a risk of dysphagia.

9) This study compared the nuclear medicine images of patients with DLB and patients with PD and extracted findings that might help discrimination. The mean specific binding ratio and difference of the right and left sides in $^{123}$I-FP-CIT single-photon emission computed tomography possibly differentiate DLB from PD.

10) In patients with untreated PD and nocturnal hypertension, the striatum uptake ratio of DaTQUANT was significantly decreased in the anterior predominance of the striatum. The reason may be that the central autonomic network containing the vagus nerve efferent nucleus has a projection path to the caudate nucleus.

2. Neuromuscular immune-related adverse events associated with immune checkpoint inhibitors
We reviewed cases of neuromuscular immune-related adverse events. They included 4 cases of myopathy and 2 cases of neuropathy. All 4 cases of myopathy presented features of both myasthenia gravis and polymyositis. Overlap of myasthenia gravis and polymyositis is apparently a feature in neuromuscular immune-related adverse events.

*Basic research*
1. Parkinson's disease
We analyzed human induced pluripotent stem cell (iPSC)-derived neurons from patients who have PD with the VPS35 retromer complex component gene (*VPS35*) D620N mutation and addressed relevant disease mechanisms. These results suggest that this mutation causes endosomal dysfunction in neural cells in PARK17.
2. Amyotrophic lateral sclerosis
In this study, we first made iPSCs into which mutations of 43-kDa transactivation response DNA-binding protein (TDP-43) were introduced with gene editing technology, the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats [CRISPR]/CRISPR-associated protein 9) system. Next, we differentiated motor neurons and sensory neurons between the healthy iPSCs cells and the TDP-43 mutated iPSCs. Finally, we made models promoting progress by stress loads, such as oxidative stress.
3. Stroke
To verify a transarterial regeneration therapy, our project is aimed at developing a new focal stroke model with a microcatheter. We present a new rat model of focal stroke using a microcatheter under fluoroscopic control. The model is capable of repeated superselective administration of therapeutics directly to the cerebral artery and practicing the 3Rs principle of replacement, reduction, and refinement in experimental animals because of minimal invasiveness.

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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 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 are widely accepted in Japan. A post-hoc analysis of retrospective multicenter large-scale study is in progress to validate treatments for advanced cases of immunoglobulin A nephropathy when diagnosed at biopsy.

*Studies of total nephron number counting*
We have performed a study of stereology-based total nephron number (TNN) counting using 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 computed tomography imaging and biopsy specimens, a study to examine TNN in clinical settings is in progress.

*In vivo regeneration of interspecies chimeric kidneys using a nephron progenitor cell replacement system*
Kidney regeneration is expected to be a new alternative treatment to the currently limited treatments for chronic kidney disease. By transplanting exogeneous nephron progenitor cells (NPCs) into the metanephric mesenchyme of a xenogeneic fetus, we aimed to regenerate neo-kidneys that originate from transplanted NPCs. Previously, we generated a transgenic mouse model (the Six2-iDTR mouse) enabling drug-induced ablation of NPCs. We demonstrated that eliminating existing native host NPCs allowed their 100% replacement with donor mouse or rat NPCs, which could generate neo-nephrons on a cul-

ture dish. To apply this method to humans in the future, we examined the possibility of the in vivo regeneration of nephrons between different species via NPC replacement. We injected NPCs-containing rat renal progenitor cells and diphtheria toxin below the renal capsule of E13.5 metanephroi of Six2-iDTR mice; the injected metanephroi were then transplanted into recipient rats treated with immunosuppressants. Consequently, we successfully regenerated rat/mouse chimeric kidneys in recipient rats receiving the optimal immunosuppressive therapy. We revealed a functional connection between the neo-glomeruli and host vessels and proper neo-glomeruli filtration. In conclusion, we successfully regenerated *in vivo* interspecies kidneys that acquired a vascular system. This novel strategy might represent an effective method for human kidney regeneration.

*Studies of CKD mineral and bone disorder*

We previously reported that the DNA methylation patterns in calcium sensing receptor gene (*CASR*) and the vitamin D receptor gene (*VDR*) were modified in the parathyroid glands (PTGs) of CKD-mineral and bone disorder (MBD) and that the glial cells missing transcription factor 2 gene (*GCM2*) plays an essential role in adult PTG cell proliferation and maintenance. Furthermore, we are investigating how the CKD environment and a high-phosphorus diet affects early changes in gene expression and cell cycle acceleration in PTGs. Because glycometabolism is attracting the most attention in various fields, we then investigated insulin resistance in patients with CKD. As a result, we elucidated the association between insulin resistance and *fibroblast growth factor 23* (*FGF23*) in patients with CKD (Scientific Reports 2018). To clarify the association insulin resistance and all-cause mortality, cardiovascular events, and CKD-MBD in patients undergoing hemodialysis, we are performing a conduct cohort study. In addition, because vascular calcification is the main cause of cardiovascular disease events in patients with CKD, establishing a treatment strategy is important. We are investigating the association between vascular calcification and CKD-MBD, especially magnesium, in patients with CKD. Our purpose is to prevent and regress vascular calcification.

*Study of renal transplantation*

We participated in the Japan Academic Consortium of Kidney Transplantation, which is composed of Tokyo Women's Medical University and Kyushu University and now continue to investigate hyperuricemia and diabetic nephropathy. In our single center analysis, we investigated the association between donor fibroblast and posttransplant anemia, and the following themes are currently in progress: (1) denervation, (2) the effect of tonsillectomy for immunoglobulin A nephropathy, and (3) endoplasmic reticulum stress. As for basic science *in vivo*, we established rat kidney transplant models and analyzed renal endothelial cell transformation and the role of pericyte in kidney fibrosis.

*Studies of peritoneal dialysis*

We reported that the prevalence of peritoneal dialysis (PD)-associated peritonitis and outcome including patient survival and technical survival were not significantly different between patients who had or did not have diabetes while undergoing PD. We reported that the lipid profile was associated with the deterioration of residual renal function in incident

PD patients. We conduct clinical research on the bicarbonate/lactate-buffered neutral PD solution, the clinical efficiency of incremental PD, the management for PD-associated peritonitis, and pathologic changes of the peritoneal membrane. Additionally, we started to use a new ultrafine laparoscope to evaluate peritoneal injury.

*Renal protective effects of T-type calcium channel blockade via the blood brain barrier in a rat model of CKD*

We are investigating the mechanism for differences in an agent's capacity to penetrate the blood-brain barrier by examining new T-type calcium channel blocker agents that can or cannot penetrate the blood-brain barrier.

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

For simplicity diagnosis and decision of method of treatment for primary aldosteronism (PA), we evaluated with various criteria the characteristics of patients with positive results of the captopril challenge test. We also evaluated the relationship between clinical characteristics of PA and the results of various confirmatory tests or adrenal venous sampling. We are evaluating with several plasma markers the reactiveness to medications for treating PA.

**Publications**

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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 (DM) 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 DM and that angiogenesis is observed in fasciitis associated with DM. 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 DM than of 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 of gene expression in the fascia and muscles of patients with DM compared with those of patients with polymyositis and are attempting to detect the location of the highest gene expression.

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. 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. Last year, we detected changes in the medulla oblongata of mice with collagen-induced arthritis (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 using a detection system for citrullinated PAD we recently developed to measure citrullinated PAD concentration in the synovial fluid of 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 (prokineticin 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. Last year, we established a tissue-specific prokineticin receptor 2 knockout mouse to analyze whether the effect of this antagonist depends on prokineticin receptor 1 or prokineticin receptor 2.

## Publications

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# Department of Internal Medicine
# Division of Cardiology

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Seiichiro Matsuo, *Assistant Professor*
Yosuke Kayama, *Assistant Professor*
Michifumi Tokuda, *Assistant Professor*

## 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.

*Heart Failure Research Group*

We are constructing and updating a database of patients who have been hospitalized for treatment, such as cardiac catheterization and heart failure. Using this database, we are conducting analysis by Covariance Structure Analysis or Structural Equation Modeling to elucidate the relationship between factors based on actual clinical data that has not been able to be expressed until now. In the path diagram used for this, the relationship between each factor is easy to understand visually, and the dependency relationship and the correlation relationship can be numerically expressed. Furthermore, as a method of visually expressing the relationship, structural equation modeling by Bayesian estimation can be used. Subsequently, detailed data analysis of pathological changes due to chronic heart failure will be conducted, various conditions related to heart failure will be verified, analytic results useful for understanding and treatment of pathological changes will be published, and a wide range of clinical research will be promoted. In addition, we have supported these revisions of the "Acute and Chronic Heart Failure Treatment Guidelines" (2017 revised edition) and "Cardiomyopathy Treatment Guidelines" (2018 revised edition) published jointly by the Japanese Circulation Society and the Japan Heart Failure Association. We are also helping to translate these guidelines into English.

*Imaging Research Group*

Before transcatheter aortic valve implantation, a procedure that continues to be performed more often, cardiac computed tomographic and echocardiographic examinations are important for evaluating the aortic valve. The data of these examinations significantly contribute to the treatment of patients with aortic valvular stenosis. Moreover, new clinical research subjects are being sought for valuable case information. Other imaging methods, such as cardiac magnetic resonance imaging and myocardial isotopes, are being

investigated and analyzed for clinical research studies of such conditions as cardiomyopathy and arrhythmia. In particular, we are conducting research on cardiac function in lysosomal disease (particularly Fabry disease) and report the research results in collaboration with the Department of Pediatrics. In addition, in our university's new outpatient building, positron emission tomography-computed tomography has begun to be performed and is expected to be an important diagnostic tool for myocardial disease.

*Molecular Biology Research Group*

Glucose becomes an important preferential substrate for myocardial energy metabolism under acute conditions of ischemia-reperfusion injury (IRI). We reported that cardiac sodium glucose co-transporter 1 (SGLT1) plays a compensatory protective role during IRI via enhanced glucose utilization, particularly under insulin resistance condition, in which IRI-induced glucose transporter 4 (GLUT4) upregulation is compromised. The hearts from mice fed a high-fat diet (HFD) or a normal-fat diet (NFD) were perfused with the non-selective SGLT-inhibitor phlorizin during IRI using the Langendorff model. After IRI, a functional recovery was impaired with a HFD compared with a NFD. Although phlorizin perfusion impaired left ventricular developed pressure recovery in a NFD, recovery was further impaired in HFD with phlorizin perfusion. The immunoblotting with plasma membrane fractionations revealed that GLUT4 expression was significantly increased after IRI with a NFD, which was substantially attenuated with a HFD, associated with a significant reduction in myocardial glucose uptake. In contrast, SGLT1 expression was remained constant during IRI regardless of diet conditions. Of note, SGLT1 inhibition by phlorizin considerably attenuated myocardial glucose uptake after IRI, particularly in a HFD.

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 an activate thermogenic program. We established a direct intracellular temperature measurement system using a fluorescent thermoprobe and reported that the thermogenic effects of A-type (atrial) natriuretic peptide (ANP) on brown adipocytes. We are now investigating whether ANP exerts significant effects on adipose tissues *in vivo*.

*Cardiac Physiology Research Group*

By examining human hearts obtained at autopsy, we have found, with an immunohistological method, the presence of thrombin, the final product of the coagulation cascade. Coagulability is increased in patients with dilated cardiomyopathy. In knock-in mice with a cardiac troponin T deletion mutation that causes human dilated cardiomyopathy (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 dilated cardiomyopathy and that thrombin inhibition can be beneficial for its treatment. 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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# Department of Internal Medicine
# Division of Diabetes, Metabolism and Endocrinology

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## General Summary

The number of patients we see in our division is more than 15,000 a month and is increasing every year. The patients we see most often have diabetes (including 10% with type 1 diabetes) but some have endocrinological disorders.
We attempt to provide the best healthcare to our patients on the basis of 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. In addition, we strongly encourage our investigators to write manuscripts and publish their findings.

## Research Activities

*Epidemiology*
1. Clinical trials of the treatment of patients with diabetes using continuous 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 important 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

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

*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. In addition, the precise molecular mechanism of glucagon secretion from α cells need to be elucidated. 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. Elucidation of the molecular mechanism(s) of Pkcδ-dependent β cell mass reduction induced by glucolipotoxicity model using β cell specific Pkcδ knockout mice
2. Elucidation of the molecular mechanism(s) of Pkcδ-dependent glucagon secretion in glucagon-secreting cell line
3. Construction glucagon-secreting cell line

*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 transporter (SGLT) 2 inhibitor in a rat model of diabetes

4) Effect of aldosterone in macula lutea degeneration

2. Clinical research

1) Effect of a SGLT2 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 with 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 the goals of HbA1c, blood pressure, and low-density lipoprotein cholesterol in patients with type 2 diabetes (the Japan Diabetes Clinical Data Management Study Group)

**Publications**

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# Department of Internal Medicine
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## General Summary

The immediate goals of our clinical and basic research are to investigate basic and clinical aspects of malignant diseases and to try to improve outcomes for patients with hematological malignancies and solid tumors, leading to the ultimate goals of improving the natural history of malignant diseases. We have also been performing several clinical trials and basic research studies successfully throughout 2019.

## Research Activities

*Leukemias*
Many patients with previously untreated hematological disorders have been referred to our department. The patients treated in 2019 included 24 patients with acute myeloid leukemia or acute lymphoblastic leukemia and 2 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 in 1987 in Japan for clinical research and the treatment of such disorders. We have investigated gene analysis of leukemia cells to understand biological mechanisms of leukemia. A number of molecular directed treatment options have recently emerged and have made comprehensive diagnostics an important pillar of clinical decision-making.

*Lymphomas*
Lymphomas are a heterogenous group of lymphoproliferative malignancies with differing patterns of behavior and responses to treatment. In 2019 we registered 102 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*
In the last 20 years, many new therapeutic agents for multiple myeloma (MM) have been introduced. We registered 11 patients with newly diagnosed MM in 2019. 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 MM. We have participated in multi-institutional research studies to develop optimal chemotherapy for patients with newly diagnosed MM or relapsed/refractory MM.

*Hematopoietic stem cell transplantation*

Hematopoietic cell transplantation (HCT) is a well-established treatment to control many malignant and nonmalignant diseases involving the hematopoietic system and tumors. To investigate and establish safer and more effective methods of HCT, we have performed serial clinical studies examining umbilical cord blood transplantation, reduced-intensity stem cell transplantation from haploidentical donors, and an investigation of the mechanisms of graft-versus-host disease. We have participated in multi-institutional research studies and published many articles about HCT.

*Solid tumors*

*Esophageal cancer*

Esophageal cancer is the 14$^{th}$ most prevalent cancer in Japan. The incidence of esophageal cancer is rapidly increasing, and the overall 5-year survival rate ranges from 15% to 25%, with the best outcomes expected for esophageal cancer diagnosed at an early stage. 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.

*Breast cancer*

Breast cancer is the most frequently diagnosed cancer in women and the 4$^{th}$ most prevalent cancer in Japan. The most important risk factors for breast cancer include age, genetic predisposition, exposure to estrogens, low parity, a Western style diet, obesity, and alcohol consumption. The choice of treatment strategy is based on the tumor's burden, location, and biology and on the patient's menopausal status, general health status, and preferences. Our clinical analysis of long-term outcomes in cases of a particular oligometastatic breast cancer showed that this cancer reflects a distinct subgroup with long-term prognosis superior to that of metastatic breast cancer, with a reasonable probability of clinical cure.

*Pancreatic cancer*

Pancreatic cancer remains a leading cancer-related cause of death worldwide and is generally characterized by a dismal prognosis and limited potential for oncologic treatment. The 5-year overall survival rate has increased over the past decade from 5% to 9%. This increased survival was achieved mainly through recent improvements in neoadjuvant and adjuvant therapeutic strategies and perioperative care. However, therapeutic options are still limited, and the tumor often develops resistance to current treatments. We have investigated combination chemotherapies with both neoadjuvant and adjuvant settings to improve the prognosis of pancreatic cancer.

*Genomic medicine*

From June 2019, 2 different cancer genome profiling test panels (OncoGuide NCC Oncopanel and FoundationOne CDx) were chosen to be covered by the National Insurance System in Japan. To run the precision medicine based on cancer gene panels, the Ministry of Health, Labour and Welfare of Japan assigned 11 certified core hospitals for genomic medicine. The Jikei University Hospital has been one of the sub-core hospitals. We have collected genomic and clinical data from patients with cancer and participated in expert panels with medical oncologists, pathologists, genomic counselors, and bioinformaticians.

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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) pathogenesis concerning cellular senescence and autophagy. We also collaborate with the National Cancer Research Center concerning extracellular vesicles in various lung diseases and immune checkpoint inhibitors in the treatment of lung cancer.

## Research Activities

*Chronic obstructive pulmonary disease*

The condition known as COPD is caused by the noxious inhalation of tobacco smoke, which leads to airway epithelial cell injury and inflammation, and the phenotypic changes. Such changes as squamous metaplasia and cellular senescence of epithelial cells 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.

Ferroptosis is a necrotic form of regulated cell death mediated by phospholipid peroxidation in association with free iron-mediated Fenton reactions. Disrupted iron homeostasis resulting in excessive oxidative stress has been implicated in the pathogenesis of COPD. Our *in vivo* and *in vitro* models show labile iron accumulation and enhanced lipid peroxidation with concomitant nonapoptotic cell death during cigarette smoke exposure. Treatment with deferoxamine and ferrostatin 1, in addition to peroxidase (GPx) 4 knockdown, illuminate the role of ferroptosis in cigarette-smoke-treated lung epithelial cells. Nuclear receptor coactivator 4 (NCOA4)-mediated ferritin selective autophagy (ferritinophagy) is starts during ferritin degradation in response to cigarette smoke treatment. Models of cigarette smoke exposure, using both GPx4-deficient and overexpressing mice, clarify the pivotal role of GPX4-regulated cell death during COPD. These findings support a role for cigarette smoke-induced ferroptosis in the pathogenesis of COPD.

*Bronchial asthma*

Mepolizumab, an anti-interleukin 5 monoclonal antibody, is effective for patients with

severe eosinophilic asthma who show exacerbation or require systemic corticosteroid maintenance therapy. To elucidate the predictive factors of the response to mepolizumab for patients with severe eosinophilic asthma. To determine the predictive factors, we reviewed patient characteristics, comorbidities, biomarkers, pulmonary function, maintenance dose of systemic corticosteroids, and the number of exacerbations in 28 patients with bronchial asthma treated with mepolizumab. The response rate to mepolizumab treatment was 70%. Compared with 11 patients without eosinophilic chronic rhinosinusitis (ECRS), 16 patients with ECRS showed significantly improved systemic corticosteroid-sparing effects, change from baseline FeNO, and symptoms. Multivariate logistic regression analysis identified ECRS as a predictive factor of the response to mepolizumab. Both groups of patients showed improved symptom scores and a decreased number of exacerbations. Mepolizumab substantially improved the clinical variables of patients with eosinophilic asthma complicated with ECRS.

*Idiopathic pulmonary fibrosis*

We have produced evidence that lungs with IPF show enhanced epithelial cell senescence, including aberrantly re-epithelialized bronchial cells. Playing important regulatory roles 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.

The imbalanced redox status in lung has been widely implicated in IPF pathogenesis. To regulate redox status, hydrogen peroxide must be adequately reduced to water by GPx. Among GPx isoforms, GPx4 is a unique antioxidant enzyme that can directly reduce phospholipid hydroperoxide. We sought to examine the involvement of GPx4-modulated lipid peroxidation in regulating transforming growth factor (TGF) β-induced myofibroblast differentiation. Immunohistochemical evaluations for GPx4 and lipid peroxidation were performed in IPF lung tissues. Immunohistochemical evaluations showed reduced GPx4 expression levels accompanied by increased 4-hydroxy-2-nonenal in fibroblastic focus in IPF lungs. The TGF-β-induced myofibroblast differentiation was enhanced by GPx4 knockdown with concomitantly enhanced lipid peroxidation and SMAD2/SMAD3 signaling. Heterozygous GPx4-deficient mice showed enhancement of bleomycin-induced lung fibrosis, which was attenuated in GPx4-transgenic mice in association with lipid peroxidation and SMAD signaling. These findings suggest that increased lipid peroxidation resulting from reduced GPx4 expression levels may be causally associated with lung fibrosis development through enhanced TGF-β signaling linked to myofibroblast accumulation of fibroblastic focus formation during IPF pathogenesis.

*Lung cancer*

Prostaglandin E2 (PGE2) is metabolized to prostaglandin E-major urinary metabolite (PGE-MUM). We aimed to elucidate the clinical usefulness of measuring PGE-MUM as an indicator of tumor burden in patients with lung adenocarcinoma. PGE-MUM was measured by a radioimmunoassay in control healthy volunteers ($n$ = 124) and patients with lung adenocarcinoma ($n$ = 54). The PGE-MUM levels were significantly elevated in

patients with lung adenocarcinoma. A PGE-MUM level of 14.9 μg/g·Cr showed 70.4% sensitivity and 67.7% specificity for the diagnosis of lung adenocarcinoma. PGE-MUM levels tended to be positively correlated with cancer progression as determined by the TNM staging system. Advanced stage (stage III, stage IV, and recurrence) was significantly associated with high PGE-MUM levels by logistic regression analysis. No apparent correlation was demonstrated between PGE-MUM and carcinoma embryonic antigen (CEA) levels. PGE-MUM can be a promising biomarker reflecting the systemic tumor burden of lung adenocarcinoma.

## Publications

***Yamakawa H, Sato S, Tsumiyama E, Nishizawa T, Kawabe R, Oba T, Kamikawa T, Horikoshi M, Akasaka K, Amano M, Kuwano K, Matsushima H.*** Predictive factors of mortality in rheumatoid arthritis-associated interstitial lung disease analysed by modified HRCT classification of idiopathic pulmonary fibrosis according to the 2018 ATS/ERS/JRS/ALAT criteria. *J Thorac Dis.* 2019 Dec; **11**(12): 5247-5257. doi: 10.21037/jtd.2019.11.73. PubMed PMID: 32030242; PubMed Central PMCID: PMC6987998.

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***Numata T, Nakayama K, Utsumi H, Kobayashi K, Yanagisawa H, Hashimoto M, Minagawa S, Ishikawa T, Hara H, Araya J, Kuwano K.*** Efficacy of mepolizumab for patients with severe asthma and eosinophilic chronic rhinosinusitis. *BMC Pulm Med.* 2019 Oct 12; **19**(1): 176. doi: 10.1186/s12890-019-0952-1. PubMed PMID: 31606052; PubMed Central PMCID: PMC6790020.

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***Yoshida M, Minagawa S, Araya J, Sakamoto T, Hara H, Tsubouchi K, Hosaka Y, Ichikawa A, Saito N, Kadota T, Sato N, Kurita Y, Kobayashi K, Ito S, Utsumi H, Wakui H, Numata T, Kaneko Y, Mori S, Asano H, Yamashita M, Odaka M, Morikawa T, Nakayama K, Iwamoto T, Imai H, Kuwano K.*** Involvement of cigarette smoke-induced epithelial cell ferroptosis in COPD pathogenesis. *Nat Commun.* 2019 Jul 17; **10**(1): 3145. doi: 10.1038/s41467-019-10991-7. PubMed PMID: 31316058; PubMed Central PMCID: PMC6637122.

***Kawamoto H, Hara H, Araya J, Ichikawa A, Fujita Y, Utsumi H, Hashimoto M, Wakui H, Minagawa S, Numata T, Arihiro S, Matsuura T, Fujiwara M, Ito S, Kuwano K.*** Prostaglandin E-Major Urinary Metabolite (PGE-MUM) as a Tumor Marker for Lung Adenocarcinoma. *Cancers (Basel).* 2019 Jun 3; **11**(6). pii: E768. doi: 10.3390/cancers11060768. PubMed PMID: 31163629; PubMed Central PMCID: PMC6627988.

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## Reviews and Books

***Minagawa S, Yoshida M, Araya J, Hara H, Imai H, Kuwano K.*** Regulated Necrosis in Pulmonary Disease: A Focus on Necroptosis and Ferroptosis. *Am J Respir Cell Mol Biol.* 2020 Feb 4. doi: 10.1165/rcmb.2019-0337TR. [Epub ahead of print] PubMed PMID: 32017592.

# Department of Internal Medicine
# Division of General Medicine

Jun Hiramoto, *Professor*
Joji Otsuki, *Professor*
Kazushige Hanaoka, *Associate Professor*
Tatsuhiro Joki, *Associate Professor*
Masami Nemoto, *Professor*
Yasuhiko Miura, *Associate Professor*
Nobuyuki Furutani, *Associate Professor*
Hideo Okonogi, *Associate Professor*

## 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*
1) We investigated patients with syncope in collaboration with the Departments of Cardiology, Neurology, and Emergency Medicine.
2) We have been constructing a database of our outpatient clinics which includes information concerning the reason for visiting, symptoms, initial diagnoses, and initial treatments. The most frequent reasons for consultation were abdominal pain, cough, and fever. The most frequent initial diagnoses were upper respiratory tract infection, infectious gastroenteritis, and headache. We expect this data to be useful for analyzing trends in primary care at large general hospitals.
3) We are organizing a continuing professional development program for physicians to acquire skills for general practice required in the community. We are conducting qualitative research on changes in participants' awareness and behavior.

*Division of General Medicine, The Jikei University Katsushika Medical Center*
1) We presented case reports at academic conferences.
2) We analyzed minor components detected in the exhaled air of patients with collagen disease (such as rheumatoid arthritis, polymyalgia rheumatica, and antineutrophil cytoplasmic antibody–related vasculitis). At the onset of a disease with severe inflammation, the presence of characteristic components in the exhaled air was revealed. Our findings suggest that the severity of an inflammatory condition can be evaluated and determined with the components of exhaled air. We hope to eventually identify these exhaled minor components.

*Division of General Medicine, The Jikei University Daisan Hospital*
We found that the presepsin concentration is sometimes high in the urine of patients with urinary tract infection.

*Division of General Medicine, The Jikei University Kashiwa Hospital*

1) Yasuhiko Miura is performing research concerning the Physician Order for Life-sustaining Treatment, Japanese version, in a Japanese hospital setting, and advanced care planning in rural areas. Data collection was completed in 2018.

2) Concerning the management of 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.

3) We conducted open seminars related to clinical ethics 4 times in 2019.

4) Development and validation of an educational program to enhance the ability to practice child rights advocacy, with a special focus on pediatric care, was performed with a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology.

5) Comprehensive research for dialogue with a focus on "human dignity" in medical and care settings was supported by a Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Culture, Sports, Science and Technology.

6) The ethical issues faced by healthcare professionals in home care facilities and nursing homes and the ethics support they need were studied with a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology.

Nobuyuki Furutani classified fibromyalgia into several clinical categories and investigated suitable treatment for each category.

### Publications

***Seki M, Fujinuma Y, Matsushima M, Joki T, Okonogi H, Miura Y, Ohno I.*** How a problem-based learning approach could help Japanese primary care physicians: a qualitative study. *Int J Med Educ*. 2019 Dec 26; **10**: 232-240. doi:10.5116/ijme.5de7.99c7. PubMed PMID: 31877111.

# Department of Psychiatry

Masahiro Shigeta, *Professor*
Hisatsugu Miyata, *Professor*
Kazutaka Nukariya, *Professor*
Wataru Yamadera, *Associate Professor*
Yuuki Inoue, *Associate Professor*
Shinsuke Kito, *Associate Professor*
Masanori Kawakami, *Assistant Professor*
Keisuke Inamura, *Assistant Professor*
Kei Nakamura, *Professor*
Hironari Sue, *Professor*
Akihiko Nunomura, *Professor*
Ayumu Tateno, *Associate Professor*
Shunichiro Shinagawa, *Associate Professor*
Tatsuhiko Itoh, *Assistant Professor*
Fumitoshi Kodaka, *Assistant Professor*

## 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 in elderly patients with psychiatric disorders. We are focusing on the neuroprotective stress response regulator — repressor element 1-silencing transcription factor (REST) — and oxidative stress in the process of neurodegeneration. We also continue to study changes in the DNA methylation level as a biomarker of neurodegenerative diseases; now focusing on the effects of DNA methylation on the appearance of neuropsychiatric 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 effects of neuropsychiatric symptoms on the decline of activities of daily living.

*Morita therapy group*
We started to study the effect of outpatient Morita therapy in collaboration with other facilities. We have continued the following studies this year: (1) practical research towards obsessive compulsive disease 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 medicine.

*Psychopharmacological group*
In basic research, Professor Hisatsugu Miyata has studied neural mechanisms underlying substance dependence, especially focusing on the effects of the aversive stimulus on development and tolerance of the rewarding property of a substance of abuse (supported by a grant from Smoking Research Foundation) in collaboration with Department of Psychology of Teikyo University. In clinical research, Professor Miyata and postgraduate student Risa Yamada have conducted research on the clinical significance of psychiatric comorbidity in gambling disorders (supported by a grant from the Ministry of Health, Labour and Welfare. Assistant Professor Fumitoshi Kodaka has studies the change in functional connectivity after repetitive transcranial magnetic stimulation in patients with treatment-resistant depression (supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology). J. Ishii has conducted a study of predictive factors of functional recovery of schizophrenia.

*Clinical electroencephalography group*
We discussed a patient who had nonconvulsive status epilepticus and psychic problems and are planning to submit a case report for publication. We have studied epilepsy in adults taking Resilience into consideration. We examined the safety and efficacy of psychotropic drugs in several forms of psychosis associated with epilepsy.

*Psychophysiology group*
Studies examined: (1) the empirical research regarding the efficacy of individual and group cognitive behavioral therapy for primary and comorbid insomnia, (2) the effects of Chinese herbal medicine on sleep disorders, and (3) the biomarkers of fatigue in obstructive sleep apnea syndrome.

*Neuromodulation group*
Neuromodulation is to relieve symptoms by modifying nerve functions with electricity, magnetism, and drugs. In neuropsychiatry, neuromodulation often refers to methods of treatment with electricity and magnetism. The mission of our group is to use repetitive transcranial magnetic stimulation, which is less invasive, and to promote the development of medical devices and to perform regulatory science research according to unmet needs in cooperation with domestic and overseas companies. The results of our group activities have been submitted to and accepted for publication in the journals *Neuropsychopharmacology Reports*, *Neuropsychobiology*, *Psychogeriatrics*, and *Psychiatry and Clinical Neurosciences*.

*General hospital psychiatry group*
In a study of interventional treatment based on cognitive-behavioral therapy aimed at preventing depression from recurring, a computer system and sleep evaluation methods were introduced with a previous evaluation system for more effective presentations and for more precise estimation. Also investigated were new indications for this treatment for patients with 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 and Psychotherapy Study Group*
In November 2019, we held a study group entitled "Reports from 2019 Conference Presentations" and had a very active discussion session. We are planning to hold an annual study group starting in the next fiscal year. We will continue to plan open study groups to further develop the fields of psychopathology and psychotherapy within the Department of Psychiatry. Assistant Professor Masanori Kawakami discussed some of his ongoing research in a presentation entitled "Current Concept of Desire for Life (Masatake Morita)" at the 42nd Annual Meeting of the Japanese Society of Psychopathology (Tokyo). He plans to prepare a manuscript based on his presentation.

*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, 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 course on clinical psychology.

*Developmental/Behavioral Neuroscience Group*
In our group, doctors and psychologists who are interested in developmental disorders and behavioral medicine have study meetings and journal club meetings on "Cognitive-Behavioral Therapy for Adult ADHD" (Solanto, 2013). Our research interest is focused on the neurophysiological response of patients with attention deficit hyperactivity disorder (ADHD) measured with near-infrared spectroscopy. We have begun a study project that seeks biomarkers of the effects of ADHD pharmacotherapy.

**Publications**

***Miyata H, Takahashi M, Murai Y, Tsuneyoshi K, Hayashi T, Meulien D, Sørensen P, Higuchi S.*** Nalmefene in alcohol-dependent patients with a high drinking risk: Randomized controlled trial. *Psychiatry Clin Neurosci.* 2019 Nov; **73**(11): 697-706. doi: 10.1111/pcn.12914. Epub 2019 Aug 5. PubMed PMID: 31298784; PubMed Central PMCID: PMC6899457.

***Kito S, Miyazi M, Nakatani H, Matsuda Y, Yamazaki R, Okamoto T, Igarashi Y.*** Effectiveness of high-frequency left prefrontal repetitive transcranial magnetic stimulation in patients with treatment-resistant depression: A randomized clinical trial of 37.5-minute vs 18.75-minute protocol. *Neuropsychopharmacol Rep.* 2019 Sep; **39**(3): 203-208. doi: 10.1002/npr2.12066. Epub 2019 Jun 25. PubMed PMID: 31240870.

***Nagata T, Shinagawa S, Yoshida K, Noda Y, Shigeta M, Mimura M, Nakajima S.*** Early Improvements of Individual Symptoms With Antipsychotics Predict Subsequent Treatment Response of Neuropsychiatric Symptoms in Alzheimer's Disease: A Re-Analysis of the CATIE-AD Study. *J Clin Psychiatry.* 2020 Feb 11; **81**(2). pii: 19m12961. doi: 10.4088/JCP.19m12961. PubMed PMID: 32074412.

***Nagata T, Shinagawa S, Shigeta M.*** The time-dependent trajectory of neuropsychiatric symptoms in patients with Alzheimer's disease. *Psychogeriatrics.* 2020 Feb 7. doi: 10.1111/psyg.12525. [Epub ahead of print] PubMed PMID: 32032460.

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*Geriatr Gerontol Int.* 2020 Jun; **20**(6): 584–588. doi: 10.1111/ggi.13918. Epub 2020 Mar 31. PubMed PMID: 32232948.
***Matsuda Y, Kito S, Igarashi Y, Shigeta M.*** Efficacy and Safety of Deep Transcranial Magnetic Stimulation in Office Workers with Treatment-Resistant Depression: A Randomized, Double-Blind, Sham-Controlled Trial. *Neuropsychobiology.* 2020; **79**(3): 208–213. doi: 10.1159/000505405. Epub 2020 Jan 17. PubMed PMID: 31955155.

### Reviews and Books

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# Department of Pediatrics

Hiroyuki Ida, *Professor*
Mitsuyoshi Urashima, *Professor*
Ichiro Miyata, *Professor*
Yoko Kato, *Professor*
Yoshihiro Saito, *Associate Professor*
Hiroshi Tachimoto, *Associate Professor*
Masaharu Akiyama, *Assistant Professor*
Daishi Hirano, *Assistant Professor*
Ken Sakurai, *Assistant Professor*
Toya Ohashi, *Professor*
Yasuyuki Wada, *Professor*
Toshio Katsunuma, *Professor*
Hiroshi Kawame, *Professor*
Hiroshi Kobayashi, *Associate Professor*
Masahisa Kobayashi, *Associate Professor*
Noriko Takahata, *Assistant Professor*
Norimichi Higurashi, *Assistant Professor*

## 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 final 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 continued research mainly on gene therapy for patients with mucopolysaccharidosis type II and GM1 gangliosidosis. This year, we optimized gene transfer into human hematopoietic stem cells. We purchased the CliniMACS Prodigy (Miltenyi Biotec), a device that can semiautomatically introduce genes into cells. As a result, we have established a protocol that can efficiently introduce a mucopolysaccharidosis type II defective enzyme gene into CD34-positive cells by using a lentivirus vector. We also conducted joint research with JCR Pharma Co., Ltd., developed an adeno-associated virus vector expressing a blood-brain barrier crossing enzyme, tested the vector in GM1 ganglioside-cis model mice, and received promising results. We also created guidelines, constructed a registry, and conducted a questionnaire survey on the latest treatments for patients.

*Neurology group*
We are conducting basic research on the pathomechanisms of developmental epileptic encephalopathies. In 2019, by using a manganese-enhanced magnetic resonance imaging technique and a rat model, we have successfully characterized a novel pathogenesis of Dravet syndrome. We also found an alteration in a neuronal maturation pattern in cerebral organoids generated from protocadherin 19 gene (*PCDH19*)-related epilepsy-specific induced pluripotent stem cells. In clinical research, we have elucidated a variety of findings on pediatric neurological diseases: risk factors for intravenous immunoglobulin-related adverse effects, clinical predictors of recurrent febrile seizures during the same febrile illness, the neurological outcome of autoimmune encephalitis, and significant

associations between ripple activities in scalp electroencephalograms and absence status epilepticus and between serum levels of matrix metallopeptidase-9 and tissue inhibitor of metalloproteinase-1.

*Nephrology group*
We have performed several epidemiologic studies for rare renal diseases as a member of the Japanese Society for Pediatric Nephrology. 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 preventive effect of tonsillectomy on recurrence of Henoch-Schonlein purpura nephritis after intravenous methylprednisolone pulse therapy.

*Neonatology group*
We conduct the neonatal medical training for young pediatricians working in university hospital and Saitama Children's Medical Center. The training help the high-risk neonate care in the university hospital and the university association institution. The main research is followed: 1) renal glomerulus development in the baby with small-for-date (SFD), 2) respiratory support device CPAP element by the new air flow body mechanism using the clinical model lung, 3) brain tissue oxygen saturation concentrations assay by the transmissive time resolved near infrared spectroscopy, 4) LOX-1 as a severity marker of the neonatal hypoxic-ischemic encephalopathy and 5) the treatment innovative drug development in AMED study with National Institute of Neuroscience.

*Infectious diseases and Immunologic Disorders group*
Our research projects were associated with infectious diseases, autoinflammatory diseases, autoimmune diseases and primary immunodeficiency diseases. We investigated the mechanisms of primary immunodeficiency diseases including chronic granulomatous disease, and autoinflammatory diseases. Moreover, our group actively undertook the investigator-initiated clinical trial that lead to the development of the anti-inflammatory therapy for chronic granulomatous disease associated colitis. As clinical research, we examine sensitivity and specificity of pathogenic genome sequence analysis in childhood patients with severe infection, and the mechanisms of soluble PD-L1 in induction of immune tolerance. We also contributed to make guidelines for autoinflammatory disease.

*Hematology and Oncology group*
The use of thrombopoietin analogue for pediatric refractory immune thrombocytopenia purpura (ITP) was investigated as the activity of the platelet committee of The Japanese Society of Pediatric Hematology/Oncology. Moreover, the treatment guide for refractory ITP in children 2019 was proposed. We are conducting a phase I/II clinical trial of a dendritic cell therapy for refractory childhood brain tumors. We and Dr. Nagayoshi of the Department of nursing, The Jikei University, in collaboration with the National Cancer Center, created materials to support long-term follow-up of retinoblastoma.

*Cardiology group*

We evaluated mechanism of reverse remodeling in the status of heart failure during growth period, calculation of the shunt flow in aorto-pulmonary collateral artery model rat with left pulmonary artery ligation under hypoxia environment, 2nd group pulmonary hypertension of left atrium stenosis model rat, utility of urine titin to detect pediatric myocardial damage, evaluation of right ventricular fibrosis using 2D-speckle tracking and Diffusion tensor imaging in right ventricular pressure overload mouse, the role of HIF-1α in pulmonary artery smooth muscle of hyperoxia-induced neonatal lung injury mice We had performed following studies; drug stress test utility of LQTS.

*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 demonstrated that cow's milk, hen's egg and wheat allergy, even anaphylaxis, could be primarily prevented using simple methods, ie, avoiding cow's milk formula or changing to amino acid-based elemental formula for at least the first 3 days of life in addition of breastfeeding. We are performing some multicenter randomized controlled trials: DIFTO study (Daily versus intermittent Inhaled fluticasone in toddlers with recurrent wheezing), MADEC study (Efficacy of a moisturizing cream in the treatment of atopic dermatitis in children) and ABC II study (Primary prevention of food allergy by restricting maternal intake of processed meat and others during first month after birth).

*Endocrinology group*

We conducted the first alanine scanning mutagenesis study, in which 132 alanine variants located in the paired domain of thyroid-specific transcription factor PAX8 were created and systematically evaluated *in vitro*. We found that 76 alanine variants (55%) were loss of function variants, and the distribution of LOF variants were skewed, with more frequently observed in the N-subdomain than in the C-subdomain. Twelve out of 13 alanine variants in residues that have been affected in patients with congenital hypothyroidism were actually LOF, suggesting that the alanine scanning data can be used to evaluate the functional importance mutated residues. On the other hand, we reported the first case of a BBS patient with biallelic splice-site BBS1 variants in the Japanese population. In our study, it was suggested that disparity between funduscopic and ERG findings might be a feature of BBS1-associated rod-cone dystrophy.

## Publications

***Fujita S, Suzuki R, Sagara N, Aota A, Akashi K, Katsunuma T.*** Three cases of diffuse panbronchiolitis in children with a past history of difficult-to-treat bronchial asthma: A case report from a single medical facility. *Allergol Int.* 2020 Mar 23. pii: S1323-8930(20)30019-8. doi: 10.1016/j.alit.2020.02.007. [Epub ahead of print] PubMed PMID: 32217024.

***Hirano D, Inoue E, Sako M, Ashida A, Honda M, Takahashi S, Iijima K, Hattori M; Japanese Society of Pediatric Nephrology.*** Clinical characteristics at the renal replacement therapy initiation of Japanese pediatric patients: a nationwide cross-sectional study. *Clin Exp Nephrol.* 2020 Jan; **24**(1): 82-87. doi: 10.1007/s10157-019-01788-5. Epub 2019 Sep 20. PubMed PMID: 31541336.

***Hirano D, Ishikawa T, Inaba A, Sato M, Shinozaki T, Iijima K, Ito S.*** Epidemiology and clinical features of childhood-onset anti-neutrophil cytoplasmic antibody-associated vasculitis: a clinicopathological analysis. *Pediatr Nephrol.* 2019 Aug; **34**(8): 1425-1433. doi: 10.1007/s00467-019-04228-4. Epub 2019 May 10. PubMed PMID: 31076873.

***Ikemoto S, Hamano SI, Yokota S, Koichihara R, Hirata Y, Matsuura R.*** High-power, frontal-dominant ripples in absence status epilepticus during childhood. *Clin Neurophysiol.* 2020 Jun; **131**(6): 1204-1209. doi: 10.1016/j.clinph.2020.02.024. Epub 2020 Mar 19. PubMed PMID: 32299003.

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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, and 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) has been impaired in individual patients. Phototherapy is also 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, brodalmab, 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*
Psychosocial factors have recently 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 to evaluate the degree of QOL impairment. We are also doing 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 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 (MPNSTs). To accurately diagnose 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 patients registered in our clinic is the largest in Japan, we concentrate on long-term follow-up and improving impaired QOL by means of accurately diagnosing and then resecting neurofibromas. The estimated lifetime risk of MPNSTs in patients with neurofibromatosis 1 is approximately 10%. We have used the methylation-specific and real-time 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 viruses 1 and 2 and varicella-zoster virus are performed in this clinic. After the diagnosis is confirmed, suppressive therapies with varaciclovir are started to improve the impaired 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 reduces the incidence of PHN. We prescribe pregabalin, tricyclic antidepressants, selective serotonin reuptake inhibitors, and opioid analgesics, such as Tramcet® (Grunethal Ltd., Stokenchurch, UK), which contains tramadol hydrochloride and acetominophen.

*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.

**Publications**

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# Department of Radiology

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## Research Activities

*Division of diagnostic imaging*

1. A study of enhancement patterns of glioblastomas on contrast-enhanced magnetic resonance imaging

Glioblastomas frequently present with a ring enhancement on contrast-enhanced magnetic resonance imaging (MRI). However, it can present with varying patterns of enhancement. Therefore, we investigated whether any enhancement pattern has not been previously reported. In this study, a new contrast pattern was identified in several cases.

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

Human papillomavirus-positive oropharyngeal cancer is frequently associated with cystic lymph node metastasis. We evaluated differences in imaging features among cystic lymph node metastasis from human papillomavirus-positive oropharyngeal cancer and 2 nonmalignant cystic neck lesions, the second branchial cyst, and tuberculous lymphadenitis.

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

Eight hundred 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. Cardiac CT as a viable alternative to echocardiography to detect vegetations and perivalvular complications in patients with infective endocarditis

We considered CT as a possible alternative to echocardiography for assessing infective endocarditis. We evaluated the diagnostic capability of preoperative CT.

5. MRI findings of ovarian mucinous tumors with mural nodules

We evaluated 3 cases of mucinous tumors with mural nodules, which could be malignant. Mural nodules in multilocular cystic ovarian tumors might be significant to identify.

6. Preoperative MRI with full diagnostic protocols

Preoperative MRI is more accurate with full diagnostic protocols than with abbreviated protocols alone for estimating tumor extent in patients with pure ductal carcinoma in situ. The presence of B3 lesions, low-grade ductal carcinoma in situ, and moderate/marked background parenchymal enhancement lowered the rate of concordance between MRI

and pathology.
7. Study of quantification of rheumatoid arthritis using dual-energy CT
We will examine the usefulness of dual-energy CT quantitative evaluation in the activity of rheumatoid arthritis compared with semiquantitative evaluation by contrast-enhanced MRI.
8. The anatomical evaluation of the findings of dual-energy CT of psoriatic arthritis using a normal cadaver finger
We compare dual-energy CT indine mapping findings of psoriatic arthritis with high-resolution MRI imaging and macroscopic appearance of a normal cadaver finger.

*Division of Nuclear Medicine*
Relapse-free survival after adjuvant radioactive iodine therapy in patients with differentiated thyroid carcinoma with a microscopically positive tumor margin
Thyroid carcinoma recurred in 52.9% of patients in the low-dose (1110 MBq) group and 22.5% of patients in the high-dose (3700 MBq) group. The most common type of recurrence was lymph node metastasis.

*Division of Interventional Radiology*
1. Evaluation of the efficacy of radiotherapy with super-selective cisplatin arterial infusion for maxillary sinus cancer
Although maxillary sinus carcinoma is rare, it is often detected at an advanced stage owing to the lack of clinical symptoms at an early stage. The treatment of advanced maxillary sinus cancer is usually surgical resection; however, surgery has many complications, such as facial deformity, removal of the eye, and severe functional impairment. For carcinoma of the T4b stage, surgery is not indicated and systemic chemotherapy combined with radiotherapy is the standard treatment; however, the treatment effect is not satisfactory. Our division started performing radiotherapy with super-selective cisplatin arterial infusion in 2016, with a number of cases.
In particular, our research focuses on preoperative imaging, complications of this type of radiology, and indicators of treatment effects.
2. Detection of the cystic artery using an automated tumor-feeder detection software
We evaluated cystic artery detectability with an automated tumor-feeder detection software program and cone-beam CT.

*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 surgically treated cases of breast cancer. Given nonuniform radiosensitivity to the model tumors, estimate local control rate by the general linear quadratic model.
2. Dose findings and confirmatory trial of superselective intra-arterial infusion of cisplatin and concomitant radiotherapy for patients with locally advanced maxillary sinus cancer

We have started new concept research and evaluate local control for more advanced local tumors, normal-tissue conservation rates, and late complications.

### Publications

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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 have started to investigate the effect of preoperative chemotherapy with fluorouracil, cisplatin, and docetaxel for advanced esophageal cancer. We still focus on reducing complications after esophagectomy; specifically used are an intraoperative thermal imaging system to evaluate the gastric tube and a recurrent nerve integrity monitoring to confirm vocal cord movement. In addition, we are planning to investigate the change of body composition in relation to esophagectomy.

Esophageal manometry and 24-hour pH study were used to evaluate pathophysiology of benign esophageal disorders. We have proposed “laparoscopic circumferential Heller myotomy” as a novel approach to relieve chest pain in patients with achalasia and are conducting a prospective research study to expand evidence of this new surgical technique.

We are continuing to evaluate the usefulness of sentinel lymph node navigation and reduction surgery for early gastric cancer. We are aiming for radical cure and functional preservation by using a sentinel lymph node search method with a fluorescent infrared endoscope and radioisotope. In order to elucidate the mechanism of lymph node metastasis(LNM) in gastric cancer, immunohistochemical staining of lymphatic vessels is performed using past surgical specimens to examine the lymphatic vessel infiltration form of cancer cells, and risk factors of LNM were reexamined. The risk factors for postgastric resection syndrome and examination according to the surgical method are also important issues, and patients who have undergone gastrectomy more than 1 year ago are evaluated with the C13 breath test. We are investigating risk factors for metastasis by performing various types of immunostaining and the real-time reverse-transcriptase polymerase chain reaction focusing on the expression of various vascular endothelial growth factors.
Treatment for obesity is performed by a team (dietician, pharmacist, and physicians), and patients with resistant to weight loss despite nutritional guidance undergo surgery. The change of gastroesophageal reflux before and after sleeve gastrectomy is assessed with 24 hours pH monitoring.

*Lower gastrointestinal surgery*
In collaboration with the Department of Internal Medicine we hold regular conferences 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 manometory 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 researches.
In collaboration 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 the involvement of dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 (DYRK2) in the induction of apoptosis and the control of the cell cycle. By correlating with the database, we investigated the relationship of DYRK2 expression and associated genes. We prepared 3-dimensional cultures with colorectal cancer specimens to form so-called organoids, with which we started basic research on the mechanism of drug action. Our aim is to develop methods to choose the appropriate medicine before treatment.
Tumors are believed to have cancer stem cells, which have self-replication ability and multipotency and to cause recurrence, metastasis, and resistance to anticancer drugs and radiotherapy. We pay attention to provirus integration site for Moloney murine leukemia virus 1 (Pim-1), which is identified from mouse T-cell lymphoma, and analyzed its function in colorectal cancer, particularly the mechanism about the characteristics acquisition of stem cells.

*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 controlling 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 preoperative treatment of eltrombopag on 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 24 patients, including 4 patients who underwent ABO-incompatible LDLT. All 24 recipients were discharged in good condition on postoperative days 15 to 146, and donors were discharged on postoperative days 7 to 32 and 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 investigate the risk factors and effective treatments for postoperative portal vein thrombosis and venous thromboembolism. We have performed clinical trials for pancreatic cancer (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) and biliary tract cancer (chemotherapy with S-1 every other day in combination with gemcitabine/cisplatin). We have also performed extended liver resections 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, 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 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 with the Institute for High Dimensional Medical Imaging Research Center. With regard to nutritional therapy for patients who have 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 university hospitals. Since 2014, we have had 6 articles published in English.

Because surgical infection is 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
# Divisions of Thoracic Surgery and of Breast and Endocrine Surgery

Thoracic Surgery
Takashi Ohtsuka, *Professor*
Tadashi Akiba, *Professor*
Shuji Sato, *Associate Professor*
Makoto Odaka, *Associate Professor*

Breast and Endocrine Surgery
Hiroshi Takeyama, *Professor*
Yasuo Toriumi, *Professor*
Kazumi Kawase, *Associate Professor*
Isao Tabei, *Associate Professor*
Hiroko Nogi, *Associate Professor*

## General Summary

The Divisions of Thoracic Surgery and Breast and Endocrine Surgery were established in June 2005. Since then, we provide comprehensive diagnostic and therapeutic services for patients with surgical diseases of the chest, breast, and endocrine systems. We are leaders in our field of surgery, with a focus on minimal 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. 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*

I. Breast

A. Clinical study

1. We have evaluated the pathological characteristics of microcalcification in breast cancer and its predictive value of the pathological response after neoadjuvant chemotherapy.
2. We performed a retrospective clinical study to evaluate the short- and long-term outcomes of immediate breast reconstruction after mastectomy following neoadjuvant chemotherapy in patients with breast cancer.
3. We have performed a multicenter clinical trial to evaluate the oncological safety of patients with 1 or 2 sentinel lymph nodes positive for metastases after axillary lymph node dissection has been omitted.
4. Aromatase inhibitors have been established as the gold-standard therapy for postmenopausal patients. We have performed a multicenter clinical trial to evaluate the efficacy of denosumab in the treatment of aromatase inhibitor-associated bone loss.
5. For 30 years, we have analyzed patients with metastatic breast cancer. The analysis has indicated that oligometastatic breast cancer is a distinct subgroup with a long-term prognosis superior to that of other metastatic breast cancers. We have performed prospective studies to characterize oligometastatic breast cancer and to evaluate the efficacy of multidisciplinary strategies, including medication, radiotherapy, and resection, to improve prognosis.
6. Because of recent progress in the diagnosis and treatment of breast cancer and the development of fertility preservation, oncofertility has become more important. With close cooperation among health care providers, we have analyzed in a multicenter clinical trial how information about fertility preservation is given to young patients with breast cancer.

B. Research study

1. We have recently established a conditionally reprogrammed cell system that enables us to examine heterogeneity, drug sensitivity, and cell function in patient-derived tumor samples. Furthermore, we have revealed the mechanism of drug resistance using conditionally reprogrammed cells obtained from the metastatic lesions of patients with HR+/HER2 − breast cancer.
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. We explored the mechanism of DYRK2 in cancer progression using breast cancer tissue from patients.

II. Endocrine

A. Clinical study

1. The effect of lenvatinib on thyroid carcinoma metastasis

Lenvatinib, a tyrosine kinase inhibitor, prolongs the progression-free survival of patients with thyroid cancer. We have participated in multicenter clinical trial to evaluate the anti-

tumor effects and side effects of lenvatinib for patients with thyroid carcinomas, including papillary carcinoma, medullary carcinoma, and undifferentiated carcinoma.

2. Tumor marker for thyroid differentiated carcinomas

A monoclonal antibody, designated JT-95, was made against a thyroid papillary carcinoma obtained by our Department of Breast and Endocrine Surgery. We have investigated, in collaboration with the Division of Molecular Cell Biology of The Jikei University, the clinical usefulness of JT-95. With the permission of the institutional review board of The Jikei University, we have performed a clinical study in which we have detected the antigen of JT-95 in the sera of patients with thyroid papillary carcinoma and of patients with breast tumor but no thyroid mass.

3. Endoscopic surgery for thyroid and parathyroid diseases

Starting this year, we have evaluated the usefulness and feasibility of endoscopic thyroidal and parathyroidal surgery for thyroid carcinoma, benign thyroid tumor, and parathyroid tumors.

We believe that endoscopic thyroidal and parathyroidal surgeries have almost the same surgical results, comparable to those of conventional surgery, with excellent cosmetic outcomes.

B. Research study

1. The detection of antigens of thyroid carcinoma in sera.

A monoclonal antibody, designated JT-95, was made against a thyroid papillary carcinoma obtained by our Department of Breast and Endocrine Surgery. We are attempting, 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 JT-95 antigens is higher in patients with papillary carcinoma, especially those with metastasis to the 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 antigen.

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# Department of Surgery
# Division of Pediatric Surgery and Vascular Surgery

Takao Ohki, *Professor*
Shuichi Ashizuka, *Associate Professor*
Naoki Toya, *Assistant Professor*

## 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 clinical trials and the development of the endovascular repair of aortic aneurysms and the treatment of peripheral arterial diseases with new techniques and devices.

## Research Activities

*Pediatric Surgery*
I. 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 collect blood via the venous route from pediatric patients, (2) understanding blood test results and fluid therapy for pediatric patients, and (3) learning basic techniques for pediatric surgery.
Education for surgical residents: They train to act as lead surgeons or assistants for pediatric surgery.
II. Clinical study
Minimally invasive and scarless surgeries. That is how we make our mark.
1. The Nuss procedure is currently the most common operation for pectus excavatum. Recently, it is performed throughout the world as a minimal invasive procedure. We have already performed more than 500 Nuss operations. However, some fatal complications, such as cardiac injury, have been reported in connection with mediastinal dissection. To safely perform the Nuss procedure, we reported and recommended a new approach to the mediastinal detachment method using endoscopic surgical instruments with a lifting hook.
a. We will evaluate the safety (intraoperative and postoperative complications) of our new Nuss operation.
b. The effect of our Nuss operation will be analyzed using the volume analyzer "SYNAPSE VINCENT," a 3‐dimensional image analysis system.

2. Analysis of the pathogenesis of pneumothorax after the Nuss operation is performed for pectus excavatum.
After the Nuss operation, pneumothorax often occurs, but its pathogenesis has not been analyzed in detail. After the Nuss operation we have observed postoperative pneumothorax in more than 10 patients, mainly in their late teens. We will analyze the pathogenesis of pneumothorax after the Nuss operation for pectus excavatum as follows.
a. Analysis of changes in CT value and lung volume on pre‐ and post‐operative CT images will be performed using volume analyzer "SYNAPSE VINCENT", a 3D image analysis system.
b. We will analyze lung specimens removed by pneumothorax surgery.
3. Comparative study on the effect of three adhesion prevention agents in pediatric laparotomy and laparoscopic surgery
We will compare 3 adhesion prevention agents about convenience and efficacy in pediatric surgery.
4. We will investigate the relationship between pediatric surgery cases under 1 year old and the pathogenesis of allergies.
The association between laparotomy and allergies in childhood is occasionally reported, but there are few reports in detail. We also have many cases of allergic complications including gastrointestinal tract after laparotomy in children, and sometimes their treatment may be difficult. We will carry out an allergy survey after laparotomy at pediatric surgery certified facilities in nationwide. From the results of these survey, we analyze the relationship and pathogenesis of laparotomy and allergy in children.
III. Basic study
A. Accidental ingestion of coin cell batteries causes esophageal ulcers and strictures, tracheoesophageal fistulas and sometimes fatal gastrointestinal damage.
However, the mechanism of injury is not known in detail, so we made three hypotheses.
1. Compression pressure by the weight of the battery
2. Electrode leakage
3. Short circuit formation between the positive case and the negative cap
To reduce damage to the esophagus and gastrointestinal wall, we modified a new battery that covered the edge of the battery by using heat shrink tube. This improvement had some positive effects. As a result, mucosal damage was reduced. However, when observed over a long period of time, the degree of damage was severe, and the effect was limited in the end. Therefore, we are starting a new experiment using a metal cap that is difficult to dissolve.

*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 perfu-

sion 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 an approval of IRB. Although stent graft repair for TAAAs requires long operative and fluoroscopic time, this treatment is feasible and safe.

2. Development of endovascular repair of aortic arch aneurysms: Retrograde in-situ branched surgery; 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 thorough one side of a carotid artery, after which a covered stent is inserted as a branch and deployed into the stent graft (in an in-situ retrograde fashion). We have examined this retrograde in-situ branched surgery in an in-vitro study and have applied it clinically. This operation is expected to be a less invasive surgery for aortic arch aneurysms. We also use Branched Thoracic Arch stent grafts those are commercially available in Europe for endovascular repair of aortic arch aneurysms after an approval of IRB.

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# Department of Orthopaedic Surgery

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## General Summary

*Basic Research*

Our studies of bone metabolism have been highly acclaimed both in Japan and abroad. Within the research on bone quantity, we reported results of quantitative analysis of advanced glycation end-products (AGEs) in bone tissue and elucidated the harmful effects AGEs on osteoblastic cells.

The research on rheumatoid arthritis has been focused on Wnt proteins expressed in the synovium based on analyses with fibroblastic cell lines and rheumatoid arthritis models. We previously reported that synovial Wnt proteins enhance osteoclastogenesis and promote joint destruction. The present studies address the roles of synovial Wnt proteins in cartilage metabolism.

*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, hand surgery, foot surgery, trauma, osteoporosis, rheumatic diseases, and sports medicine. All teams maintain a high level of expertise and are actively involved in scientific activities.

## Research Activities

*Treatment of proximal humeral fractures and their sequelae with the Aequalis fracture prosthesis*

The present study examined the clinical results of hemiarthroplasty with the Aequalis fracture prosthesis (Tornier, Wright Medical Group) for proximal humeral fracture or its complications. The patients were 1 man and 3 women with a mean age of 69 years. The mean postoperative follow-up period was 8 months. In all patients the pain disappeared soon after the operation, and no complications were noted. In 2 patients younger than 70 years, a good range of motion was obtained with a good tuberosity healing. This prosthesis has a slim shape with potential bone grafting and reduction of tuberosities is relatively easy. As a result, the repair of the rotator cuff function was obtained, which led to a good

result.

*The current activities of the hand surgery division*
We reported the usefulness of dual energy computed tomography (DECT) for the diagnosis of gouty tophi located in the carpal tunnel. DECT is a CT method wherein a target is imaged with X-rays of two different energies. The technique of using the difference in the attenuation coefficient of each energy enables discrimination of the materials. DECT not only helps with the diagnosis of and surgical planning for atypical tophi but is also extremely useful for postoperative evaluation to determine whether the lesion has been removed. Schwannoma is derived form a peripheral nerve. Its enucleation is surgical. After operation, Neurological deficits appearing after operation remain a clinically challenging issue. We analyzed risk factors of neurological deficits following enucleation. The identified risk factors were the radial nerve origin, proximal nerve origin and 25 mm or more of tumor size of.

*Pelvic anchor screw fixation for spinal deformity in adult patients*
In corrective surgery for adult spinal deformities, strong fixation with pelvic anchor screws is important. Since 2016, we have been percutaneously inserting 2 sacral-alar-iliac screws on each side with a modified J-probe under X ray fluoroscopy. There were no complications observed (organ/vessel/nerve damage) after surgery. The duration of surgery and intraoperative irradiation could be effectively shortened. While being minimally invasive, the surgery is expected to provide strong and lasting fixation.

*Clinical results of a 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 (mean postoperative follow-up period 5.3 years). Favorable clinical functional outcomes were obtained, including radiographically confirmed biological fixation with all stems. Regarding complications, split fracture of the femoral calcar region during stem insertion did not occur, and postoperative dislocation occurred only in 1 case (0.4%). The flexibility of the flat-shaped short low-volume stem allows more accurate stem positioning in patients with hip dysplasia. Better and easier positioning might reduce fractures when sufficient and secure fixation is achieved.

*The current activities of the knee team*
To improve the surgical technique of total knee arthroplasty, the accuracy of a new intraoperative 3D navigation system was assessed by comparison with preoperative 3D CT data. In another study, it was demonstrated through multivariate analysis of postoperative patient data that preoperative anemia and use of sedative hypnotics are main risk factors for postoperative delirium that strongly affects postoperative rehabilitation and recovery time.
The knee team has also used a miniature swine model to demonstrate that a titanium-web

implants improve tendon-bone healing and collagen maturation in reconstructed anterior cruciate ligaments (ACL). A new technique with an originally designed rectangular retrodilator for bone tunnel preparation enabled safe and anatomical reconstructions of the ACL attachment sites.

*A study on the pathogenesis of hallux rigidus using CT*
There are still many uncertain issues concerning the pathogenesis of hallux rigidus, and we conducted various investigations to clarify some of them. In 2019, we recorded and examined the osteoarthritic changes in hallux rigidus using CT. The CT results showed narrowing of the joint space on the dorsal side of the metatarsophalangeal joint, and location of the proximal phalanx on the plantar side relative to the metatarsal head. This suggested that restricted dorsiflexion and arthritic changes in the metatarsophalangeal joint may be caused by plantar contracture. In our previous review, osteophytes and residual cartilage on the dorsal metatarsal head were observed more dorsally to the cartilage deficit, consistent with the current findings.

*Quantitative analysis of AGEs in bone tissue, and elucidation of detrimental effects AGEs on osteoblastic cells*
Many reports indicate that accumulation of AGEs in bone collagen deteriorates bone quality. We quantitatively analyzed various AGEs including pentosidine with liquid chromatography mass spectrometry (LC-MS). Analysis of 182 specimens of human cancellous bone dissected during total knee replacement revealed that MG-H1 and CML, AGEs newly determined in our research, were at levels approximately 100-200 times as high as pentosidine but showed similar tendencies of accumulation. A multiple linear regression analysis identified the independent determinants of high AGE levels to be male sex, ageing, low turnover, high HbA1c, and obesity. We also analyzed detrimental effects of AGEs on osteoblasts by incubating MC3T3-E1 cells with glycolaldehyde to induce CML accumulation within cells. The results showed that the accumulation of CML in osteoblasts causes apoptosis, which is mediated by endoplasmic reticulum (ER)-related stress.

*Treatment of unstable intertrochanteric femoral fractures using an injectable complex of beta-TCP, hyaluronic acid, and FGF-2*
We evaluated effects of an injectable complex of beta-TCP granules, hyaluronate, and FGF-2 on repair of unstable intertrochanteric fractures. We used the complex clinically to treat intertrochanteric fractures of AO classification 31-A2 fractures in 7 patients; intramedullary nails were inserted after injection of the complex. Fracture union occurred in all cases and union of the displaced lesser trochanter to the shaft was obtained in 6 cases by 12 weeks. The complex can facilitate callus formation and may be useful in treatment of other long bone fractures with displaced fragments and its usage may reduce invasiveness of the surgical procedure.

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# Department of Neurosurgery

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## General Summary

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

## Research Activities

*Cerebrovascular diseases・Endovascular surgery*

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 study include (1) development of novel parameters, (2) elucidation of relationship between the hemodynamic patterns vs. rupture risk, and (3) the development of a novel software program for computational fluid dynamics dedicated to the image workstation for angiographic machines.

3. Newly development techniques of neuroradiological imaging: intravenous 3-dimensional digital subtraction angiography, Neuro PBV, and metal artifact removal

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 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) are software applications for evaluating cerebral blood flow with a cerebral angiography device. These applications are used for patients who have had a stroke to evaluate cerebral blood flow with only 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 artifacts. We conduct these clinical studies in collaboration with Siemens.
4. Development of a novel intracranal stent device for the treatment of brain aneurysms
A novel intracranial stent device for the treatment of brain aneurysms 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 for smartphones
The telemedicine software application “Join” has recently become available for smartphone users. The 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 neo-antigens using 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 survival 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 using C-arm CT

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 to 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 opera-

tions 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 2000. 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.

### Publications

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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 experience 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 an important 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 collapsed completely under forced inspiration owing to weak cartilagenous support. Other than narrowing of both nostrils, no nasal deformity was present. Preoperative computed tomography 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 septal 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.

*Use of unsintered hydroxyapatite and poly-L-lactic acid composite sheets for management of orbital wall fracture*

Although unsintered hydroxyapatite and poly-L-lactic acid (u-HA/PLLA) composite sheets have various applications, including in craniomaxillofacial fractures, orthognathic surgery, and orthopedic surgery, and have been proven to be safe and effective, no studies have reported the use of u-HA/PLLA composite sheets for orbital wall reconstruction with long-term follow-up. This study reports our preliminary results using the u-HA/PLLA composite sheet for orbital wall fractures. The SuperFIXSORB® MX sheet (u-HA/PLLA composite sheet, Takiron, Japan), measuring 30 × 50 mm and 0.5 mm thick, was used for hard reconstruction of orbital bone defects. Seventy-two patients with acute orbital wall fractures (within 2 weeks after sustaining the injury) treated at The Jikei University from January 2014 through August 2016 were included. We evaluated postoperative complications and the operability of the material. With the use of the u-HA/PLLA composite sheet, we observed no postoperative complications, such as infection, postoperative diplopia, or enophthalmos. In pure type orbital fractures (orbital fractures only), the operation time was significantly longer with combined inferior and medial wall fractures (n = 11; mean = 201.1 minutes; standard deviation [SD] = 36.6) than with inferior wall or medial wall fractures only (n = 51; mean = 135.0 minutes; SD = 54.4) (Mann-Whitney U test, $P < 0.001$). The u-HA/PLLA composite sheet is safe and can be used for reconstructing orbital wall fractures. We believe that further long-term functional and aesthetic assessments are necessary for infection, ocular movement disorder, enophthalmos, and any other complication.

*Augmented external fixation of ulnar carpometacarpal joint fracture dislocations*

Fracture dislocations of the ulnar-sided carpometacarpal (CMC) joint are uncommon, are frequently associated with fractures of the metacarpal base or hamate or both, and are often sustained by young men who have struck a hard object. Because such fracture dislocations are complex, anatomic alignment and joint congruency must be carefully restituted for mobility and stability. Miniaturization has allowed external fixation devices to be applied to the hand. Spanning external fixation utilizes the principles of "ligamentotaxis," in which capsule-ligamentous structures are indirectly reduced through distraction forces. Treatment options for ulnar-sided CMC fracture dislocations are varied. We reviewed 10 patients with ulnar-sided CMC fracture dislocations who had most recently undergone acute surgical repair and intra-articular comminution with external fixation devices. The surgical technique included percutaneous Kirschner wire fixation and spanning with a miniature external fixation device. The follow-up period was at least 1 year. Radiographs from the patient's most recent appointments were evaluated by 2 independent reviewers. Patients rated their level of pain and assessed functions using a subjective

outcome instrument. Mean total active motion, when comparing a traumatized digit to the same digit in the contralateral hand, was 100%. All fractures healed primarily with maintenance of the congruent joint space and without radiographic displacement or arthrosis. Patients had high satisfaction and all returned to their preinjury level of function. Our study demonstrates that in the management of ulnar-sided CMC fracture dislocations the use of an external fixation device is effective in reestablishing and maintaining normal hand anatomy, reducing pain, increasing function, and preserving motion.

*Pharyngoesophageal reconstruction with the anterolateral thigh free flap*
In Japan, pharyngoesophageal defects traditionally have ben reconstructed with a jejunal flap. However, because of its advantages over the jejunal flap for reconstructing head and neck defects, the anterolateral thigh flap is often used. We recently introduced the anterolateral thigh flap for pharyngoesophageal reconstruction. The purpose of this retrospective study was to analyze the clinical and functional outcomes achieved with the anterolateral thigh flap. The medical records of 50 patients who had undergone pharyngoesophageal reconstruction with an anterolateral thigh flap were reviewed. Outcomes analyzed were perioperative mortality, morbidity, fistula, neck abscess, and other complications, swallowing, and tracheoesophageal speech function. Before reconstruction, 9 patients had undergone radiotherapy and 11 had undergone open abdominal surgery. The donor site of the anterolateral thigh flap was closed primarily in 1 patient, skin grafted in 42 patients, and closed with a local flap in 7 patients. Flap necrosis was partial in 1 patient and total in 1 patient. Pharyngocutaneous fistula occurred in 1 patient, and strictures occurred in 2 patients. This study demonstrates that with the anterolateral thigh flap, excellent clinical and functional outcomes can be achieved. The anterolateral thigh flap offers many advantages for pharyngoesophageal reconstruction and is as good as the free jejunal flap. We should further investigate which reconstructive method is superior.

*Analysis of postoperative speech function after oropharyngeal cancer reconstruction*
Speech and swallowing are the important postoperative functions of oropharyngeal cancer surgery. However, speech function after reconstruction in patients with lateral wall oropharyngeal cancer has rarely been reported. We analyzed speech function in 10 patients in whom lateral wall oropharyngeal cancer was resected and free flap reconstruction was performed at our institution from 2011 through 2015. The best evaluation scores were for Hirose's 10-point scoring system and Taguchi's method. The average score of the 100 monosyllable list was 82% (range, 70% to 95%). The most frequent incorrect answer was for plosive sounds. The resected area, speech function, and nasopharyngeal closure function were not significantly correlated. The score of 100 monosyllable list was found to be significantly correlated with nasal leakage during soft blowing ($r = 0.82$, $p < 0.01$). Although patients with lateral wall oropharyngeal cancer who had undergone reconstruction could generally obtain good speech intelligibility, a decrease in syllable articulation centered on plosive sounds was observed. These findings suggest that a cause is nasopharyngeal closure dysfunction.

## Publications

***Iimura J, Miyawaki T, Kikuchi S, Tsumiyama S, Mori E, Nakajima T, Kojima H, Otori N.*** A new "J septoplasty" technique for correction of mild caudal septal deviation. *Auris Nasus Larynx.* 2020 Feb; **47**(1): 79–83. doi: 10.1016/j.anl.2019.04.009. Epub 2019 May 9. PubMed PMID: 31078357.

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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, multi-center analysis of surgical treatment for ischemic mitral regurgitation 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) 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 by the quantitative analysis of left ventricular functional recovery after ischemia-reperfusion. Subsequent study was conducted to establish the novel formulation of the modified Del Nido solution, which can be clinically applied in Japan.
2) The atrioventricular conduction system in human heart specimens with various types of cardiac anomaly including atrioventricular septal defect, corrected transposition of the great arteries, and right isomerism heart was visualized by the high-resolution phase contrast CT imaging, and the special anatomy of the cardiac conduction bundle in various types of malformations was reconstructed in 3D imaging.
3) 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 22 AVPs in 2019 and achieved good early outcomes. Kunihara have published many articles with regard to AVP. 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.

4) 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 in 2018 (Circ J. 2018; 82: 2530-4) and we have used this method also in 2019.
5) 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.
6) 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.
7) Registry study of early outcomes after valve-sparing root replacement and composite valved-graft replacement for aortic regurgitation:
From 2008 to December 31, 2017, the first elective aortic root replacement (excluding infective endocarditis) was performed for 5,303 cases with aortic regurgitation and registered in the JCVSD database. It was found that valve-sparing root replacement surgery was preferred for relatively young patients with mild aortic regurgitation, such as Marfan syndrome, compared with total root replacement surgery. After propensity-score matching (n=1164 each), the valve-sparing root replacement surgery was associated with longer operation time but less postoperative cerebral infarction, shorter ventilation time, and less hospital death (0.8% vs. 1.8%).
8) Investigating the mechanism of inner mitochondrial collapsing by acute overstretch, and the mechanism of heart failure by acute volume overload:
The pulmonary artery of a male SD rat was ligated for 30 seconds and the right ventricular papillary muscle was extended to 120% of the length of Lmax within 2 seconds and maintained for 5 minutes. As a result, it was found that acute and transient hyperextension disrupted the inner mitochondrial membrane, but no subsequent decline in cardiac function was observed.

9) Japanese Study of Bidirectional Evaluation of Surgical Performance on Cardiovascular Surgery (jBLADE Study-0)

Background: The cardiac surgery procedure consists of meticulous steps including: (1) opening the chest; (2) establishment of cardiopulmonary bypass (CPB); (3) harvesting saphenous vein graft; (4) harvesting the internal mammary artery for coronary artery bypass grafting (CABG), (5) main procedures, (6) cessation of CPB; and (7) closing the chest.

Included in this study were board-eligible and board-certified trainees before their first renewal who agreed to participate in the jBLADE-0 study.

Basic technical skills of these participants were monitored with video recording. A total of 155 video records of each case were blinded and evaluated by members of the evaluation committee and standardized evaluation was confirmed with over 90% consistency.

### Publications

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# Department of Obstetrics and Gynecology

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## General Summary

The main research topics of the Department of Obstetrics and Gynecology are oncology, perinatology, reproductive endocrinology, and women's health. Researchers for each field commit to basic and clinical research to overcome clinically or biologically relevant issues with the latest technology and experimental methods.

## Research Activities

*Gynecologic Oncology*

1. Identifying common targetable gene alteration among different tumor locations in advanced ovarian clear cell carcinoma

Ovarian clear cell carcinoma (OCCC) is a tumor that is resistant to conventional chemotherapy and demands novel molecularly targeted therapy. To identify common targetable mutations we perform gene sequencing of specimens of primary tumors from different locations and a metastasized tumor from 1 patient. This research may clarify the true target of OCCC.

2. Exploring novel target gene related to ovarian cancer carcinogenesis

Employing genome-scale clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 screens against 4 OCCC cell lines, we identified 1 candidate gene postulated to be involved in the growth of OCCC. Although the viability of cell lines with these mutations was reduced upon small interfering RNA- or short hairpin RNA-mediated knockdown of the candidate gene, this knockdown did not cause any significant changes in gene expression. On the basis of this finding, we postulate that the candidate gene induces cell death in a way not characterized in the literature. Therefore, we are focusing on changes in cell metabolites and protein function upon knockdown of the candidate gene.

3. Therapeutic preferability of gemcitabine for AT-rich interactive domain-containing protein 1A-deficient OCCC

A unique genomic feature of OCCC is frequent deficiency of the AT-rich interactive domain 1A gene (*ARID1A*). The present study was performed to investigate standard chemotherapeutic options suitable for *ARID1A*-deficient OCCC. Drugs with selective toxic-

ity to *ARID1A*-deficient OCCC cells were identified among 6 cytotoxic drugs used in standard chemotherapy for OCCC. Both *ARID1A*-knockout and *ARID1A*-deficient OCCC cells had selective sensitivity to gemcitabine. Growth of OCCC xenografts with *ARID1A* deficiency was inhibited by the administration of gemcitabine. Furthermore, of 7 patients of a retrospective cohort who had OCCC and received single-agent therapy with gemcitabine, 3 patients with *ARID1A*-deficient OCCC had significantly longer progression-free survival after gemcitabine treatment than did 4 patients with *ARID1A*-proficient OCCC. Patients with *ARID1A*-deficient OCCC might benefit from gemcitabine treatment.

4. Profiling of genomic alterations and clinicopathological factors with cervical cancer in the Japanese population

A potentially curative resection procedure was performed at the National Cancer Center Hospital for 154 patients with cervical cancer. Genomic DNA samples were analyzed with targeted sequencing, copy number assays, and human papillomavirus genotyping. Specific genomic alteration profiles were observed in patients with cervical cancer. These profiles were correlated with certain histological types and human papillomavirus genotypes. We detected actionable genomic alterations in 54 (35%) patients with cervical cancer. Furthermore, alterations of the serine/threonine kinase 11 gene (*STK11*) caused a poorer prognosis in patients with cervical cancer and in The Cancer Genome Atlas dataset. We have proposed the prognostic value of *STK11* genomic alterations.

5. The role of human epidermal growth factor receptor 3 expression in the resistance of ovarian cancer to chemotherapy

Human epidermal growth factor receptor (HER) 3, a member of the HER family, which also includes epidermal growth factor receptor and HER2, is expressed in approximately 50% of patients with ovarian cancer and has been reported to be a negative prognostic factor. A preclinical study showed that HER3 expression and chemoresistance are corelated. Therefore, we are investigating the relationship of HER3 expression and chemotherapy by means of clinical tumor specimens. This study might provide important information for overcoming the chemoresistance of ovarian cancer and, hopefully, for making HER3 an appropriate treatment target. This study is in collaboration with National Cancer Center Hospital East and Daiichi-Sankyo.

6. Gene function analysis of VUS (variant undertermined significance)

Genetic panel tests have become a basis of cancer precision medicine. However, few patients have received molecularly targeted therapy matched to known mutations; therefore, more mutations related to the efficacy of existing anticancer drugs must be identified. We are focusing on mutations in the extracellular domain of REarranged during Transition (RET) kinase as a model of mutants whose significance needs to be clarified. In some mutants, a gain-of-function property, such as transforming ability and growth factor-independent growth, was observed in tumor cell lines. The findings were confirmed by the result that RET tyrosine kinase inhibitors inhibit cell growth. Thus, the therapeutic significance of many druggable mutations might not have been investigated because of their rarity and unclustered features. The significance and promise of uncharacterized mutations in molecularly targeted therapy will be investigated by employing RET as a model.

7. Exploring novel genetic factors of adult granulosa cell tumor related to tumorigenesis and treatment
Adult type granulosa cell tumor (aGCT) is a rare ovarian tumor whose treatment is based on insufficient evidence or on the treatment of other ovarian cancers. The aGCT is characterized by late recurrence, but the treatment of aGCT other than complete resection has not been established. A recent study has shown that more than 90% of aGCTs harbor forkhead transcription factor L2 C402G mutations and telomerase reverse transcriptase promoter C228T mutations are associated with a poorer prognosis, but the validation in the Japanese cohort is not sufficient. The aim of this study is to detect the novel mechanism of tumorigenesis and to establish the effective treatment of this tumor through the complete genomic analysis of clinical samples.
8. MicroRNA as a 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 study, we provided the rationale for microRNA-34a replacement being a promising therapeutic strategy for this ovarian carcinoma.
9. Antiangiogenic therapy for OCCC with high interleukin 6 expression
Interleukin (IL) 6 is reported to be a potential treatment biomarker for antiangiogenic drugs against ovarian cancer. Despite OCCC having high IL-6 expression, whether antiangiogenic therapy is suitable remains unclear. We found that IL-6 is related to the effect of an antiangiogenic agent *in vivo* and that both vascular endothelial growth factor, which is the main target of the antiangiogenic agent, and other factors, such as angiopoietin, play roles between IL-6 and antiangiogenic therapy for OCCC.

*Perinatology*
1. Protective effect of ferroptotic cell-derived blebs
Ferroptosis is a nonapoptotic, iron-dependent form of programmed cell death caused by depleting glutathione and inactivating the phospholipid peroxidase glutathione peroxidase 4. Morphological characteristics of ferroptosis include cell rounding followed by plasma membrane rupture at the end stage of the cell death process. Plasma membrane changes include the formation of blebs, which are usually created via the local detachment of the cortex from the membrane with a spherical protrusion followed by plasma membrane rupture. Blebs reportedly have dynamic features connected to dramatic cellular reorganization with roles in the cytokinesis, cell spreading, virus uptake, apoptosis, and locomotion of tumors and embryonic cells. However, little has been reported on the functional analysis of blebs in ferroptosis. BeWo cells form blebs during the process of ferroptosis. We examined the functional role of blebs. We collected conditioned medium containing blebs from ferroptotic BeWo cells, treated the recipient cells with the conditioned medium, and examined cell viability with a lactate dehydrogenase releasing assay. We found that cells treated with the conditioned medium showed greater viability than did control cells, indicating that ferroptic cells might secret factors that have protective effects against cell death into blebs.
2. Genomics and epigenetics research in the 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
3. Amplicon sequencing-based noninvasive fetal genotyping for *RHD*-positive D antigen-negative alleles
Cell-free DNA-based fetal Rh blood group D antigen gene (*RHD*) genotyping might eliminate the necessary of routine anti-D immunoglobulin administration to RhD-negative pregnant women to avoid infant hemolytic disease from maternal anti-fetal Rh antigen alloantibodies. However, current *RHD* deletion detection methods do not address the higher *RHD*-positive D antigen-negative allele rates in non-white populations. We developed an amplicon-sequencing method to estimate the paternally inherited fetal *RHD* allele from 4 major *RHD* alleles in the Japanese population: D antigen-positive (*RHD**01, 92.9%) and D antigen-negative (*RHD**01N.01, 6.6%; *RHD**01EL.01, 0.3%; *RHD**01N.04, 0.1%) alleles, using cell-free DNA from the blood plasma of pregnant women (Takahashi K, et al: Clinical Chemistry 65:10 1307-1316, 2019). This method allows targeted anti-D immunoglobulin to be administered in East Asian countries and increases the accuracy of fetal *RHD* genotyping implemented nationally in several European countries. We have started a prospective study.
4. Development of cell therapy for a mouse model of hypophosphatasia
The aim of this research is to develop a new approach of treatment for hypophosphatasia. We established a mouse model of hypophosphatasia and are developing an alkaline phosphatase overexpressing cell line. This study is supported by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science in 2019.

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# Department of Urology

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Tatsuya Shimomura, *Assistant Professor*

## General Summary

We performed both basic and clinical research in the following areas: oncology, involving such sites as the kidney, bladder, prostate and testes; anatomy, physiology, and pharmacology of the bladder and urethra; imaging and radiology; infections and inflammation of the genitourinary tract, such as interstitial cystitis and prostatitis; infertility; andrology and sexual function; 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 the treatment of 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 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), for example, multifocal IOLs, toric IOLs, and yellow IOLs. We implant these new IOLs through microincisions and evaluate subsequent visual function.

*Neuro-ophthalmology*
1. We performed a large-scale cohort study of the clinical and epidemiologic characteristics of optic neuritis in Japan. Serum samples from patients with optic neuritis were tested for antieaquaporin-4 antibodies (AQP4-Abs) and anti-myelin oligodendrocyte glycoprotein antibodies (MOG-Abs) and were correlated with the clinical findings. Among 531 serum samples from patients with optic neuritis, 12% were AQP4-Ab-positive and 10% were MOG-Ab-positive. Pretreatment visual acuity worsened to more than a median 1.0 log minimum angle of resolution in patients. Patients who were AQP4-Ab-positive were more likely to be a woman and exhibit diverse visual field abnormalities and 22% of patients demonstrated concurrent spinal cord lesions on MRI. Patients who had optic neuritis and were positive for AQP4-Ab had poor visual outcomes. Patients who were positive for MOG-Ab had rates of optic disc swelling and pain with eye movement which were significantly higher than those in the AQP4-Ab-positive patients. Patient who were MOG-Ab-positive manifested severe clinical findings of optic neuritis before treatment but generally showed good treatment responses with favorable visual outcomes. Most patients showed isolated optic neuritis lesions on MRI. Multivariate logistic regression analysis of all patients identified age and presence of antibodies as significant factors

affecting visual outcome. These findings indicate that autoantibody measurement is useful for the prompt diagnosis and proper management of cases of optic neuritis that tend to become refractory.
2. We reported the usefulness of a head-mounted perimeter in unilaterally nonorganic visual loss, spontaneous recovery in cases of Leber's hereditary optic neuropathy with the 11778 mutation, demyelinating optic neuritis associated with golimumab, and intravenous immunoglobulin treatment for steroid-resistant optic neuritis.

*Ocular oncology and histopathology*
1. Optic nerve sheath meningioma (ONSM) is a rare tumor. We examined the efficacy and complications associated with intensity-modulated radiation therapy (IMRT) for ONSM in 15 patients and compared visual function before and after treatment. After IMRT, tumor enlargement was not detected in any eyes. At the final posttreatment follow-up visit, eyes with fusiform and globular growth were found to have maintained better visual acuity. Final posttreatment visual field abnormalities improved in 11 eyes. All adverse events identified during IMRT improved rapidly during the treatment period. The use of IMRT for patients with ONSM improved and preserved visual function. In particular, early IMRT before the appearance of optic disc edema and atrophy can be more effective for improving visual function.
2. We reported a case of sebaceous gland carcinoma with perineural spread in the orbital apex and recurrent and giant pilomatricoma.

*Glaucoma*
1. Improve efficiency and precision of glaucoma checkup examination
Glaucoma is a progressive and irreversible disease that causes blindness. Early detection with checkup examinations and early treatment is essential for preventing severe visual impairment because early symptoms are rarely reported by patients. We evaluated the efficiency of a plurality of examination items using big data and the Markov model.
2. Quality of vision after glaucoma surgery
Although the purpose of glaucoma treatment is to protect visual function, glaucoma surgery decreases quality of vision and increases both regular and irregular astigmatism. We are investigating causes of the development of astigmatism by using corneal topographies and anterior optical coherence tomography.
3. Personality traits of glaucoma patient
Glaucoma is an incurable and irreversible disease requiring endless eye drop therapy. To prevent disease progression, patients must adhere to constant therapy. To study five-factor model of personality traits of patients with glaucoma, we have used TIPI-J, a Japanese version of the Ten Item Personality Inventory. To improve patient adherence, we evaluated the relationship between patients' adherence and five-factor model.
4. Basic research on neuroprotection and regeneration
Of patients with glaucoma in Japan, approximately 70% have normal-tension glaucoma. Glaucoma can progress even if intraocular pressure is sufficiently decreased. Studies are urgently needed to develop a radical cure for direct nerve protective or reproduction treatment.

5. Improve diagnosis and detection of progression methods
Because many patients with glaucoma report no symptoms until the late stage, both undiagnosed cases at an early stage and progressive glaucomatous changes after diagnosis are important to detect. We have attempted to improve clinical examinations, such as the visual field test and optical coherence tomography.

*Functional neuroimaging*
Many researchers have recently applied graph theory to estimate the efficiency of complex brain networks. Graph theory is a mathematical theory which uses a graph consisting of a pairwise nodes and edges. Our team has succeeded in constricting cerebral functional and morphological connectivity matrices by using a MR scanner. We are now ready to examine brain networks by means of graph theory analysis.

*Developmental functional abnormality*
We compared patients with strabismus and control groups by using graph theory and by constructing brain morphological connectivity matrices from diffusion MRI. Some graph theory indices show predominance for control group, whereas patients with strabismus also exceed than normal control with several graph theory indices. These results are suggested to reflect a functionally compensated mechanism for patients with strabismus despite fragile binocular function.

*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 an active field analyzer, which that can be used to 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 23‑, 25‑, and 27‑gauge transconjunctival vitrectomy systems to treat cases 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 25‑ and 23‑gauge transconjunctival vitrectomy systems might effectively reduce operative times of select cases that do not require the full capability of conventional vitrectomy.

To evaluate clinical efficacy of a 7-mm IOL (ETERNITY®, Santen Pharmaceutical Co. Ltd.) for combined pars plana vitrectomy, phacoemulsification, and IOL implantation, we observed the visibility of the retina during vitrectomy and measured the depth of anterior chamber preoperatively and postoperatively with the PENTACAM® corneal tomographic scanner (Oculus Optikgeräte GmbH).
We are planning to evaluate the changes in regular and irregular corneal astigmatism after 25- and 23-gauge transconjunctival sutureless vitrectomy.
We investigated changes in corneal thickness following vitreous surgery and determined whether such changes can be used as a criterion for evaluating the invasiveness of vitrectomy.
As a method of treating a dropped lens nucleus that occurred during cataract surgery, we removed the dropped lens nucleus through the corneal wound without performing pars plana vitrectomy.

*Electrophysiology*
We are recording electroretinograms to evaluate whether there are 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 has 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 tomographic 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 a Abca4(−/−)Rdh8(−/−) mouse model of Stargardt disease and a 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 of target genes and whole-exome sequencing for these disorders and successfully identified several novel pathogenic variants. In addition, among patients with congenital color blindness, we analyzed genetic variations for 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 epinephrine contained in local anesthetics on upper eyelid height in transconjunctival blepharoptosis surgery.
2. We examined the relationship between the postlevator aponeurosis fat-pad and the results of phenylephrine testing.

**Publications**

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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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# Department of Anesthesiology

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## 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 2019 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 2019.

## 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.

Each year the investigators have successfully obtained peer-reviewed research grants. For one thing, Grants-in-Aid for Scientific Research (*kakenhi*) were awarded to 6 members of our faculty in 2019. 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 Anesthesiologists in Kobe and the American Society of Anesthesiologists in Orlando, Florida. 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.

Doctor Ikeda has been investigating the protective effects of sedatives in ischemic encephalopathy. Doctor Kida's research has been focused on the mechanism of spinal cord ischemia during aortic cross clamping, which might lead to new therapeutic interventions, such as inhalational carbon dioxide.

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. Doctor Abukawa was awarded for creating a new model to practice cricothyroidotomy in an infant. As a new faculty member, Dr. Kurata was recruited to establish an outpatient pain clinic. He has a good track record of publications related to neuroimaging techniques for patients with chronic pain.

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

### Publications

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# Department of Rehabilitation Medicine

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Satoshi Takagi, *Assistant Professor*
Shu Watanabe, *Professor*
Nobuyuki Sasaki, *Associate Professor*
Masanori Funakoshi, *Associate Professor*
Kun Suk Chung, *Assistant Professor*
Kouhei Miyamura, *Assistant Professor*
Naoki Yamada, *Assistant Professor*

## General Summary

The main research topics of our department are as follows: (1) investigation of the effects of repetitive transcranial magnetic stimulation (rTMS) on N-methyl-d-aspartate receptor (NMDAR)-associated amino acids, (2) association between recovery of upper limb paralysis in patients after stroke and an interhemispheric imbalance in cortical brain activity, (3) whether a 2-minute spontaneous swallowing frequency measurement can make predictions about the need for tube feeding for dysphagia.

## Research Activities

*Investigation of the effects of rTMS on NMDAR-associated amino acids*
The rTMS improves depressive symptoms and motor function in stroke patients. While metabolic derangement of the kynurenine pathway has been reported in stroke patients, the effect of rTMS on this pathway remains unknown. The aim of this study was to investigate the effects of rTMS on NMDAR-related amino acids in serum of patients after stroke. The results suggest that rTMS can modulate NMDAR-related amino acids in blood and produce beneficial effects.

*Association between recovery of upper limb paralysis in stroke patients and an interhemispheric imbalance in cortical brain activity*
This study was designed to determine the association between motor functional recovery and interhemispheric imbalance in cortical brain activity in patients with subcortical stroke and moderate-to-severe upper limb hemiparesis admitted to the convalescent rehabilitation ward. Our results suggest that activation of the nonlesional hemisphere in subacute stroke is associated with motor recovery in moderate-to-severe upper limb hemiparesis. A multidisciplinary rehabilitation of patients with moderate-to-severe upper limb hemiparesis after stroke might enhance the compensatory movements and pre-existing motor network from the nonlesional motor cortex.

*Whether a 2-minute spontaneous swallowing frequency measurement can make predictions about the need for tube feeding for dysphagia*
The present study investigated whether measuring the frequency of spontaneous swallowing for 2 minutes can predict independence on enteral feeding 1 week after admission in patients with acute stroke. The results suggest that the 2-minute spontaneous swallowing

screening predicts independence on enteral feeding 1 week after admission in patients with acute stroke.

### Publications

***Hamaguchi T, Abo M, Murata K, Kenmoku M, Yoshizawa I, Ishikawa A, Suzuki M, Nakaya N, Taguchi K.*** Association of Long-Term Treatment by Botulinum Neurotoxins and Occupational Therapy with Subjective Physical Status in Patients with Post-Stroke Hemiplegia. *Toxins (Basel).* 2019 Aug 2; **11**(8): 453. doi: 10.3390/toxins11080453. PMID: 31382438; PMCID: PMC6723584.

***Hara T, Momosaki R, Niimi M, Yamada N, Hara H, Abo M.*** Botulinum Toxin Therapy Combined with Rehabilitation for Stroke: A Systematic Review of Effect on Motor Function. *Toxins (Basel).* 2019 Dec 5; **11**(12): 707. doi: 10.3390/toxins11120707. PMID: 31817426; PMCID: PMC6950173.

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***Niimi M, Fujita Y, Ishima T, Hashimoto K, Sasaki N, Hara T, Yamada N, Abo M.*** Role of D-serine in the beneficial effects of repetitive transcranial magnetic stimulation in post-stroke patients. *Acta Neuropsychiatr.* 2020 Jan 29: 1-22. doi: 10.1017/neu.2020.4. Epub ahead of print. PMID: 31992382.

***Niimi M, Hashimoto G, Hara T, Yamada N, Fujigasaki H, Ide T, Abo M.*** The 2-Minute Spontaneous Swallowing Screening Predicts Independence on Enteral Feeding in Patients with Acute Stroke. *J Stroke Cerebrovasc Dis.* 2020 Feb; **29**(2): 104508. doi: 10.1016/j.jstrokecerebrovasdis.2019.104508. Epub 2019 Nov 20. PMID: 31759914.

***Tanaka T, Hamaguchi T, Suzuki M, Sakamoto D, Shikano J, Nakaya N, Abo M.*** Estimation of Motor Impairment and Usage of Upper Extremities during Daily Living Activities in Poststroke Hemiparesis Patients by Observation of Time Required to Accomplish Hand Dexterity Tasks. *Biomed Res Int.* 2019 Nov 7; **2019**: 9471921. doi: 10.1155/2019/9471921. PMID: 31828151; PMCID: PMC6885294.

# Department of Emergency Medicine

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

## 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

***Takeda S.*** "AED mapping and application to community in Japan" in a part of the workshop "CPR and AED education in community". *Jeju National University Journal.* 2019 December; 1–12.

***Kamioka H, Mori Y, Nagata K, Iwanaga S, Uzura M, Yamaguchi S.*** Relationship of daily hot water bathing at home and hot water spa bathing with underlying diseases in middle-aged and elderly ambulatory patients: A Japanese multicenter cross-sectional study. *Complement Ther Med.* 2019 Apr; **43**: 232–239. doi: 10.1016/j.ctim.2019.02.003. Epub 2019 Feb 10. PMID: 30935536.

***Suzuki K, Komukai K, Nakata K, Kang R, Oi Y, Muto E, Kashiwagi Y, Tominaga M, Miyanaga S, Ishikawa T, Okuno K, Uzura M, Yoshimura M.*** The Usefulness and Limitations of Point-of-care Cardiac Troponin Measurement in the Emergency Department. *Intern Med.* 2018 Jun 15; **57**(12): 1673–1680. doi: 10.2169/internalmedicine.0098-17. Epub 2018 Feb 9. PMID: 29434124; PMCID: PMC6047987.

***Mitsunaga T, Hasegawa I, Uzura M, Okuno K, Otani K, Ohtaki Y, Sekine A, Takeda S.*** Comparison of the National Early Warning Score (NEWS) and the Modified Early Warning Score (MEWS) for predicting admission and in-hospital mortality in elderly patients in the pre-hospital setting and in the emergency department. *PeerJ.* 2019 May 16; **7:** e6947. doi: 10.7717/peerj.6947. PMID: 31143553; PMCID: PMC6526008.

# Department of Laboratory Medicine

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

## General Summary

We performed a wide range of research in clinical laboratory medicine, including practical research on 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, 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, clinical, and epidemiological research on viral hepatitis, severe acute respiratory syndrome coronavirus 2, and *Streptococcus pneumoniae* was conducted, and our manuscript about the usefulness of pneumococcal vaccination was accepted for publication in the *Journal of Infection and Chemotherapy*. We also investigated the effects of metoformin, a widely used pharmacotherapy for the treatment of type 2 diabetes, on intestinal microflora in mice. Moreover, we attempted to perform the chromatographic analysis of the volatile organic compound patterns in exhaled breath from patients with inflammatory diseases.

*Clinical Chemistry*

We established a new method to evaluate high-density lipoprotein-mediated cellular cholesterol efflux capacity with stable isotope and reported this methodology and the related study results (J Lipid Res 2019; 60: 1959-67). Our clinical research provided the significant relevance of uric acid and homocysteine to renal function (estimated glomerular filtration rate), suggesting the possibility of these markers for the presumption of vascular disorder risk.

We also 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, after function-preserving gastrectomy.

Although we had previously reported that the fasting $^{13}$C-glucose breath test (FGBT) was useful for the diagnosis of hepatic insulin resistance (HIR), there has been no report in an actual clinical setting. We, therefore, performed the FGBT in patients with heart disease to assess the difference in the diagnostic ability of HIR between the FGBT and homeostasis model assessment insulin resistance. We also assessed the relationship between the FGBT and known cardiovascular risk factors. These results of this study indicated the FGBT is more sensitive than homeostasis model assessment insulin resistance for evaluating HIR as a cardiovascular risk factor and is likely useful for managing patients to prevent cardiovascular disease.

*Clinical Hematology & Oncology*

We studied the pathophysiology of bone marrow failure syndrome.

We analyzed the clinical and laboratory data of aquired aplastic anemia (AA) patients who received immunosuppressive therapy to assess the optimal treatment for AA.

The Jikei University School of Medicine is a collaborative hospital of the National Cancer Center Hospital, which is a core hospital for cancer genomic medicine. Therefore, the following training was provided to the Information Utilization Strategy Office, Center for Cancer Genomics and Advanced Therapeutics (C-CAT) in National Cancer Center. The aims of training were 1) launch of molecular dynamics simulation for reducing gene mutation information to protein structural information, 2) significance of cancer genome mutation and review of cancer genome information management training survey results, and 3) source of genome analysis participation in expert panel conference to extract useful information for medical treatment from data. The knowledge and experience gained through the above training to the clinical laboratory technologists of the university hospital, and at the same time, utilize them for the education of medical students.

*Clinical Psychiatry*

We discussed a case of non-convulsive status epilepticus suffering from psychic problems and plan the submitting of the case report. We have conducted the study on epilepsy in adult taking Resilience into consideration. We examined the safety and efficacy of psychotropic drugs in several forms of psychosis associated with epilepsy.

*Clinical Physiology*

Continuing from last year, we conducted research on the accuracy of arrhythmia diagnosis by electrocardiogram automatic analysis, and started to compare the program software used in our hospital with the latest program software. In the area of arrhythmia, we continued our research related to catheter ablation of atrial fibrillation.

In addition, we developed a new scoring method for delayed angiography in cardiac MRI, analyzed the delayed angiography rate in patients with Fabry disease, and proved its usefulness.

*Clinical Pathology & Clinical Cell Biology*

An earthquake struck Japan on March 11, 2011. The Fukushima Daiichi nuclear power plant was severely damaged and emitted large amounts of radioactive pollutants world-

wide. We collected samples of animals, plants, fungi and lichens from Arctic and measured the radioactivity of $^{134}$Cs and $^{137}$Cs. Though no radioactivity of $^{134}$Cs was observed, radioactivity of $^{137}$Cs was observed in some samples of lichens and fungi.
Fibrosis of the liver is triggered by the production of TGF-β from sinusoidal cells and its activation. TGF-β released from cells is anchored mainly by Latency associated protein (LAP) in the extracellular matrix. LAP is cleaved by tissue-specific proteases to activate TGF-β. When the LAP fragment in blood is measured by ELISA using an antibody that recognizes the cleaved free LAP, the liver fibrosis activation can be evaluated by a blood test. In collaboration with RIKEN (Souichi Kojima, Visiting Professor; Yutaka Furuya, Senior Researcher), we were able to construct a new LAP-D antibody and construct a highly sensitive ELISA system (patent application).

*Psychological Safety in Clinical Laboratory*
We investigated psychological safety (PS) of clinical laboratory. As a result, an environment easy to ask for help was present, but no attitude toward activity was felt, a difference between the departments was present, the department with low PS was strongly aware of its' necessity, the number of mid-career engineers was the lowest. Since there is a difference in PS, it is necessary to think about what to do to improve PS.

*Data Management on Clinical Examination*
A major impact of a Fukushima nuclear disaster on hospitals was caused by lack of vision to retain human resources. To utilize point-of-care testing (POCT) in disaster settings, validation of the test results and secure data sharing system is a key.
A retrospective cohort study of rheumatoid arthritis patients revealed there seem two types of treatment unresponsiveness among the patients.

**Publications**

***Yokoyama H, Masaki T, Inoue I, Nakamura M, Mezaki Y, Saeki C, Oikawa T, Saruta M, Takahashi H, Ikegami M, Hano H, Ikejima K, Kojima S, Matsuura T.*** Histological and biochemical evaluation of transforming growth factor-βactivation and its clinical significance in patients with chronic liver disease. *Heliyon.* 2019 Feb 16; **5**(2): e01231. doi: 10.1016/j.heliyon.2019.e01231. PMID: 30815603; PMCID: PMC6378908.
***Arihiro S, Nakashima A, Matsuoka M, Suto S, Uchiyama K, Kato T, Mitobe J, Komoike N, Itagaki M, Miyakawa Y, Koido S, Hokari A, Saruta M, Tajiri H, Matsuura T, Urashima M.*** Randomized Trial of Vitamin D Supplementation to Prevent Seasonal Influenza and Upper Respiratory Infection in Patients With Inflammatory Bowel Disease. *Inflamm Bowel Dis.* 2019 May 4; **25**(6): 1088-1095. doi: 10.1093/ibd/izy346. PMID: 30601999; PMCID: PMC6499936.
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***Shimizu T, Miyazaki O, Iwamoto T, Usui T, Sato R, Hiraishi C, Yoshida H.*** A new method for measuring cholesterol efflux capacity uses stable isotope-labeled, not radioactive-labeled, cholesterol. *J Lipid Res.* 2019 Nov; **60**(11): 1959-1967. doi: 10.1194/jlr.D086884. Epub 2019 Aug 27. PMID: 31455616; PMCID: PMC 6824490.
***Tokutake K, Tokuda M, Yamashita S, Sato H, Ikewaki H, Okajima E, Oseto H, Yokoyama M, Isogai R, Yokoyama K, Kato M, Narui R, Tanigawa S, Matsuo S, Miyanaga S, Sugimoto K, Yoshimura M, Yamane T.*** Anatomical and Procedural Factors of Severe Pulmonary Vein Stenosis After Cryoballoon Pulmonary Vein Ablation. *JACC Clin Electrophysiol.* 2019 Nov; **5**(11): 1303-1315. doi: 10.1016/j.jacep.2019.08.003. Epub 2019 Oct 30. PMID: 31753437.

***Yamashita S, Tokuda M, Matsuo S, Mahida S, Hachisuka EO, Sato H, Ikewaki H, Oseto H, Yokoyama M, Isogai R, Tokutake K, Yokoyama K, Narui R, Kato M, Tanigawa S, Sugimoto K, Yoshimura M, Yamane T.*** Comparison of atrial arrhythmia recurrence after persistent atrial fibrillation ablation between patients with or without tachycardia-induced cardiomyopathy. *J Cardiovasc Electrophysiol.* 2019 Nov; **30**(11): 2310-2318. doi: 10.1111/jce.14144. Epub 2019 Sep 22. PMID: 31452290.

***Isshi K, Matsuhashi N, Joh T, Higuchi K, Iwakiri K, Kamiya T, Manabe N, Ogawa M, Arihiro S, Haruma K, Nakada K.*** Proton pump inhibitor monotherapy is effective to attenuate dyspepsia symptoms associated with gastroesophageal reflux disease: a multicenter prospective observational study. *J Gastroenterol.* 2019 Jun; **54**(6): 492-500. doi: 10.1007/s00535-019-01546-0. Epub 2019 Jan 23. Erratum in: J Gastroenterol. 2019 May; **54**(5): 480-483. PMID: 30673836.

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***Kutsuma T, Katagiri S, Hayashi T, Yoshitake K, Iejima D, Gekka T, Kohzaki K, Mizobuchi K, Baba Y, Terauchi R, Matsuura T, Ueno S, Iwata T, Nakano T.*** Novel biallelic loss-of-function KCNV2 variants in cone dystrophy with supernormal rod responses. *Doc Ophthalmol.* 2019 Jun; **138**(3): 229-239. doi: 10.1007/s10633-019-09679-6. Epub 2019 Mar 15. PMID: 30877594.

***Mezaki Y, Kato S, Nishikawa O, Takashima I, Tsubokura M, Minowa H, Asakura T, Matsuura T, Senoo H.*** Measurements of radiocesium in animals, plants and fungi in Svalbard after the Fukushima Daiichi nuclear power plant disaster. *Heliyon.* 2019 Dec 24; **5**(12): e03051. doi: 10.1016/j.heliyon.2019.e03051. PMID: 32083202; PMCID: PMC7019073.

***Saeki C, Takano K, Oikawa T, Aoki Y, Kanai T, Takakura K, Nakano M, Torisu Y, Sasaki N, Abo M, Matsuura T, Tsubota A, Saruta M.*** Comparative assessment of sarcopenia using the JSH, AWGS, and EWGSOP2 criteria and the relationship between sarcopenia, osteoporosis, and osteosarcopenia in patients with liver cirrhosis. *BMC Musculoskelet Disord.* 2019 Dec 26; **20**(1): 615. doi: 10.1186/s12891-019-2983-4. PMID: 31878909; PMCID: PMC6933666.

***Ochi S, Leppold C, Kato S.*** Impacts of the 2011 Fukushima nuclear disaster on healthcare facilities: A systematic literature review. *International Journal of Disaster Risk Reduction.* 2020; **42:** 101350. doi: 10.1016/j.ijdrr.2019.101350.

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## Reviews and Books

***Yoshida H.*** Clinical Impact and Significance of Serum Lipoprotein (a) Levels on Cardiovascular Risk in Patients With Coronary Artery Disease. *Circ J.* 2019 Apr 25; **83**(5): 967-968. doi: 10.1253/circj.CJ-19-0221. Epub 2019 Apr 4. PMID: 30944264.

***Yanai H, Yoshida H.*** Beneficial Effects of Adiponectin on Glucose and Lipid Metabolism and Atherosclerotic Progression: Mechanisms and Perspectives. *Int J Mol Sci.* 2019 Mar 8; **20**(5): 1190. doi: 10.3390/ijms20051190. PMID: 30857216; PMCID: PMC6429491.

***Hirowatari Y, Yoshida H.*** Innovatively Established Analysis Method for Lipoprotein Profiles Based on High-Performance Anion-Exchange Liquid Chromatography. *J Atheroscler Thromb.* 2019 Dec 1; **26**(12): 1027-1040. doi: 10.5551/jat.RV17037. Epub 2019 Sep 20. PMID: 31548491; PMCID: PMC6927812.

# Department of Endoscopy

Kazuki Sumiyama, *Professor*
Hiroshi Arakawa, *Associate Professor*
Hirobumi Toyoizumi, *Assistant Professor*
Keiichi Ikeda, *Associate Professor*
Masayuki Kato, *Associate Professor*
Naoto Tamai, *Assistant Professor*

## 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 (AFI), 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 patients, 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 ($\times 90$) 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 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 the confocal laser endomicroscopy over conventional endoscopy for differentiating gastric neoplasia from nonneoplastic 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 patients with 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 knife 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 during their early stages, 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 modality 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.

## Publications

***Furuhashi H, Ohya TR, Matsui H, Sumiyama K.*** A novel diathermy knife with suction function capable of keeping clear visibility while controlling bleeding. *VideoGIE.* 2019 Apr 5; **4**(5): 197–199. doi: 10.1016/j.vgie.

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### Reviews and Books

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# Department of Clinical Pharmacology and Therapeutics

Tsuyoshi Shiga, *Professor and Director* Yasuhiro Arakawa, *Assistant Professor*

## General Summary

The principle of drug treatment is to maximize pharmacological effects while preventing adverse events. Clinical Pharmacology is the scientific discipline that involves all aspects of the relationship of drugs and patients. Research in clinical pharmacology is usually interdisciplinary and is often performed in collaboration with other professionals and with clinical researchers from other medical specialties. Clinical pharmacokinetics is the basis for determining the dose of a drug and optimizing the results achieved in each patient, so-called personalized medicine. Clinical pharmacology has an important role in the development of new drugs, repositioning drugs, and reevaluating drug efficacy on the basis of exploratory hypotheses from cohort studies.
In addition to conducting research, another important part of clinical pharmacology is the teaching of clinical pharmacology to medical students and new physicians, nurses, and pharmacists.

## Research Activities

*Cardiotoxicity of anticancer drugs*
We are conducting a retrospective observational study of more than 2,000 patients receiving anticancer drugs at 4 affiliated Jikei University Hospitals in 2018 and 2019.

*Pharmacokinetic/pharmacodynamic studies in disease states*
We are evaluating the predictive performance of a pharmacokinetic-pharmacodynamic model of digoxin in patients with heart failure and renal impairment.

*Clinical pharmacology of cardiovascular drugs*
We are conducting a cohort study and are planning clinical trials to examine the effects of novel drugs in patients with heart failure.

### Publications

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# Department of Infection Control

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

## General Summary

This year we investigated the seroprevalence of *Toxoplasma gondii* among patients with human immunodeficiency virus (HIV) infection and the rate of enterocolitis when HIV infection is diagnosed. These studies will contribute to the early diagnosis and appropriate treatment of HIV infection. In addition, we demonstrated the safety and tolerability of medicinal *Trichuris suis* ova (TSO), a result that should accelerate further studies of new strategy for inflammatory bowel diseases.

## Research Activities

*Safety and tolerability of medicinal TSO in healthy Japanese volunteers: a randomized, double-blind, placebo-controlled trial*

Background: Medicinal use of TSO, and its potential for improving the immunomodulatory capacity of the human immune system, has been verified in several studies. To the best of our knowledge, earlier research has been limited to only European and American subjects, among which Asian subjects are poorly documented. Therefore, we performed a clinical trial to reveal the safety and tolerability of TSO therapy among the Japanese population.

Methods: The study was a randomized, double-blind, placebo-controlled trial held at The Jikei University Hospital. Twelve subjects were stratified into 3 TSO dose-dependent groups (TSO 1,000, 2,500, and 7,500), and 1 subject in each group was randomly assigned to the control group. Subjects were limited to healthy Japanese men aged at least 20 years. Single doses of medicinal TSO or placebo were given, and all subjects were followed up for 56 days after ingestion. During the follow-up period, clinical practitioners checked each subject at the clinic at postingestion days (PIDs) 7, 14, 28, and 56. Subjects were asked of clinical symptoms with a questionnaire-based self-report, which was filled out at every visit. Blood samples were drawn at PIDs 7, 14, 28, and 56. Stool samples were collected at PIDs 28 and 56.

Results: During the study period, no severe adverse events occurred in any subject. However, during the observational period participants in each TSO group had mild to moderate abdominal symptoms: diarrhea, bloating, and appetite loss. One subject in the placebo group had mild diarrhea. Stool samples were fully collected, and microscopic examinations detected no TSO in samples. Blood samples showed raised eosinophil count in several subjects, especially in the groups with a higher dose of TSO. No extra-abdominal symptoms developed in any subject.

Conclusions: All doses of TSO were well tolerated, without severe adverse events. On the

other hand, mild to moderate abdominal symptoms developed in several subjects. We believe that medicinal use of TSO would be safe for Japan, but detailed follow-up is recommended for sustainable usage.

*Seroprevalence and associated factors of* Toxoplasma gondii *among HIV-infected patients in Tokyo: A cross sectional study*

An HIV infection, particularly in patients in whom acquired immunodeficiency syndrome (AIDS) has developed, carries a risk of toxoplasmosis with encephalitis, which is mostly caused by a form (bradyzoite) of the protozoan parasite *Toxoplasma gondii*. HIV/AIDS in Japan has been recognized as a serious health issue in recent years. In this study, to elucidate *T. gondii* seroprevalence in HIV-positive patients in Japan and associated characteristics with Toxoplasma parasite infection, the titer of *T. gondii* immunoglobulin (Ig) G (Tg-IgG) was measured in 399 HIV-positive patients who visited a hospital in Tokyo from 2015 through 2017. A questionnaire survey was also conducted to investigate associations of lifestyle and customs. The overall prevalence of Tg-IgG-positive serum was 8.27% (33 of 399 subjects). Positivity for Tg-IgG was confirmed with the Sabin-Feldman dye test; the titers between each examination with strongly correlated ($p < 0.001$, $r = 0.6$). The *T. gondii* infection rate was found to be correlated with age ($p < 0.001$) but was not significantly correlated with lifestyle customs, such as consuming undercooked meat and having a pet cat. An association was observed between *T. gondii* infection and the experience of living in Hokkaido ($p = 0.001$). These results suggest that the rate of previous exposure to *T. gondii* parasites is similar among HIV-positive and HIV-negative populations in Japan and provides clear information about the potential risk of *T. gondii* encephalitis.

*Enterocolitis in the patients with HIV infection*

The clinical course of HIV infection is divided into acute HIV infection, clinical latency, and AIDS. Because the most common symptom of acute HIV infection is fever, following lymphadenopathy, throat pain, and skin eruption, physicians should consider HIV infection when patients have infectious mononucleosis, measles, or rubella. In addition, 30% of HIV patients have diarrhea as a symptom in the acute infection phase. Immunosuppression and microbial dysbiosis in the intestine due to HIV infection cause diarrhea in patients in the acute phase of HIV infection. In this study, we investigated the reason HIV infection was diagnosed in The Jikei University Hospital. We found that HIV infection was diagnosed in 30 of 667 patients when they had diarrhea or bloody stool. In these patients, the most common pathogens were *Entamoeba histolytica,* in 11 patients, and cytomegalovirus, in 5 patients. *Criptosporidium* infection, salmonellosis, giardia intestinalis, *Campylobacter* infection were each diagnosed in 1 patient. These results suggest that physician should notice digestive symptom in HIV patients and consider HIV infection in patients with enterocolitis.

## Publications

***Hoshina T, Horino T, Saiki E, Aonuma H, Sawaki K, Miyajima M, Lee K, Nakaharai K, Shimizu A,***

***Hosaka Y, Kato T, Sato F, Nakazawa Y, Yoshikawa K, Yoshida M, Hori S, Kanuka H.*** Seroprevalence and associated factors of *Toxoplasma gondii* among HIV-infected patients in Tokyo: A cross sectional study. *J Infect Chemother.* 2020 Jan; **26**(1): 33-37. doi: 10.1016/j.jiac.2019.06.012. Epub 2019 Jul 23. PubMed PMID: 31350182.

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***Watanabe N, Saito K, Kiritani A, Fujimoto S, Yamanaka Y, Fujisaki I, Hosoda C, Miyagawa H, Seki Y, Kinoshita A, Takeda H, Endo Y, Kuwano K.*** A case of invasive pulmonary aspergillosis diagnosed by transbronchial lung biopsy during treatment for diabetic ketoacidosis in a type 1 diabetic patient. *J Infect Chemother.* 2020 Feb; 26(2): 274-278. doi: 10.1016/j.jiac.2019.08.011. Epub 2019 Sep 19. PubMed PMID: 31542205.

***Izumisawa T, Kaneko T, Soma M, Imai M, Wakui N, Hasegawa H, Horino T, Takahashi N.*** Augmented Renal Clearance of Vancomycin in Hematologic Malignancy Patients. *Biol Pharm Bull.* 2019 Dec 1; **42**(12): 2089-2094. doi: 10.1248/bpb.b19-00652. Epub 2019 Sep 18. PubMed PMID: 31534058.

### Reviews and Books

***Horino T, Hori S.*** Metastatic infection during *Staphylococcus aureus* bacteremia. *J Infect Chemother.* 2020 Feb; **26**(2): 162-169. doi: 10.1016/j.jiac.2019.10.003. Epub 2019 Oct 30. Review. PubMed PMID: 31676266.

# Department of Dentistry

Katsuhiko Hayashi, *Professor*
Shigeru Suzuki, *Associate Professor*
Takeshi Takayama, *Assistant Professor*

## General Summary

1. Measuring the condylar height in patients with anterior disc displacement (ADD) without reduction using panoramic radiographs
2. Quantitative analysis of a new anatomical indicator of articular surface of condyle (ASC) in the diagnostic image
3. Clinical study of oral appliance (OA) in the treatment of obstructive sleep apnea (OSA) in our hospital

## Research Activities

*Measuring with panoramic radiographs of condylar height in patients undergoing ADD without reduction*

The condylar height of affected side was significantly lower than on the unaffected side in patients who have ADD without reduction.

The aim of the study was to measure the condylar height with panoramic radiographs in patients without osteoarthritis who had undergone ADD without reduction (208 patients, 416 joints). These diagnoses were confirmed with magnetic resonance imaging at the Department of Oral and Maxillofacial Radiology and Diagnosis in Tsurumi University Hospital. We quantitatively and statistically compared differences of right and left condylar heights of these patients. We found that condylar height was significantly lower on the affected side than on the unaffected side. According to the study, panoramic radiographs are useful for screening patients who have undergone ADD without reduction.

*Quantitative analysis of a new anatomical indicator of the ASC (anterior condylar ridge) in the diagnostic image.*

The ASC is a site where morphological changes occur in temporomandibular disorders, but its anatomical indication is unclear. The anterior edge of the ASC coincided with the anterior part of the condyle on the basis of double-contrast enhanced computed tomography (CT) of the temporomandibular joint (TMJ) and has been named the "anterior condylar ridge." The aim of the study was to quantify the position of the anterior condylar ridge with sagittal CT images of the double-contrast enhanced CT of the TMJ.

The joints of 20 patients (3 male and 17 female, age, 13 to 59 years; median age, 28 years) were examined through double-contrast enhanced CT of the TMJ. The reconstructed sagittal images were used to mark the mandibular condyle attachment in the lower joint space. As a position by the bone morphology, we drew a perpendicular line from the connecting line between the lowest point of the eminence and the tympanic fissure (posterior process) to the anterior part of the condyle; this line was marked as the

anterior edge of ASC. The distance between the these marked points were measured twice by 2 observers on different dates, and statistical processing was performed on its reliability.

As a result of measurements by the 2 observers, the intraclass correlation coefficient within the 2 observers was 0.994 to 1.000. In addition, the intraclass correlation between each of the 2 observers was as high as 0.996, which means that the measured values are reliable.

The mandibular condyle attachment in the lower joint space and the anterior ridge of the condyle showed almost the same position on the image, and the anterior condylar ridge was suggested to be an effective index for image diagnosis.

*Clinical study of OA in the treatment of OSA in our hospital*

We perform OA treatment for patients with OSA in cooperation with the departments of otolaryngology, psychiatry, and respiratory medicine in dentistry of The Jikei University School of Medicine. We conducted a clinical study to clarify the characteristics of patients with OSA when OA treatment has problems.

The subject consisted of 146 patients who we treated with OA for 5 years from January, 2014 through December 2018. The OSA had been diagnosed at specialized medical institutions. We examined sex, age, severity of the sleep breathing disorder, treatment regimen, and treatment effect.

Of the 146 patients, 116 were men and 30 were women. Of the patients, 73% were aged 40 to 69 years. The severity of OSA was mild in 42 patients, moderate in 70 patients, and severe in 34 patients. The treatment regimen was OA only in 127 patients, of whom 9 had discontinued continuous positive airway pressure (CPAP) treatment, and a combination of OA and CPAP in 19 patients. Of all patients who had undergone OA treatment, 32 (22%) never visited the hospital for follow‐up, and 65 (44%) underwent polysomnography for determining effects. The OA treatment was considered to have been effective if the apnea‐hypopnea index (AHI) became less than 5 times/hour or became less than 50% of that before treatment. Therefore, OA treatment was considered effective in 43 patients (66%) of 65 patients who underwent polysomnography.

In the comparison of AHI before and after OA treatment, AHI decreased significantly in all severity. When the multiple imputation that considered missing values as a sensitivity analysis was performed, the results were similar.

About 20% of the whole never visited the hospital for follow‐up, and less than half of the whole underwent PSG for determining effects. Therefore, it is important to patients that we enlighten the need of a follow‐up and determining effects effect for OA treatment. AHI before and after OA treatment significantly decreased and we confirmed high usefulness of the OA.

# Department of Transfusion Medicine and Cell Therapy

Tetsunori Tasaki, *Professor* Tomohiko Sato, *Associate Professor*

## General Summary

In 1963, the Blood Transfusion Service division was established in The Jikei University Hospital. In 2018, the division was renamed the Department of Transfusion Medicine and Cell Therapy. The theme of the department's research has gradually changed over 57 years. The research initially dealt with transfusion testing and proper blood transfusion practices, but it recently shifted its focus to autologous blood transfusion and cell therapy using hematopoietic stem cells or dendritic cells. The present subjects of our research are categorized as follows: (1) improvement of transfusion testing, (2) proper storage and use of blood products, (3) adequate treatment or investigation of the cause of transfusion-related adverse events, (4) donation and transfusion of autologous blood, (5) collection, evaluation, and storage of hematopoietic stem cells, (6) support of dendritic cell therapy, and (7) education in the field of transfusion medicine.

## Research Activities

*Critical Reviews*

Associate Professor Sato reviewed 2 articles that were published in *The New England Journal of Medicine*. In the first correspondence, he emphasized the importance of using ABO-matched platelet transfusions to decrease the risk of infrequent but preventable hemolytic transfusion, although the priority for donor-recipient ABO compatibility of plasma seems to be low in Western countries. In the second correspondence, he insisted that in the treatment of severe childhood anemia due to malaria, controlling the underlying disease with antimalarial agents should be given priority over increasing the red blood cell volume administered. Dr. Sato has advised medical students based on his research experience to read medical journals critically, which will upgrade their knowledge and motivate research.

*Prevent bacterial contamination of blood products*

Blood products for transfusion must be aseptically prepared. If saliva has adhered to the plastic needle of the transfusion set, the blood can be contaminated with bacteria. Therefore, to prevent such medical problems, we must wear masks and no talk when handling blood products for transfusion at a nurses' station or bedside. This information was presented by Yuta Furukawa, a medical technologist, at the 56$^{th}$ annual Kanto Kosienetsu regional congress of the Japanese Association of Medical Technologists in 2019.

*A patient with a hemolytic reaction to antibiotics*

We reported on a 42-year-old man with back pain and dyspnea a few minutes after receiving an antibiotic, micafungin sodium hydrate (MCFG). Because gross hematuria

was observed 4 hours later, a hemolytic reaction to MCFG was suspected. *In vitro* examination demonstrated that immune complexes, which included MCFG, had activated complement and induced hemolysis. This case was reported by Miyuki Ishibashi, a qualified medical technologist in transfusion medicine, at the 67th annual meeting of the Japan Society of Transfusion Medicine and Cell Therapy in 2019.

*Multicenter collaborative research*

1. Platelet transfusions for a patient with broad anti-HLA antibodies

Platelets matched for human lymphocyte antigen (HLA) are effective in elevating platelet counts of patients with platelet transfusion refractoriness due to anti-HLA antibodies. In an emergency situation, however, crossmatch-positive platelets are unavoidable. A 74-year-old woman with myelodysplastic syndrome received 30 consecutive HLA-matched platelet concentrate (PC) products, of which 23 were crossmatch-negative. The results of a crossmatch test for 7 PCs were inconclusive. The effectiveness of PCs was evaluated by calculating the 1-hour post-transfusion corrected count increment. Although crossmatch-negative PCs (n = 23) were more effective than other PCs (n = 7), the difference was no statistically significant. In conclusion, HLA-matched PCs other than crossmatch-negative HLA-matched PCs should be used without hesitation in emergency circumstances. This case report was published in the journal *Platelets* by Dr. Takeshi Hagino (Tama-Hokubu Medical Center).

2. The percentage of children having alloantibodies to red blood cells

We sought to clarify the percentage of children who received transfusions and were positive for alloantibodies against red blood cells using a cohort consisting of 17,376 children (1 to 19 years). The data were collected from 51 facilities in Japan. The rates of positive findings were categorized by age group: 1.93% (1 to 4 years), 1.89% (5 to 9 years), 3.01% (10 to 14 years), and 2.34% (15 to 19 years); the mean rate was 2.21%. The rates of production of alloantibodies by alloimmunization were 0.72% (1 to 4 years), 0.82% (5 to 9 years), 0.94% (10 to 14 years), and 1.56% (15 to 19 years). Among alloantibodies, anti-E was seen most frequently (39%). These findings were reported by Dr. Yoshiko Tamai (Hirosaki University) at the 67th annual meeting of the Japan Society of Transfusion Medicine and Cell Therapy in 2019.

## Publications

***Sato T, Goto N, Tasaki T.*** Hemolytic Transfusion Reactions. *N Engl J Med.* 2019 Oct 3; **381**(14): 1396-1397. doi: 10.1056/NEJMc1910551. PubMed PMID: 31577896.

***Sato T, Takahashi K, Tasaki T.*** Transfusion Timing and Volume in African Children with Severe Anemia. *N Engl J Med.* 2019 Oct 24; **381**(17): 1686-1687. doi: 10.1056/NEJMc1911668. PubMed PMID: 31644853.

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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 cellular phenomena associated with proteostatic disruption and (2) subcellular localization analysis of endogenous adjuvant molecules in Japanese cedar pollen.

## Research Activities

*Analysis of cytotoxicity and cell senescence using polyubiquitin as an index (Takada)*
Two complementary proteolytic pathways of ubiquitin-proteasome system and autophagy are involved in intracellular protein homeostasis (proteostasis). Because proteasome recognizes polyubiquitin tags as degradation signals, and the autophagic pathways preferably degrade polyubiquitin-containing aggregates in a p62-dependent manner, the state in which polyubiquitin accumulates in cells indicates proteostatic disruption. We have shown that when epithelial cells are exposed to Cd and methylmercury for 48 hours, both of which are equivalent to the half-lethal concentration ($LC_{50}$), a marked increase in the hardly-soluble polyubiquitinated proteins precedes cell death. In the present study, to find chemicals that exhibit this type of cytotoxicity, we compare the cytotoxic events of chemicals containing metal elements ($FeCl_3$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $AgNO_3$, $ZnSO_4$, and $CdCl_2$) in human renal proximal tubular human kidney-2 cells by using an efficient system comprised of cell culture, cytotoxicity assay, protein assay and polyubiquitin enzyme-linked immunosorbent assay. As a result, exposure to $LC_{50}$ levels of Co, Ag, and Zn significantly increased the cellular amounts of hardly-soluble polyubiquitin, similar to Cd, but no such phenomenon was observed upon exposure to Fe, Ni, and Cu. These results suggest that the cytotoxicity of metal ions can be classified into at least 2 types: those with or without the proteostatic disruption.
Because proteasome and autophagy activities decrease with aging (Sands *et al*, J. Physiol. 595: 6383-90, 2017), senescence will affect proteostasis. In this study, we focused on the methyltransferase enzyme Su(var)3-9, Enhancer-of-zeste, and Trithorax domain-containing protein 8 (SETD8), which regulates cellular senescence (Tanaka *et al*. Cell Reports 18; 2148-61, 2017), and studied the effect of an SETD8 inhibitor (UNC0379) on cellular polyubiquitin levels in human epidermal keratinocyte HaCaT cells. After 6-day culturing with 10 μM UNC0379, staining of senescence-associated beta-galactosidase activity, a marker of cell senescence, was observed. The enzyme-linked immunosorbent assay-estimated polyubiquitin levels in the easily-soluble and hardly-soluble fractions prepared from the cells were significantly increased by the treatment with UNC0379. These results suggest that the cell senescence due to SETD8 suppression is accompanied by proteos-

tatic disruption.

*The immunostimulatory effect of β-1,3-D-glucan contained in Japanese cedar pollen (Hiratsuka)*
Many Japanese have cedar pollinosis. Under these circumstances, cedar pollen allergens have often been analyzed, but there are many unclear points regarding auxiliary substances involved in promoting allergic reactions. Therefore, we are analyzing the immunostimulatory effect of β-1,3-D-glucan (β glucan) contained in cedar pollen. As a result, the localization of β glucan in the outer wall of pollen and the germ cell wall was clarified, and the relationship with the β glucan receptor Dectin-1 is currently being analyzed.

# 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 or modulating 1-dimensional photonic crystal. We are investigating the relation between the conditions of the amplification and 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 condensed matter theory. The phenomena interested in are phase transitions and critical phenomena.

## Research Activities

1. We consider amplification of an incident electromagnetic wave within a photonic crystal, which is made by stacking dielectric plates arranged in parallel with equi-intervals. We have confirmed that an incident electromagnetic wave is resonantly amplified when the dielectric constants of the plates are artificially modulated in time and space.
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

effect of scattering by the skull on the phononic structure will be discussed.
3. The optimized lens constructed by arranging point-like scatterers on a lattice has been designed in terms of the concept of the Fresnel lens. The arrangement of the scatterers has been optimized so that the wave intensity vanishes at any point except the focal point within the object domain and to make the wave intensity convex around the focal point. We have confirmed that the optimized lens focuses an incident plane wave sharply on the focal point, whereas the wave intensity at the points except the focal point is constant and small enough.
4. We have made Monte Carlo simulations to study systems with phase transitions. Multigrid cluster update simulations are used to study $q$-state ferromagnetic Potts models on square lattices. We calculated the relaxation times of order parameters and energy.

### Publications

***Dincel O, Ueta T, Kameoka J.*** Acoustic Driven Microbubble Motor Device. *Sensors & Actuators A: Physical.* 2019; **295:** 343-347. doi: 10.1016/j.sna.2019.05.013.
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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

*Conformation analysis of anti-gauche transformation of butane*
The anti-gauche conformational energy change of butane was reanalyzed with the second-order Møller-Plesset method and the large basis functions because of the differences (2.5-3.8 kJ/mol) among organic chemistry textbooks. The calculated energy difference between anti conformation and gauche conformers was 2.3 kJ/mol. This smaller difference is due to the additional van der Waals interaction between the methyl groups.

*Phosphorescent molecules that resist concentration quenching in the solution state*
The first phosphorescent molecules that do not exhibit concentration quenching in the homogeneous solution state throughout the entire range of concentrations were discovered in this study. A series of newly designed polymethylene-vaulted trans-bis[2-

(iminomethyl)imidazolato]platinum(II) complexes was prepared by treating $[PtCl_2(CH_3CN)_2]$ with the corresponding imino ligands. The trans coordination and vaulted structures of the complexes have been unequivocally established from X-ray diffraction studies. When the concentration of a clear homogeneous solution of the complexes in organic solvents increases from the diluted to the saturated state, the emission intensity and quantum efficiency increase continuously without concentration quenching at the ambient temperature. The present concentration-driven emission enhancement is observed more intensely in a solution of a racemic mixture in comparison to that of the optically pure solution.

### Publications

***Le NH, Inoue R, Kawamorita S, Komiya N, Naota T.*** Phosphorescent Molecules That Resist Concentration Quenching in the Solution State: Concentration-Driven Emission Enhancement of Vaulted *trans*-Bis[2-(iminomethyl)imidazolato]platinum(II) Complexes. *Inorg Chem.* 2019 Jul 15; **58**(14): 9076-9084. doi: 10.1021/acs.inorgchem.9b00608. Epub 2019 Jun 24. PMID: 31247829.

## Social Science (Law)

Ryuichi Ozawa, *Professor*

### General Summary

Problems of Constitutional Law in present-day Japan

### Research Activities

Professor Ozawa published articles and books cited in Japanese Research Activities 2019.

## 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 a subject of thought, is still a popular and paradigmatic image for the human subject: to be a mature human means that one can think indepen-

dently 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 show how important the relationship of the baby with his mother is at the first stage of the ego. George Herbert Mead considers 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 experiences in Auschwitz*
From another respect, the "inhuman" situations in the Auschwitz concentration camp showed various elements needed to be "human." From the experiences in 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 the review conversation in the closing psychotherapy session.

### Research Activities

The characteristics, contents, and expressions of the review conversation in the closing psychotherapy session by an expert therapist was examined by applying the Roter Interaction Analysis System (RIAS), a quantitative method to analyze medical communication.
The study was presented at the 20th annual meeting of the Japanese Federation for Psychotherapy held in Yokohama on May 11, 2019.

# Mathematics

Katsuya Yokoi, *Professor*
Yasuko Hasegawa, *Assistant Professor*

### General Summary

I. To study dimension theory and topological dynamics
II. Applications of automorphic forms to number theory

### Research Activities

I. We studied omega-limit sets, (strong) chain recurrent sets on topological dynamics, Conley index theory, and Lusternik-Schnirelmann category.
II. Many analytical properties of a real analytic Siegel-Eisenstein series, which is one of several variable automorphic forms, have been studied for a long time. We were able to prove some analytical properties of certain Dirichlet series through the relation of the Siegel-Eisenstein series to the Dirichlet series.

### Publications

***Yokoi K.*** Lusternik-Schnirelmann category based on the discrete Conley index theory. *Glasgow Mathematical Journal.* 2019; **61**(3): 693-704. doi: 10.1017/S0017089518000447.

## English

Alan Hauk, *Professor*

Tetsuro Fujii, *Professor*

### General Summary

*Medical English education and materials development (Hauk)*
*English Language communication and education: material analysis and development (Fujii)*
Hauk has been developing in-house medical English teaching materials for use in a new English program for 2nd-, 3rd-, and 4th-year English classes. The materials emphasize learning medical vocabulary in context and cyclical learning as a way to improve students' understanding and retention of class contents. In addition, the materials are designed to connect to what the students are studying in medical classes to make them more relevant and to improve students' motivation to learn English. Hauk has also been active as an editorial committee member of the *Journal of Medical English Education*, reviewing manuscripts submitted for publication.
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

Hauk researched what overseas medical students are learning in standard medical textbooks to make materials for teaching medical English in the university. Also, research was conducted in how to improve understanding and retention of study materials by students. The goal is to produce a textbook for medical English that will meet the students' needs in the future as physicians in the global medical community.

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 issued by the Ministry of Education, Culture, Sports, Science and Technology. The new textbook series will be published in 2020. In addition, Fujii studied bible verses as materials to learn English and published *Eigo de Ajiwau Seisho no Kotoba* (*Appreciating Bible Verses in English*. Tokyo: Inochi No Kotoba Sha; 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 with the novels of Sherko Fatah. His father is Kurdish-Iraqi, and his mother is Polish-German. In fact, he is a native writer with the background as 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.

# Laboratories
# 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 on preventive medicine against sarcopenia and metabolic syndrome in terms of exercise physiology.

## Research Activities

*Age-related difference in autophagic adaptation and the effect of resistance exercise in rat soleus muscle atrophied with unloading*

We have reported that unloading selectively affected type I fibers in aged rats disrupts myofibrils with a decrease in sarcomeric proteins, forming inclusion bodies and accumulating abnormal mitochondria. In the present study we aimed to clarify age-related differences in autophagic adaptation and the effects of intermittent resistance-exercise, which is known to ameliorate sarcopenia, in unloaded muscles of rats. Unloading-induced atrophy with a degenerative decrease in myofibrillar protein concentration was more prominently observed in aged rats (2 years old) than in young rats (4 months old). F-box (Fbx) 32, a muscle-specific ubiquitin ligase, increased, along with an increase in ubiquitinated protein, by unloading in both aged and young rats. Light chain 3 (LC3)-II, an autophagy marker protein, and mitochondrial calcium uniporter (MCU), a key protein activating mitochondrial biogenesis and of signaling pathways for muscle hypertrophy, increased with unloading in young rats but decreased in aged rats. Proliferator-activated receptor-gamma coactivator 1 (PGC1), playing roles similar to those of MCU, decreased with unloading to a specifically greater extent in aged rats than in young rats. Intermittent resistance-exercise ameliorated atrophy similarly in the rats of both ages, while the levels of LC3-II, MCU, and PGC1 were still lower than control levels in aged rats but not in young rats. In conclusion, autophagic adaptation and myogenic response were critically different with age in the rat soleus. These differences might be responsible for age-related muscle responsiveness to unloading and training.

*The effects of eccentric contraction on unloaded skeletal muscle.*

We have been studying the effect of eccentric contraction (ECC) on the contractile ability, protein signals, intramuscular microstructure to find optimized intensity of ECC for rehabilitation practice to induce muscle protein synthesis while suppressing deteriorative damages. Practical objective muscle of rehabilitation is generally in a catabolic process. Therefore, we studied the effects of ECC on the unloaded muscle of rats that have been tail-suspended for a short period (72 hours). Plantaris muscle received 30 sessions of ECC with stretching to the length of maximal isometric twitch tension ($L_0$) from 0.9 $L_0$

during 0.3 second tetanic contraction elicited by 50 Hz supramaximal electrical stimulation through tibial nerve under anesthetized condition. One hour after the series of ECC sessions, muscle was dissected for analysis with x-ray diffraction and electrophoresis. Significant structural changes were not observed in control and unloaded muscles. Incomplete isometric tetanus tension elicited by 40 Hz stimulation was significantly reduced in the tail-suspended muscle compared with the non-suspended muscle. Therefore, catabolic processes would make skeletal muscle more susceptible to damages induced by ECC probably through deterioration in excitation-contraction coupling. It seems safe to limit ECC intensity at a lower level in general rehabilitation practice.

*Effect of 2,3-butanedione monoxime on the structure of extraocular muscle revealed by x-ray diffraction*

Extraocular muscles show diverse range of contraction modality from slow sustained contraction to fast instantaneous twitches. We performed x-ray diffraction experiments of extraocular muscle to obtain structural characteristics of the muscle. Skinned fibers of extraocular muscle lacked a sampling peak on the myosin layer line at 0.05/nm suggesting a possibility that the extraocular muscle have more mobile myosin heads with a wider range of motion compared with other muscles. To elucidate whether the higher mobility is due to a larger proportion of mobile intermediate of myosin heads hydrolyzing ATP, we examined the effect of 2,3-butanedione monoxime, which is known to shift myosin heads from a mobile intermediate to a stable one. Skinned fibers were prepared from fast type muscle, slow type muscle, and extraocular muscle of male rabbits. X-ray diffraction experiments were carried out at the BL6A station of the Photon Factory in the High Energy Accelerator Research Organization. Although 2,3-butanedione monoxime failed to induce the sampling peak on the myosin layer line at 0.05/nm, it unexpectedly enhanced the intensity at 0.04/nm in the extraocular muscle. The myosin heads in the extraocular muscle fibers might be intrinsically arranged to form a simple lattice structure.

*Effects of chronic exercise combined with dietary restriction on the ultrastructure and metabolism of soleus muscle in Wistar Bonn Kobori fatty rats*

Wistar Bonn Kobori fatty rats lack leptin receptors and develop chronic pancreatitis and diabetes with obesity. Our recent analysis of the ultrastructure and metabolism of this rat's pancreatic tissue indicated that adequate habitual exercise combined with dietary restriction improved pancreatic exocrine and endocrine functions and prevented the development of diabetes. This prevention of diabetes is in contrast with dietary restriction without exercise, which had little effect on pancreatic functions and the development of diabetes. Exercise is believed to improve pancreatic function through metabolic adaptation in the metabolism of the involved muscles or in cardiopulmonary function. Therefore, we further investigated the effects of dietary restriction and exercise on the ultrastructure and metabolism of the involved muscle and on the improvement of diabetes. Dietary restriction and exercise improved mitochondrial lipid accumulation and swelling, oxidative metabolism, and glucose uptake ability in soleus muscle. These changes might help prevent the development of diabetes in the rat. The results partly support the impor-

tance of habitual exercise in preventing diabetes in obese persons.

*Habitual exercise with dietary restriction enhances hepatic fatty acid binding protein 1 expression and ameliorates fatty liver in hyperphagic fatty rats*

Weight control solely depending on dietary restriction might cause a failure in lipid metabolism and progression of fatty liver. Fatty acid binding protein 1 (FABP1) is generally postulated to work as a fatty acid delivery controller to intracellular organelles. Therefore, we studied the dynamics of FABP1 at conditions of dietary restriction and habitual exercise combined with dietary restriction in male Zucker fatty rats. With ordinary dietary conditions, the rats became obese with a fatty liver, increases in serum free fatty acid and hepatic fatty acid translocase (FAT)/CD36, and a slight decrease in FABP1. Dietary restriction without exercise exacerbated fatty liver with a decrease in hepatic FABP1 and an increase in hepatic FAT/CD36. On the other hand, dietary restriction and exercise improved fatty liver with a decrease in serum levels of free fatty acid and hepatic FAT/CD36 and an increase in hepatic FABP1. The hepatic triglyceride content levels negatively correlated with hepatic FABP1 protein expression levels. These results suggest that hepatic FABP1 plays a crucial role in the development and progress of fatty liver in hyperphagic Zucker fatty rats.

# Laboratories
# Aerospace Medicine

Susumu Minamisawa, *Professor* Hiroki Bochimoto, *Assistant Professor*

## General Summary

Our main research interests are gravitational physiology and aerospace medicine.

## Research Activities

*Phenotypic analysis of juvenile onset dilated cardiomyopathy mouse model*
Dilated cardiomyopathy (DCM) is characterized by cardiac dilation and pump failure. However, a fundamental therapy for DCM has not been established. In particular, the DCM that develops in young persons has a poor prognosis. Troponin T amino acid mutation (ΔK210) knock-in mouse (ΔK 210-KI) generated by Morimoto et al., is considered to have a phenotype similar to that of human juvenile DCM. However, neither the neonatal period nor the weaning period was 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 the homozygous ΔK210-KI mice have already developed cardiac hypertrophy at birth. Furthermore, we are researching for the development of gene therapy by replacing mutant troponin T with normal troponin T overexpression.

*Molecular mechanisms of intracellular $Ca^{2+}$ mediated muscle atrophy*
Induced muscle atrophy by tail-suspension and denervation increased 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 KO mice and denervated wild type mice. Muscle atrophy in denervated sarcolipin KO mice was less than that in denervated wild type mice. We are now examining these molecular mechanisms.

*The effect of microgravity on the morphology of the stomach tissue of mice*
For people to live in space, nutritional mechanisms in space must be understood. We analyzed the histological changes that occurred in the fundus gland of tissues of the stomach of mice that were on the International Space Station for 35 days. We found that the cytoplasmic and nucleic areas of parietal cells were reduced after spaceflight. Currently, we are examining this intracellular ultrastructure.

*Morphological analysis in transplantation strategy of machine perfusion preservation*
The use of marginal donors and donors after circulatory death is an important way of resolving the critical shortage of the donor organ pool. We aim to develop new preservation technology of machine perfusion preservation for marginal grafts. We are analyzing

the ultrastructural characteristics of porcine livers donated after cardiac death and preserved with machine perfusion preservation.

### Publications

***Shonaka T, Matsuno N, Obara H, Yoshikawa R, Nishikawa Y, Ishihara Y, Bochimoto H, Gochi M, Otani M, Kanazawa H, Azuma H, Sakai H, Furukawa H.*** Impact of human-derived hemoglobin based oxygen vesicles as a machine perfusion solution for liver donation after cardiac death in a pig model. *PLoS One.* 2019 Dec 11; **14**(12): e0226183. doi: 10.1371/journal.pone.0226183. eCollection 2019. PubMed PMID: 31825976; PubMed Central PMCID: PMC6905570.

***Takano R, Kozuka-Hata H, Kondoh D, Bochimoto H, Oyama M, Kato K.*** A High-Resolution Map of SBP1 Interactomes in Plasmodium falciparum-infected Erythrocytes. *iScience.* 2019 Sep 27; **19**: 703-714. doi: 10.1016/j.isci.2019.07.035. Epub 2019 Jul 25. PubMed PMID: 31476617; PubMed Central PMCID: PMC6728614.

***Bochimoto H, Kondoh D, Ishihara Y, Kabir MHB, Kato K.*** Three-dimensional fine structure of feeder organelle in Cryptosporidium parvum. *Parasitol Int.* 2019 Dec; **73**: 101958. doi: 10.1016/j.parint.2019.101958. Epub 2019 Jul 9. PubMed PMID: 31299356.

***Bochimoto H, Kondoh D, Nagata R, Ishihara Y, Tomiyasu J, Han KH, Shimada K, Sasaki M, Kitamura N, Fukushima M.*** Ultrastructural changes in colonic epithelial cells in a rat model of inflammatory bowel disease. *Microsc Res Tech.* 2019 Aug; **82**(8): 1339-1344. doi: 10.1002/jemt.23285. Epub 2019 May 9. PubMed PMID: 31070847.

***Tanihata J, Nishioka N, Inoue T, Bando K, Minamisawa S.*** Urinary Titin Is Increased in Patients After Cardiac Surgery. *Front Cardiovasc Med.* 2019 Feb 8; **6**: 7. doi: 10.3389/fcvm.2019.00007. eCollection 2019. PubMed PMID: 30800662; PubMed Central PMCID: PMC6375839.

***Echigoya Y, Lim KRQ, Melo D, Bao B, Trieu N, Mizobe Y, Maruyama R, Mamchaoui K, Tanihata J, Aoki Y, Takeda S, Mouly V, Duddy W, Yokota T.*** Exons 45-55 Skipping Using Mutation-Tailored Cocktails of Antisense Morpholinos in the DMD Gene. *Mol Ther.* 2019 Nov 6; **27**(11): 2005-2017. doi: 10.1016/j.ymthe.2019.07.012. Epub 2019 Jul 26. PubMed PMID: 31416775; PubMed Central PMCID: PMC6838919.

***Hosokawa M, Takeuchi A, Tanihata J, Iida K, Takeda S, Hagiwara M.*** Loss of RNA-Binding Protein Sfpq Causes Long-Gene Transcriptopathy in Skeletal Muscle and Severe Muscle Mass Reduction with Metabolic Myopathy. *iScience.* 2019 Mar 29; **13**: 229-242. doi: 10.1016/j.isci.2019.02.023. Epub 2019 Feb 27. PubMed PMID: 30870781; PubMed Central PMCID: PMC6416966.

***Shibasaki H, Imamura M, Arima S, Tanihata J, Kuraoka M, Matsuzaka Y, Uchiumi F, Tanuma SI, Takeda S.*** Characterization of a novel microRNA, miR-188, elevated in serum of muscular dystrophy dog model. *PLoS One.* 2019 Jan 30; **14**(1): e0211597. doi: 10.1371/journal.pone.0211597. eCollection 2019. PubMed PMID: 30699200; PubMed Central PMCID: PMC6353185.

***Masaki Y, Yamamoto K, Inde T, Yoshida K, Maruyama A, Nagata T, Tanihata J, Takeda S, Sekine M, Seio K.*** Synthesis of 2'-O-(N-methylcarbamoylethyl) 5-methyl-2-thiouridine and its application to splice-switching oligonucleotides. *Bioorg Med Chem Lett.* 2019 Jan 15; **29**(2): 160-163. doi: 10.1016/j.bmcl.2018.12.005. Epub 2018 Dec 4. PubMed PMID: 30551900.

# Laboratories
# Neuropathology

Senya Matsufuji, *Professor*

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

*Caspase independent apoptosis in the central nervous system in mouse models of prosaposin deficiency disease*

Introduction: The pathophysiological changes of the central nervous system (CNS) in prosaposin knockout mice accompanies 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 increases in proportion to the number of amino-cupric-silver-impregnated neurons. However, caspase-dependent apoptosis has not been evaluated with active caspase 3 immunohistochemistry and the terminal deoxyribonucleotidyl transferase-mediated deoxyuridine triphosphate-biotin fluorescein nick-end labeling (TUNEL) method. Although much emphasis has been laid on the role of caspase in cell death, recent data indicate that, in many instances, mammalian cell death is caspase-independent. Apoptosis-inducing factor (AIF) is a new mammalian, caspase-independent death effector which, upon apoptosis induction, translocates from its normal localization, the mitochondrial intermembrane space, to the nucleus. Once in the nucleus, AIF causes chromatin condensation and large-scale DNA fragmentation to fragments of approximately 50 kbp. This study investigated the AIF in the CNS of prosaposin knockout mice using anti-AIF antibodies.

Material and methods: We analyzed the central CNS of mouse models of prosaposin deficiency with the amino-cupric-silver method and immunohistochemical methods with antibodies against SCMAS and AIF.

Results: From 8 days of age, the neurons of the spinal cord, brain stem, deep cerebellar gray matter, diencephalon, striatum, and cerebrum in prosaposin knockout mice immunoexpressed SCMAS in proportion to the amino-cupric-silver-impregnated neurons. Subsequently, the distribution of SCMAS immunoreactive and the amino-cupric-silver-impregnated neurons spread in the CNS in general. The AIF migrated into the nuclei of neurons in the spinal cord, brainstem nuclei, deep cerebellar gray matter, and striatum from 21 days of age and was persistently expressed in the nuclei from approximately 30 days of age until death.

Discussion: In the CNS neurons of prosaposin knockout mice, swollen lysosomes accumulated. The number of structurally preserved peroxisomes and Golgi apparatuses decreased slightly. In contrast, the number of mitochondria, endosomes, endoplasmic reticulum, and ribosomes decreased markedly. After sorting in endosomes, most of the proteins are rapidly recycled. Degraded proteins are packaged into lysosomes and then processed into ubiquitin–proteasome system or autophagy–lysosome system. In lysosomal storage diseases, recycled endosomes were inhibited and degraded proteins accumulated in the lysosomes. Mitochondria depletion developed the energy crisis and decreased the activity of protein and lipid synthesis in ribosomes and endoplasmic reticulum.
We also reported the accumulation of SCMAS in the neuronal cytoplasm of neuronal ceroid lipofuscinoses; mucopolysaccharidoses types I, II, and VII; Niemann–Pick disease type C; Fabry disease; mucolipidoses; and methylenetetrahydrofolate reductase deficiency and that SCMAS is a candidate for amino–cupric–silver–impregnated material in the CNS of prosaposin knockout mice. The increase of ATP synthase might be induced by the depletion of ATP because of the organelles' dysfunction and the activations of ubiquitin–proteasome and autophagy–lysosome systems. However, caspase–dependent apoptosis has not been evaluated with active caspase 3 immunohistochemistry and the TUNEL method. The AIF is released from mitochondria during cell death and migrates into the nucleus and might be involved in DNA aggregation and fragmentation in a mouse model of prosaposin deficiency disease.

### Publications

***Kawamura M, Sato S, Matsumoto G, Fukuda T, Shiba–Fukushima K, Noda S, Takanashi M, Mori N, Hattori N.*** Loss of nuclear REST/NRSF in aged–dopaminergic neurons in Parkinson's disease patients. *Neurosci Lett.* 2019 Apr 23; **699**: 59–63. doi: 10.1016/j.neulet.2019.01.042. Epub 2019 Jan 23. PubMed PMID: 30684677.

***Noda S, Sato S, Fukuda T, Tada N, Uchiyama Y, Tanaka K, Hattori N.*** Loss of Parkin contributes to mitochondrial turnover and dopaminergic neuronal loss in aged mice. *Neurobiol Dis.* 2020 Mar; **136**: 104717. doi: 10.1016/j.nbd.2019.104717. Epub 2019 Dec 15. PubMed PMID: 31846738.

***Noda S, Sato S, Fukuda T, Tada N, Hattori N.*** Aging–related motor function and dopaminergic neuronal loss in C57BL/6 mice. *Mol Brain.* 2020 Mar 23; **13**(1): 46. doi: 10.1186/s13041–020–00585–6. PubMed PMID: 32293495; PubMed Central PMCID: PMC7092461.

# Laboratories
# Sports Medicine

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

## General Summary

*Clinical Research*
The ongoing research in our division 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

*Pain of the anterior superior iliac spine in the athletes in growth period*
Physical, roentgenographical, and magnetic resonance findings, and duration of absence were evaluated in 10 patients in the growth period (average age: 14 years old) who had pain of anterior superior iliac spine. Symptoms were presented at the side of the pivot foot in 6 of 7 soccer players. The pain had a relatively chronic onset. Fat suppressed magnetic resonance imaging showed high signal intensity at the epiphysis, bone marrow, and sounding muscles. The average duration of absence was approximately 7 weeks.

*Recovery process of neuromuscular coordination after a return to sports in patients who had undergone anterior cruciate ligament reconstruction: Comparison of 1 month after returning to sports and 20 months after surgery*
The recovery process of neuromuscular coordination after the return to sports of 41 patients who had undergone anterior cruciate ligament reconstruction was evaluated with the switching silent period (SSP). One month after the return to sports, SSP was significantly longer in the operated side than in the nonoperated side. The SSP 20 months after surgery had significantly improved in the operated side and 1 month after the return to sports did not differ statistically between the nonoperated side and the operated side. Nerve-muscle coordination of the operated side was decreased 1 month after the return to sports but had significantly recovered 20 months after surgery.

*Platelet-rich plasma therapy for sports-related tendon and ligament injuries*
We have started a clinical trial of platelet-rich plasma therapy for sports-related tendon and ligament injuries. So far, we have performed this therapy for 8 patients. We have verified that this therapy is safe and effective for medial collateral ligament injury of the knee, tendinopathy of Achilles tendon, and lateral epicondylar of the elbow.

*Evaluation of muscle training method for the rotator cuff: Comparison between closed kinetic chain and open kinetic chain*
The effectiveness of muscle strength training between closed kinetic chain (CKC) and

open kinetic chain (OKC) cuff-exercises was compared in 42 patients with disabled throwing shoulder. We found that the impossibility rate was significantly lower with the CKC cuff exercise than with the OKC cuff exercise. In addition, the degree of increased muscle strengthening with the CKC cuff exercise was equal to or higher than that with OKC cuff exercise. The CKC cuff-exercise is suggested to be effective and can be performed even in the acute phase.

*A case of a osteochondral lesion at the patellar side of the femur treated with open reduction and internal fixation*

We reported a rare case of an osteochondral lesion at the patellar side of the femur in a 13-year-old boy. The lesion was treated with open reduction and internal fixation. Magnetic resonance images showed a $1.7 \times 1.6$-cm cartilage defect on the patellar side of the lateral condyle. A freely isolated fragment of the articular cartilage was reduced and fixed with 2 bioabsorbable pins. Second-look arthroscopy 5 months after initial surgery revealed the fragment was included in a stable union.

*Simultaneous avulsion fracture at the tibial tubercle and the inferior pole of the patella occured in a growth period: A case report*

We reported a simultaneous avulsion fracture at the tibial tubercle and inferior pole of the patella in a 13-year-old soccer player. Radiologic images showed patella alta and an avulsion fracture at inferior-medial pole of the patella. Magnetic resonance imaging showed the patella tendon attached to the bone fragment. Open reduction and internal fixation was performed with suture bridge technique, an bioabsorbable pin, and suture anchors. Five months after surgery, the patient returned to play at soccer practice. This type of fracture is so rare that only 5 cases have been reported.

*Exercise-induced arrhythmia in a professional soccer player which had difficulty in diagnosis: A case report*

We reported on a 27-year-old professional soccer player who had exercise-induced arrhythmia that was difficult to diagnosis at an early stage. At first, the symptoms were of low frequency and short duration. Although examinations at rest had normal finding, an electrocardiographic event recorder during exercise for several days first demonstrated abnormal findings. Exercise-induced arrhythmia was finally diagnosed with a high load during treadmill testing. One year 6 months after catheter ablation, the patients has returned to play with no recurrence.

## Publications

***Hayashi H, Kurosaka D, Saito M, Ikeda R, Kubota D, Kayama T, Hyakutake T, Marumo K.*** Positioning the femoral bone socket and the tibial bone tunnel using a rectangular retro-dilator in anterior cruciate ligament reconstruction. *PLoS One*. 2019 May 2; **14**(5): e0215778. doi: 10.1371/journal.pone.0215778. PMID: 31048889; PMCID: PMC6497238.

# Endowed Departments
# 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*

Molecular targeted therapies, such as monoclonal antibodies, were widely used for various cancers recently, leading to improve patients' outcomes. Photoimmunotherapy (PIT) is a new class of molecular targeted cancer theranostics, which employs monoclonal antibody conjugated to a photosensitizer, IR700, that is activated by focal near-infrared (NIR) light irradiation leading to necrotic cell death by cell membrane rapture where antibody-IR700 conjugates binds to target membrane proteins specifically. A global phase 3 clinical trial of PIT for recurrent head and neck cancer patients has been conducted utilizing anti-EGFR monoclonal antibody, cetuximab-IR700 conjugate. We have recently developed a new type of PIT agents targeting VEGFR-2 expressed on vascular endothelium in a tumor. VEGFR-2 expressing NCI-N87 tumors were successfully treated with VEGFR-2-targeting DC101-IR700 conjugate and NIR light by damaging microvessels in the tumors. As VEGFR-2 is upregulated in many types of solid cancers, this method may be considered as being applicable to various types of cancers. Here, we tried to examine the feasibility of endoscopic molecular imaging of inducible murine colon cancer with DC101-IR700 in DSS-treated Apc/min+ mice. In contrast to cell line derived xenograft

models, the tumor lesions of this mouse possess the histological complexity and heterogeneity. DSS-treated mice were monitored for 2 weeks to obtain the recovery of colitis, then endoscopic examination was performed under anesthesia. Pediatric fiberoptic bronchoscope was used as mouse colonoscopy, and distal side of the colonic mucosa was examined with white light imaging as well as IR700 fluorescence imaging. Tumor lesions of were successfully detected both imaging methods. Additionally, pathological examination revealed that tumor lesions were adenocarcinoma. We plan to examine a treatment effect in response to endoscopic NIR-PIT by using this model to prove a concept of endoscopic molecular theranostics.

*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 States 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 has been available in Europe since 2018. In 2019, Tajiri gave advice to preparing application materials to Pharmaceuticals and Medical Devices (PMDA) for introducing the instrument to Japan, as well as being responsible in shared writing the educational English textbook for its global diffusion. Since the motorized spiral enteroscope needs less procedure time compared to the conventional scope, we anticipate that this technology will be applied to colonoscope in the near future and revolutionize endoscopic medicine.

*Endoscopic Research utilizing Japan Endoscopy Database (JED) and AI*

Japan Endoscopy Database (JED) Project, started in 2015, started the operation of the automatic registration and is in the process of making data entry mandatory at all facilities, with the support of nearly 1,450 educational facilities. JED Institute was established on 1 March, 2018, and Tajiri has served the first President. Activities of operation and deployment related to JED project are conducted and collected data is analyzed, then given feedback to medical institution to improve the quality of endoscopy and standardize the technology. Japan Agency for Medical Research and Development (AMED) is conducting joint research with National Institute of Informatics (NII), as public offered research project, progressing the research of endoscopic image diagnosis using AI. In 2019, we conducted joint research to evaluate the quality of medicine and the practicality of ensuring safety focusing on unique AI research such as "deviation monitoring during screening", which solves the urgent problems that the endoscopists need in clinical scene. Since collecting large amounts of data matters, it is desired to construct a system fostering study mind to cooperate in collecting data, reducing the burden for the work and facilitating verification of AI. Japanese technology of GI endoscopy is a world-class proud, that precise manufacturing environment and the dexterity of endoscopists of this country has accomplished. By combining Japanese technology with AI, which is quite advanced,

innovation never before is convinced to be possible.

*Educational Activity*

Since 2014 the Japan Gastroenterological Endoscopy Society has been leading hands-on courses in China, and in 2019, Tajiri visited Peking, Dairen and Zunyi, where Tajiri gave lectures and performed therapeutic manipulation with animal models and live demonstrations. In addition, new hands-on courses have been conducted in Cambodia and Mongolia in 2019. The young physicians being trained made remarkable progress in early cancer detection and diagnosis and endoscopic treatment. In Russia, Vietnam, Myanmar, and Indonesia, we have been conducting similar activities. Hands-on courses have already been held in rural regions of Japan. Tajiri visited and will visit the regions directly, to improve the environment for facilities, responsible for community medicine, to standardize endoscopic diagnosis and treatment. Tajiri will continue to conduct these supportive activities.

*Inspection/Evaluation*

Remarkable results have been achieved up to the clinically applicable in the research of molecular diagnosis of cancer using fluorescence probe and development of endoscopic molecular theranostics. The development of devices to realize endoscopic molecular theranostics is in progress. As the first President of JED Institute, Tajiri is promoting close joint research with related parties to bring about innovation never before, by combining advanced GI endoscopy technology with AI in Japan.

**Publications**

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# Endowed Departments
# Department of Environmental Allergy

Saburo Saito, *Professor*

## General Summary

This department was established in April 2019 with the aim of disseminating cedar pollen rice, which has few side effects and can be an effective immunotherapy against cedar pollinosis; elucidating the antigen recognition mechanism of T cells for contact dermatitis caused by small molecules, such as drugs and metals; and further evaluating the usefulness of plaster used as a building material from the viewpoint of antiallergic effects.

## Research Activities

*Rice-based oral peptide vaccine for Japanese cedar pollinosis*

Previously transgenic rice seeds contains a hybrid peptide called 7Crp peptide consisting of 7 linked dominant human T-cell epitopes derived from Cry j 1 and Cry j 2, the 2 major allergens of Japanese cedar pollen. Recent clinical studies have shown that oral administration of transgenic rice seeds significantly suppresses specific T cell responses without side effects. This finding suggests that genetically modified rice will be an effective immunotherapy that can improve clinical symptoms.

To evaluate the oral tolerance mechanism and the improvement of clinical symptoms, we performed a randomized, placebo-controlled study of oral immunotherapy with the transgenic rice seeds for Japanese cedar pollinosis. The subjects were divided into 3 groups that were evaluated after having orally ingested 5 g of TG seeds plus 45 g of control rice seeds, 20 g of transgenic rice seeds plus 30 g of control rice seeds, or 50 g of control rice seeds. The transgenic rice was orally administered for 6 months from before to after pollen season for 2 years.

We found that the reactivity of allergen-specific T cells was significantly suppressed. Interestingly, oral intake of transgenic rice seeds reduced medication usage during the Japanese cedar pollen season, although clinical symptom during 2 Japanese cedar pollen seasons did not differ significantly among the 3 groups.

These results suggest that the administration of transgenic rice seeds will clinically improve pollen symptoms. Further studies are needed to analyze the use of transgenic rice seeds by increasing the number of subjects and the administration period.

*Antigen-specific recognition mechanism of paraphenylenediamine-specific T-cells*

Allergic contact dermatitis due to paraphenylenediamine (PPD) has recently increased in both hairdressers who dye hair and in people whose hair is dyed. Exposure to PPD, a central component of most permanent hair dye formulations, is associated with the development of T-cell-mediated allergic contact dermatitis. To analyze the characteristics of antigen-specific T cells, the characteristics need to be examined in the presence of autologous

antigen-presenting cells; therefore, analysis with human T cells is difficult.
The aim of the present study was to generate PPD-specific T-cell lines and explore the mechanism of antigen presentation to T cells in a mouse model. Mice of the C57BL/6 strain were immunized with PPD via subcutaneous injection once a week for 6 weeks, and antigen-specific T-cell proliferation of spleen cells was analyzed 7 days after the last immunization. After more than 7 cycles of antigen stimulation once every 2 or 3 weeks in the presence of the antigen-presenting cells, PPD-specific T-cell lines were established, and their antigen specificity was investigated with structurally related chemicals.
Established T-cell lines were CD4-positive, secrete interleukin 4, and cross-react with the oxidoconjugation product Bandrowski's base. In addition, the reactivity of PPD-specific T cells was suppressed in the presence of the chemicals with a thiol group. It remains unclear how PPD is presented to T cells, but the results suggest how PPD associates with self-proteins.

## Publications

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# Research Center for Medical Sciences
# Division of Gene Therapy

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

## General Summary

*Education and research outline*
In terms of education, graduate students were instructed in basic gene manipulation techniques by group, undergraduate students were instructed in reading medical English specialized literature, and in the lab, were instructed in practical research for 6 weeks. In terms of research, the entire laboratory is working on the practical application of gene therapy targeting hematopoietic stem cells (HSCs) of mucopolysaccharidosis type II (MPS II). Receiving budget allocation from Japan Agency for Medical Research and Development, we confirmed a significant effect in model mice by gene transfer using lentiviral vector, and applied for a patent. In addition, the lab members' own research was conducted on themes of lysosomal diseases such as MPS II, GM1 gangliosidosis, Fabry disease, and malignant tumors.

## Research Activities

*Establishment of gene therapy protocol to human HSCs using lentiviral vector*
In the practical application research of the gene therapy method targeting HSCs of MPS II, a third-generation lentiviral vector (MPSV LTR, NCR deleted, dl587 PBS [MND] vector) that was outsourced to Takara Bio Inc. Gene transfer was performed *in vitro* with KG1a cells (acute myeloid leukemia-derived lymphocyte line) and cells positive for human CD34 (derived from healthy human bone marrow). As a result, significant increases in iduronate 2-sulfatase (IDS) enzyme activity and provirus copy number were observed in a dose-dependent manner. In addition, the *Escherichia coli* master cell bank of each plasmid, which is the material for preparing the third-generation lentiviral vector, was also prepared at the guanosine monophosphate level in anticipation of preclinical studies.

*Ex-vivo HSC gene therapy for murine MPS II model*
We generated a new type of third-generation self-inactivating lentiviral vector with a MND promoter to treat a mouse model of MPS II with *ex-vivo* HSC gene therapy. The mouse HSC was transduced with the lentiviral vector and transplanted to MPS II mice. Our lentiviral vector achieved high-level IDS enzyme activity and a significant reduction of glycosaminoglycan storage in both peripheral tissues and the central nervous system (CNS) of the MPS II mouse.

*$CD34^+$ HSC gene therapy for MPS II*
This year we transplanted human $CD34^+$ cells, transfected with a lentiviral vector carry-

ing human (h) IDS, to NOG/MPS II mice, and analysed them. Increased enzymatic activity of IDS and decreased levels of glycosaminoglycans in several tissues were observed in the gene therapy group. This result indicates that $CD34^+$ cells transfected with our lentiviral vector can bring therapeutic efficacy to an animal model of MPS II.

*Development of HSC-targeted 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. Because no effective treatments are available, we aimed to develop HSC-targeted gene therapy. We constructed a lentiviral vector expressing β-galactosidase under control of the MND promoter and performed gene therapy in a mouse model of GM1 gangliosidosis. The results showed a significant elevation of β-galactosidase activity in serum but only limited elevation in CNS tissue.

*Pathological and behavioral analysis of a mouse model of GM1 gangliosidosis*
We revealed the precise pathological condition of GM1 gangliosidosis in the brain by using a mouse model. Microglia, resident immune cells of the brain, were increased in number and most of them were activated. Astrocytes also increased, indicating inflammation was induced in the brain. Interestingly, the neural stem cells in the hippocampus severely decreased. The mouse model of GM1 gangliosidosis showed a loss of motor function in the rotarod performance test and also showed demyelination in the brain.

*Development of a novel preconditioning method for HSC-targeted gene therapy for Fabry disease*
Fabry disease is a hereditary X-linked metabolic disorder characterized by a deficiency or absence of lysosomal α-galactosidase A activity. It causes accumulation of globotriaosylceramide. Because preconditioning with chemotherapy is essential for HSC-targeted gene therapy, we aimed to develop a new preconditioning method that was safer and had effects equivalent to those of chemotherapy. This time, we used an antibody-based method. Six months after treatment, we observed a 77.2% ± 14.3% engraftment rate and a 33.0% ± 7.3% vector transduction rate.

*Artificial intelligence research on Fabry disease*
We are extracting RNA from peripheral blood of a male patient with Fabry disease and will measure promoter activity by comprehensively identifying the transcription start site with an outsourced cap analysis of gene expression system. By dividing the male patient group into 2 groups with and without afferent cardiac hypertrophy, we will identify the gene groups that show significant changes in promoter activity and input image data, such as myocardial magnetic resonance imaging and T1 mapping, and blood test data to the computer. By deep learning, we analyze the correlation between changes in the genetic environment other than pathogenic genes and cardiac hypertrophy and fibrosis and aim to create a diagnostic algorithm for Fabry disease. Currently, 24 patients and 5 normal blood samples have been collected, and RNA extraction is in progress. Data conversion and analysis will be gradually started.

*Administrative study of lysosomal disease*

With funding by the Intractable Diseases Policy Research Program (Ministry of Health, Labour and Welfare), we created clinical practice guidelines for lysosomal disease and constructed a disease registry. In particular, we have enrolled more than 100 patients in a Fabry disease registry.

*New strategy of cancer gene therapy with suppression of lysosome enzymes*

Lysosomes are involved in cancer proliferation and survival through various mechanisms, such as autophagy. Lysosomes contain several enzymes, which might be novel therapeutic targets in cancers. We investigate the antitumor effects induced by knocking down several lysosomal enzymes in pancreatic cancer cell lines and a mouse model of subcutaneous tumors.

## Publications

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# Research Center for Medical Sciences
# Division of Oncology

Mutsunori Murahashi, *Associate Professor and Director*
Masaki Ito, *Assistant Professor*
Yuko Kamata, *Assistant Professor*

## General Summary

*Tumor immunology*
We are conducting research to develop novel cancer immunotherapies; an adjuvant-free artificial antigen vaccine that induces tumor immunity using synthetic biological techniques; bispecific antibodies that bind cancer cells, and $CD8^+$ T cells to exert strong cytotoxicity.

*Cancer genomics*
Many gene mutations occur in tumor cells, and the mutant peptides resulting from the gene mutations are believed to act as neoantigens and to induce tumor-specific immune responses. In collaboration with clinical departments, we are searching for new target molecules, including neoantigens, for tumor immunity by gene mutations and expression analyses.

## Research Activities

*Possibility of augmentation of responses by anti-programmed cell death 1 antibodies against pancreatic cancer using Patched 1-interacting peptide (Mutsunori Murahashi)*
Pancreatic ductal adenocarcinoma (PDAC) is resistant to immunotherapy. As a factor of resistance, the dense fibrosis of this cancer acts as a barrier to inhibit immune cell infiltration into a tumor. We examined the influence of a Hedgehog signal inhibitor, Patched 1-interacting peptide, on fibrosis, infiltration of immune cells, and the immunotherapeutic effects on PDAC. We found that this peptide inhibited proliferation and migration of cancer-associated fibroblasts and cancer cells. Furthermore, this peptide reduced the production of extracellular matrix and transforming growth factor β1 in cancer-associated fibroblasts and induced expression of HLA-ABC in PDAC cells and interferon-γ in lymphocytes. *In vivo*, the peptide suppressed fibrosis of PDAC and increased immune cell infiltration into tumors. The combination of this peptide and an antibody against programmed death 1 augmented the antitumor effect and showed the same effect in experiments with cancer cells and autologous lymphocytes. These results indicate that, in addition to the direct effect of tumor suppression, the Patched 1-interacting peptide increases the infiltration of immune cells by reducing fibrosis of PDAC and consequently enhances the effects of immunotherapy. Therefore, treatment with this peptide might be a novel therapy with 2 different mechanisms: direct tumor suppression and enhancing the immune response against PDAC.

*Functional analysis of endogenous antigen presentation in cancer cells (Masaki Ito)*
Cancer cells presenting somatically mutated peptides (neoepitope) on major histocompatibility complex I (MHC-I), known as human leucocyte antigen (HLA), are eliminated by the immune surveillance system in the early stage of tumor development. However, some cancer cells acquire the capability of immune evasion and subsequently develop malignant cancers. To investigate the mechanism of immune evasion of cancer, we have functionally analyzed the ability of endogenous antigen presenting in cancer cells using reporter T cells that recognize the Wilms Tumor 1 (WT1) peptide epitope on HLA-A*24. The ACC-MESO-4 (malignant mesothelioma) cells, which strongly express both WT1 and MHC-I, showed high endogenous antigen-presenting activity. By treatment with interferon gamma, the endogenous antigen presentation was induced in SW480 cells (colon cancer) and MIA Paca-2 cells (pancreatic cancer) with low WT1 expression. Despite expressing both WT1 and MHC-I, NCI-H460 cells (lung cancer) and Hep G2 cells (liver cancer) showed no antigen presentation. However, antigen presentation was observed when these cells were exogenously pulsed with the WT1 epitope peptide. This result suggests an abnormality in the process of endogenously processing the antigen in NCI-H460 cells and Hep G2 cells. Further analysis of the antigen processing pathway of endogenous antigens in cancer cells that do not show antigen presentation might improve the study of immune evasion mechanisms of cancer.

*Search for neoantigens for malignant brain tumors (Yuko Kamata, Jun Takei)*
Dendritic cell/tumor cell fusion vaccine therapy has been used for patients with malignant glioma. Variant peptides produced from tumor mutation are believed to play a role as tumor-specific antigens in cancer immunotherapy. To find novel mutation-derived antigens, we performed whole exome and whole transcriptome analysis of malignant glioma cells from 9 patients who have undergone dendritic cell/tumor cell fusion vaccine therapy and 1 type of glioma stem-like cell-induced malignant glioma cell. Because mutation-derived antigens differ by HLA, analysis of mutation-derived antigen was performed for HLA-A*24:02 and HLA-A*02:01, which are common HLA types in Japan. The number of candidate mutation-derived antigens detected in 5 or more samples was 46 for HLA-A*24:02 and 54 for HLA-A*02:01. Some candidate variants were validated with Sanger sequencing. The T2 cell assay showed that some candidate peptides had HLA-binding ability. For a more precise analysis, we have searched for mutation-derived antigens in an increasing number of patients with malignant glioma.

## Publications

***Sawada R, Arai Y, Sagawa Y, Nagata Y, Nishimura T, Noguchi M, Amano K, Arihiro S, Saruta M, Homma S.*** High blood levels of soluble OX40 (CD134), an immune costimulatory molecule, indicate reduced survival in patients with advanced colorectal cancer. *Oncol Rep.* 2019 Nov; **42**(5): 2057-2064. doi: 10.3892/or.2019.7304. Epub 2019 Sep 6. PMID: 31545443.

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# Research Center for Medical Sciences
# Division of Molecular Genetics

Mayumi Tamari, *Professor and Director* 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 molecular targets and improve our understanding of diseases.
We are performing collaborative research with other institutions. We also support research projects, which are conducted in clinical departments of The Jikei University School of Medicine.

## Research Activities

*Genetics of inflammatory diseases*
Recent genome-wide association studies (GWASs) have identified variants of the thymic stromal lymphopoietin gene (*TSLP*) locus that 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 those diseases. Our functional study using a super-shift binding assay suggested an allele-specific influence of rs1837253 on affinity for upstream stimulatory factors 1 and 2 in nasal fibroblasts. We reported those findings in *Allergology International* (2020 Jan; 69(1): 138-140).
Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a severe food allergy that usually develops after 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 HWP (Glupearl 19S®) through the use of the facial soap "Chano-shizuku." Glupearl 19S® is a degraded gluten made from the direct resolution of wheat by hydrochloric acid. We conducted a GWAS of WDEIA induced by HWP-containing facial soap in 464 patients and 3,099 control subjects. Single nucleotide polymorphisms at a region on chromosome 6 were associated with WDEIA induced by HWP-containing facial soap. We reported those findings in the *Journal of Allergy and Clinical Immunology* (2019 Nov; 144(5): 1354-1363).
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 research on inflammatory skin diseases. We have recruited patients with psoriasis and performed an association study of psoriasis with GWAS-discovered loci for psoriasis. We also performed a GWAS, next-generation sequencing analysis, and metabolome analysis of psoriasis. In 2019, we exam-

ined whether polymorphisms of the genes tumor necrosis factor A (*TNFA*), TNF receptor superfamily member 1B (*TNFRSF1B*), and TNF alpha induced protein 3 (*TNFAIP3*) contribute the positive response to drug treatment in Japanese patients with psoriasis, but there was no significant association of the 3 single nucleotide polymorphisms with response to treatment against tumor necrosis factor β. We have submitted these findings to *Journal of Dermatology*.

*An effective strategy for the research of allergic and immunological diseases*

Professor Tamari has served as the principal investigator of a group established to make plans for the next 10 years of allergy and clinical immunology research. The hope is to establish a stable society in which people can have long, healthy lives, as free as possible from allergic and immunological diseases at each stage of life. This work is supported by Health Science Research Grants from the Ministry of Health, Welfare and Labour of Japan. We have reported a manuscript on an effective strategy for the research of allergic and immunological diseases (Arerugi 2020; 69(1): 23–33) and launched a website for the research plans, ENGAGE-TF toward 2030 (https://www.engage-tf.jp).

## Publications

***Nakayama T, Hirota T, Asaka D, Sakashita M, Ninomiya T, Morikawa T, Okano M, Haruna S, Yoshida N, Takeno S, Tanaka Y, Yoshikawa M, Ishitoya J, Hizawa N, Isogai S, Mitsui C, Taniguchi M, Kojima H, Fujieda S, Tamari M.*** A genetic variant near TSLP is associated with chronic rhinosinusitis with nasal polyps and aspirin-exacerbated respiratory disease in Japanese populations. *Allergol Int.* 2020 Jan; **69**(1): 138–140. doi: 10.1016/j.alit.2019.06.007. Epub 2019 Jul 17. PMID: 31326260.

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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. The main research projects of the division have been focused on polymer drug carrier systems, or polymeric micelle drug carrier systems, for efficient therapeutic and diagnostic treatments. One project aims to develop a polymer-based magnetic resonance imaging (MRI) contrast-agent carrier system for the precise diagnosis of disease states. Polymer-based MRI contrast agents have great potentials to improve diagnostic accuracy and to provide pathophysiological states of diseases, which general low-molecular-weight MRI contrast agents cannot exhibit. A specific characteristic of polymer-based MRI contrast agents is their long blood half-live, which is a main role in polymer-based MRI contrast agents' specific features described above. However, the long half-life might increase the risk of free gadolinium ions (Gd3+) being released from gadolinium (Gd) chelates. Therefore, we have developed new stable polymer-based MRI contrast agents exhibiting appropriate blood half-lives. Furthermore, a comparison method to evaluate the stability of Gd chelates and an established high-performance liquid chromatography (HPLC) method are needed. We have confirmed that prepared polymer-based MRI contrast agents show greater stability than low-molecular-weight MRI contrast agents. The other project aims to develop a new poly(ethylene glycol) (PEG) conjugation method (PEGylation) for biopharmaceutic agents. Because the characteristics of PEG, which are safety, nontoxicity, and very low immunogenicity, have been commonly accepted, PEG has been widely used for biopharmaceutics, as well as cosmetics. However, inductions of antibodies against PEG have been found in patients who have been treated with PEGylated biopharmaceutics, and anti-PEG antibodies significantly affect the therapeutic efficacy of PEGylated biopharmaceutics. We have studied PEG-related immunological issues and, as a result of the study, have suggested a new PEGylation for therapeutic proteins to reduce antibody responses against both the proteins and PEG.

## Research Activities

*Development of polymer-based Gd chelates for the safety of MRI contrast agents*

A common clinical method for diagnostic purposes is MRI, and contrast agents for MRI have been widely used to visualize blood vessels. Paramagnetic metal ions, such as a Gd ion, have been used for MRI contrast agents and need chelate groups, such as the diethylenetriamine pentaacetic acid (DTPA) group and the 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacatic acid (DOTA) group, to form a stable metal ion complex, and Gd chelate complexes are, in general, low-molecular-weight complexes. Normally, Gd chelates

exhibit short blood half-lives and are immediately diffused into the bloodstream after intravenous injection. Therefore, high volumes of Gd chelates, as the MRI contrast agent, are administered with a single injection, and we repeatedly inject MRI contrast agents for each diagnostic examination. Therefore, the risks of Gd-based MRI contrast agents are increased, as is the incidence of nephrogenic systemic fibrosis, in patients who have decreased renal function. The risks of Gd chelates are caused by the release of free Gd3+ from Gd chelates (Gd toxicity), which we believe are in stable chelate complexes. Furthermore, although their clinical significance has not been understood, repeatedly injected Gd chelates have enhanced signals in both the nucleus dentatus and globus pallidus. These facts indicate that an appropriate design is necessary for stable Gd chelates.

We have shown attractive functions of polymer-based MRI contrast agents for precise MR images which are not generally provided by low-molecular-weight Gd chelates. Although attractive functions have been achieved by a characteristic of polymer-based MRI contrast agents, they have long blood half-lives. These long half-lives will be an issue regarding Gd toxicity, and the stability of polymer-based MRI contrast agents must be proved.

Long-term exposure of the body to polymer-based MRI contrast agents increases the likelihood of interactions between Gd chelates and endogenous substances; therefore, also increased is the risk of Gd3+ release from Gd chelates. What must be developed are new polymer-based MRI contrast agents exhibiting stable Gd chelates and methods for evaluating chelate stability. We have noticed that Gd chelates with low stability have been used as linear-type chelates and in 7-coordinated Gd chelate groups. Therefore, we have focused on macrocyclic chelate ligands, which have a chemical structure more rigid than liner-type chelates. In addition to the chemical structure of chelate groups, we also considered the number of chelate sites. We selected a 1,4,7,10-tetraazacyclododececane,1-(glutaric acid)-4,7,10-triacetic acid (DOTAGA) chelate group, which is a macrocyclic and 8-coordinated Gd chelate group, as a stable polymer MRI contrast agent. The 8-coordinated DOTAGA group is expected to have greater stability than the 7-coordinated DOTA group.

Poly(glutamic acid)-based MRI contrast agents possessing DOTAGA chelate groups have been synthesized by our division, and we used the HPLC method to evaluate free Gd3+ released from Gd chelates. We have evaluated polymer-based MRI contrast agents and several low-molecular-weight Gd complexes, such as Gd-DTPA, and Gd-DTPA-bismethylamide (BMA). We evaluated the stability of Gd chelates in 50% serum conditions. High phosphate concentration (0.05 M) in a 50% serum condition increased in the release of Gd3+ from a low-molecular-weight Gd chelate. Poly(glutamic acid)-based Gd chelates exhibited Gd chelate stability similar to that of Gd-DOTA. Previously prepared polymeric micelle MRI contrast agents exhibited the greatest Gd chelate stability, although they have been used in a 7-coordinated 1,4,7-tris(carboxymethylaza)cyclododecane-10-azaacetylamide (DO3A) chelate group. We believe that the polymeric micelle structure is an important advantage for protecting Gd3+ from proteins, other metal ions, and other substances. We have started to prepare new polymeric micelle MRI contrast agents, which possess 8 coordinated chelates.

*Development of a new PEGylation technology*

The most widely used polymer for biopharmaceutics is PEG. The method of PEG conjugation called PEGylation is a simple, versatile way to produce therapeutic proteins. In addition, PEGylation has been used to cover the surfaces of drug carriers. PEGylation has various merits for the development of biopharmaceutics, such reducing protein immunogenicity and improving protein pharmacokinetics. However, because of the usefulness of PEGylation, a significant issue is immunogenicity, namely antibody responses against PEG. For one example, PEGylated uricase exhibited anti-PEG antibody responses in patients. Antibodies against PEG has been clinically reported and found in patients who had repeatedly received PEGylated biotherapeutic agents. In fact, strong anti-PEG antibody responses have been found in nonresponsive patients, whereas weak anti-PEG antibody responses have been found in responsive patients. To date, more than 10 PEGylated therapeutic proteins have been under clinical trials. The above example, as well as other clinical reports regarding anti-PEG antibodies, indicate the importance of anti-PEG antibodies for therapeutic efficacy; therefore, the generation of anti-PEG antibodies has attracted much attention. Although the standard belief against PEG is thought to be a non-immunogenic, or weakly immunogenic polymer, researchers have recently noticed that PEGylated agents become, somehow, immunogenic.

We have examined the immunogenicity of synthetic PEG-block copolymers and have suggested a concept that reduces antibody responses against PEG by interfering interactions between a PEG chain and PEG-specific molecules. We have started a new project, which has been funded by the Japan Society for the Promotion of Science (funded for the promotion of Joint International Research), to develop a new PEGylation method and have collaborated with researchers at Utrecht University. We have succeeded in preparing several examples of PEGylated proteins, which native proteins are highly immunogenic proteins. Initially, we optimized conditions to activate lysine terminal amine groups in proteins and coupled PEG derivatives to synthesize a series of new PEGylated proteins. Coupling reactions were followed by gel permeation chromatography, and an excess amount of PEG derivatives was removed by ultracentrifugation. We characterized the new form of PEGylated proteins derivatives by means of $^{1}$H nuclear magnetic resonance spectroscopy and gel permeation chromatography. Further characterization and *in vivo* experiments will be performed next year.

## Publications

***Yokoyama M, Shiraishi K.*** Stability evaluation of Gd chelates for macromolecular MRI contrast agents. *MAGMA.* 2020 Aug; **33**(4): 527-536. doi: 10.1007/s10334-019-00805-8. Epub 2019 Dec 10. PubMed PMID: 31823277.

# Research Center for Medical Sciences
# Division of Ultrasound Device Development and Application

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

## Research Activities

*Outline of education and research*

1. Research on the development of a breast ultrasound diagnostic support system by deep learning

In this study, we aim to develop a diagnostic support system using deep learning, a type of machine learning, to determine benign or malignant characteristics of B-mode mammary ultrasound images by means of artificial intelligence (AI). Required for this study are more than 1,000 ultrasound mammary images (data for teacher training) from cases with pathological diagnostic results, and we are currently developing an AI with the approval of the university's ethics committee to collect cases and install a deep learning program. This study is expected to improve the diagnostic efficiency of breast ultrasound examiners.

2. Development of computer system for AI development

Japan Agency for Medical Research and Development (AMED), Research on the construction of a national database of digital ultrasound images and the development of an artificial intelligence-assisted ultrasound diagnosis system, adopted in the "ICT Infrastructure Construction and Artificial Intelligence Implementation Research Project for Clinical Research, etc." in FY2019 (principal investigator: Dr. Kudo, president of the Japanese Society of Ultrasound Medicine) As a research participant, we are working on the development of ultrasound image processing and prototype diagnostic support. In this fiscal year, as a study of AI transfer learning effects using image sets from other fields, we are evaluating the effects of transfer learning using various image data sets and preparing computer hardware and software environments for the development of artificial intelligence.

3. Educational activities to promote AI application in diagnostic imaging

We educated the members of the Japan Society of Ultrasonography and the Japan Society of Radiology about the future of AI applications and the principles of its application for supporting diagnostic radiology. We also wrote and published articles in the *Journal of the Japanese Society of Nephrology* and the *Journal of the Society for Medical Imaging Information*.

4. In vitro study of ultrasound combined with microbubbles for rapid resumption of acute critical vessel occlusion

Early reopening of the occluded vessel is the most fundamental treatment for acute stroke. Transcranial ultrasound and microbubbles have been shown to promote thrombolysis of recombinant tissue-type plasminogen activator (rt-PA). However, the frequent failure of

clinical thrombolysis of completely occluded vessels is thought to be due to the difficulty or inability of rt-PA itself to reach the site of the thrombus in a vessel that has completely stopped blood flow. We have theoretically demonstrated that the combination of ultrasound and microbubbles may not only promote local acceleration of rt-PA thrombolysis, but also have a long-distance delivery effect on rt-PA. In the present study, we are investigating the existence and magnitude of this delivery effect and its relationship to various ultrasound parameters through in vitro experiments. We are also investigating the precise optical measurement of microbubble size to validate the simulation study.

5. Study on the prevention of vascular occlusion by ultrasound

Vascular reocclusion often occurs immediately after hyperacute vascular reopening, i.e., rt-PA treatment after the onset of cerebral vascular embolism. Vascular reocclusion is a critical problem because anticoagulation is prohibited within 24 hours after rt-PA treatment. In this study, investigators have shown that noninvasive ultrasound can control thrombus growth. Safe and simple ultrasound irradiation could be used to prevent reocclusion after rt-PA treatment for hyperacute stroke, and basic research is underway for further clinical applications.

6. Research Department Name Change in the Next Fiscal Year (FY2020)

Since the establishment of the Ultrasonic Technology Research Division in April 2015, we have taken over the research on the application of ultrasound to the treatment of brain infarction from the former Medical Engineering Research Division (MERI). However, due to the changes in research after that, most of the research in our department has been focused on artificial intelligence in medicine, and both Grant-in-Aid for Scientific Research (KAKENHI) 1 and AMED 1 of the public research funds (KAKENHI 2 and AMED 1) that our department currently receives are for research on artificial intelligence. has become a research theme. Nowadays, researches on artificial intelligence other than ultrasound have been increasing, such as research activities of AI-related societies such as the Japanese Medical AI Society (Nakata) and the Japanese Society for Medical Imaging Engineering (Nakata), and the researches on artificial intelligence in medicine including ultrasound have become the research subjects and targets of our division. Therefore, from April 1, 2020, the name of our laboratory will be changed from “Ultrasound Science and Development” to “Artificial Intelligence Laboratory.”

# Research Center for Medical Sciences
# Division of Neuroscience

Fusao Kato, *Professor and Director*

## General Summary

*Historical overview*

The Laboratory of Neurophysiology, created as the second division of the Department of Neuroscience in 2001, was the predecessor of the present Department of Neuroscience. Fusao Kato was appointed as the Director of the Laboratory and remained so after its renewal as the Department of Neuroscience in 2014. Since the beginning, this division has been the core of research and education in non-clinical neuroscience at Jikei University. The number of the people who have belonged to this Department, including the staffs and students, counts more than 120 and, notably, 21 PhD students wrote their thesis based on the research done in this division and have been doctorated in these 20 years.

*Missions*

We have the following three missions. 1) Education of neuroscience, neurophysiology in particular, to the undergraduate medical school students. 2) Education and training of PhD students in graduate school to help them advance their research and make an independent researcher in the medical field. 3) Advancing the state-of-art top-class neuroscience research, which would allow collaborations with the researchers in clinical areas to address unsolved issues in the clinics. These issues include, for example, chronic pain, neuronal plasticity and chronic diseases caused by stress. As many functions in the peripheral organs are monitored, regulated and integrated by the brain, approaches from neuroscience are necessary to solve a wide range of remaining problems associated with diseases in the whole body.

*Scientific goals*

A particular example of this unsolved clinical problem is pain. In particular, more than 15% of the population in advanced countries suffer from chronic pain, defined as pain lasting or recurring for more than three months. Without doubt, pain 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. This situation is more difficult in patients with chronic primary pain, which is pain without an identifiable cause in the site where one feels pain. Lines of evidence including those from our Department indicate that the pain-associated neuronal plasticity the pain network in the brain is one of the mechanisms underlying such chronic pain, which is the central subjects of the Department of Neuroscience. To identify the mechanisms underlying the plastic changes, we use approaches at the molecular, cellular, synaptic, and network levels. These approaches include the patch-clamp analysis of synaptic transmission, the high-frame rate $Ca^{2+}$ imaging, and behavioral anal-

yses combined with most updated “optogenetic” and “chemogenetics” approaches. These latter approaches enable to artificially control the activity of specific neuron ensemble, which we connect brain activity imaging with the high-magnetic field (9.4 T) small-animal MRI.

*Social engagement*
In addition to these missions, we also participate in a wide range of social activities, including board members of scientific societies, the member of the Science Council of Japan and committee members of the International Association for the Study of Pain.

## Research Activities

In the 2019 fiscal year, we have examined the following subjects.
1. 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
2. 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
3. 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
4. Development of methods of selective gene expression in the trigeminal ganglion using adeno-associated viruses
5. Evaluation of spontaneous/voluntary behaviours in animals with collagen-induced rheumatoid arthritis using a wheel-running paradigm and temperature-dependent choice
6. Visualization of neuronal activation and dopamine receptor expression in the brain reward system in response to acute itch using multiple single RNA imaging
7. 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
8. Behavioral analysis of the relationships of the social rank order of individual mice to glucose tolerance and insulin release regulation

### Publications

***Oto Y, Takahashi Y, Kurosaka D, Kato F.*** Alterations of voluntary behavior in the course of disease progress and pharmacotherapy in mice with collagen-induced arthritis. *Arthritis Res Ther.* 2019 Dec 12; **21**(1): 284. doi: 10.1186/s13075-019-2071-z. PMID: 31831067; PMCID: PMC6909634.
***Arimura D, Shinohara K, Takahashi Y, Sugimura YK, Sugimoto M, Tsurugizawa T, Marumo K, Kato F.*** Primary Role of the Amygdala in Spontaneous Inflammatory Pain-Associated Activation of Pain Networks - A Chemogenetic Manganese-Enhanced MRI Approach. *Front Neural Circuits.* 2019 Oct 1; **13**: 58. doi: 10.3389/fncir.2019.00058. PMID: 31632244; PMCID: PMC6779784.

# Research Center for Medical Sciences
# Division of Molecular Epidemiology

Mitsuyoshi Urashima, *Professor and Director*

## General Summary

Despite having the same disease diagnosis, some patients might be cured but some might 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 disease 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 will be started from the principles of epidemiology and biostatistics by reading textbooks and by analyzing real clinical data with the STATA software program (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

***Urashima M, Ohdaira H, Akutsu T, Okada S, Yoshida M, Kitajima M, Suzuki Y.*** Effect of Vitamin D Supplementation on Relapse-Free Survival Among Patients With Digestive Tract Cancers: The AMATERASU Randomized Clinical Trial. *JAMA.* 2019 Apr 9; **321**(14): 1361-1369. doi: 10.1001/jama.2019.2210. PMID: 30964526; PMCID: PMC6459116.

***Momosaki R, Abo M, Urashima M.*** Vitamin D Supplementation and Post-Stroke Rehabilitation: A Randomized, Double-Blind, Placebo-Controlled Trial. *Nutrients.* 2019 Jun 7; **11**(6): 1295. doi: 10.3390/nu11061295. PMID: 31181657; PMCID: PMC6628052.

***Igarashi G, Segawa T, Akiyama N, Nishino T, Ito T, Tachimoto H, Urashima M.*** Efficacy of Brazilian Propolis Supplementation for Japanese Lactating Women for Atopic Sensitization and Nonspecific Symptoms in Their Offspring: A Randomized, Double-Blind, Placebo-Controlled Trial. *Evid Based Complement Alternat Med.* 2019 Sep 11; **2019**: 8647205. doi: 10.1155/2019/8647205. PMID: 31611924; PMCID: PMC6755297.

***Imakita T, Suzuki Y, Ohdaira H, Urashima M.*** Colonoscopy-assisted percutaneous sigmoidopexy: a novel, simple, safe, and efficient treatment for inoperable sigmoid volvulus (with videos). *Gastrointest Endosc.* 2019 Sep; **90**(3): 514-520. doi: 10.1016/j.gie.2019.04.246. Epub 2019 May 8. PMID: 31077700.

***Yonaga H, Okada S, Akutsu T, Ohdaira H, Suzuki Y, Urashima M.*** Effect Modification of Vitamin D Supplementation by Histopathological Characteristics on Survival of Patients with Digestive Tract Cancer: Post

Hoc Analysis of the AMATERASU Randomized Clinical Trial. *Nutrients.* 2019 Oct 22; **11**(10): 2547. doi: 10.3390/nu11102547. PMID: 31652554; PMCID: PMC6835362.

***Urashima M, Mezawa H, Okuyama M, Urashima T, Hirano D, Gocho N, Tachimoto H.*** Primary Prevention of Cow's Milk Sensitization and Food Allergy by Avoiding Supplementation With Cow's Milk Formula at Birth: A Randomized Clinical Trial. *JAMA Pediatr.* 2019 Oct 21; **173**(12): 1137–45. doi: 10.1001/jamapediatrics.2019.3544. Epub ahead of print. PMID: 31633778; PMCID: PMC6806425.

***Urashima M, Okuyama M, Akutsu T, Ohdaira H, Kaji M, Suzuki Y.*** Effect of Vitamin D Supplementation on Survival of Digestive Tract Cancer Patients with Low Bioavailable 25-Hydroxyvitamin D levels: A Post Hoc Analysis of the AMATERASU Randomized Clinical Trial. *Cancers (Basel).* 2020 Feb 4; **12**(2): 347. doi: 10.3390/cancers12020347. PMID: 32033150; PMCID: PMC7072519.

***Akutsu T, Okada S, Hirooka S, Ikegami M, Ohdaira H, Suzuki Y, Urashima M.*** Effect of Vitamin D on Relapse-Free Survival in a Subgroup of Patients with p53 Protein-Positive Digestive Tract Cancer: A *Post Hoc* Analysis of the AMATERASU Trial. *Cancer Epidemiol Biomarkers Prev.* 2020 Feb; **29**(2): 406–413. doi: 10.1158/1055-9965.EPI-19-0986. Epub 2019 Dec 23. PMID: 31871108.

# 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 "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 1 regional hospital and more than 10 teaching clinics in Tokyo, Kanagawa, and Saitama. The follow-up period was until January 31, 2017. This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number JP24590819. In May 2019, a member of this study group, who is a visiting researcher and a former graduate student of the Division of Clinical Epidemiology, presented some of the study results at the 10th annual

meeting of the Japan Primary Care Association and was awarded the Hinohara Prize.

*Development of a Japanese version of the 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 developed a Japanese version of the Patient Centered Assessment Method to evaluate patient complexity.

*Relationship between drinking habit and patient complexity: a cross-sectional study at a remote island*
As aging progresses, not only the frequency of multimorbidity, but also social and psychological problems might increase; therefore, understanding the complexity of patients from the bio-psycho-social perspective is absolutely necessary.
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 problems with alcohol 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 was 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 (LGBT) related contents 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 has revealed that the knowledge and attitudes of members of the medical staff such as physicians, and medical students were improved by education about LGBT-specific health issues.
Therefore, in this research, we conducted a questionnaire survey to clarify the current status of time and educational contents spent on education about LGBT related contents at medical schools throughout Japan. Moreover, by comparing our survey data with the situation in Canada and the United States, we aimed to find problems in education about LGBT related contents at medical schools in Japan.

## Publications

***Yodoshi T, Matsushima M, Taniguchi T, Kinjo S.*** Utility of point-of-care Gram stain by physicians for urinary tract infection in children ≤36 months. *Medicine (Baltimore).* 2019 Apr; **98**(14): e15101. doi: 10.1097/MD.0000000000015101. PubMed PMID: 30946373; PubMed Central PMCID: PMC6456128.
***Kaneko M, Van Boven K, Takayanagi H, Kusaba T, Yamada T, Matsushima M.*** Multicentre descriptive cross-sectional study of Japanese home visit patients: reasons for encounter, health problems and multimorbidity. *Fam Pract.* 2020 Mar 25; **37**(2): 227-233. doi: 10.1093/fampra/cmz056. PubMed PMID: 31586446.
***Seki M, Fujinuma Y, Matsushima M, Joki T, Okonogi H, Miura Y, Ohno I.*** How a problem-based learning approach could help Japanese primary care physicians: a qualitative study. *Int J Med Educ.* 2019 Dec 26;

**10**: 232–240. doi: 10.5116/ijme.5de7.99c7. PubMed PMID: 31877111; PubMed Central PMCID: PMC7246125.
***Hayashi T, Matsushima M, Wakabayashi H, Bito S.*** Association between delivery methods for enteral nutrition and physical status among older adults. *BMC Nutr.* 2020 Jan 14; **6**: 2. doi: 10.1186/s40795-019-0318-3. eCollection 2020. PubMed PMID: 32153976; PubMed Central PMCID: PMC7050869.
***Sato T, Sato S, Yamagami H, Komatsu T, Mizoguchi T, Yoshimoto T, Takagi M, Ihara M, Koga M, Iwata H, Matsushima M, Toyoda K, Iguchi Y.*** D-dimer level and outcome of minor ischemic stroke with large vessel occlusion. *J Neurol Sci.* 2020 Jun 15; **413**: 116814. doi: 10.1016/j.jns.2020.116814. Epub 2020 Mar 31. PubMed PMID: 32259707.

# Research Center for Medical Sciences
# Division of Regenerative Medicine

Hirotaka James Okano, *Professor and Director* Junichi Hata, *Assistant Professor*

## General Summary

Regenerative medicine might soon be 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 be 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.
Magnetic resonance imaging (MRI) is a powerful and flexible imaging tool for diagnosis in clinical practice. Sophisticated MRI hardware enables image assessment from small experimental animals, such as mice, rats and marmosets, at a resolution of several tens of microns. In particular, diffusion tensor imaging (DTI) is a promising method for characterizing structural differences with neuropathology because DTI is highly sensitive to changes at the microstructural level. We use DTI, structural MRI, and MR spectroscopy to investigate brain structure and functions.

## Research Activities

*Anatomical and surgical evaluation of the common marmoset as an animal model in hearing research*
Sensorineural hearing loss (SNHL) is most often associated with impairments of the cochlear hair cells. Cochlear hair cell dysfunction can be caused by aging, infection, exposure to loud noises, and ototoxic drug abuse. Mammalian cochlear hair cells do not spontaneously regenerate, and current treatments for chronic SNHL are ineffective. However, basic research with rodent models has contributed to the understanding of the underlying cause of hearing loss and the development of potentially effective therapies. Direct administration of viral vectors or small compounds to the inner ear might aid in the treatment of SNHL. However, owing to species differences between humans and rodents, translating experimental results into clinical applications remains challenging. Therefore, when evaluating potential interventions for hearing loss, research involving nonhuman primate models is required to minimize the species gap. Moreover, for the successful delivery of potential therapies, the appropriate surgical approach must be accurately defined in such a model. The common marmoset (*Callithrix jacchus*), a New World monkey, is a valuable nonhuman primate model because of its small size, high reproductive efficiency, and the cross-reactivity of its cytokines and hormones with their human coun-

terparts. We describe morphometric data acquired from the temporal bone of the common marmoset to define the routes of topical drug administration to the inner ear. Dissection and DTT were performed on the fixed cadaverous heads of 13 common marmosets. To investigate potential routes for drug administration to the inner ear, we explored the anatomy of the round window, oval window, semicircular canal, and endolymphatic sac. Of these routes, an approach *via* the round window with posterior tympanotomy appeared feasible for delivering drugs to the inner ear without manipulating the tympanic membrane and thereby minimizing the chances of conductive hearing loss. The courses of 4 critical nerves, including the facial nerve, were visualized with 3-dimensional DTT, which might help nerve damage to be avoided during surgery. Finally, to investigate the feasibility of actual drug administration, we measured the volume of the round window niche, which was approximately 0.9 μL. The present findings might help establish experimental standards for evaluating new therapies in this primate model (Kurihara S. et al. Front Neuroanat. 2019).

*Cell biological study of hereditary motor and sensory neuropathy with proximal dominant involvement*

Hereditary motor and sensory neuropathy with proximal dominant involvement (HMSN-P) is an autosomal-dominant neurodegenerative disorder characterized by late-onset progressive muscle weakness of proximal limbs followed by distal sensory involvement. A large family affected by HMSN-P has been reported from the Okinawa Islands. Pathological studies of HMSN-P revealed 43-kDa transactivation response DNA-binding protein (TDP-43)-positive cytoplasmic inclusion bodies in the spinal and cortical motor neurons, shared with findings in amyotrophic lateral sclerosis. Furthermore, a heterozygous mutation in Tropomyosin-receptor kinase Fused Gene (TFG) was identified as the responsible gene for HMSN-P. A research group of Okinawa National Hospital, our collaborator, found a large family with HMSN-P in an island of Okinawa. The researcher obtained informed consent from participants and collected blood samples of affected and unaffected individuals in the family. Currently, we generated iPSC lines from 3 affected and 3 unaffected individuals of the pedigree and differentiated iPSCs into motor 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.

*Primate brain image analysis using high-field MRI and technological development for comparative neuroscience*

The development of nuclear magnetic resonance (NMR) imaging techniques has enabled us to extract macroscopic biological information and achieve three-dimensional analysis of rodent and primate brains. In recent years, there has been a growing momentum to create three-dimensional brain maps (MRI images, tissue images) of human and closely related primate models and to use them to elucidate higher brain functions and psychiatric and neurological disorders in humans. In collaboration with RIKEN, Kyoto University Primate Research Institute, Johns Hopkins University, and Keio University, we have developed a brain imaging database last year by collecting brain anatomy and brain circuit data of primates using our high-field MRI system (9.4T). (Sakai et al, Primates.

2018) (http://www.j-monkey.jp/BIR/index.html). In this fiscal year, we achieved the acquisition of knowledge and technological development to further accelerate the analysis of brain images, such as the development of high-precision segmentation techniques for brain structure using deep learning (Ito R, et al, Neural Networks. 2019), the development of contrast techniques with pathological images (Huo B, et al, Eur J Neursci. 2019), and the development of visualization techniques for biological information and brain function, such as the development of cell type differentiation methods using MRI (Hata J, et al. PLos One. 2019), the evaluation of brain ischemic status by high-precision restrictive tissue structure analysis (Ohki A, et al, Magn Reson Imaging. 2019), and the detection of neural circuit activity disorders in autism (Tsurugizawa T, et al. Science Advances. 2020). This will lower the hurdle for many researchers and experts in medical and biological sciences as well as in mathematical statistics, deep learning, etc. to engage in primate brain science research. It is also expected to bring a new frontier in primate science research as basic and bridging research.

## Publications

***Fujimoto T, Yamanaka S, Tajiri S, Takamura T, Saito Y, Matsumoto K, Takase K, Fukunaga S, Okano HJ, Yokoo T.*** In vivo regeneration of interspecies chimeric kidneys using a nephron progenitor cell replacement system. *Sci Rep.* 2019 May 6; **9**(1): 6965. doi: 10.1038/s41598-019-43482-2. PMID: 31061458; PMCID: PMC6502858.

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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

Yoshinobu Manome, *Professor and Director*
Takeo Iwamoto, *Professor*
Yumi Kanegae, *Associate Professor*
Yuji Ohno, *Assistant Professor*
Akihito Tsubota, *Professor*
Toshiaki Tachibana, *Professor*
Tadayuki Iwase, *Associate Professor*
Keiichi Ikeda, *Assistant Professor*

## General Summary

The Research Center for Medical Sciences of The Jikei University School of Medicine has been reorganized, and in April 2019 the Division of Molecular Cell Biology and the Division of Molecular Genetics of the Core Research Facilities for Basic Science were integrated and are now known as the Core Research Facilities. This integration has consolidated the facilitation of on-campus research support.

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 2019, 168 researchers registered at our annual registration system, and we provided research support 244 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, or perform high-performance liquid chromatography and mass spectrometry. The service fee is minimal because services are limited to the university.

## Research Activities

*Possibility of nicotinamide phosphoribosyltransferase suppression as a molecular target*

Although brain tumors, particularly gliomas, are intractable and resist many first-line treatments, candidate target molecules have recently been identified by analyzing what is known about genes and proteins. Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme in the nicotinamide adenine dinucleotide ($NAD^+$) biosynthetic salvage pathway which converts nicotinamide to nicotinamide mononucleotide. The converted nicotinamide mononucleotide is further metabolized to $NAD^+$ and serves as a coenzyme of various types of dehydrogenation. Previous research suggests that increases in NAMPT transcription and expression correlate with the growth or clinical grade of glioma. Therefore, because NAMPT modulation can be directly applicable as an adjuvant

remedy for radiotherapy or chemotherapy, we established cell lines that suppressed NAMPT expression with short hairpin interfering RNA. As a result, NAMPT inhibition alone suppressed cell growth and increased radiosensitivity, but the effects were transient. Furthermore, inhibition did not alter the sensitivity of glioma to the antineoplastic agent temozolomide. This involved other salvage pathways. We are creating a system to enhance and prolong the effect of NAMPT suppression.

*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 also used chimeric mice to create an animal model of hepatitis B or C virus infection. We are intensely performing research on the efficacy of novel antiviral agents, the mechanism of progression to chronic infection, and ultrastructural alterations of intrahepatocellular organelle after viral eradication.

*Single nucleotide polymorphisms, and resistant-associated variants in the treatment of chronic hepatitis C virus infection*
Direct-acting antiviral agents are the first-line treatment for chronic hepatitis C virus infection. We are investigating the association of single nucleotide polymorphisms of the 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 an intrahepatic feeding artery, proper hepatic artery, and peripheral vein when we perform transcatheter arterial chemoembolization (TACE) for patients with hepatocellular carcinoma (HCC), and are investigating the association between serum microRNA expression levels and treatment outcome/prognosis in patients who have HCC and were treated with TACE/radiofrequency ablation (RFA).

*Comprehensive gene expression profiling analysis of microRNA/messenger RNA*
We are profiling and analyzing the expression of microRNA/messenger RNA in the liver tissue of hepatitis B virus (HBV)-infected human hepatocyte chimeric mice. We have found the novel interaction between microRNA and messenger RNA in HBV replication and lifecycle. We are also investigating the association between serum microRNA expression level and treatment outcome/prognosis in HCC patients who were treated with TACE/RFA.

*A new method for measuring cholesterol efflux capacity using liquid chromatography-ultrahigh-resolution mass spectrometry with stable isotope-labeled cholesterol*
The incidence of cardiovascular events correlates inversely with cholesterol efflux capacity (CEC) more than with the high-density lipoprotein cholesterol level. The measurement of CEC is used to qualify cardiovascular disease risk and is conventionally per-

formed with radioisotope-labeled cholesterol. So, we established a CEC measurement technique using stable isotope-labeled cholesterol as an alternative, and we compared our method with radioisotope- and fluorescence-labeled cholesterol methods using cells and patient serum. We incubated J774 cells labeled with [d 7] cholesterol (d 7-C) with patient serum, and d 7-C extracted from the cell culture medium was quantified by liquid chromatography-quadrupole time-of-flight mass spectrometry. The assay coefficient of variation of five consecutive measurements of three sets of samples ranged from 7.3% to 9.5%, and the interassay coefficient of variation determined by measuring 3 samples four times ranged from 4.1% to 8.5%, both indicating good precision. The CEC levels were measured for 41 outpatients with serum high-density lipoprotein cholesterol levels of 36 to 94 mg/dl (mean, 61.7 ± 18.0 mg/dl) under cyclic adenosine monophosphate. Results were suggested that positive correlation between CEC levels using the stable isotope and radioisotope methods. It was stronger than the correlation between measurements using the fluorescence and radioisotope methods ($r = 0.73$, $P < 0.0001$ vs. $r = 0.55$, $P < 0.001$). Therefore, our newly developed using stable isotope method can be considered useful as a non-radioisotope method and thus deserves evaluation in future clinical studies.

*Effect of smoking inflammatory response to smoking cessation on human gingival fibroblast and periodontal ligaments cells*

The purpose of this study was to investigate the inflammatory response of human gingival fibroblasts and periodontal ligament cells during smoking (during nicotine stimulation) and the effect of repair period during smoking cessation (interruption of nicotine stimulation).

Both cells were obtained from healthy periodontal tissue. The cells were cultured until they reached confluence, replaced with a medium containing 1 μg/ml nicotine, and cultured for 24 hours. After that, the supernatant was replaced with a nicotine-free medium and the culture was carried out for 48 hours. Culture supernatants at each time point after nicotine stimulation and after nicotine discontinuation were collected, and interleukin 6 production was measured with enzyme-linked immunosorbent assay. Interleukin 6 production increased significantly ($p < 0.001$) in both cells after nicotine stimulation, but decreased significantly after nicotine discontinuation ($p < 0.001$). Scanning electron microscopy revealed many depressions on the surface of the cell membrane due to nicotine stimulation.

From these facts, it was demonstrated that smoking probably had an adverse effect on cells, and the possibility of a cell repair effect by smoking cessation was shown.

*Development of the adenovirus vector systems*

We have developed a protocol for curing HBV infection with an adenovirus vector (AdV). We established the efficient detection system of HBV genome replication applying AdVs (HBV103-AdV system) and identified several promising compounds. Furthermore, we succeeded in efficient cleavage of the HBV genome using a hepatocyte-specific genome editing system by AdV and we identified several promising genomic RNA candidates.

*Rapid identification and quantification of* Lactobacillus rhamnosus*-targeting real-time polymerase chain reaction using a TaqMan probe*

*Lactobacillus rhamnosus* is a gram-positive, rod-shaped bacterium and is commonly used as a probiotic to maintain intestinal health. Recently, surveillance of Lactobacillus bacteremia was conducted using biochemical or conventional polymerase chain reaction (PCR) assay; however, these assays are unable to quantify the target, and might detect a small number of DNA fragments or yield a false-positive result. In this study, we developed an *L. rhamnosus*-targeting quantitative PCR assay, which produces accurate and reproducible results based on the specificity of a TaqMan probe targeting the unique 16S ribosomal DNA sequence of *L. rhamnosus*. The assay specifically detected the targeted bacterium, *L. rhamnosus*, and no non-specific signals were generated under the study conditions. Using genomic DNA from the bacterial cells of *L. rhamnosus* (101 to 106 cells), the cycle threshold value showed a linear trend (R2 = 0.9993). This *L. rhamnosus*-targeting quantitative PCR assay can contribute to advance research into the effects of the organism on microflora, microbial infections, and the host.

*Protective actions of urocortin family peptide on pancreatic β-cells*

It has been reported that urocortin family peptides exert cellular protective actions. We are now investigating actions of urocortin family peptides, especially, urocortin III, against toxic actions to pancreatic β-cells, such as hyperglycemic condition and nicotine exposure which resulted in reduced insulin release. As the first step of these approach, we tried to investigate the action of urocortin III on such conditions by insulin release. Urocortin III facilitate insulin release at the hyperglycemic condition and recovered the suppressive effect on insulin release by nicotine.

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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 2019, 868 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*. To investigate the dynamics of parasite infection of the host body, we generated a parasite that overexpressed a reporter gene (luciferase gene fused with enhanced green fluorescent protein gene. Using this genetically modified parasite, we plan to conduct *in vivo* imaging research for analyzing infection dynamics of the parasite and for evaluating the efficacies of vaccine candidates against it.

*Study of postoperative nausea and vomiting in common marmosets*
Common marmosets (*Callithrix jacchus*) are small primates with a high degree of sociability and genetic homology to humans. marmosets often vomit as a complication of anesthetic during induction and awakening. We have previously searched for risk factors for postoperative nausea and vomiting in marmosets by multivariate analysis and found that inhalation anesthesia and prolonged general anesthesia were associated with a significant increase in vomiting. In addition, an anesthesia protocol with the addition of malopitant citrate, a neurokinin-1 receptor antagonist, was investigated to control vomiting. The results showed that preoperative administration of malopitant significantly inhibited vomiting under both injection and inhalation anesthesia (poster presentation at the 66th annual meeting of the Japanese Association for Laboratory Animal Science). We continue to

investigate the effective prevention of postoperative nausea and vomiting in marmosets through the use of several antiemetic agents with mechanisms that differ from those of malopitant.

*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 better understanding of host-*Plasmodium* interactions, we focused on plasma amino acids and performed "aminogram analysis," 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 malaria, we have shown that aminogram modification, by adjusting amino acid intake with isoleucine-deficient diet, prolonged survival without inhibiting parasite proliferation (cerebral malaria tolerance). Interestingly, the observation with optical microscopy of a Giemsa-stained thin blood smear demonstrated that erythrocytes are smaller in mice fed an isoleucine-deficient diet. However, in the case of *Plasmodium falciparum*, erythrocyte did not differ between a control medium and an isoleucine-free medium. These results indicate the possibility that the size of erythrocyte is involved in the progression of cerebral malaria. We are now studying the effect of isoleucine deficiency on erythrocyte size and the severity of cerebral malaria in a murine model.

## Publications

***Kawahata K, Cordeiro IR, Ueda S, Sheng G, Moriyama Y, Nishimori C, Yu R, Koizumi M, Okabe M, Tanaka M.*** Evolution of the avian digital pattern. *Sci Rep*. 2019 Jun 12; **9**(1): 8560. doi: 10.1038/s41598-019-44913-w. PubMed PMID: 31189916; PubMed Central PMCID: PMC6561939.

# 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, medical students, and graduate students. In 2019, the laboratory of this facility was used by 31 researchers from 9 departments and by 10 students of 2 curriculums. Major nuclides used for experiments were $^{51}Cr$, $^{125}I$, $^{14}C$, and $^{3}H$. Education related to radiation is also an interest.

## Research Activities

*The mechanism that induced epithelial-mesenchymal transition in proteasome inhibitor-resistant cells*

Ishikawa endometrial cancer cells induced epithelial-mesenchymal transition by acquiring resistance to the proteasome inhibitor epoxomicin (EXM). A decrease in microRNA-200 (miR200) expression induced transcriptional repressor zinc finger E-box-binding homeobox 1 (ZEB1) expression and caused E-cadherin expression to disappear. The regulation of E-cadherin expression by miR200 was also apparent from the fact that introduction of miR200 into EXM-resistant Ishikawa cells suppressed the expression of ZEB1 and restored the expression of E-Cadherin.

In EXM-resistant Ishikawa cells, the expression of dual-specificity protein phosphatase 6 (DUSP6) disappeared, and the phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 was enhanced. This increased phosphorylation of ERK1/2 increased expression of FOS like 1, antigen-1 transcription factor (FOSL1). It was found that the expression of miR200 was suppressed by controlling the expression of FOSL1.

On the other hand, CD44 expression was enhanced in EXM-resistant Ishikawa cells, but not cells in non-EXM-resistant Ishikawa cells. When Ishikawa cells overexpressed CD44, DUSP6 expression disappeared. Accompanying DUSP6 disappearance, expression of FOSL1, suppression of miR200 expression, and enhancement of ZEB1 expression were observed.

These results show that in Ishikawa cells without CD44 expression, the phosphorylation level of ERK1/2 was reduced by DUSP6 expression, miR200 was expressed, ZEB1 expression was suppressed, and E-cadherin was expressed with decreased FOSL1 expression. On the other hand, in EXM-resistant Ishikawa cells, CD44 was expressed, and the suppression of DUSP6 expression by CD44 increased the phosphorylation level of ERK1/2 and induced FOSL1 expression. As a result, miR200 disappeared and E-cadherin

expression was suppressed along with ZEB1 expression.

*Chemotherapy with curcumin and prodrug curcumin for drug-resistant cancer*
Mutation of the Kirsten ras proto-oncogene, GTPase gene (*KRAS*), which is found in about 40% of intestinal cancers, is thought to be a cause of resistance and causes the activation of the nuclear factor kappa B pathway. This pathway is reported to be inhibited by curcumin, which can thus be an effective drug for oxaliplatin-resistant colorectal cancer. However, conventional oral curcumin has low bioavailability and has difficulty reaching a blood concentration that has a sufficient therapeutic effect. The newly developed prodrug-type curcumin, curcumin monoglucuronide (CMG), succeeded in solving this problem. That is, curcumin is hydrophobic and slightly soluble in water and, so, cannot be administered intravenously, but CMG is water-soluble and can be administered intravenously. A high concentration of curcumin in blood can be achieved by intravenous injection of CMG.
Therefore, the antitumor effect of CMG was examined in a mouse xenograft model in which human colon adenocarcinoma HCT116 cells (*KRAS* mutation/p53 wild type) had been transplanted. As a result, CMG was shown to have remarkable anticancer activity without the weight loss, bone marrow suppression, and liver damage observed with oxaliplatin administration. We also found that CMG, when used in combination with oxaliplatin, had an additive anticancer effect and did not worsen the side effects of oxaliplatin.

*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 50 to 200 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. Environmental samples, such as soil and plants, were collected from Fukushima Prefecture and the Kanto region, and the concentration of radiocesium and radiation images were analyzed with an imaging plate. In indoor dust samples collected from about 40 points within 10 km from the Fukushima Daiichi Nuclear Power Plant the concentrations of $^{134}Cs$ and $^{137}Cs$ were measured. From the $^{134}Cs/^{137}Cs$ ratio at the time of the accident, the mixing ratios of materials from Units 1, 2, and 3 were obtained. We have obtained important basic data that can be used as a reference when releasing the evacuation order in the difficult-to-return area. Furthermore, we examined a safe, simple, and rapid method of analyzing radioactive strontium in seawater. The analytical method using the strontium adsorbent (Pureceram® MAq, Ebara Co./Nippon Chemical Industrial Co., Ltd.) was examined with $^{85}Sr$ and $^{90}Sr$. We found that strontium was adsorbed only by stirring without a complicated procedure. This adsorbent can be used for screening sea-

water or purifying contaminated water.

## Publications

***Mezaki Y, Kato S, Nishikawa O, Takashima I, Tsubokura M, Minowa H, Asakura T, Matsuura T, Senoo H.*** Measurements of radiocesium in animals, plants and fungi in Svalbard after the Fukushima Daiichi nuclear power plant disaster. *Heliyon.* 2019 Dec 24; **5**(12): e03051. doi: 10.1016/j.heliyon.2019.e03051. eCollection 2019 Dec.PubMed PMID: 32083202; PubMed Central PMCID: PMC7019073.

***Mimoto R, Yogosawa S, Saijo H, Fushimi A, Nogi H, Asakura T, Yoshida K, Takeyama H.*** Clinical implications of drug-screening assay for recurrent metastatic hormone receptor-positive, human epidermal receptor 2-negative breast cancer using conditionally reprogrammed cells. *Sci Rep.* 2019 Sep 16; **9**(1): 13405. doi: 10.1038/s41598-019-49775-w. PubMed PMID: 31527634; PubMed Central PMCID: PMC 6746954.

# Research Center for Medical Sciences GMP Production Facilities for Cell Therapy and Gene Therapy

Mutsunori Murahashi, *Associate Professor and Director*

## General Summary

*Clinical trials*

A Good Manufacturing Practice-grade Cell Processing Factory has been operating for a long time in the Research Center for Medical Sciences. Until now, middle ear mucosa regeneration by grafting artificial mucosa for intractable middle ear disease by otolaryngology, and clinical trials of immune cell therapy for brain tumors by neurosurgery and pediatrics have been performed. Middle ear mucosa regeneration by grafting artificial mucosa has been adopted by the Japan Agency for Medical Research and Development (AMED) as a research project for practical application of regenerative medicine and is currently undergoing nonclinical safety tests. Immune cell therapy for brain tumors has been safely performed by neurosurgery and pediatrics this year, and cases are accumulating. We are aiming to be included in a AMED practical research project for innovative cancer medical treatment.

*Opening of a new Cell Processing Factory in 2020*

The new outpatient building of 2020 will include a cell processing facility that applies the concept of manufacturing control and the quality control standards of Good Manufacturing Practice/Good Gene, Cellular, and Tissue-based Products Manufacturing Practice Ministerial Ordinance. We believe that our mission is to the perform the first clinical trials in human patients and investigator-initiated clinical trials developed from academia seeds. We would like to focus on the findings and problems that can be discovered for the first time in clinical research during this exploration period and connect them to the period of development. On the other hand, the Cell Processing Factory will also play roles in such hospital functions as storage, in the quality control of such cell processing products as chimera antigen receptor T cells, and in regenerative medicine. Making the most of the benefits of the region, we will also aim to support and revitalize research in cancer immunotherapy and regenerative medicine through industry-academia collaboration.

## Research Activities

*Department of Neurosurgery (Yasuharu Akasaki)*

We are conducting clinical studies of immunotherapy for malignant glioma using tumor cells and cells fusing tumorigenic cells and dendritic cells. This is a study of immunotherapy using dendritic cells, known as specialized antigen-presenting cells, fused with tumor

cells as a tumor vaccine. Furthermore, the fused cells are activated by polyinosinic: polycytidylic acid/interleukin 10–short interfering RNA–embedded cationic liposomes to promote secretion of endogenous interleukin 12.

*Department of Pediatrics (Masayoshi Yamaoka)*
We are conducting autologous dendritic cell therapy for refractory pediatric brain tumors jointly by pediatrics and neurosurgery. In 2019 cell therapy was performed 12 times for 4 children with brain tumors. No adverse events were observed in any patient, and the effects of therapy could be confirmed in all 4 patients. Relapse-free survival occurred in 2 patients; tumors recurred in 2 patients, but the recurrence pattern was changed and 1 patient was in remission after undergoing a second operation. We will continue to accumulate cases and report at the International Society for Childhood Brain Tumors held in December 2020.

# Research Center for Medical Sciences
# Institute for High Dimensional Medical Imaging

Asaki Hattori, *Associate Professor and Director*

## 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. We are developing a 4D motion system for analyzing human activities, such as the motions of the whole body. The system is driven by motion data obtained from anatomical and skeletal muscle models reconstructed from X-ray CT data sets.

This year, in collaboration with the Department of Plastic Surgery, we started research and development of a method to analyze the 4D changes of joints by focusing on the movement of the fingers in the upper limbs, measuring the magnetic resonance images of the fingers during movement.

*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.

Continuing from last year, we developed a drive mechanism using a shape memory alloy for an overtube to control the posture of a robot in the abdominal cavity and presented the research results at an international conference.

*Development of a surgical simulator for various surgical techniques*

We are developing a simulator that can deal with various surgeries, such as laparotomy and endoscopic surgery, using preoperative X-ray CT data of a patient.

This year, we developed a system that sets the resection plane on preoperative X-ray CT

data of the patient, performs intraoperative navigation with the set data, records the procedure of the operator, and analyzes and evaluates it after surgery. In addition, we worked for the third year on a 4D image display system in real space, with a Japan Society for the Promotion of Science Grant-In-Aid for Scientific Research (A). As we developed the structure of the experimental machine last year, this year we improved the structure and the function of an experimental machine to improve the display function. We also applied for a patent for the display method and device configuration of this 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-tech navigation operating room of Daisan Hospital as a semiroutine procedure.

This year, by using a system that we developed last year to navigate surgical procedures based on preoperatively planned excision surface data, we performed clinical trials during actual partial hepatectomy procedures and evaluated the system. In addition, to perform gynecological laparoscopy, we continue to develop a navigation system that does not use preoperative X-ray CT or magnetic resonance imaging 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. Regarding the X-ray CT data analysis of the cause of traffic accidents deaths of nationally protected animals, at an international symposium we presented the results obtained from accident data with analytical methods we developed.

## Publications

***Yasuda J, Okamoto T, Onda S, Fujioka S, Yanaga K, Suzuki N, Hattori A.*** Application of image-guided navigation system for laparoscopic hepatobiliary surgery. *Asian J Endosc Surg.* 2020 Jan; **13**(1): 39-45. doi: 10.1111/ases.12696. Epub 2019 Apr 3. PMID: 30945434.

# Research Center for Medical Sciences
# Institute of Clinical Medicine and Research

Toya Ohashi, *Professor and Director*
Midori Kono, *Assistant Professor*
Ayako M. Watabe, *Professor*

## General Summary

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 continued to engage in an educational laboratory course program with the assignment of third-year medical students. We also fulfill research support duties for registered researchers of The Jikei University Hospital at Kashiwa campus so that physician-researchers can make the best achievements.

## Research Activities

*Elucidating the circuitry mechanisms underlying aversive and appetitive learning*
Avoiding pain and harm is fundamental for the survival of human and animals. Aversive stimuli, therefore, potently induce adaptive behaviors and memory formation. Clarifying neuronal circuitry mechanisms underlying such adaptive behaviors is fundamental for understanding brain functions. Furthermore, the dysregulation of the neuronal circuitry of such aversive behaviors leads to various anxiety disorders, such as posttraumatic stress disorders, 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 the parabrachial nucleus (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 memory formation, suggesting that the PB-CeA pathway might be involved in some emotional aspects of pain.
As for our research in 2019, 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, and, therefore, that the 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).

Regarding collaborative research, we have contributed to a study of lysosomal storage diseases performed by Professor Toya Ohashi (Division of Gene Therapy, Department of Pediatrics). We found that a mouse model of MGII with lysosomal storage disease knock-out exhibited impaired fear memory formation and that cell-targeted gene therapy with strong preconditioning significantly improved the phenotype to the level comparable to that of wild-type mice (in preparation). These works were supported by a Grant-in-Aid for Scientific Research (B), Strategic Research Program, the Japan Agency for Medical Research and Development (AMED) and Core Research for Evolutional Science and Technology to Professor Watabe; AMED and a Grant-in-Aid for Scientific Research (B) to Professor Ohashi.

*Predicted markers of overall survival in patients with pancreatic cancer receiving dendritic cells pulsed with Wilm's tumor protein 1 peptide*

We evaluated predictive markers of survival on patients with pancreatic ductal adenocarcinoma (PDA) treated with multiple MHC class I/II-restricted Wilm's tumor protein 1 (WT1) peptide-pulsed dendritic cell vaccines in combination with chemotherapy. The plasma levels of soluble factors (myeloperoxidase, matrix metalloproteinase 9, and transforming growth factor β1) derived from granulocytes in 7 eligible patients with PDA were examined. Compared with the 4 non-super responder patients (overall survival < 1 year), the remaining 3 super responder patients (overall survival ≥ 1 year) showed significantly lower plasma levels of matrix metalloproteinase 9 throughout long-term therapy. Prolonged low levels of a granulocyte-related systemic inflammatory response after the early period of therapy and low WT1 cytoplasmic expression in PDA cells might be predictive markers of survival for patients with PDA receiving WT1-targeting immunochemotherapy. This study was supported by a Grant-in-Aid for Scientific Research (C) to SK.

*Development of a qualitative analysis method for high-density lipoprotein capacity and investigation of biomarkers for cardiovascular diseases in diabetic kidney disease*

We have established a new method for evaluating high-density lipoprotein-mediated cellular cholesterol efflux capacity with a stable isotope and reported this method and the related study results (J Lipid Res 2019; 60: 1959-67). Our clinical research indicated the significant relevance of uric acid and homocysteine to renal function (estimated glomerular filtration rate), suggesting the possibility that they are markers for the presumption of vascular disorder risk. This research was supported by Grant-in-Aid for Scientific Research (C) to HY.

*Mechanism of islet injury and beta cell regeneration in diabetes mellitus*

Pancreatic islet β cells have a unique function to secrete insulin depending on blood glucose concentration (glucose-stimulated insulin secretion, GSIS). Under *in vivo* circumstances, this function is finely regulated by the nervous system, the microcirculation system, hormones, and metabolites, whereas the failure of this function causes type 2 diabetes mellitus. Furthermore, insulinoma, in which regulatory functions, such as GSIS, are also lost, shows inappropriate hypersecretion. To identify abnormalities of insulin secretion machinery, in a study of the current fiscal year approved by The Jikei University

Ethics Committee, we extracted genomic DNA, total RNA, and protein components from 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 (1.65 billion reads, 248 billion bases) and in the insulinoma (1.92 billion reads, 287.9 billion bases) and compared to the international standard University of California at Santa Cruz reference sequence human genome 19 generic annotation file (UCSC hg19), mutation of 1.3 million blood cells and insulinomas were found. When analysis was limited to high-precision reads of the sequence, 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.

## Publications

***Matsumura K, Seiriki K, Okada S, Nagase M, Ayabe S, Yamada I, Furuse T, Shibuya H, Yasuda Y, Yamamori H, Fujimoto M, Nagayasu K, Yamamoto K, Kitagawa K, Miura H, Gotoda-Nishimura N, Igarashi H, Hayashida M, Baba M, Kondo M, Hasebe S, Ueshima K, Kasai A, Ago Y, Hayata-Takano A, Shintani N, Iguchi T, Sato M, Yamaguchi S, Tamura M, Wakana S, Yoshiki A, Watabe AM, Okano H, Takuma K, Hashimoto R, Hashimoto H, Nakazawa T.*** Pathogenic POGZ mutation causes impaired cortical development and reversible autism-like phenotypes. *Nat Commun.* 2020 Feb 26; **11**(1): 859. doi: 10.1038/s41467-020-14697-z. PMID: 32103003; PMCID: PMC7044294.

***Hirowatari Y, Yoshida H.*** Innovatively Established Analysis Method for Lipoprotein Profiles Based on High-Performance Anion-Exchange Liquid Chromatography. *J Atheroscler Thromb.* 2019 Dec 1; **26**(12): 1027-1040. doi: 10.5551/jat.RV17037. Epub 2019 Sep 20. PMID: 31548491; PMCID: PMC6927812.

***Yoshida S, Ito Z, Suka M, Bito T, Kan S, Akasu T, Saruta M, Okamoto M, Kitamura H, Fujioka S, Misawa T, Akiba T, Yanagisawa H, Sugiyama H, Koido S.*** Clinical Significance of Tumor-Infiltrating T Cells and Programed Death Ligand-1 in Patients with Pancreatic Cancer. *Cancer Invest.* 2019; **37**(9): 463-477. doi: 10.1080/07357907.2019.1661427. Epub 2019 Sep 18. PMID: 31490702.

***Yoshida H, Tada H, Ito K, Kishimoto Y, Yanai H, Okamura T, Ikewaki K, Inagaki K, Shoji T, Bujo H, Miida T, Yoshida M, Kuzuya M, Yamashita S.*** Reference Intervals of Serum Non-Cholesterol Sterols by Gender in Healthy Japanese Individuals. *J Atheroscler Thromb.* 2020 May 1; **27**(5): 409-417. doi: 10.5551/jat.50187. Epub 2019 Sep 5. PMID: 31484845; PMCID: PMC7242229.

***Shimizu T, Miyazaki O, Iwamoto T, Usui T, Sato R, Hiraishi C, Yoshida H.*** A new method for measuring cholesterol efflux capacity uses stable isotope-labeled, not radioactive-labeled, cholesterol. *J Lipid Res.* 2019 Nov; **60**(11): 1959-1967. doi: 10.1194/jlr.D086884. Epub 2019 Aug 27. PMID: 31455616; PMCID: PMC6824490.

***Yanai H, Yoshida H.*** Beneficial Effects of Adiponectin on Glucose and Lipid Metabolism and Atherosclerotic Progression: Mechanisms and Perspectives. *Int J Mol Sci.* 2019 Mar 8; **20**(5): 1190. doi: 10.3390/ijms20051190. PMID: 30857216; PMCID: PMC6429491.

***Yoshida H.*** Clinical Impact and Significance of Serum Lipoprotein (a) Levels on Cardiovascular Risk in Patients With Coronary Artery Disease. *Circ J.* 2019 Apr 25; **83**(5): 967-968. doi: 10.1253/circj.CJ-19-0221. Epub 2019 Apr 4. PMID: 30944264.

***Ito Z, Kan S, Bito T, Horiuchi S, Akasu T, Yoshida S, Kajihara M, Hokari A, Saruta M, Yoshida N, Kobayashi M, Ohkusa T, Shimodaira S, Okamoto M, Sugiyama H,Koido S.*** Predicted Markers of Overall Survival in Pancreatic Cancer Patients Receiving Dendritic Cell Vaccinations Targeting WT1. *Oncology.* 2019; **97**(3): 135-148. doi: 10.1159/000500359. Epub 2019 Jun 19. PMID: 31216557.

# Centers of Advanced Medicine
# Center for Neuroscience of Pain

Fusao Kato, *Professor and Director*
Toya Ohashi, *Professor and Core Leader*
Shoichi Uezono, *Professor and Core Leader*

## 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-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 as a central site to develop and advance clinical and nonclinical studies and to teach about the neuroscience of pain.
In particular, 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

As the research activities of the center are promoted as collaborations of departments and research teams, the publication lists are provided in the pages for each department and division. These are some examples of such collaborations promoted in FY2019:
1) Brain mechanisms underlying the rheumatoid arthritis-associated emotional and cognitive complications
2) Mechanisms underlying the pain chronification in the rodent model of low back pain, with a particular interest in the involvement of the amygdala network: a study using high-magnetic field magnetic resonance imaging
3) Brain mechanism underlying widespread/ectopic sensitization in primary chronic pain
4) Brain mechanisms for the pleasantness of scratching as an exacerbating factor of dermatitis
5) Delivery-dependent modulation of the central oxytocin receptors as a neural basis for the modulation of labor pain

The following are 2 important publications from the Department of Neuroscience, where the headquarters of the JCNP are located.

## Publications

***Oto Y, Takahashi Y, Kurosaka D, Kato F.*** Alterations of voluntary behavior in the course of disease progress and pharmacotherapy in mice with collagen-induced arthritis. *Arthritis Res Ther.* 2019 Dec 12; **21**(1): 284. doi: 10.1186/s13075-019-2071-z. PMID: 31831067; PMCID: PMC6909634.

***Arimura D, Shinohara K, Takahashi Y, Sugimura YK, Sugimoto M, Tsurugizawa T, Marumo K, Kato F.*** Primary Role of the Amygdala in Spontaneous Inflammatory Pain-Associated Activation of Pain Networks - A Chemogenetic Manganese-Enhanced MRI Approach. *Front Neural Circuits.* 2019 Oct 1; **13**: 58. doi: 10.3389/fncir.2019.00058. PMID: 31632244; PMCID: PMC6779784.

# Centers of Advanced Medicine
# Center for Medical Entomology

Hirotaka Kanuka, *Professor and Director*
Tatsuya Sakurai, *Assistant Professor*
Kenji Ishiwata, *Professor*
Manabu Ote, *Assistant Professor*

## General Summary

Arthropod vectors are organisms that play a role in the transmission of pathogens from humans or from animals to humans. Vectors tend to be blood-sucking insects that ingest the disease-causing organism with blood from an infected host and then inject 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. We have previously discovered that *Wolbachia* targets the RNA-protein complex processing body (P-body) in *Drosophila* female germline cells and controls the translation of host RNAs. We discovered that the same mechanism might be working against virus RNAs and thereby suppress virus replication. P-body proteins were recruited to virus replication sites and supported virus amplification. The *Wolbachia* factor Toxic manipulator of oogenesis (TomO) controlling the P-body targeted virus RNAs and suppressed virus replication. We hypothesize that the P-body proteins and the *Wolbachia* effector protein TomO are the common factors underpinning diverse *Wolbachia*-mediated phenomena by targeting different types of RNA.

*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 the 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. To elucidate the function of *Capa* in diapause, we produced deletion heterozygous mutants in *A. albopictus* using the CRISPR/Cas9 system (clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 system) in both tropic strains and temperate strains. We are now attempting to establish *Capa* homozygous mutants to evaluate the function of Capa protein in the formation and maintenance of diapause eggs.

### Publications

***Badolo A, Sombié A, Pignatelli PM, Sanon A, Yaméogo F, Wangrawa DW, Sanon A, Kanuka H, McCall PJ, Weetman D.*** Insecticide resistance levels and mechanisms in *Aedes aegypti* populations in and around Ouagadougou, Burkina Faso. *PLoS Negl Trop Dis.* 2019 May 23; **13**(5): e0007439. doi: 10.1371/journal.pntd.0007439. PMID: 31120874; PMCID: PMC6550433.

***Sato K, Ahsan MT, Ote M, Koganezawa M, Yamamoto D.*** Calmodulin-binding transcription factor shapes the male courtship song in *Drosophila*. *PLoS Genet.* 2019 Jul 25; **15**(7): e1008309. doi: 10.1371/journal.pgen.1008309. PMID: 31344027; PMCID: PMC6690551.

***Ote M, Yamamoto D.*** Impact of *Wolbachia* infection on *Drosophila* female germline stem cells. *Curr Opin Insect Sci.* 2020 Feb; **37**: 8-15. doi: 10.1016/j.cois.2019.10.001. Epub 2019 Oct 17. PMID: 31726321.

# 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 mechanism of fatigue and fatigue-related diseases.

Resulting from stress — whether related to work or not — or insomnia, 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. 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

*Attenuation of HHV-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.

*Clinical significance of cancer-related fatigue 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.

## Publications

***Kobayashi N, Nishiyama T, Yamauchi T, Shimada K, Suka M, Kondo K, Yanagisawa H.*** Attenuation of human herpesvirus 6B reactivation by aging. *J Med Virol.* 2019 Jul; **91**(7): 1335-1341. doi: 10.1002/jmv.25434. Epub 2019 Feb 27. PMID: 30788852.

# 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 and analyze 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.
In addition, we are attempting simultaneous analysis of vitamin D metabolites in human serum using an automatic high performance liquid chromatography-mass spectrometer.

### Publications

***Taki T, Hoya Y, Nakada K, Kawamura M, Iwasaki T, Murakami K, Okamoto T, Mitsumori N, Yanaga K.*** Gastric Emptying Improved Significantly After PRG Compared to Billroth-I Reconstruction: Assessment of Gastric Emptying With a $^{13}$C-Breath Test. *Anticancer Res.* 2019 Jun; **39**(6): 3227-3230. doi: 10.21873/anticanres.13463. PMID: 31177172.

***Mezaki Y, Kato S, Nishikawa O, Takashima I, Tsubokura M, Minowa H, Asakura T, Matsuura T, Senoo H.*** Measurements of radiocesium in animals, plants and fungi in Svalbard after the Fukushima Daiichi nuclear power plant disaster. *Heliyon.* 2019 Dec 24; **5**(12): e03051. doi: 10.1016/j.heliyon.2019.e03051. PMID: 32083202; PMCID: PMC7019073.

***Ezaki H, Matsuura T, Ayaori M, Ochi S, Mezaki Y, Masaki T, Taniwaki M, Miyake T, Sakurada M, Ikewaki K.*** The fasting $^{13}$C-glucose breath test is a more sensitive evaluation method for diagnosing hepatic insulin resistance as a cardiovascular risk factor than HOMA-IR. *Clin Chim Acta.* 2020 Jan; **500**: 20-27. doi: 10.1016/j.cca.2019.09.014. Epub 2019 Oct 10. PMID: 31606399.

# 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*
Tetsuya Horino, *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*
Shinya Sugimoto, *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

*Imaging of biofilms in solution by atmospheric scanning electron microscopy*
In this study, we visualized aqueous biofilms formed by the Gram-positive coccus *Staphylococcus aureus* and the Gram-negative bacillus *Escherichia coli* by means of recently developed atmospheric scanning electron microscopy (ASEM). In addition, ASEM reveals polyfunctional nanofibril appendages that mediate attachment, filamentation, and filament adaptability in Fe/Mn-oxidizing bacterium *Leptothrix cholodnii*. Collectively, our results suggest that ASEM is a broadly applicable approach for microbial research.

*Functional analysis for periplasmic proteases that degrade bacterial amyloid precursor proteins*
Bacterial extracellular amyloid fibers, called Curli, are involved in biofilm formation and the colonization of *E. coli*. Previously, we reported that cytoplasmic molecular chaperone DnaK kept CsgA and CsgB, the major and minor structural components of Curli, in a translocation-competent state by binding to their signal peptides prone to aggregation. We also found that certain periplasmic proteases degraded CsgA and CsgB in the periplasmic

space. These findings provide new insights into the regulation of bacterial amyloid fibers.

*Microscopic and bacterial analyses of biofilms formed in clogged biliary stents from patients*

Endoscopic biliary stenting is the most common treatment for patients who have jaundice associated with malignant hepatobiliary tumors. However, recurrent jaundice is a major complication of biliary endoprosthesis insertion. Thus, stent removal and replacement with a new stent frequently occur as a consequence of device blockage caused by microbial biofilm formation and biliary sludge accumulation in the lumen. In this study, we are analyzing microbial biofilms formed in the stents by ASEM and confocal laser scanning microscopy. We are also characterizing bacteria isolated from a biliary stent removed from a patient.

*Screening of* S. aureus *biofilm inhibitors and mechanism of action studies*

Formation of *S. aureus* biofilms on medical devices can cause severe or fatal infectious diseases. To develop new methods for preventing and treating biofilm-associated infections, we have performed high-throughput screening of small compounds that inhibit biofilm formation by *S. aureus*. One of the screened compounds, JK1, suppressed production of extracellular polysaccharide that is important for biofilm formation. A pull-down assay of JK1-immobilized beads revealed that JK1 specifically binds to a protein that is involved in cell-wall synthesis of *S. aureus*. Substitution of amino acids in the active center of this protein significantly reduced both enzymatic activity and JK1-binding activity. Therefore, JK1 appears to bind to the active center of the target protein. We also evaluated the biofilm inhibitory activity of JK1 using a continuous flow biofilm model on a microfluidic device and revealed that JK1 was effective under flowing conditions.

*Promotion of biofilm formation by external RNA*

*S. aureus* often causes life-threatening infections, such as biofilm-associated infections, due to formation of robust biofilm. Understanding the molecular mechanisms of how biofilms form is important for developing medical countermeasures for 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 a polysaccharide-dependent manner. Microscopic, biochemical, and molecular interaction analyses showed that RNA directly binds with and co-localizes with polysaccharides in the biofilm. Additionally, RNA purified from human blood promoted biofilm formation under static and flow conditions. These results suggest that RNA serves as a biofilm scaffold during infection and that *S. aureus* might utilize blood RNA to form a strong biofilm on implanted medical devices or tissues, causing chronic infections.

**Publications**

***Yonemoto K, Chiba A, Sugimoto S, Sato C, Saito M, Kinjo Y, Marumo K, Mizunoe Y.*** Redundant and Distinct Roles of Secreted Protein Eap and Cell Wall-Anchored Protein SasG in Biofilm Formation and Pathogenicity of *Staphylococcus aureus*. *Infect Immun.* 2019 Mar 25; **87**(4). pii: e00894-18. doi: 10.1128/IAI.00894-18. Print 2019 Apr. PMID: 30670553.

***Abe M, Nakamura S, Kinjo Y, Masuyama Y, Mitsuyama J, Kaku M, Miyazaki Y.*** Efficacy of T-2307, a novel arylamidine, against ocular complications of disseminated candidiasis in mice. *J Antimicrob Chemother.* 2019 May 1; **74**(5): 1327-1332. doi: 10.1093/jac/dkz020. PMID: 30753506.

***Okai C, Itani Y, Furuta A, Mizunoe Y, Iwase T.*** Rapid Identification and Quantification of *Lactobacillus rhamnosus* by Real-Time PCR Using a TaqMan Probe. *Jpn J Infect Dis.* 2019 Sep 19; **72**(5): 323-325. doi: 10.7883/yoken.JJID.2019.102. Epub 2019 Apr 26. PMID: 31061362.

***Lopes AA, Yoshii Y, Yamada S, Nagakura M, Kinjo Y, Mizunoe Y, Okuda K.*** Roles of lytic transglycosylases in biofilm formation and β-lactam resistance in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother.* 2019 Sep 30; **63**(12): e01277-19. doi: 10.1128/AAC.01277-19. Epub ahead of print. PMID: 31570396; PMCID: PMC6879220.

***Ueno K, Yanagihara N, Otani Y, Shimizu K, Kinjo Y, Miyazaki Y.*** Neutrophil-mediated antifungal activity against highly virulent *Cryptococcus gattii* strain R265. *Med Mycol.* 2019 Nov 1; **57**(8): 1046-1054. doi: 10.1093/mmy/myy153. PMID: 30668754.

***Kunoh T, Morinaga K, Sugimoto S, Miyazaki S, Toyofuku M, Iwasaki K, Nomura N, Utada AS.*** Polyfunctional Nanofibril Appendages Mediate Attachment, Filamentation, and Filament Adaptability in *Leptothrix cholodnii*. *ACS Nano.* 2020 May 26; **14**(5): 5288-5297. doi: 10.1021/acsnano.9b04663. Epub 2019 Dec 5. PMID: 31804801.

# School of Nursing

# Fundamental Nursing

Sachiko Tanaka, *Professor*
Kiyoko Fukai, *Professor*
Chieko Hanyu, *Assistant Professor*
Noriko Aoki, *Assistant Professor*
Noriko Sato, *Professor*
Hiroko Yatsu, *Professor*
Sumiko Satake, *Assistant Professor*

## General Summary

Major study areas in fundamental 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, (5) nursing diagnosis, and (6) developmental research of symptom management.

## Research Activities

Sachiko Tanaka studied the healthy work environment of nurses in hospitals.
Hiroko Yatsu published a revised edition of a book with 2 co-workers about maternity nursing skill. She presented a poster about an integrative literature review on global nursing at an academic conference held in Chang Mai. The 2 manuscripts she wrote in 2019 about nursing theory and animal rights will be published in 2020.
Chieko Hanyu studies the theme of "clinical judgment in nursing." She has done pilot tests of a clinical judgment program for nurses in their second year of work.
Sumiko Satake studies 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.
Kiyoko Fukai has been preparing manuscripts on symptom management nursing with the doctoral students of her former university and continues her research on pain management using physiological methods.

### Publications

***Win MMTM, Fukai K, Nyunt HH, Hyodo Y, Linn KZ.*** Prevalence of peripheral neuropathy and its impact on activities of daily living in people with type 2 diabetes mellitus. *Nurs Health Sci.* 2019 Dec; **21**(4): 445-453. doi: 10.1111/nhs.12618. Epub 2019 Jun 18. PMID: 31215149.
***Win MMTM, Fukai K, Nyunt HH, Linn KZ.*** Hand and foot exercises for diabetic peripheral neuropathy: A randomized controlled trial. *Nurs Health Sci*. 2020 Jun; **22**(2): 416-426. doi: 10.1111/nhs.12676. Epub 2019 Dec 26. PMID: 31876991.
***Kubo Y, Kajii F, Takahashi K, Satake S, Ishikawa J, Mochizuki R, Shimasawa J, Kita M.*** Clarification of Self-Motivated Learning Behaviors among Undergraduate Nursing Students in Japan. *Jikeikai Medical Journal.* 2019 Dec; **66**(1-4): 17-29.

# Adult Nursing

Misuzu Nakamura, *Professor*
Midori Nagano, *Professor*
Miwako Fukuda, *Associate Professor*
Tetsuya Myojin, *Assistant Professor*
Masami Sato, *Professor*
Ruka Mochizuki, *Associate Professor*
Yoko Murooka, *Assistant Professor*
Itsuko Yamamoto, *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.

3. Research on difficulties in nursing practice in critical care nursing

This study developed a scale for difficulties in practice in critical care nursing and presented it at an academic conference in Oita in 2019. In the future, I would like to promote research for creating assessment indicators and exploring the ideal way of supporting nurses involved in critical care.

*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 was performed to examine a model of collaboration of pharmacists and nurses for patients who have cancer and their family members who have gone to a pharmacy. We performed focus group interviews of 15 pharmacists who are had supported such patients and their families. We will present the results 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 analyzed the interview results of 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 continue conduct Internet surveys from last year.

*Research on other topics*

1. Development of support tools for people living in their own residence by judging upon hospitalization patients with pressure injuries at home

This research received a grant from the Ministry of Education, Culture, Sports, Science and Technology (19K10963/Grant‐in‐Aid for Scientific Research (C); KAKENHI). We received the cooperation of 10 nurses certified in Wound, Ostomy, and Continence nursing from The Jikei University Hospitals who served as expert meeting members. Data were collected from electronic medical records of 261 patients who had a pressure injury at the time of hospital admission. We gave a presentation on the characteristics of patients with pressure injuries from home upon hospitalization in society (APETNA 2019: 8th Asia Pacific Enterostomal Therapy Nurse Association). We are analyzing the characteristics of patients who had died during admission, patients with cancer who had undergone chemotherapy, and patients who live at nursing homes.

2. Measuring pressure and shear force on the heel region and the reduction effect using dressing materials

The pressure and misalignment force of the heel was measured when the head was raised, and the data were analyzed to see if the pressure and misalignment force could be reduced by the dressing. As a result, it was possible to measure the pressure and displacement force simultaneously, and both values tended to increase and maintain as the head was raised. The pressure and displacement force applied to the skin surface of three types of dressings: film (group A), low_friction hydrocolloid (group B), and silicone foam (group C) were measured with a three_axis tactile sensor. As a result, the pressure and misalignment forces were reduced for B and C materials compared to A materials. Based on these results, we presented the results at a conference and are now preparing a paper.

### Publications

***Nakano M, Nakamura M, Furushima S, Sato M, Hasegawa N, Sasaki A***. Educational effect of a nursing training conference adopting the world café method: Medical care teams. *Journal of Nursing Education and Practice.* **9**(8): 91–98.
***Kanda K, Fujimoto K, Mochizuki R, Ishida K, Lee B.*** Development and validation of the comprehensive assessment scale for chemotherapy-induced peripheral neuropathy in survivors of cancer. *BMC Cancer.* 2019 Sep 10; **19**(1): 904. doi: 10.1186/s12885-019-6113-3. PMID: 31506070; PMCID: PMC6734590.
***Kubo Y, Kajii F, Takahashi K, Satake S, Ishikawa J, Mochizuki R, Shimasawa J, Kita M.*** Clarification of Self-Motivated Learning Behaviors among Undergraduate Nursing Students in Japan. *Jikeikai Medical Journal.* 2019 Dec; **66**(1-4): 17-29.

## Gerontological Nursing

Fumiko Kajii, *Professor* Yoshie Nakajima, *Associate Professor*

### General Summary

The present study was performed in 2019 and was joint research by The Jikei Third Hospital Dementia Medical Center and The Jikei University. A survey examined the problems of family caregivers for patients with dementia, and this work was supported by the nursing school research expenses.

### Research Activities

Purpose of the study: To clarify the contents of problems of family caregivers of persons with dementia, who had medical examinations at the outpatient departments of Psychiatry, Neurosurgery, and Neurology, and to improve the quality of services for dementia medical care at The Jikei Third Hospital Dementia Medical Center.
Study method: The study involved a cross-sectional survey with anonymous self-administered questionnaires returned by postal mail. From February through May 2019, family caregivers of patients with dementia who consulted with outpatient physicians of the departments of Psychiatry, Neurosurgery, and Neurology for consultation, diagnosis, and treatment of dementia. A comparative analysis was performed among family caregivers who were younger than 70 years or 70 years or older. The $\chi^2$ test was used to analyze nominal variables.
Results: Of 185 questionnaires that were sent to caregivers, 97 (52.4%) were returned. The average age of the 97 family caregivers was 69.1 years (standard deviation, 12.94). Difficulty to give care was due to “response to symptoms of dementia, such as forgetfulness and loitering” (40 caregivers, 41.2%); “compliance assistance” (27 caregivers, 27.8%); and “outpatient assistance” and “money management” (24 caregivers, 24.7%). There results did not differ significantly between the age groups. A difficulty in daily life was reported by a significantly higher percentage of caregivers 70 years or older (26 caregivers, 70.3%) than of caregivers younger than 70 years (12 caregivers, 29.3%, $p = 0.001$). Frustration was reported by a significantly higher percentage of caregivers

younger than 70 years (35 caregivers, 94.6%) than of caregivers 70 years or older (38 caregivers, 80.8%, p = .045). Opportunities to obtain information about caregiving were reported by a significantly higher percentage of caregivers younger than 70 years (32 caregivers, 86.5%) than of caregivers 70 years or older (31 caregivers, 67.4%, p = .037).
Discussion: Family caregivers younger than 70 years often obtain information via the Internet and specialized books in addition to family members and friends. For this reason, a method of transmitting information via the Internet, such as websites and social media, should be devised in the medical center. Therefore, this method is necessary for distributing information that can be used to balance work and as a two-way place where we can discuss concerns and concerns.

### Publications

***Kubo Y, Kajii F, Takahashi K, Satake S, Ishikawa J, Mochizuki R, Shimasawa J, Kita M.*** Clarification of Self-Motivated Learning Behaviors among Undergraduate Nursing Students in Japan. *Jikeikai Medical Journal.* 2019 Dec; **66**(1-4): 17-29.

## Mental Health and Psychiatric Nursing

Yasuko Koyano, *Professor*
Junko Ishikawa, *Assistant Professor*
Mayuko Yamashita, *Associate Professor*

### General Summary

First, we give lectures to teach students about medical systems and social resources based on mental health and welfare acts. Second, we also give lectures to teach students about methods to assess patients who have mental problems during treatment.

### Research Activities

Yasuko Koyano: *Noncomprehensive Dialectical Behavior Therapy with a focus on mindfulness and skill training*
The author has been conducting the program mainly for a skill training of Dialectical Behavior Therapy by the psychiatric outpatient practice with night care since 2011. Here is a report on the usefulness of Noncomprehensive Dialectical Behavior Therapy with interviews performed thus far of individual participants.
The program results were as follows.
1. Mindfulness with repeatedly various variations each time as an introduction to the program became a method of training for patients to notice their own emotions and to enhance sensitivity.
2. A rush of positive emotions was effectively enhanced through sharing, with a focus on subsequent positive events. The skill training followed by the enhancement allowed patients to generate an ease of mind by not being influenced by emotions owing to appro-

priately understanding their own emotions.
3. It was clarified that variations through a change in how to comprehend to the emotions of others by appropriately understanding one's own emotions was effectively developed for further variation to solve a problem.

Mayuko Yamashita: *Development of a Self-Care Competency Assessment Scale for Persons with Mental Disabilities*
We created a scale proposal based on concept analysis and qualitative research. I conducted a third-party assessment-type questionnaire of 191 persons with mental disabilities hospitalized at a dedicated mental health hospital and then validated the reliability and validity of the scale.
We confirmed that the developed scale fulfilled specific standards for reliability and content validity, convergent validity, and construct validity and that the scale would be used to simply and adequately assess self-care competence among persons with mental disabilities.

### Publications

***Kubo Y, Kajii F, Takahashi K, Satake S, Ishikawa J, Mochizuki R, Shimasawa J, Kita M.*** Clarification of Self-Motivated Learning Behaviors among Undergraduate Nursing Students in Japan. *Jikeikai Medical Journal.* 2019 Dec; **66**(1-4): 17-29.

## Child Nursing

Kinu Takahashi, *Professor* Michie Nagayoshi, *Lecturer*

### General Summary

The lectures given to undergraduates included an introduction, methods, practice, and educational evaluation. The lectures promoted the learning of practical abilities in pediatrics, through training in outpatient clinics, 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 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

*Clarification of self-motivated learning behaviors among undergraduate nursing students in Japan*
The aim of this study was to clarify specific contents regarding self-motivated learning

behaviors among undergraduate nursing students in Japan. The method to be used was that 23 nursing students participated in semistructured interviews. Data were analyzed with descriptive qualitative methods. A total of 273 codes related to self‐motivated learning behaviors were received and were divided into 19 categories and 66 subcategories. Jikeikai Med J 2019; 66: 17‐29.

*Motivation and challenges of making career choices during the growth process after treatment completion in child cancer survivors*

The purpose of this study was to identify motivation and challenges faced when making career choices during the growth process after the completion of treatment in child cancer survivors receiving in‐hospital education and to explore an ideal method for long‐term psychosocial follow‐up. Fourteen subjects aged 18 to 26 years were enrolled, and 1‐hour‐long semistructured interviews and qualitative descriptive analysis were conducted. With respect to the motivation for making career choices, six categories were extracted. Hospitalization experience, their relationship with care staff and teachers, and interaction with friends helped motivate participants to make career choices. Hindering factors included late stage complications, reduced physical strength, delayed learning, and social factors.

The 51th Congress of the International Society of Pediatric Oncology (Lyon, France)

*Development and verification of educational programs for nurses involved in children to enhance the practice of advocacy for children*

After ratifying the Convention on the Rights of the Child, the importance of advocating for the rights of the child has been made known. Education on advocacy of patients is conducted at general hospitals. However, education is focused on adults, who make up many patients. Nurses involved with children have very few opportunities to learn about advocacy for children. This study develops and tries the “educational program to improve the practice of advocacy for children for nurses involved with children”, clarifies and improves the issues, and then the nurses involved with the children. It is intended to be delivered to their facilities.

The program was constructed by a research team consisting of nurses, doctors, nursing teachers, and ethics researchers, and the program was tested and evaluated. The program 1) Relax and get to know the other person 2) What is happening around the advocating for the rights of the child 3) Experience collaborating with other occupations in the advocating for the rights of the child ― What to do when in trouble No. 4) take‐home message‐Training experience to be taken to the ward. As a result of the trial, the average of the program goals (5‐grade evaluation) of all the participants was 4.7±SD. The effectiveness of the program will be clarified by tracing the status of the practice of advocacy for children by nurses who participated in the program.

*The development of the ability scale of regarding child advocacy for pediatric nurses (revised version)*

This study reviews the issues of the previous study ‘Development of the ability scale of regarding child advocacy for pediatric nurses’ (2019 Takahashi, Takita), The purpose is to

improve reliability and validity and to create a revised version. The 30-item draft was prepared by reexamining the draft of the scale item of the nurse's practice ability scale of advocating for the rights of the child, involved in the first stage. In the future, the validity of the contents of the proposed scale will be examined, the reliability and validity of the scale will be verified, and the development of the ability scale of regarding child advocacy for pediatric nurses (revised version) will be planed.

### Publications

***Kubo Y, Kajii F, Takahashi K, Satake S, Ishikawa J, Mochizuki R, Shimasawa J, Kita M.*** Clarification of Self-Motivated Learning Behaviors among Undergraduate Nursing Students in Japan. *Jikeikai Medical Journal.* 2019 Dec; **66**(1-4): 17-29.

## Maternity Nursing

Yasuko Hososaka, *Professor* Mayumi Hamada, *Assistant Professor*

### General Summary

Studies have been performed to examine the various health issues in each of the lifestyle stages of women and to explore how nursing assistance should be extended in maternal nursing.

### Research Activities

*Development of a mother and father discipline self-triage scale*
We examined the reliability and validity of a discipline self-triage measure of the mother and father. A questionnaire survey was conducted for parents of 191 children who attend kindergarten in the Tokyo metropolitan area. Item reliability, exploratory factor analysis, and Cronbach's α coefficient were examined to confirm the scales reliability and validity. The Cronbach's α coefficient of each subscale was 0.70 to 0.92 and 0.76 to 0.80, and the reliability and validity of the scale were verified.

*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 (J-QATQS), a scale used to comprehensively evaluate quantitative research from the standpoint of the quality of the research, was created while ensuring the equivalence of the 2 versions, and the reliability of J-QATQS was demonstrated. The J-QATQS was created in a chart format to ensure ease of use. Of the nursing research studies written in Japanese and published by 2 researchers on the Ichushi-Web, 10 studies of comparative testing and comparative research were evaluated with the J-QATQS, and the interrater reliability (κ coefficient) was calculated. In all cases, the κ coefficient satis-

fied the level of significance, and reliability was found, on the basis of the following scores, to be high for all of the studies: selection bias, $\kappa = 0.94$; research design, $\kappa = 0.86$; confounding factor, $\kappa = 1$; blinded, $\kappa = 0.67$: data collection method, $\kappa = 0.68$; and target dropout rate, $\kappa = 1$. The equivalence of the J-QATQS created in the present study was confirmed by the original authors, and the results of the reliability study showed that the interrater reliability of the tool was high, indicating that the effectiveness of published studies can be objectively evaluated.

*Difficulty for midwives to provide support to husbands when their wives give birth in hospitals and maternity clinics*

To clarify the difficulties that midwives face in building a relationship with husbands present during their wives' childbirth. We applied a qualitative descriptive research design and conducted a semistructured interview with 5 midwives who worked at hospitals in the Tokyo area. We obtained research ethics approval from The Jikei University Research Ethics Committee in 2018. Difficulties for the midwives were classified into the following 6 categories: (1) frustration with husbands who are disinterested in sharing the birthing experience, (2) annoyance while providing support to husbands, (3) failure to build relationship with husbands, (4) uncomfortable feeling about support for husbands caused by difference in standing positions of physicians and midwives, (5) inconsolable feeling about insufficient support for husbands because of lack of staff, and (6) limitations on continuous support to husbands. The study also suggested that for midwives to express their feelings of difficulty is important.

*Factors related to the nutritional method at discharge of preterm infants admitted to the neonatal intensive care unit: Comparison of complete breastfeeding and mixed nutrition*

The nutritional method at discharge was statistically analyzed for 360 mothers who care for preterm infants whose were hospitalized at 12 facilities registered at perinatal medical care centers in Japan. The most significant factor contributing to being a mother at the time of the infant's discharge from the neonatal intensive care unit was that the lactation volume was maintained before discharge.

*A survey of postpartum care needs for mothers at the time of discharge from the Jikei Daisan Hospital*

We investigated whether puerperal mothers want postnatal care after discharge from the hospital. The average age of 45 mothers during childbirth was $31.9 \pm 4.7$ years, and the average length of pregnancy was $38.8 \pm 1.8$ weeks. 18 mothers (40.0%) were aware of postnatal care facilities, 15 mothers (33.3%) were willing to use these facilities without assistance and 22 mothers (48.9%) were willing to use then if they had assistance.

There was no significant difference in the $\chi^2$ independence test. We plan to continue studying in the future.

# Community Health Nursing

Junko Shimasawa, *Professor*
Yumiko Shimizu, *Assistant Professor*
Yoshiko Kubo, *Assistant Professor*

## General Summary

The faculty's research has been 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) clarification of self-motivated learning behaviors among undergraduate student nurses in Japan, (4) health and welfare in patients undergoing hemodialysis who live in 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 visiting nursing care to promote continued community life by mentally ill patients living at home. In this study, such assistance was considered to be 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. The results suggested 9 categories and 23 subcategories.

*Clarification of self-motivated learning behaviors among undergraduate student nurses in Japan*
This study aimed to clarify specific contents regarding self-motivated learning behaviors among undergraduate student nurses in Japan. Twenty-three nursing students participated in semistructured interviews. Data were analyzed using descriptive qualitative methods. In total, 273 codes relating to self-motivated learning behaviors emerged, falling into 66 subcategories across 19 categories.

*Health and welfare in patients undergoing hemodialysis and living 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, Fumiko F, Takahashi K, Satake S, Ishikawa J, Mochizuki R, Shimasawa J, Kita M.*** Clarification of Self-Motivated Learning Behaviors among Undergraduate Student Nurses in Japan. *Jikeikai Medical Journal.* 2019; **66**(1-4): 17-29.
***Sugisawa H, Shinoda T, Shimizu Y, Kumagai T, Sugisaki H.*** Psychosocial Mediators between Socioeconomic Status and Dietary Restrictions among Patients Receiving Hemodialysis in Japan. *Int J Nephrol.* 2019 Apr 17; **2019**: 7647356. doi: 10.1155/2019/7647356. PMID: 31139469; PMCID: PMC6500646.
***Sugisawa H, Shinoda T, Shimizu Y, Kumagai T, Sugisaki H, Sugihara Y.*** Caregiving for Older Adults Requiring Hemodialysis: A Comparison Study. *Ther Apher Dial.* 2020 Aug; **24**(4): 423-430. doi: 10.1111/1744-9987.13453. Epub 2019 Dec 10. PMID: 31693297.

## Home Care Nursing

Motoko Kita, *Professor*
Hiroko Toyama, *Assistant Professor*
Yuri Sugiyama, *Assistant Professor*

### General Summary

Since 2011, our undergraduate course, Home Care Nursing, has focused on students becoming able 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 2019, we launched an educational assessment study. We also did research aligned with the theme 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.

*The effect of lectures, exercises, and practical training on home nursing in basic nursing education on the construction of perspectives for home nursing*

The construction of a community-based comprehensive care system is being promoted, and the importance of nursing for patients who have completed inpatient treatment and continue medical treatment at home is increasing. At our university, we provide home nursing lectures, exercises, and practical training centered on 2 types of transitional nursing and home-visit nursing. How is the basic manner of thinking about home-nursing learned in basic education applied to practice in postgraduate clinical settings? The purpose of this study was to conduct a survey of nurses in the first to fifth years of clinical experience to obtain suggestions for educational content.

*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.

**Publications**

***Kubo Y, Kajii F, Takahashi K, Satake S, Ishikawa J, Mochizuki R, Shimasawa J, Kita M.*** Clarification of Self-Motivated Learning Behaviors among Undergraduate Nursing Students in Japan. *Jikeikai Medical Journal.* 2019 Dec; **66**(1-4): 17-29.

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**Research Activities 2019**

The Jikei University School of Medicine

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