RESEARCH ACTIVITIES OF SAPPORO MEDICAL UNIVERSITY 1997 – 2000

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Edited by the Committee for International Affairs and Medical Exchanges, Sapporo Medical University

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I OUTLINE OF SAPPORO MEDICAL UNIVERSITY

SAPPORO MEDICAL UNIVERSITY

Sapporo Medical University was founded as Sapporo Medical College in 1950 by the Hokkaido Prefectural Government, located in the center of Sapporo. Since its establishment, the college, endowed with a progressive spirit, has been providing advanced medical services while contributing to the promotion of the health of Hokkaido citizens.

After the School of Health Sciences – Nursing, Physical Therapy, Occupational Therapy—was established in 1993, the college became a medical University. The Graduate School has been expanded since 1956 to train specialists in advanced medical treatment and researchers. The Ph.D. Course of Medicine has 3 programs with 11 divisions, and the School of Health Sciences has 3 master courses and 2 Doctor courses. In 1999, the Basic Medical Research Building was completed including the University Library, the Information Center of Computer Communication and the Biomedical Research, Education and Instrumentation Center, all of which facilitate a wide range of advanced research. In addition, highly qualified professors with a wide range of knowledge offer high quality education based on close communication with their students.

We actively promote exchange activities with Northern Region countries whose climates and living conditions are similar to those of Hokkaido to improve the health and welfare of people living in these regions. Since 1977, the university has established medical exchange programs with universities in Finland, Canada, China and the U.S.A. Moreover the university has many participants in various international cooperation projects to help developing countries.

In 2000, Sapporo Medical University celebrated its 50th anniversary. On this occasion, we signed “The Declaration of Sapporo on the Joint Development of Northern Region Medicine Towards the 21st Century” along with other overseas universities to establish a common goal and to contribute to the improvement of health and welfare at a global level.

Through this brochure, we hope to introduce our research activities to scientists around the world and we hope that it provides you with an opportunity to collaborate with us.

AIM OF THE UNIVERSITY

Sapporo Medical University aims to contribute to the improvement of the healthcare of the local community as well as to the cultural development of mankind by teaching theories and applications regarding medicine and health sciences, researching in depth, fostering student’s intellectual and moral abilities and their capacity for application.

HISTORY

As part of the Hokkaido's comprehensive development, Sapporo Medical University was founded in 1950 based on Hokkaido Women's Medical College. The most recent development was the establishment of the School of Health Sciences in April 1993 in accordance with the reorganization of the Health Sciences Junior College – which opened in April 1983 – attached to Sapporo Medical College. In June, 2001, the University celebrated its 50th anniversary.

Chronology of Hokkaido Women's Medical College

April 1945	Hokkaido Women's Medical College was founded.

Chronology of Sapporo Medical College

April 1950	Sapporo Medical College opened.
June 1950	Opening ceremony held - June 25 designated as the college foundation day.
September 1955	Cancer Research Institute established as an affiliated research institution.
March 1955	Establishment of the Graduate School of Medicine approved - enrollment capacity is 25 students.
January 1958	Premedical course provided.
September 1968	Marine Biomedical Institute established.
April 1979	Divided courses - premedical and special courses abolished.
April 1983	Health Sciences Junior College attached to Sapporo Medical College opened.

Chronology of Sapporo Medical University

April 1993	School of Health Sciences - Departments of Nursing, Physical Therapy and Occupational Therapy - established to accept 90 students.
April 1998	Graduate School of Health Sciences - Nursing, Physical Therapy and Occupational Therapy - established - enrollment capacity is 24 students.
April 1999	Information Center of Computer Communication established.
April 2000	Doctoral course for Physical Therapy and Occupational Therapy established in the Graduate School of Health Sciences - enrollment capacity is 6 students.
April 2001	Ph.D. course of Medicine for three programs reorganized in the Graduate School of Medicine – the total enrollment capacity is 50.

ORGANIZATION

SCHOOL OF MEDICINE

The School of Medicine offers 11 subjects and 33 courses. Its affiliated institutions include the University Hospital, the Cancer Research Institute, which consists of three departments, and the Marine Biomedical Institute. It also hosts the Biomedical Research, Education and Instrumentation Center, which consists of three departments, and the Animal Experimentation Center.

BIOMEDICAL RESEARCH, EDUCATION AND INSTRUMENTATION CENTER

Due to the rapid progress of the technology in molecular biology, the techniques used for medical treatment and biological research have rapidly improved. For this reason, the Biomedical Research, Education and Instrumentation Center is supplied with the latest research equipment so that the most advanced research in the world can be conducted. This equipment can be shared by researchers. The collaboration between basic researchers and clinical researchers is expected to result in significant contributions to the world's scientific community.

UNIVERSITY HOSPITAL

The University Hospital consists of 22 departments and is provided with 994 in-patient beds. The hospital treats as many as 1,800 outpatients a day. It serves as a center for providing clinical education and conducting research. In addition, the hospital is designated as a "disaster base hospital" and "HIV regional hospital"; thus it plays a great role as a core medical institution of Hokkaido.
In 1996, the University Hospital was approved by the Minister of Health and Welfare as a specialty function hospital – a hospital which is capable of administering advanced medical treatment, developing medical procedures and providing training.

CANCER RESEARCH INSTITUTE

The Cancer Research Institute was founded in 1955. Now it has three departments, Pathology, Molecular Biology, and Biochemistry. Each department participates in the education of medical students with a responsibility for interdisciplinary teaching subjects: tumor pathology (Dept. of Pathology), molecular biology (Dept. of Molecular Biology), and molecular medical science (Dept. of Biochemistry). Each department also accepts graduate students and research fellows interested in joining in with the ongoing research and related subjects.

ANIMAL RESEARCH CENTER

Animal research has greatly contributed to advanced research and basic research on highly advanced medical treatment. The Animal Experimentation Center offers the facilities and technology to conduct and support advanced research, which includes organ transplantation, gene knock out animals and a variety of molecular investigations.

MARINE BIOMEDICAL INSTITUTE

In order to promote medical research on marine animals living around Rishiri Island and on measures for providing medical treatment for people on solitary islands, the Marine Biomedical Institute was established in September 1968. A director of the institute, full-time associate director and scientists are stationed at the institute to engage in research.

SCHOOL OF HEALTH SCIENCES

In compliance with the increasing demand for health care, the School of Health Sciences was established in April 1993 aiming to train humane, highly skilled practitioners who have learned practical theory and procedures in the fields of nursing, physical therapy and occupational therapy, as well as to build a foundation for contributing to development in each field as educators and researchers.

GRADUATE SCHOOL OF MEDICINE

The Graduate School of Medicine was established in 1956 to provide students with the opportunity to independently conduct their own research and acquire basic knowledge necessary to further engage in advanced specialized medical science and technologies. Since its establishment, 671 students have completed the required courses (as of March 2001) and 1,325 students have obtained doctorates after presenting their theses (as of March 2001). Our graduates are actively engaged in a wide range of medical professional activities. Since April 2001, the graduate school has started a new program that consists of 3 major fields of study [(i) community health and comprehensive medicine, (ii) molecular and organ regulation and (iii) signal transduction medicine] and 49 major courses. This has involved a reorganization of the Graduate School of Medicine as previously there were 5 major fields (physiological studies, pathology studies, sociomedical studies, the science of

internal medicine and the science of surgery) in which 31 students participated. The newly developed program, which has 50 participating students, has brought remarkable opportunities for our graduate students to study highly advanced medical science and therapeutic approaches.

GRADUATE SCHOOL OF HEALTH SCIENCES

Master's level courses were established at the Graduate School of Health Sciences in April 1998 aiming at providing students with in-depth knowledge from various viewpoints, and researching the techniques in their chosen fields necessary to engage in specialized professions. The doctoral level course was established at the Graduate School of Health Sciences in April 2000. The students conduct research to acquire sophisticated technical know-how and the profound basic knowledge necessary to engage in specialized professions.

NUMBER OF TEACHING STAFFS & FELLOWS

(as of March 31, 2001)

SCHOOL OF MEDICINE

BASIC MEDICAL SCIENCES

	Prof.	Assoc. Prof.	Assist. Prof.	Instructor	Research Fellow	Total
Anatomy (I)	1	0	1	2	1	5
Anatomy (II)	1	0	0	4	4	9
Physiology (I)	1	0	1	3	0	5
Physiology (II)	1	2	0	1	0	4
Biochemistry (I)	1	0	1	2	2	6
Biochemistry (II)	1	1	1	2	3	8
Pathology (I)	1	1	2	1	10	15
Pathology (II)	1	1	2	1	8	13
Microbiology	1	0	2	2	1	6
Pharmacology	1	1	1	1	0	4
Hygiene	1	1	1	1	1	5
Public Health	1	1	1	2	1	6
Legal Medicine	1	1	1	1	0	4
Total	13	9	14	23	31	90

CLINICAL MEDICAL SCIENCES

	Prof.	Assoc. Prof.	Assist. Prof.	Instructor	Clinical Fellow	Total
Internal Medicine (I)	1	2	2	4	91	100
Internal Medicine (II)	1	2	4	3	40	50
Internal Medicine (III)	1	2	2	5	32	42
Internal Medicine (IV)	1	1	2	5	72	81
Surgery (I)	1	0	3	6	46	56
Surgery (II)	1	0	4	4	10	19
Orthopedic Surgery	1	2	1	6	24	34
Neurosurgery	1	2	2	3	5	13
Obstetrics & Gynecology	1	2	4	3	25	35
Pediatrics	1	1	4	4	29	39
Ophthalmology	1	0	3	4	27	35
Dermatology	1	2	3	2	5	13
Urology	1	1	1	6	22	31
Otolaryngology	1	0	2	6	17	26
Neuropsychiatry	1	1	2	5	26	35
Radiology	1	1	2	5	10	19
Anesthesiology	1	2	4	2	42	51
Community & General Medicine	1	0	0	3	0	4
Clinical Labora- tory Medicine	1	1	1	1	0	4
Oral Surgery	1	1	2	6	40	50
Total	20	23	48	83	563	737

BIOMEDICAL RESEARCH, EDUCATION AND INSTRUMENTATION CENTER

	Prof.	Assoc. Prof.	Assist. Prof.	Instructor	Research Fellow	Total
Molecular Medicine	1	0	0	4	3	8
Cell & Tissue Engineering	(1)	0	0	0	0	(1)
Radioisotope Research	(1)	0	0	0	0	(1)
Total	1(2)	0	0	4	3	8(2)

UNIVERSITY HOSPITAL

	Prof.	Assoc Prof.	Assist. Prof.	Instructor	Research Fellow	Total
Hospital Pharmacy	1	0	0	1	2	4
Laboratory Diagnosis	(1)	0	0	0	1	1(1)
Diagnostic Ultrasound & Medical Electronics	1	0	1	1	5	8
Clinical Pathology	(1)	1	1	1	2	5(1)
Radiology & Nuclear Medicine	(1)	0	0	0	0	(1)
Rehabilitation	(1)	1	1	0	1	3(1)
Traumatology & Critical Care Medicine	1	1	1	14	9	26
Neurology	1	1	1	1	16	20
Perinatal Medicine	(1)	1	0	1	0	2(1)
Plastic & Reconstructive Surgery	(1)	0	2	1	4	7(1)
Total	4(6)	5	7	20	40	76(6)

CANCER RESEARCH INSTITUTE

	Prof.	Assoc. Prof.	Assist. Prof.	Instructor	Research Fellow	Total
Biochemistry	1	0	2	1	0	4
Molecular Biology	1	0	0	3	0	4
Pathology	1	1	0	0	2	4
Total	3	1	2	4	2	12

ANIMAL RESEARCH CENTER

	Prof.	Assoc. Prof.	Assist. Prof.	Instructor	Research Fellow	Total
Total	(1)	1	0	0	0	1(1)

MARINE BIOMEDICAL INSTITUTE

	Prof.	Assoc. Prof.	Assist. Prof.	Instructor	Research Fellow	Total
Total	(1)	1	0	1	0	2(1)

LIBERAL ARTS AND SCIENCES

	Prof.	Assoc. Prof.	Assist. Prof.	Instructor	Research Fellow	Total
Philosophy & Ethics	1	0	0	0	0	1
Psychology	1	1	0	1	0	3
Jurisprudence & Sociology	0	1	0	0	0	1
Information Sciences	0	0	1	0	0	1
Mathematics	0	1	0	0	0	1
Physics	1	0	1	1	0	3
Chemistry	1	1	1	1	0	4
Biology	1	1	1	1	0	4
English	1	0	1	0	0	2
German	0	0	0	0	0	0
Exercise Science	0	1	0	0	0	1
Total	6	6	5	4	0	21

SCHOOL OF HEALTH SCIENCES

NURSING

	Prof.	Assoc. Prof.	Assist. Prof.	Instructor	Research Fellow	Total
Medical & Behavioral Subjects	3	1	0	0	0	4
Foundamen-tal & Adult Nursing	1	2	2	3	0	8
Maternal & Child Nursing	3	1	2	1	0	7
Community Health, Gerontologi-cal&Psychiatr ic Nursing,	2	2	3	3	0	10
Total	9	6	7	7	0	29

PHYSICAL THERAPY

	Prof.	Assoc. Prof.	Assist. Prof.	Instructor	Research Fellow	Total
Physical & Therapeutic Sciences	2	3	1	1	0	7
Applied Physical Therapy	3	2	1	1	0	7
Total	5	5	2	2	0	14

OCCUPATIONAL THERAPY

	Prof.	Assoc. Prof.	Assist. Prof.	Instructor	Research Fellow	Total
Occupational &Therapeutic Science	2	2	1	2	0	7
Applied Occupational Therapy	3	1	2	1	0	7
Total	5	3	3	3	0	14

LIBERAL ARTS AND SCIENCES

	Prof.	Assoc. Prof.	Assist. Prof.	Instructor	Research Fellow	Total
Biology	0	1	0	0	0	1
Sociology	0	0	0	0	0	0
Physics	1	0	0	0	0	1
Chemistry	1	0	0	0	0	1
Psychology	0	1	0	0	0	1
Information Sciences	0	1	0	0	0	1
English	1	0	0	0	0	1
Total	3	3	0	0	0	6

NUMBER OF STUDENTS (as of March 31, 2001)

Undergraduate	School of Medicine	616
	School of Health Sciences	412
Graduate	School of Medicine	123
	School of Health Sciences	38
Total		1,189

PUBLICATION

The Sapporo Medical Journal (bimonthly)

Tumor Research (annually)

Journal of Liberal Arts and Sciences, School of Medicine Sapporo Medical University (annually)

Bulletin of the Marine Biomedical Institute, Sapporo Medical University, School of Medicine (triennially)

Annual Report, School of Health Sciences, Sapporo Medical University (biennially)

Journal of Health Sciences, Sapporo Medical University

II RESEARCH ACTIVITIES

A SCHOOL OF MEDICINE
1 Basic Medical Sciences

Anatomy (I)

IT (Information Technology) is challenging us to work out a new methodology of anatomy, which will bring a variety of panoptic techniques of comprehension from molecular to mankind levels. Taking advantage of IT, especially the Internet in the biomedical field, we search and research the eternal truth and to elucidate the mystery of life from the viewpoint of anatomy.

Professor
Haruyuki Tatsumi, M.D., Ph.D.
Interests:
Computerized Anatomy & Histology, 3D reconstruction using applied information & Internet technology, Object database

Assistant Professor:
Takafumi Ninomiya, B.S., Ph.D.
Interests:
Neuroanatomy

Instructor:
Masahiro Nakamura, M.D.
Masamichi Goudge, M.D.

1. Aiming at a paradigm shift: A Renaissance of Anatomy

Anatomy is considered to be the basis of science, especially in medicine. We would like to expand the spectrum of anatomy to "Information Science", as this will help our understanding of living organisms. Full use should be made of the advantages of information sciences, and anatomy, which provides basic techniques for understanding complicated matters, is no exception. To use an analogy: when observing and taking apart a black box which is difficult to understand, we may discover the fact that the box consists of two small boxes. This is the first step and a basic technique in science. Based on the information, we integrate fragmental facts into concepts: systems, organs and tissues. Anatomy is one of the morphological sciences, however, it is also a kind of metaphysics beyond the morphology, because we deduce various things from the visual information.

We, anatomists, dissect human bodies to discover muscles, bones and so on. Anatomy has been developed from macroscopic to microscopic, and nowadays, molecular levels by increasing visual information. The methods of study are to dissect, simplify, and visualize things by removing obstacles or by magnification. Therefore the development of anatomy is dependent on instruments of observation, namely, magnifying glass, microscopes, and electron microscopes, which increase visual information. And then what is the next? As we mentioned above, integration comes after disintegration and observation to obtain full comprehension of living organisms. Taking advantage of information technology in our research fields, we would like to create a comfortable research and teaching environment, which is also a kind of renaissance of anatomy.

We have been making every effort to develop the infrastructure of the research environment, resulting in a high performance network and increased computing power. One of the incarnations of our efforts is the Information Center of Computer Communication in Sapporo Medical University, of which we make the most to accentuate our anatomical research and medical education.

2. Internet Compliant System for Morphological Study

In order to integrate fragmental visual information, we are developing a three-dimensional reconstruction system, which includes multimedia anatomical digital databases. Some of them are included in the Information G7 Global Healthcare Application Projects (subproject 8, multi-language anatomical digital database): http://www.sapmed.ac.jp/anat/

3D reconstruction system (2.3.6.7)

a. **Input**: full computer-controlled microscope.
b. **Processing**: outline tracing , object modeling with meta-balls, rendering with CG(computer graphics)
c. **Output**: Image database with computer network

Application of the system to morphological study (4-7)

a. Morphological studies on spontaneous nephrotic (ICGN) mice
b. Ultrastructural and immunohistochemical studies on distribution of key enzymes.

3. VHP Viewer for Anatomy Education and Research

VHP (Visible Human Project) is a worldwide project started in 1986 by NLM (National Library of Medicine, USA), planning to build a digital image library of volumetric data representing a complete, normal adult male and female. We developed the Visible Human Project data viewer for anatomical collaboration (2.3.6.7) and education (1) using the NGI (Next Generation Internet).

For the real-time retrieval of dissections along arbitrary directions from the VHP image data, we need powerful computers to handle the large data sets of up to 15-40GB. Then we developed a network multi-parallel computing system, which is implemented on low--cost PCs connected with a high performance network, instead of expensive super computers. Experiments on distributed computing with 35 PCs proved to be useful, taking about 2 seconds to respond to a request to retrieve a 12MB image from about 15GB VHP of image data. This is over 1000 times faster than a straightforward method on one PC (6.7). This system also proved to be useful between Japan and the USA via the NGI using a satellite link or APAN(Asia Pacific Advanced Network) [www.nren.nasa.gov/workshop4.html].

4. Development and apoptosis of neuronal cells in culture.

In the development and differentiation of cultured neuronal cells, the mechanisms of neurite elongation and apoptosis are analyzed using morphological, immunocytochemical and biochemical techniques. The development of dorsal root ganglion cells was observed and it was seen that the appearance of calbindin- or substance P-immunoreactive neurons was influenced by various culture conditions and neurotrophic factors. The neurite formation of peripheral and central neurons was also influenced by various neurotrophic factors (NGF, NT3, BDNF) (8). Veratridine neurotoxicity in sympathetic neurons is dramatically altered as a function of incubation time in vitro. Veratridine was not toxic to 1 day of cultured neurons, but became toxic to 7 days of cultured neurons, which underwent both apoptotic and necrotic cell death as judged by staining with bisbenzimidem, the TUNEL and by electron microscopic examinations. In contrast, nuclear features of apoptosis were greatly reduced in 21 days of cultured neurons. These results suggest that cellular and nuclear vulnerability to veratridine is subject to independent regulation during development in vitro (9.10).

List of Main Publications from 1997 to 2000

1) Tatsumi H, Nakamura M, et al. Medical education at a distance - a future vision for the way to study medicine. The 3rd International Conference on the Medical Aspects of Telemedicine. Kobe, Japan. Program and Book of Abstracts, 85 (1997).
2) Nogawa H, Ohkawa Y, Nakamura M, Tatsumi H. Prototype of Sophisticated System for 3D reconstruction. Proceedings of The Second China-Japan Joint Symposium on Medical Informatics 140-144 (1997).
3) Nogawa H, Tatsumi H, et al. An Application of End-User-Computing Environment for VHP. The Second Visible Human Project Conference 99-100 (1998).
4) Matsumoto A, Hanayama R, Nakamura M, Suzuki K, Fujii J, Tatsumi H, Taniguchi N. A High Expression of Heme Oxygenase-1 in the Liver of LEC Rats at the Stage of Hepatoma: The Possible Implication of Induction in Uninvolved Tissue. Free Radic. Res 28: 383-392 (1998).
5) Nakamura M, Tatsumi H, Nogawa H. Immunostaining of Mn-SOD, CuZn-SOD and NOS. In: Taniguchi N, Gutteridge MC, (eds) Experimental Protocols for reactive oxygen and nitrogen species. Oxford University Press Inc. New York. 126-135 (2000).
6) Aoki F, Tatsumi H, Nogawa H, Akashi H, et al. A Parallel Approach for VHP Image Viewer. International Workshop 2000, Proceedings on Medical Session-I, pp.209-214 (2000).
7) Aoki F, Akashi H, Goudge M, Toyota M, Sasaki Y, Guo X, Li S, Tokino T, Tatsumi H: Post-Genome Applications Based on Multi-Parallel Computing over High Performance Network. International Workshop on Next Generation Internet and its Applications: BioMedical Applications Pre-Proceedings, 61-67 (2001).
8) Ninomiya T: Nerve growth factor affecting neurite formation of aging mouse dorsal root ganglion cell in vitro. Europ J Neurosci S10: 407 (1998).
9) Koike T, Ninomiya T : Alteration of veratridine neurotoxicity in sympathetic neurons during development in vitro. Neuroreport 11: 151-155 (2000).
10) Koike T, Tanaka S, Oda T, Ninomiya T: Sodium overload through voltage-dependent Na^+ channels induces necrosis and apoptosis of rat superior cervical ganglion cells in vitro. Brain Res Bull 51: 345-355 (2000).

Anatomy (II)

Our department is characterized by its high level of activity in clinical anatomy as well as a famous paleoanthropological collection of human skeletons. The numerous bone specimens of Ainu and Okhotsk populations provide a valuable chance for observation by many researchers. More than 3 surgeons come to our department every year from other universities, in addition to the 5-10 surgeons and rehabilitation therapists in our university, to study regional anatomy using human viscera and other parts collected from our university and from others.

Professor
Gen Murakami, M.D., Ph.D.
Interests:
Human regional anatomy, Comparative anatomy

Instructor
Toshio J Sato, M.D., Ph.D.
Mizuyo H Tsugane, M.D., Ph.D.
Futoshi Nakajima, M.D.
Masato Abe, DDS.

1. Clinical Anatomy (directed by G.M.)

Our research field extends over the entire human body, i.e., from the base of the skull to the toes. Research fellows come from almost all surgical departments using surgical techniques. With the aid of other universities, we always prepare 50-100 specimens within a year for one research project. Beyond a simple description of human anatomical variations, first, we try to provide definite guidelines for decision making before and during surgery. With this aim, we attempt to find some significant correlations between a superficial form identified easily and another, deep morphology which makes the surgery difficult or which is connected to prognosis of the patient. Morphometrical and typological points of view are useful to find the correlations. Second, we are very interested in research to indicate a specific surgical procedure such as how to remove a part or how to identify a margin. To maintain the reproducibility of the research, usual dissections and macroscopic observations should be matched with routine histology, immunohistochemistry and/or kinematic investigations, including measurements of loading force case by case. Third, we are now trying to evaluate certain surgical procedures proposed very recently, such as Belghiti's conventional hanging maneuver of the liver without mobilization, before they are accepted widely, i.e., before surgeons finish evaluating the methods according to their trials on patients. Those newly developed fields of clinical anatomy require excellent ideas and concentrated efforts of aggressive young surgeons. We always welcome such persons from all universities and hospitals.

2. Paleoanthropology (directed by Hajime Ishida, M.D., Ph.D., Visiting Professor)

a) Population history in the circum-Pacific and Pacific areas

Nonmetric cranial variation of the Okhotsk and their neighboring peoples was investigated. Estimates of biological distances were carried out with Konigsberg's method (1990) using 22 nonmetric traits. The neighbor-joining method was applied to the distance matrix to generate an unrooted tree. The Hokkaido Ainu and Jomon are grouped together and join a cluster with the Okhotsk and Sakhalin Ainu. That cluster also join with the Neolithic Baikal, Amur, Yayoi and modern Japanese. Another three distinct branches emerge from the center; the first consists of the Marianas and Hawaii, a branch which is closest to the Asian and especially Southeast Asian Islanders; a second branch culminates in Russian, Tagar and Kazakh; a third includes an isolated arctic branch.

1) Ishida H, Kondo O. Human cranial variations based on different sets of measurement variables. Anthropol Sci 106:41-60 (1998).
2) Hanihara T, Ishida H, Dodo Y. Os zygomaticum bipartitum: the frequency distribution in major human populations. J Anat 192:539-555 (1998).
3) Hanihara T, Ishida H, Dodo Y. Place of the Hokkaido Ainu among circumpolar and other peoples of the world -A comparison of the frequency variations of discrete cranial traits. International Journal of the Circumpolar Health 57:257-275 (1998).
4) Ishida H. Ancient peoples of the North Pacific Rim: Ainu biological relationship with their neighbors. In: W.W. Fitshugh and C.O.Dubreuil, editors. Ainu: Spirit of a Northern People. Washington, D.C., National Museum of Natural History Smithsonian Institution, University of Washington Press, pp

52-56 (1999).
5) Ishida H, Kondo O. Nonmetric cranial variation of the Ainu and neighboring human populations. Perspectives in Human Biology 4:127-138 (1999).
6) Yamaguchi B, Ishida H. Human skeletal remains of the Heian period from the Tekiana cave site on Tobi-shima Island, Yamagata Prefecture. Bull Natn Sci Mus, Tokyo, ser. D. 26:1-16 (2000).
7) Ishida H. Skeletons and lifeways around the Sea of Okhotsk. Interdisciplinary Study on the Origins of Japanese Peoples and Cultures Newsletter Special Issue, pp 15-16 (2000).

b) Neanderthal child

In addition to the first discovery of a Neanderthal child in 1993, the partial skeleton of another child was recovered from the Middle Paleolithic layer at Dederiyeh cave, Syria in 1997-1998. The skeleton consists of an almost complete cranial vault, a partial facial skeleton including the mandible, bodies of vertebrae, right clavicle, left ilium, right and left femora, right and left tibiae, left fibula and other elements. The degree of dental calcification suggests an age around two years old. The child skeleton is a Neanderthal sharing many morphological features with European Neanderthal children and is similar to the first Neanderthal child from Dederiyeh cave.

List of Main Publications from 1997 to 2000

1) Isobe M, Murakami G, Kataura A. Variations of the uncinate process of the lateral nasal wall with clinical implications. Clin Anat 11:295-303 (1998).
2) Taguchi K, Tsukamoto T, Murakami G. Anatomical studies of the autonomic nervous system in the human pelvis by the whole-mount staining method: left-right communicating nerves between bilateral pelvic plexuses. J Urol 161:320-325 (1999).
3) Koshino T, Murakami G, Morishita K, Mawatari T, Abe T. Does the Adamkiewicz artery originate from the larger segmental arteries? J Thorac Cardiovasc Surg 117:898-905 (1999).
4) Murakami G, Hirata K, Takamuro T, Mukaiya M, Hata F, Kitagawa S. Vascular anatomy of the pancreaticoduodenal region: a review. J Hep Bil Pancr Surg 6:55-68 (1999).
5) Hirose K, Murakami G, Kura H, Tokita F, Ishii S. Cartilage degeneration in facets of the ankle joints from Japanese cadaveric donors. Orthopedic Science 4:273-285 (1999).
6) Sato TJ, Murakami G, Tsubota H, Isobe M, Akita K, Kataura A. Morphometric study of the medial aspect of the human maxillary sinus with special reference to the nasal fontanelle. Auris Nasus Larynx 27: 121-130 (2000).
7) Ishibashi Y, Murakami G, Honma T, Sato TJ, Takahashi M. A morphometric study of the sphincter of Oddi with special reference to the configuration of the submucosal portion of the sphincter muscle mass. Clin Anat 13: 159-167 (2000).
8) Hamamoto M, Murakami G, Kataura A. Topographical relationships among the facial nerve, chorda tympani nerve and round window with special reference to the approach route for cochlear implant surgery. Clin Anat 13:251-256 (2000).
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Physiology (I)

The department is pursuing the mechanism of physiological function at the cellular and subcellular level. Particular attention is paid to ion channels and their regulatory systems in order to understand their physiological function. Electrophysiology, including the patch clamp method, and confocal fluorescence imaging of calcium are fundamental tools for us. Beacause the function of ion channels is closely related to their structure, we analyse the gene structure of ion channels using techniques of molecular biology.

Professor
Noritsugu Tohse, M.D., Ph.D.
Interests:
Signal transduction for regulation of ion channels, Development of cardiac ion channels and excitation-contraction coupling

Assistant Professor
Masato Nagashima, M.D., Ph.D.
Interests:
Modulation of cardiac ion channels based on structure-function relationship, Trafficking for subcellular distribution of ion channels

Instructor
Yoichi Yamada, M.D., Ph.D.
Sumihiko Seki, M.D., Ph.D.
Masaaki Tsutsuura, B.S.

1. Developmental change in cardiac ion channels

Ion channels change during both the embryonic/fetal period and the neonatal period (1.2). These developmental changes include changes in the types of, number of, and kinetic properties of the ion channels. The developmental changes in ion channels are clearly observed in excitable cells because their resting potential and action potential are greatly altered progressively during the developmental stages.

In the early fetal period, the heart is tubular in shape. In the middle fetal period, the heart tube twists and the cardiac loop is constructed. In this period, the ventricular portion becomes distinguished from the atrial portion. The resting membrane potential of rat ventricular myocytes dramatically hyperpolarizes in the later phase of the fetal period. In order to investigate the mechanisms of this hyperpolarization, we examined the electrophysiological properties and molecular structure of the inwardly rectifying background K^+ channels of rat fetal ventricular myocytes (3.4). In a patch clamp experiment the whole-cell current of the inwardly rectifying background K^+ channel increased 12 fold from between 12 days and 18 days after impregnation. In the single channel recording, large-conductance channels were mainly observed in the 18-day fetal ventricular myocytes. In the 12-day cells, the large-conductance channels were not observed although the low-conductance channels were infrequently observed. During the fetal period, the expression of Kir 2.2 mRNA increased tremendously, whereas the increase in the expression of Kir 2.1 mRNA was not so great. These results show that hyperpolarization in the late fetal period seems to be mainly due to the dramatic increase in expression of Kir 2.2 mRNA rather than expression of Kir 2.1 mRNA.

2. Noise analysis of membrane current in cardiomyocytes

Although outward current through inward-rectifierK^+ channels in cardiomyocytes has been observed in the whole-cell mode of the patch-clamp technique, no outward unitary current in single-channel studies has been recorded in physiological conditions. Hence , the relationship between single-channel activities and the inward-rectification of the whole-cell current has been examined by the noise analysis of the K^+ current using the whole-cell patch clamp method (5). The unitary conductance of the inward-rectifier K^+ channel exhibited an inward-rectification, and tended to be smaller when the pipette contained high Mg^{2+} concentration. These results suggest that the inward rectification in the whole-cell current was due to the inward-rectifying property of the unitary conductance of the K^+ channels.

3. Function of cardiac ion channel coded by mutated gene

We studied the characterization of channel function and its structure in the pathophysiological condition. Mutations in a human cardiac Na^+ channel gene (SCNSA) are responsible for chromosome 3−1inked congenital long QT Syndrome (LQT3), producing lethal arrhythmias. Here, we characterized a denovo missense mutation（R1623Q, S4 segment of domain 4) identified in an infant japanese girl with a severe form of LQT3 (6). When expressed in oocytes, mutant Na^+ channels exhibited only minor abnormalities in channel activation, but had significantly delayed macroscopic inactivation. Single channel analysis revealed that R1623Q channels have significantly prolonged open times with

bursting behavior, suggesting a novel mechanism of pathophysiology in Na^+ channel-linked long QT syndrome.

4. Function of vascular endothelium and NO

It has been reported that human umbilical artery (HUA) at term pregnancy released endothelium-derived relaxing factor (EDRF), using a superfusion bioassay system. However, other reports showed that endothelium-dependent relaxation was not observed in isometric tension studies using HUA ring with intact endothelium. Thus, we intended to clarify whether vascular smooth muscle of HUA at term is sensitive to EDRF (7). Sodium nitroprusside (SNP), a nitric oxide (NO) donor drug, relaxed HUA rings precontracted by 5 ー hydroxytryptamine (5-HT) in a dose-dependent manner. Histamine, substance P, carbachol, or the calcium ionophore A23187, which are considered to be EDRF-releasing agents, did not relax the HUA rings. By immunohistochemical study, it was confirmed that endothelial cells were present in the luminal surface of the HUA rings after the isometric tension recording. These findings suggest that HUA at term is sensitive to NO but not EDRF. These findings suggest that HUA is lacking the conversion system from EDRF to NO.

The possible role of endothelium-dependent pulmonary vasodilation was examined in lung autotransplantation (8). Left lung autotransplantation (LLA) results in a chronic attenuation in endothelium-dependent, nitric oxide (NO)-mediated pulmonary vasodilation. Therefore, this abnormality seems to involve a decrease in the effective concentration of NO due to inactivation by superoxide anion. Endothelium-dependent relaxation to acetylcholine (ACh) was inhibited after LLA, with reduction of the maximum vasorelaxation response. In contrast, after pretreatment with the superoxide anion scavengers tiron or superoxide dismutase (SOD), the dose-response relationships for Ach were similar in control and LLA rings. Oxypurinol, which inhibits superoxide anion production by endothelial xanthine oxidase, also restored the vasorelaxation response to Ach in LLA rings. These results indicate that the attenuated responses to these pulmonary vasorelaxants post-LLA involve inactivation of NO by superoxide anion generated by endothelial xanthine oxidase.

5. Effect of pharamacological tools on cardiac ion channel

Thiamine tetrahydrofurfuryl disulfide (TTFD), a derivative of thiamine (vitamine B_1) produces positive inotropic and negative chronotropic effects, through its action of prolongation of action potential duration. Therefore, we examined the effect of TTFD on the electrophysiological characteristics of single atrial myocytes of guinea-pig (9). TTFD prolonged action potentials at cycle lengths from 250 to 10,000 ms. The degree of TTFD-induced prolongation was similar among these cycle lengths. TTFD inhibited the delayed rectifier K^+ current without affecting Ca^{2+} current and inward-rectifier K^+ current. TTFD blocked the delayed rectifier K^+ current in a voltage- and time-independent manner, indicating that TTFD blocked both subtypes of the delayed rectifier K^+ current (rapid and slow components).

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Physiology (II)

In our department, experimental studies on mammals are being conducted from cellular to behavioral levels using neurophysiological and neuroanatomical methods. Several projects have been under way to elucidate the neuronal mechanisms of respiration, plasticity of neural circuits after spinal cord injury and regional differences of synaptic transmissions in the hippocampus. Several species of animals are used for experiments, such as cats, monkeys, rats and pond snails.

Professor
Mamoru Aoki, M.D., Ph.D.
Interests:
Central neural mechanisms of respiration,
Neural plasticity and motor recovery, Synaptic transmissions

Associate Professor
Yutaka Fujito, M.S., Ph.D.
Interests:
Synaptic plasticity, Neural mechanisms of learning

Kiyoji Matsuyama, M.D., Ph.D.
Interests:
Motor control of posture and locomotion in mammals

Instructor
Suguru Kobayashi, M.S., Ph.D.

1. Central neural mechanisms of respiration in vivo

Studies are being conducted on the central nervous system of respiration in cats, rats and monkeys. Electrophysiological techniques are combined with anatomical identification of the neurons examined and tracings of the afferent and efferent connections of these respiration-related neurons in the spinal cord and the brainstem. Our recent study has demonstrated that the specific projection from the pontine pneumotaxic center to the nucleus raphe magnus is involved in respiratory control via raphe-spinal pathways. In more recent studies, we have shown that stimulation in the nucleus raphe magnus (NRM) produced marked inhibitory effects on respiratory activities in cats and other animals. We provided evidence for the involvement of GABA in raphe-induced responses. We have recently demonstrated that there are some axonal projections to the Botzinger complex from the upper cervical cord and the other respiratory related structures in the brain stem (1). We have also provided evidence that GABAergic neurons in the NRM project to the phrenic motor nucleus using a combined method of retrograde WGA-HRP labeling and anti-GABA immunostaining (2).

2. Regional differences of synaptic transmissions in the rat hippocampal slices.

Regional differences of synaptic transmissions between the CA1 region and the dentate gyrus in the hippocampus are being studied. The effects of benzodiazepines on evoked potentials on CA1 pyramidal cells (CA1-PCs) and granule cells (DG-GCs) are analyzed by intracellular recordings and patch clamp techiques (3-6). The mechanisms of the differential effects could be partly due to the different types of $GABA_A$ recptors between CA1-PCs and DG-GCs.

3. Neural mechanism of learning and memory

Studies are being conducted on neural mechanisms of learning and memory in mammals and invertebrates. Synaptic plasticity in the red nucleus after crossinnervation of distal forelimb muscles in the cat is considered to underlie motor learning, because monosynaptic connections between the rubrospinal tract and motoneurons innervating distal forelimb muscles have been demonstrated. It was demonstrated that the pond snail, Lymnaea stagnalis, exhibits associative learning. Observation of the real-time changes of three-dimensional fine neuronal terminals was performed in living cultured neurons using an atomic force microscope (AFM). We showed the long term enhancement of an inhibitory input to the feeding pattern generator after acquisition of conditioned aversion learning (7).

4. Neural plasticity and recovery of motor function

Electrophysiological and histological studies are being combined in an effort to understand the ability of the central nervous system to compensate for motor disturbances produced by spinal cord lesions (8). The mechanisms of functional reorganization in corticospinal and other descending tracts in rats

and monkeys with the spinal cord chronically hemisected at the thoracic level are being investigated using anterograde and retrograde HRP methods. An attempt is being made to restore the respiratory descending tracts by a peripheral nerve autograft bridging the spinal lesion at the cervical level. Nerve discharges from a grafted nerve and/or the phrenic nerve are recorded (9).

5. Brainstem-spinal cord mechanisms in the control of locomotion in mammals

This study aims to characterize the functional role of the brainstem-spinal cord system in the control of locomotion in cats. For this purpose, the morphology of neural elements of the brainstem descending pathways and the spinal interneuronal circuits is investigated with neural tracing techniques using antero-and/or retrograde neural tracers and intra-axonal and/or intracellular tracer injections. The discharge characteristics of the neural elements are also studied with the extracellular unit recording technique and the intra-axonal and/or intracelluar recording technique (10.11).

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11) Matsuyama K, Drew T. Discharge characteristics of vestibulospinal and reticulospinal neurons during locomotion in the intact cat: II. Walking on an inclined plane. J Neurophysiol 84: 2257-2276 (2000).

Biochemistry (I)

Our department has been investigating the molecular mechanisms of the regulation of protein functions and has applied biochemical studies to clinical sciences to understand the pathophysiology of various diseases. We now focus on the molecular mechanisms of innate immunity and structure-function analysis of pathogen receptors and host defense molecule collectins, of phospholipid- and calcium- binding protein annexin and of photoreceptors.

Professor
Yoshio Kuroki, M.D., Ph.D.
Interests:
Molecular mechanism of innate immunity, Structure-function analysis of collectins, Biomedical and clinicopathological studies

Assistant Professor
Hitoshi Sohma, M.S., Ph.D.
Interests:
Protein chemisty, Structure-function analysis of calcium-binding protein

Instructor
Daisuke Iwaki, M.S., Ph.D.
Seiji Murakami, M.D.

1. Lung collectins and innate immune host defense

Pulmonary surfactant is the first biological interface encountered by airborne organisms that reach the alveolar compartment. Surfactant proteins A and D (SP-A and SP-D) are the members of multifunctional lectins called collectins along with mannose-binding proteins (MBP), possessing the amino-terminal collagenous domain. Collectins are believed to play important functions in the innate immune host defense system. We have found that SP-A interacts with distinct serotypes of bacterial LPS and modulates LPS-induced cellular responses. Our study demonstrates that the direct interaction of SP-A with CD14, a receptor for LPS, constitutes a likely mechanism by which SP-A modulates LPS-elicited cellular responses (1). In addition, we have found that SP-D binds CD14 by a mechanism different from SP-A (2). The SP-A neck domain and SP-D lectin domain participate in CD14 binding. SP-A recognizes a peptide component and SP-D recognizes a carbohydrate moiety of CD14. We have found that MBP also interacts with CD14 (3), demonstrating that the interaction with an LPS receptor is an important property common to the collectin family.

2. Molecular mechanism of innate immunity

Toll-like receptors (TLRs) have recently been implicated in signaling by bacterial components and CD14. We have been investigating the essential regions of the extracellular domain of TLR for transmitting the signal of the bacterial cell wall component, peptidoglycan (PGN). We have found that the extracellular domain of CD14 cannot functionally replace that of TLR2 in PGN signaling and that amino acid residues 40-64 of an N-terminal TLR2 region is critical for PGN recognition.

3. Structure-function analysis of lung surfactant proteins

We have studied the structural requirements of surfactant proteins (lung collectins) in interactions with lipids and alveolar type II cells. SP-A, SP-D and MBP exhibit distinct functions although they share common structural features. Analysis by SP-A/MBP chimeric proteins reveals that the MBP region of Glu185-Ala221 can functionally replace the SP-A region of Glu195-Phe228 without loss of interaction with lipids and alveolar type II cells (4). The studies with SP-A/SP-D chimeric proteins also reveal that the SP-A region of Leu219-Phe228 is required for liposome aggregation and type II cell interaction, that the SP-A region of Cys204-Cys218 is required for dipalmitoyl-phosphatidylcholine binding, that the SP-D regions of Cys331-Phe355 is essential for phosphatidylinositol (PI) binding, and that the structural requirement for the binding of SP-D to PI is different from that for glucosylceramide, demonstrating the importance of the carboxyl terminal regions of lung collectins (5.6). The study with SP-A/MBPchimera has further demonstrated that substituting the MBP region of Thr164-Asp184 for the SP-A region of Thr174-Gly194 introduces MBP-type PI recognition into SP-A (7). We have also studied the structure-function relationship of SP-A by site-directed mutagenesis and have found that the SP-A-mediated lipid uptake is not directly correlated with phospholipid vesicle aggregation and that the specific interactions of SP-A with alveolar type II cells are involved in the lipid uptake process (8.9).

4. Clinicopathological studies of lung diseases

We established a sensitive assay system for the determinations of SP-A and SP-D and found that the concentrations of SP-A and SP-D dramatically increase in sera from patients with idiopathic pulmonary fibrosis (IPF) and alveolar proteinosis. We have found that serum levels of SP-A and SP-D are also useful biomarkers for interstitial lung diseases (ILD) in patients with progressive systemic sclerosis (10). Elevated levels of serum SP-A and SP-D reflect well the presence of ILD complicated with collagen vascular diseases and that the combination of SP-D and X-ray contributes to reduce the risk of clinicians overlooking ILD. In addition, we have shown that serum SP-A and SP-D can be used as prognostic factors to predict the outcome of patients with IPF.

Because surfactant proteins are expressed in lung adenocarcinomas, we have established a detection system of micrometastasis of lung adenocarcinomas using ELSA and RT-PCR.

Some of the patients with IPF possess autoantibody against lung epithelial cells. We have been investigating the autoantigen, which may be important for the pathophysiology of the development of lung fibrosis.

5. Structure-function analysis of annexin

The annexin family possesses four consesus repeats of about 70 amino acids and a unique N-terminal tail and binds acidic phospholipids. We have previously shown that SP-A binds annexin IV in a Ca^{2+}-dependent manner. Site-directed mutagenesis has revealed that Ca^{2+} binding to domain 3 of annexin IV is required for Ca^{2+}-dependent binding by SP-A and that Arg197 of SP-A is important in this binding (11).

6. Biochemical study of photoreceptors

We have been attempting to elucidate the mechanism of phototransduction in vertebrate photoreceptors. We purified cone arrestin from bovine retinas for the first time and have found that the biological activity of cone arrestin to bind rhodopsin and heparin is different to that of rod arrestin (12). We have also found that the retina-specific calcium-binding protein recoverin is expressed in more than 50 % of a variety of cancer cells and may play a significant role in the cell proliferation of tumor cells and be involved in the pathophysiology of cancer-associated retinopathy.

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1) Sano H, Sohma H, Muta T, Nomura S, Voelker DR, Kuroki Y. Pulmonary surfactant protein A modulates the cellular responses to smooth and rough lipopolysaccharides by interaction with CD14. J Immunol 163: 387-395 (1999).
2) Sano H, Chiba H, Iwaki D, Sohma H, Voelker DR, Kuroki Y. Surfactant proteins A and D bind CD_{14} by different mechanisms. J Biol Chem 275: 22442-22451 (2000).
3) Chiba H, Sano H, Iwaki D, Murakami S, Mitsuzawa H, Takahashi T, Konishi M, Takahashi H, Kuroki Y. Rat mannose-binding protein A binds CD_{14}. Infect Immun 69: 1587-1592 (2001).
4) Honma T, Kuroki Y, Tsunezawa W, Ogasawara Y, Sohma H, Voelker DR, Akino T. The mannose-binding protein A region of Glu185-Ala221 can functionally replace the surfactant protein A region of Glu195-Phe228 without loss of interaction with lipids and type II cells. Biochemistry 36: 7176-7184 (1997).
5) Sano H, Kuroki Y, Honma T, Ogasawara Y, Sohma H, Voelker DR, Akino T. Analysis of chimeric proteins identifies the regions in the carbohydrate recognition domain of lung collectins that are essential for interactions with phospholipids, glycolipids and alveolar type II cells. J Biol Chem 273: 4783-4789 (1998).
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10) Takahashi H, Kuroki Y, Tanaka Y, Saitoh T, Kurokawa K, Chiba H, Sagawa A, Nagae H, Abe S. Serum levels of surfactant proteins A and D are useful markers for interstitial lung disease in patients with progressive systemic sclerosis. Am J Respir Crit Care Med 62: 258-263 (2000).
11) Sohma H, Creutz CE, Saitoh M, Sano H, Kuroki Y, Voelker DR, Akino T. Characterization of the Ca^{2+}-dependent binding of annexin IV to surfactant protein A. Biochem J 341: 203-209 (1999).
12) Maeda T, Ohguro H, Sohma H, Kuroki Y, Wada H, Okisaka S, Murakami A. Purification and characterization of bovine cone arrestin (cArr). FEBS Lett 470: 336-340 (2000).

Biochemistry (II)

We have been investigating at the cellular and molecular levels the action mechanisms of enzymes involved in the metabolism of lipid mediators, *i.e.*, diacylglycerol kinase and phosphatidic acid phosphatase. Quality control mechanisms of secretory pathways including the role of molecular chaperones have also been studied.

Professor
Hideo Kanoh, M.D., Ph.D.
Interests:
Molecular biology of diacyglycerol kinase and phosphatidic acid phosphatase

Associate Professor
Ikuo Wada, M.S., Ph.D.
Interests:
Quality control mechanisms of secretory pathways,
Action mechanisms of molecular chaperones like calnexin and calreticulin.

Assistant Professor
Fumio Sakane, M.S., Ph.D.
Interests:
Molecular biology of enzymes participating in the metabolic processing of lipid mediators, Gene-targeting of diacylglycerol kinase isozymes.

Instructor
Masahiro Kai, M.S., Ph.D.
Shin-ichi Imai, M.S.

1. Diacylglycerol kinase (DGK)

DGK phosphorylates the second messenger, diacyglycerol, yielding phosphatidic acid, which is also known to be a potent lipid mediator. Since our first successful cDNA cloning of DGK-alpha reported in 1990, as many as 9 members of the DGK gene family have been molecularly characterized. We classified these isozymes according to their structural features, and our naming system has been widely accepted in the research field (1). The Type I DGKs consisting of alpha, beta and gamma isozymes possess in common two sets of calcium-binding EF-hand motifs. We examined calcium binding to the EF-hands of the three isozymes and found that these EF-hands are distinct from each other with respect to affinities for calcium binding and also to calcium-dependent conformational changes(2). The commercially available DGK inhibitors such as R59022, and R59949 have been repeatedly used to explore the role of DGK in signal transduction. In collaboration with Dr. J.P. Walsh (3), we showed that these DGK inhibitors are specific to the Type I, calcium-dependent DGK isozymes. The Type I DGKs also appeared to control the function of glomeruli in diabetic rats (4). In addition to the group I DGKs, we are also investigating the physiological function of DGK-delta, that belongs to the Type II group according to our classification. In collaboration with Dr. Prescott in Utah, we succeeded in preparing KO mice of this DGK isozyme. Analysis of homozygous mice, which died within 24 h of birth, showed unexpectedly that this DGK controls differentiation of epithelial tissues through serving as a regulator of TACE (TNF alpha converting enzyme, Sakane et al. manuscript in preparation). We also observed expression of GFP-fusion protein of DGK-delta in different cell lines, and found that this isozyme resides in the endoplasmic reticulum using its C-terminal SAM domain as an ER-localization signal. DGK-delta in the E.R. participates in the control of the ER-Golgi export (Nagaya et al. manuscript in preparation). The function of the PH domain present in the N-terminal portion of DGK-delta remains unknown, although this domain was shown to bind to PIP2 (5) and myosin II (6) in in vitro studies.

2. Phosphatidic acid phosphatase (PAP)

This enzyme has long been known to catalyze the rate-limiting step of triacylglycerol synthesis and also to control cellular signal transduction mediated through the action of phospholipase D. After the initial success of first identifying the mouse PAP reported in 1996, we further cloned and characterized the two human PAP isozymes designated 2a and 2b (7). Our work demonstrated that the Drosophila Wunen, which regulates germ cell migration, and rat Dri 42, which is up-regulated during

epithelial differentiation, are indeed PAP, thus suggesting unexpectedly important biological functions of PAP (8.9). When expressed in HEK293 cells, PAP-2b dephosphorylates extracellular substrates without their prior binding to the cell surface (10).

3. Molecular mechanisms for protein maturation and dynamics in the secretory pathway

Eukaryotes has developed the endomembrane system to express protein functions in the oxidative environment. Newly synthesized proteins are matured in the endoplasmic reticulum (ER) and targeted to their final destinations. These processes are tightly regulated and their failures often associate with various diseases. Our recent research has focused on three most mysterious steps of maturation, including oxidative folding of nascent proteins, selective degradation of misfolded proteins and export of properly folded molecules from the ER.

Previously, we reported that nascent secretory proteins were found in association with a set of molecular chaperones such as BiP and a monoglucose lectin, calnexin and calreticulin. By analyzing the interactions, we reported that calnexin binding per se arrested folding while preventing misfolding and the dissociation from the chaperone complex promoted acceleration of folding. During spermatogenesis, endomembranes are dynamically rearranged. We showed that calmegin, which is a spermatogenesis-specific isoform of calnexin, was selectively required for binding of sperm to zona pellucida of eggs presumably by allowing the putative egg receptor to fold properly. Recently, we found that infertility in calmegin KO mouse was caused by improper hetero-oligomerization of a single cell surface receptor.

Generally, BiP and calnexin/calreticulin are interchangeable. The above mentioned egg receptor was the first example of cargo which absolutely requires monoglucose lectin for maturation. We next found that tyrosinase, a key enzyme of melanogenesis, was another example. Folding of tyrosinase was completely dependent on the amount of calnexin in the ER. Abnormality of this enzyme is known to associate with type I Oculocutaneous Albinism (OCA). We further reported that depigmentation in the patients of OCA I was caused by retention of various types of tyrosinase mutants and that they were rapidly disposed by proteasome upon dissociation from calnexin. Also, we reported that expression level of calreticulin in the Alzheimer's disease brain was selectively augmented.

Proteasome-mediated degradation of misfolded proteins synthesized in the ER (ERAD) has been a hot topic in cell biology. We reported that a membrane protein, EDEM, which has similarity to alpha-mannosidase but lacks catalytic activity, accelerated degradation of α_1-antitrypsin mutant which causes the elastolytic destruction of lung elastin. We postulated that EDEM may be a long-sought man_8 receptor for misfolded proteins. The first step of the maturation in the ER is oxidation of cysteine. We recently found that the oxidation in higher eukaryotes was selective for newly synthesized proteins and depended on an enzymatic reaction by flavin containing oxidase, Ero1L.

List of Main Publications from 1997 to 2000 (Project 1 and 2 only)

1) Sakane F, Kanoh H: Diacylglycerol kinase, Molecule in focus. Int J Biochem Cell Biol 29: 1139-1143 (1997).
2) Yamada K, Sakane F, Matsushima N, Kanoh H: EF-hand motifs of alpha, beta and gamma forms of diacylglycerol kinase bind calcium with different affinities and conformational changes. Biochem J 321: 59-64 (1997).
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Pathology (I)

Our department has two final aims in terms of research activity. First, a better understanding of the molecular mechanisms of human diseases is essential. To this end, the most advanced knowledge and sophisticated technology have been applied to the pathological analysis of human diseases. Second, we intend to contribute with our basic research activities to the progression and development of human pathology and medicine.

Professor
Noriyuki Sato, M.D., Ph.D.
Interests:
Pathology, Basic immunology & Tumor immunology

Associate Professor
Shinn-ichiro Kon, M.D., Ph.D.
Interests:
Molecular immunology,

Assistant Professor
Toshihiko Torigoe, M.D., Ph.D.
Interests:
Molecular pathology & Molecular biology

Hideyuki Ikeda, M.D., Ph.D.
Interests:
Molecular biology & Tumor immunology

Instructor
Shingo Ichimiya, M.D., Ph.D.

Our department's research has focused on several interests, and each project has been dedicated, to a better understanding and advancement of pathology and medicine.

1. Molecular mechanism in human tumor immunity (1-6)

We have been analyzing the MHC class I-restricted human tumor antigens by using human autologous cytotoxic T lymphocytes (CTL) and tumor lines. These tumor antigens and their molecular cloning are underway in gastric, pulmonary, pancreatic, nasopharyryeal, osteosarcoma, synovial, and brain tumors. The natural antigenic peptide of the human gastric signet ring cell carcinoma was first reported in 1999 from our lab. We also analyze MHC class II-restricted tumor antigens. In oral cancers HLA-DR8-restricted CD4 T cell epitopes were successfully determined. Candidates for future cancer vaccines were determined in these system, and these vaccines will be used in clinically-very common epithelial cancers (1-6).

2. Heat shock proteins (HSP) in immunology and cell biology (7)

HSP is considered to play an important role in the antigen processing and presentation pathway. In fact, this is important for the peptide antigen presentation in the professional antigen presenting cells (APC). HSP70 is physically associated with transporters associated with the antigen processing (TAP). This association is critically important for peptide translocation into the endoplasmic reticulum. Therefore, the association status of HSP70 and peptides as well as the interaction between HSP70 and TAP are responsible for T cell function and repertoire. Certain HSP bind the cell-surface receptors expressed on APC such as dendritic cells, resulting in the activation of APC. These APC could efficiently present or cross-present endocytosed antigen to T cells. Perhaps HSP will be used as vectors for the future MHC-bound peptide vaccines.

HSP also regulates the functional status of certain substrates as the molecular chaperones in cell cycle control. For example, HSP70 can affect the function of retinoblastoma protein pRb110 by stabilizing molecular integrity. HSP also works in the regulation and development of neurodegenerative diseases such as Alzheimer's disease. Our research intends to develop a method to inhibit the neurodegenerature process by using HSP and molecular chaperones.

3. Target structure and regulation of natural killer cells (8.9)

We showed that CD44 may play a role in the target structure of rat NK cytotoxic mechanism. Cho-1 was determined as a novel NK inhibitory molecule.

4. p73 function in the thymus and immune regulation (10)

A functional analysis of a p53-related novel molecule, p73, has been undertaken in human and animal system.

5. Molecular analysis of lymphomas and cancers (11.12)

In our lab, many monoclonal antibodies were established, including the world-famaous L26.

6. Immunology and pathology of anisakidosis and other parasitic diseases (13)

Immunological analyses have been continued, and we established an anisakidosis-specific immunological diagnostic system.

7. Development of immunosuppressive reagents from marine biomaterials and the immunobiology of transplantation

A novel immunosuppressive reagent was discovered from sea-urchin intestines in Rishiri-island.

8. Human immunology in pulmonary sarcoidosis (14)

The cytokine network and its interaction has been studied in the disease process of human sarcoidosis.

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4) Maeda A, Ohguro H, Maeda T, Wada I, Sato N, Kuroki Y, Nakagawa T. Aberrant expression of photoreceptor specific calcium binding protein (recoverin) in cancer cell lines. Cancer Res 60: 1914-1920 (2000).
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Pathology (II)

The human body includes various compartments that maintain considerable independence from blood by a continuous cell sheet. For the functions of these compartments, passage through the intercellular spaces of the sheet must be strictly regulated by tight junctions. Once tight junctions are disturbed, illnesses such as edema, jaundice, diarrhea etc. will develop. Our department has been trying to expand our understanding of molecular and human disease regulation as effectedly tight junctions.

Professor
Norimasa Sawada, M.D., Ph.D.
Interests:
Tight junction and diseases, Blood-tissue barrier,
Biology of hepato-cytes

Associate Professor
Yasuo Kokai, M.D.
Interests:
Pathology, Molecular biology, Disease models

Assistant Professor
Hideki Chiba, M.D., Ph.D.
Interests:
Nuclear receptors,Tight junctions,Gene targeting

Takashi Kojima, D.V.M., Ph.D.
Interests:
Gap junctions, Tight junctions, Cell polarity,Signal transduction

Instructor
Hirotoshi Tobioka, M.D.

1. Regulation of blood-tissue barrier

The capillary endothelium in the brain forms a highly impermeable structure between the blood and the central nervous system (CNS) called the blood-brain barrier (BBB), and plays an essential role in maintaining homeostasis of the CNS. In the BBB, highly impermeable tight junctions between endothelial cells are the most important cellular apparatus for paracellular barrier function as well as limited transcytosis of endothelial cells. Regarding the regulation of the BBB, it has been reported that astrocytes presumably secrete unknown factors differentiating capillaries to the BBB-type capillary, to form impermeable tight junctions. Recently we found that neurotrophic factor GDNF enhances the function of tight junctions of the BBB (1.2). We suggested that the barrier function of the blood-retinal barrier (BRB) is regulated by GDNF and neurturin in a paracrine dependent manner, like the BBB (3).

2. Pathologic basis of disease using in vivo molecular biology

Using molecular techniques including cloning and transgenic mice, we have been trying to understand the mechanisms involved in human disease. Cloning of human aldose reductase and 7H6 (barmotin) have provided us with an opportunity to determine how these gene products are involved in diabetic complications (4) and tight junctions (5), respectively. To understand the role and mechanism of granulocyte colony-stimulating factor, I established mice transgenic for G-CSF and determined that this particular cytokine not only inhibits allogeneic marrow rejection but also modifies hemopoietic-mesenchymal interaction, which results in severe osteoporosis in these mice. As a foundation for these research activities, I emphasize routine anatomical pathology, analysis of biopsy specimens and postmortem examination. All of our activities are based on these clinical aspects of pathology.

3. Interaction between gap and tight junctions in the liver

The two most prominent junctional types in the liver are gap junctions, which provide direct intercellular communication, and tight junctions, which serve to partition membrane domains of individual cells and to occlude extracellular space, restricting pericellular diffusion (6). Although gap and tight junctions perform very different functions, there are numerous points at which functional studies overlap. Indeed, the findings that the traditionally tight junction-associated protein ZO-1 also binds to a connexin, and that occludin is colocalized with Cx32 in transfectants, indicate the possibility for either coordinate or reciprocal regulation of macromolecular complexes containing gap and tight junction proteins (7.8). Studies of protein-protein interactions and of coordinate and subordinate regulation of gene families are expected to soon disclose the intricacies of inter- and

intracellular signaling and growth control at gap junctions and the regulatory mechanisms of the blood-biliary barrier formed by tight junctions.

4. Physiological and pathological functions of nuclear receptors and tight junctions

The tetracycline-inducible gene expression system and the site-specific Cre/*loxP* recombination system have gained increasing popularity for conditional expression and gene disruption in mammalian cells. We have generated an F9 cell line F9:rtTA:Cre-ER^T L32T2, constitutively expressing both the doxycycline-controlled transactivator rtTA and the tamoxifen-dependent Cre recombinase Cre-ER^T (9). This genetically engineered cell line, which allows, upon simple ligand addition, sophisticated genetic manipulation such as sequential inactivation of *loxP*-flanked genes and tightly controlled reexpression of their cDNAs, is a valuable tool for studying gene functions. Since F9 cells bear functional tight junctions upon retinoid treatment, we are now analyzing the functions of nuclear receptors and various tight junction-associated molecules using F9:rtTA:Cre-ER^T L32T2 cells (10). Tissue-specific expression of Cre-ER^T allows somatic mutation of the mammalian genome *in vivo* in a spatiotemporally regulated manner (11).

5. Relevance to human diseases

Tight junctions have two functions, the maintenance of cell polarity and the regulation of the paracellular pathway. The former is deeply involved in cancer biology and the latter is relevant to edema, jaundice, diarrhea and diabetic retinopathy. At present, most of the studies on tight junctions are focused on discovering new proteins and their characterization. Since our goal is to comprehend how tight junctions prevent us from getting ill, we are attempting to develop antibodies against tight junction proteins that are applicable to paraffin-embedded human materials. In addition, biological and histopathological studies have also been carried out using human materials. Finally, our wish is that the elucidation of the regulation of tight junction functions in the body will substantially contribute to the development of new therapies targeting tight junctions.

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2) Utsumi H, Chiba, H, Kamimura, Y, Osanai M, Igarashi Y, Tobioka H, Mori M, Sawada N. Expression of GFR α1, receptor for GDNF, in rat brain capillary during postnatal development of the blood-brain barrier. Am J Physiol 279: C361-C368 (2000).

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11) Li M, Chiba H, Warot X, Messaddeq N, Gerard C, Chambon P, Metzger D. RXR α ablation in skin keratinocytes results in alopecia and epidermal alterations. Development 128: 675-688 (2001).

Microbiology

It is suggested that virus infections largely contribute to the modulation of the immune system through the fluctuation of the cytokine signaling pathway. The effect of virus infection on the immune system is under investigation.

Identification of pathogenic factors (toxin, LPS and bacterial protein/antigen) is taking place in several bacteria species by molecular biological and immunological approaches.

Professor

Nobuhiro Fujii, M.S. Ph.D.

Interests:

Viral and bacterial mechanisms of immune evasion or modulation, Identification of the genes involved in pathogenesis

Assistant Professor

Noriko Yokosawa, B.S. Ph.D.

Interests:

Viral infection and IFN signaling pathway

Shin-ichi Yokota, M.S. Ph.D.

Interests:

Apoptosis and IFN system, Characterization of pathogenic factors

Instructor

Kayo Tsuzuki, B.S., Ph.D.

Toru Kubota, M.S.

1. Virology

Many functions of IFN are induced by activation of JAK/STAT signaling pathway. In IFN-α signaling pathway, STAT-2 and STAT-1α are phosphorylated by IFN-α to form the complex of ISGF-3 through activation of tyrosine kinases, Jak-1 and Tyk-2. The ISGF-3 complex formed from STAT-1α, STAT-2 and p48 (IRF-9) components strongly binds to the consensus sequence of ISRE in the promoter region of IFN-α inducible genes. IFN-γ mediates phosphorylation of STAT-1α to form homodimer, GAF complex, through activation of Jak-1 and Jak-2 caused by tyrosine phosphorylation. GAF complex binds to the GAS element in the regulatory region of IFN-γ inducible genes. However, it has been reported that some viruses have the ability to breakdown IFN functions through suppression of IFN signal transduction pathway in addition to inhibition of 2-5AS and PKR activities induced by IFNs.

We reported evidence showing a decrease of basal expression levels of STAT-1α in mumps virus infection and STAT-2 in parainfluenza virus type 2 infection. Therefore, these viruses inhibit IFN signaling pathway through suppression of ISGF-3 and GAF complex formation.

Furthermore, recent studies have shown that IFN induces or enhances a self-destructive process known as apoptosis. IFN induced apoptosis is closely correlated with activation of IFN-induced 2-5AS/RNase L and PKR. We have reported that all cells persistently infected with mumps virus show poor induction of 2-5AS and PKR as described above. Augmentation of verotoxin or chemical reagent induced apoptosis by IFNs (IFN-α or IFN-γ)is repressed in the persistently infected cells. Therefore, the augmentation of apoptosis by IFNs may be mediated by 2-5AS/RNase L and PKR. It is suggested that mumps virus can suppress apoptosis and establish persistent infection (1-3).

2. Bacteriology

a) Chemical structure and antigenic epitopes of *H. pylori* LPS from Japanese strains. We examined the chemical structure and antigenicity of the O-polysaccharide region of LPS derived from Japanese strains. The main chains were commonly constituted by polymeric [Lewis or 1-4-linked lactosamine], and various Lewis antigen structures were found in the nonreducing terminal of the polysaccharides. Type 1 Lewis antigens were more frequently found in Japanese strains than in Western strains. On the other hand, we identified two distinct epitopes dominant in humans on the basis of the reactivity of human sera to the polysaccharides of LPS. The epitopes were termed as highly-antigenic and weakly-antigenic epitopes, respectively. All smooth type strains of *H. pylori* had both epitopes. These epitopes existed independently from the Lewis antigen structures. Strains carrying the weakly-antigenic epitope were more frequently found in gastric cancer patients compared with chronic

gastritis patients. So the antigenicity of LPS are likely to be related to disease status caused by *H. pylori* (4-6).

b) Identification of the gene encoding a bacterial antigen correlated with several diseases. Serological, epidemiological and biological studies suggest that *S. sanguinis* is implicated in the etiology of Behcet's disease. The gene (bes-1)-encoding antigenic protein has been identified to clarify the immunological role of *S. sanguinis* serotype KTH-1 (uncommon serotype 1, strain 113-20). The residues in a portion of the amino acid sequence showed a 60% correspondence to those of the human intraocular peptide Brn-3 (7). Recently, *A. otitidis* is thought to be a new pathogen associated with otitis media with effusion (OME) in addition to other pathogens, *M. catarrhalis*, *S. pneumoniae* and *H. influenzae*. We showed that bacterial soluble protein/antigen can modulate the immune response in OME (8). Identification of the gene encoding the antigen is under investigation.

c) The relative importance of enterohemorrhagic *E. coli*(EHEC) infection was assessed by using germ-free mice. EHEC infection induced TNF synthesis within kidney and brain. Treatment with TNF-α or its inhibitor modified the disease in experimental animals (9).

d) Studies on toxins produced by *C. botulinum*. We determined the arrangement of genes encoding the progenitor M toxin and its upstream region in *C. botulinum* type E strain and *C. butyricum* strain BL6340. The gene arrangement of both strains is different from that of other types (10). The two epitope regions were determined in heavy chain of type E neurotoxin. One is the amino acid sequence VIKAIN, closed by the ion channel-forming domain which is associated with toxin internalization, and the other epitope is the amino acid sequence YLTHMRD, closed by the binding domain of toxin within 30 residues of the C-terminal region of heavy chain (11). Furthermore, we determined the complete gene structure for C2 toxin (ADP-ribosyltransferase) from chromosomal DNA of *C. botulinum* type C strain (12).

List of Main Publications from 1997 to 2000

1) Yokosawa N, Kubota T, Fujii N. Poor induction of interferon-induced 2',5'-oligoadenylate synthetase (2-5AS) in cells persistently infected with mumps virus is caused by decrease of STAT-1α. Arch Virol 143:1985-1992 (1998).
2) Fujii N, Yokosawa N, Shirakawa S. Suppression of interferon response gene expression in cells persistently infected with mumps virus, and restoration from its supression by treatment with ribavirin. Virus Res 65:175-185 (1999).
3) Hariya Y, Shirakawa S, Yonekura N, Yokosawa N, Kohama G, Fujii N. Augmentation of verotoxin-induced cytotoxicity/apoptosis by interferon is repressed in cells persistently infected with mumps virus. J Interferon Cytokine Res 19: 479-485 (1999).
4) Yokota S, Amano K, Hayashi S, Fujii N. Low antigenicity of the polysaccharide region of Helicobacter pylori lipopolysaccharides derived from tumors of patients with gastric cancer. Infect Immun 65: 3509-3512 (1997).
5) Yokota S, Amano K, Hayashi S, Kubota T, Fujii N, Yokochi T. Human antibody response to Helicobacter pylori lipopolysaccharide: presence of an immunodominant epitope in the polysaccharide chain of lipopolysaccharide. Infect Immun 66: 3006-3011 (1998).
6) Yokota S, Amano K, Shibata Y, Nakajima M, Suzuki M, Hayashi S, Fujii N, Yokochi T. Two distinct antigenic types of the polysaccharide chains of Helicobacter pylori lipopolysaccharides characterized by reactivity with sera from human with natural infection. Infect Immun 68: 151-159 (2000).
7) Yoshikawa K, Kotake S, Kubota T, Kimura K, Isogai E, Fujii N. Cloning and sequencing of BES-1 gene encoding the immunogenic antigen of Streptococcus sanguis KTH-1 isolated from the patients with Behcet's disease. Zentbl Bakteriol 287: 449-460 (1998).
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10) Kubota T, Yonekura N, Hariya Y, Isogai E, Isogai H, Amano K, Fujii N: Gene arrangement in the upstream region of Clostridium botulinum type E and Clostridium butyricum BL6340 progenitor toxin genes is different from that of other types. FEMS Microbiol Lett 158: 215-221 (1998).
11) Kubota T, Watanabe T, Yokosawa N, Tsuzuki K, Indoh T, Moriishi K, Sanda K, Maki Y, Inoue K, Fujii N: Epitope regions in the heavy chain of Clostridium botulinum type E neurotoxin recognized by monoclonal antibodies. Appl Environ Microbiol 63: 1214-1218 (1997).
12) Kimura K, Kubota T, Ohishi I, Isogai E, Isogai H, Fujii N. The gene for component-II of botulinum C2 toxin. Vet Microbiol 62: 27-34 (1998).

Pharmacology

Elucidation of the aging mechanism is one of the most important goals of life-science in the post-genomic generation. Our department has extensively studied K^+ channels and also signal transduction mechanisms using techniques of pharmacology, biochemistry, molecular biology and cell biology. Now, we have started a new project on aging and senescence. Our goal is to understand the molecular mechanism of aging and develop new agents to affect the aging process.

Professor
Yoshiyuki Horio, M.D., Ph.D.
Interests:
Aging, Potassium channels

Associate Professor
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Interests:
Signal transduction mechanism, Aging

Shin-ichi Hatta, Ph.D.
Interests:
Signal transduction mechanism, cAMP

Assistant Professor
Haruo Takemura, Ph.D.
Interests:
Signal transduction mechanism,
Ca^{2+} mobilization

Instructor
Jun Sakamoto, M.D., Ph.D.

1. Silent information regulator (Sir)

Silent Information Regulator (Sir) proteins have been found to suppress the transcription of the silent mating loci, telomeres and rDNA in yeast. Sirs are also implicated in repair of DNA double-strand breaks, regulation of the mitotic cell cycle, meiosis and aging. Recent studies demonstrated that over-expression and suppression of Sir2 extended and shortened the life-span of yeast, respectively. Thus Sir2 might control the life-span of yeast. To investigate whether Sir proteins may be related to aging and senescence in mammals, we have cloned several isoforms of the Sir family from the mouse brain cDNA library. We investigate the expression and function of these proteins. We are currently studying the effects of overexpression or suppression of Sir mRNA in various cultured cells and in primary cultured cells. Also we are trying to isolate proteins that interact with Sir proteins.

2. Potassium channels

We have been studying inwardly rectifying K^+ (Kir) channels. We have cloned Kir4.1 cDNA from brain and found that Kir4.1 is the predominant glial Kir channel. Kir4.1 was expressed in Müller cells, which is a principal glial cells of retina (1). Immunoelectron microscopic examination of Müller cells demonstrated the polarized expression of Kir4.1 on membranes adjacent to the vitreous body, pericytes, and endothelial cells of capillaries, suggesting that Kir4.1 participates in the net movement of K^+ ions through membranes facing blood vessels and the vitreous body (2). Kir4.1 was found to interact with SAP97 (dlg) through its C-terminal end (3). Binding of Kir4.1 to SAP97 was suggested to interact with its polarized distribution. Kir4.1 is also expressed in basal regions of microvilli, which face photoreceptor cells. Because the rectifying property of Kir4.1 is weaker than that of strong inwardly rectifying K^+ channels such as Kir2.1, Kir4.1 is believed to participate in extrusion as well as in intrusion of K^+ ions.

Expression of Kir4.1 is not limited to retinal Müller cells. Kir4.1 has been found in retinal pigment epithelial cells, which have been implicated in the transport of K^+ ions in the subretinal space. Satellite cells of cochlear ganglia, trigeminal ganglia, and superior cervical ganglia also express Kir4.1. Satellite glial cells wrap the somata of ganglion neurons with multiple layers of myelin sheaths, the structure of which is similar to that of myelin sheaths of Schwann cells. Developmental expression of Kir4.1 parallels the maturation of retinal and auditory activities. These results strongly suggest that Kir4.1 participates in the K^+ spatial buffering mechanism, which maintains extracellular K^+ concentration at a constant level.

3. Signal transduction mechanisms

a) Phospholipase C and IP_3 formation.

In order to explore the interaction between phospholipase C (PLC) isozymes and G proteins in cerebral-cortical membranes, we used a nonhydrolyzable GTP analogue and assayed activity of PLC isozymes. We have found that cortical PLC-β_1 isozyme may be regulated by both inhibitory and stimulatory G proteins.

The different G protein subunits may serve an important role in regulating the PLC-β_1 -mediated IP_3 formation (4). Furthermore, the regulation of the α_1-adrenoceptor-G protein-phospholipase C (PLC) cascade was investigated in rat cerebral cortex in adult (6-month-old) and senescent (24-month-old). Our results suggest that the altered PLC-β_1 dual regulatory systems could be involved in the pathogenesis of brain aging (5).

b) Adenylyl cyclase and cAMP formation

The function of adenylyl cyclase of the stroke-prone spontaneously hypertensive rats, a model of vascular dementia, was studied. Ca^{2+}/calmodulin-sensitive, presumably type 1, adenylyl cyclase was impaired in the hippocampus and cerebral cortex, suggesting that adenylyl cyclase possibly contributes to the behavioral impairment in the mutant rats (6).

c) Intracellular Ca^{2+} mobilization

Intracellular Ca^{2+} mobilization is one of the most important signals for living cells. We have assayed and studied the mechanism of the elevation of intracellular Ca^{2+}. We found that tyrosine kinase was involved in activation of capacitative Ca^{2+} entry in rat glioma C6 cells (7). Furthermore, we found that capacitative Ca^{2+} entry was caused by Ca^{2+} influx factor.

Acinar cells from parotid glands are one of the modes to study the function of Ca^{2+} in exocrine cells. We analyzed intracellular Ca^{2+} elevation of exocrine acinar cells by muscarinic receptor stimulation using high speed laser confocal microscopy at the millisecond interval. Muscarinic receptor stimulation caused a rapid elevation of $[Ca^{2+}]_i$ and reached a plateau within 1 sec, when acini and ducts were intact. Ca^{2+} initiation sites were the apical region or basal region (8).

Primary cultured cells from heart were used to study sarcoplasmic reticulum (SR) function and spontaneous contraction. The frequencies of spontaneous contraction and Ca^{2+} spikes were low and irregular at day 2 of culture and became high and regular at day 6. Ultrastructural observation showed that the structure of SR developed less at day 6 of culture. Thus, the degradation of SR and regularity of spontaneous contraction were related. Transforming growth factor-β1 upregulated the function and structure of SR.

d) GABAergic system.

Bilobalide is a terpene constituent of *Ginkgo biloba* L. and has been found to have an anticonvulsant activity. GABA, one of the inhibitory neurotransmitters in the brain, may interact with bilobalide. To investigate the mechanism of bilobalide, bilobalide was administered to mice and the concentration of GABA (γ-aminobutylic acid) and activity of glutamate decarboxylase (GAD), which converts glutamate to GABA, were studied in the mouse brain. Bilobalide elevated GABA levels and GAD activity in the hippocampus of the mouse. These results suggest that an increase in GABA levels by bilobalide is responsible for its anticonvulsant effects (9).

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Hygiene

Our research activity in recent years has focused on the following four subjects: 1) epidemiology of rotavirus and poliovirus infections, 2) study on reassortment of rotavirus RNA segments, 3) molecular epidemiologic analysis of bacteria causing nosocomial infections and study of drug-resistance genes, and 4) study of the effect of chemicals on early mouse embryos in vivo and in vitro.

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Interests:
Epidemiology of enteric virus infections,
Mutagenicity of environmental chemicals

Associate Professor
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Interests:
Molecular epidemiology of rotavirus infections,
Nosocomial infections, Drug-resistance genes of bacteria

Assistant Professor
Kazunobu Kojima, M.D., Ph.D.
Interests:
Epidemiology, Molecular epidemiology of rotavirus infections, Poliovirus infections

Instructor
Keiji Mise, M.S.

1. Epidemiology and molecular epidemiology of rotavirus gastroenteritis

For the last two decades, epidemiology and virology of rotavirus infections were the major subjects of study in our department. These studies have been carried out in collaboration with researchers in various countries.

Recently, group A human rotaviruses detected in stool specimens from diarrheal patients in China (1) and Bangladesh were characterized by using monoclonal antibodies specific for different serotypes and subgroups or by RT-PCR with serotype-specific primers. Group A animal rotaviruses detected in Thailand, Bangladesh and Korea were also characterized in a similar manner. Nucleotide sequence analysis of viral genome of a couple of group A human rotaviruses with unique P serotype isolated from Thai infants showed that they were closely related to a group of porcine rotaviruses prevailing worldwide, suggesting interspecies transmission of rotavirus between human and animals (2).

Human Group B rotaviruses which had been found only in China were detected in Calcutta, India, recently. Viral genes of these strains were amplified by RT-PCR (3). Based on comparative nucleotide sequence analysis among human and animal group B rotaviruses, the evolution of group B rotaviruses was discussed (4).

2. Virology of rotavirus

Reassortment is one of the important mechanisms of rotavirus evolution in nature. Viral factors affecting the reassortment of rotavirus genes were investigated using a single gene-substitution reassortant (5), and various factors involved in this phenomenon were discussed (6).

3. Study of poliovirus infections

Worldwide eradication of polio is in its final stage, and nationwide polio vaccination to children is being conducted in tropical countries. We succeeded in preparing relatively heat-stable oral poliovaccine for use in tropical countries by lyophilizing live vaccine viruses. In the process of participating in a polio eradication program in Myanmar, we pointed out the low rate of seropositivity among teenagers, i.e. the presence of a high risk population for polio in Yangon, Myanmar, based on the seroprevalence of polio in that population (7).

4. Genomic analysis of bacteria causing nosocomial infectios and study of drug-resistance genes

Methicillin-resistant *staphylococcus aureus* (MRSA) is presently recognized as the most important pathogen of nosocomial infection worldwide. Epidemiologic study of MRSA is important to understand the spread and transmission of this bacteria among patients and also among communities. In order to develop a genetic typing method for MRSA, genetic diversity of

protein A gene (Xr-region) of *S.aureus* was analyzed for a number of clinical isolates in collaboration with the department of Laboratory Diagnosis of Sapporo Medical University Hospital. In the protein A gene, a repeat number of 24-bp units and nucleotide sequences of the repeat units were found to be highly variable depending on bacterial isolates. These genetic diversities in protein A gene were shown to be useful as epidemiologic markers of MRSA (8).

Beta-lactam resistance of MRSA is essentially due to the presence of penicillin-binding protein (PBP)-2a with reduced binding affinity for beta-lactams which is encoded by a chromosomal gene *mecA*. The expression of PBP-2a is considered to be originally controlled by *mec* regulator proteins encoded by *mecI* (repressor gene) and *mecR1* genes which are located adjacent to *mecA*, and PBP-2a is produced only when beta-lactams exist around bacterial cells. However, recent MRSA isolates have been reported to be constitutively resistant to beta-lactams. To understand the genetic mechanisms associated with the constitutive resistance of the recent MRSA, the nucleotide sequence of *mecA* regulator region was analyzed for clinical isolates. In most isolates, mutations were detected in *mecI* gene or *mecA* promoter/operator region (9), and insertion of IS*1272* accompanied by deletion of *mecI* was found in a few isolates (10). These mutations or genomic changes were considered to cause inactivation of *mecI* gene product (repressor of *mecA*) and to be associated with constitutive resistance to beta-lactams of MRSA (11).

5. Study of the effect of chemicals on early mouse embryos in vivo and in vitro

Genotoxicity of three chemicals (Mitomycin C, Cyclophosphamide and N-Methyl-N-nitrosourea) in the early developmental stage of mouse embryos were investigated using sister chromatid exchange (SCE) induction as the marker and using two different ways of exposure (*in utero* and *in vitro* treatments). The three chemicals showed 50 to 100-fold higher SCE induction in *in vitro* exposure than *in utero* exposure,and the two chemicals (MMC and CP) showed higher SCE induction in the embryos of 7 to 8 days of gestation than in those of other developmental stages (12).

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12) Mise K, Kobayashi N, Urasawa S: Developmental stage specificity in sister-chromatid exchange induction by three chemicals in early mouse embryonic cells with two different ways of exposure. The 1st International Conference of Asian Society of Toxicology, J Toxicol Sci. 23:Suppl II: 316 (1998).

Public Health

Our department has been conducting epidemiological research on cancer and other diseases. The objective of this research is mostly associated with identification of risk factors for these diseases and the identification of their prognostic factors in order to reduce resulting mortality rates. Furthermore, we have carried out neurobehavioral, neurochemical, and teratological studies on environmental and industrial chemicals as well as studies related to nutritional toxicology and biochemistry of metals.

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Interests:
Neurotoxicology

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Interests:
Child health, Mass screening for neuroblastoma

Instructor
Chieko Sugawara, Ph.D.
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1. Epidemiologic studies on cancer and other diseases.

Incidences of pancreatic, hepatocellular, colorectal, breast, ovarian, and endometrial cancer have risen here in Hokkaido. Accordingly, we have conducted various epidemiological studies to identify risk factors for these cancers. For example, we conducted a case-control study of ovarian cancer to assess environmental and other risk factors for the cancer from 1994 to 1996 (1). After controlling for the effect of potential confounders with the conditional logistic regression model, the odds ratios of ovarian cancer across increasing quartiles of the heaviest body weight were 1.00, 1.15, 1.71, and 2.29 (P for trend, $P<0.01$). Significantly increased risks were noted for a history of diabetes mellitus as well as for a family history of ovarian cancer. The prevalence of diabetes mellitus has been increasing in Japan. Consequently, since 1999 we have started a cohort study to assess the association of a history of diabetes mellitus with cancer in Hokkaido in cooperation with the Second Department of Internal Medicine, Sapporo Medical University. We have also carried out a study of the relationship between dietary habits and cancer. We showed that frequent consumption of vegetables and fruits decreased the risk of pancreatic cancer (2). We have started a case-control study of prostatic cancer with special reference to dietary habits since 2000 in cooperation with the Department of Urology, Sapporo Medical University, because the incidence of prostatic cancer has increased in Japan. The community-based prospective study examined the effects of viral infections and lifestyle habits on hepatocellular carcinoma (HCC) risk as well (3). We found that positivity for hepatitis B surface antigen and positivity for high-titer hepatitis C virus antibody were significantly associated with HCC risk. There was a significant interaction on an additive scale for the risk of HCC development between high-titer hepatitis C status and a history of smoking ($p<0.05$).

Evaluation of mass screening for neuroblastoma has been performed (4). In Sapporo City a mass screening program for neuroblastoma targeting children age 6 months (6-MS) was initially introduced in 1981. Since April 1991, an additional program has been implemented, aimed at children age 14 months (14-MS). High performance liquid chromatography was employed in the 14-MS. Identification of cases was dependent on the Registry of Childhood Malignancies in the Hokkaido Prefecture. Though the incidence of cases identified by the 14-MS was much higher than expected, several true-positive cases had unfavorable histology (as defined by Shimada's classification), a high dopamine level, and/or a high homovanillic acid/vanillylmandelic acid ratio. Mass screening of children age 14 months for neuroblastoma detects cases missed or unscreened at age 6 months, though the detection rate appears to be in excess of what would be expected from natural incidence (5).

2. Neurobehavioral, neurochemical & teratological studies on the environmental or industrial chemicals

To assess potential reproductive, embryotoxic and teratogenic risks of styrene, the research was designed to determine the neurobehavioral and neurochemical adverse effects in the offspring of rats exposed during gestation. Maternal Wistar rats were exposed to 0,50 or 300 ppm of styrene for 6 hrs/day during gestation days 6 to 20. Their offspring was evaluated postnatally for growth, physical landmarks of development (body weight, ear holding, the age of eye opening, incisor eruption, surface and air righting reflexes), compared with their pair-feeding or ad lib.-feeding control rats. Significant delay on a few developmental landmarks (incisor eruption, age of eye opening and air righting reflex) in the offspring of dams exposed to 300 ppm of styrene during pregnancy was observed compared with pair-feeding, and also ad lib-feeding control rats. Significant delay of air righting reflex in the pups of dams exposed to 50 ppm of styrene was observed compared with ad lib-feeding control rats (6).

Neurotransmitter analyses showed decreases of neuroamines, especially 5-hydroxytryptamine and homovanillic acid in the cerebrum of newborn offspring of dams receiving a 300 ppm styrene exposure compared with the ad lib. fed control group and homovanillic acid was also decreased compared to the pair-feeding control. The results suggest that prenatal styrene exposure affect the developing fetal brain in terms of a few signs of neurochemical alteration (7).

3. Studies related to nutritional toxicology and biochemistry of metals

Heavy metals have been identified as environmental pollutants of public nuisance, in Japan. The toxicological effects of heavy metals were investigated in terms of their interactions with nutritional metals. Recent studies are categorized as follows: (a) testicular dysfunction by Cd compounds, (b) heavy metal excretion from the liver, correlated with serum ceruloplasmin and bile canalicular multispecific organic anion transporter, (c) Cu metabolism and metallothionein in new mutant rats (8.9). Toxicity of these heavy metals was modified with dietary constituents (amino acids; cysteine, methionine or nutritonal metals; Zn, Fe, Se) or new type chelator; tetrathiomolybdate.

To examine the effect of Fe on the progression to hepatitis, LEC female rats were fed an Fe-regular (Fe 214 microg/g; Fe(+) group) or an Fe-restricted (Fe 14 microg/g; Fe(-) group) diet from 53 days of age for 35 days. Fischer rats were also fed as control animals. Adenine nucleotide decomposition was determined as an index of oxidative stress based on xanthine oxidase activity. In the LEC rat liver, the Fe concentration in the Fe(+) group was 160% of that in the Fe(-) group and the correlation coefficient between the hepatic Fe concentration and the energy charge was significant. In this strain, an increase of xanthine oxidase activity resulted in an increase of xanthine, an oxidized metabolite of hypoxanthine in the liver. The results suggest the involvement of the Fe in the progression to hepatitis in the LEC rat, even if the dietary Fe concentration is similar to that of a commercial diet (9).

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1) Mori M, Nishida T, Sugiyama T, Komai K, Yakushiji M, Fukuda K, Tanaka T, Yokoyama M, Sugimori H. Anthropometric and other risk factors for ovarian cancer in a case-control study. Jpn J Cancer Res 89: 246-253 (1998).
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Legal Medicine

The Department of Legal Medicine is an interdisciplinary medical science department at Sapporo Medical University School of Medicine. We conduct research in molecular genetics of human minisatellites, molecular mechanisms of alcohol action, forensic application of DNA polymorphisms, and morphological and biochemical aspects of injury and asphyxia. We also perform forensic autopsy on request from Hokkaido Prefectural government.

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Interests:
Molecular genetics of human minisatellite, Forensic genetics

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Interests:
Molecular mechanisms of alcohol action,
Molecular biology of ubiquitination

Assistant Professor
Jun-ichi Azumi, Ph.D.
Interests:
Chromosomal abnormality,
Forensic application of DNA polymorphism

Instructor
Noriko Tabata, Ph.D.

1. Molecular genetics of human minisatellite

Minisatellites are a class of tandem repetitive DNA arranged in arrays ranging from 0.5 to 30 kb long. Some human minisatellites are highly polymorphic for allele length. There are also some base substitutional polymorphisms between the repeat units of minisatellite. Minisatellite variant repeat mapping using polymerase chain reaction (MVR-PCR) was devised to reveal the interspersion pattern of subtle repeat variants along minisatellite tandem arrays. MVR-PCR has revealed enormous diversity of allele structures in various populations.

Such properties can be of great advantage for forensic identification. MVR-PCR was potentially applied to a paternity case and revealed that the power of MVR-PCR at two loci is enough to establish paternity (1). However, hypervariable minisatellites can show significant germline mutation rates to new length alleles which in turn can generate false exclusion in paternity cases. We analyzed the similarity between mutant/progenitor versus mutant/non-progenitor alleles by MVR-PCR and discussed the estimation of the paternity index (2). Detailed knowledge of mutation processes with MVR analysis of allele structure by MVR-PCR can help distinguish mutation from non-paternity.

MVR-PCR also can investigate minisatellite mutation processes by analyzing the internal structure of new mutant alleles. We analyzed one of the most unstable human minisatellites, B6.7 (3). Male germline instability by small pool PCR of sperm and blood DNA varied per allele and increased with tandem array size. A highly informative MVR-PCR system revealed a wide variety of changes in allele structure, as seen at other human minisatellites. The main mode of sperm mutation, however, resulted in extremely complex allele reorganization with evidence of inter-allelic transfer plus the generation of novel repeats by rearrangement at the sub-repeat level.

Since minisatellites show high frequencies of germline mutation rates, they have been used to monitor the effects of ionizing radiation on genomes. To explore this further, we have used a single molecule approach to quantify the frequencies of mutation at the hypervariable minisatellites in the sperm of three seminoma patients following hemipelvic radiotherapy (4). We show no evidence for mutation induction in any of the patients and discuss this finding in the context of previous population studies using minisatellites as reporter systems.

2. Molecular mechanisms of alcohol action

Biomedical Research on alcohol has developed by using the animal model. It is very important to extrapolate from animal experimental data to human. We estimated the relationship of ethanol elimination kinetics in mammals using an allometric principle (5). We got the allometric equations of the two-compartment Michaelis-Menten model. The good fit of its simulated curves to the blood data sets proves that ethanol

elimination in various species can be predicted quantitatively.

Several lines of investigation indicate that endotoxin and oxidative stress are important pathogenic mechanisms in alcohol-induced liver injury. One pathway by which endotoxiemia and oxidative stress can cause liver injury is via NF-κB. We evaluated the relationship between pathological liver injury and NF-κB activation (6). Activation of NF-κB and increased expression of cytokines and chemokines was seen in alcoholic liver injury models. Then the question remained as to whether ethanol alone or other compounds induced by chronic ethanol administration causes activation of NF-κB. We found that ethanol alone causes activation of NF-κB and that the metabolism of ethanol via CYP2E1 contributes to acute activation of NF-κB by ethanol in perfused rat livers. Pretreatment with an inhibitor of Kupffer cells prevented activation of NF-κB. AP-1 activation occurred in the same manner as NF-κB. Therefore Kupffer cells play an important role in NF-κB activation. We also suggest that ethanol may activate NF-κB via MEKK1, an upstream protein kinase for NF-κB and AP-1 activations.

Nitric oxide (NO) activates NF-κB and ethanol administration causes NO release in blood. The pretreatment with $GdCl_3$ reduced the increase in portal vein pressure during the early phase of ethanol perfusion, but did not affect the release of NO (7). These findings suggest that Kupffer cells play an important role in liver microcirculation during the early stage of ethanol intake, which mechanism which may not be regulated by NO release. Therefore, the release of NO by ethanol did not contribute to activation of NF-κB in Kupffer cells.

3. DNA polymorphisms and personal identification

In forensic practice, we are often asked to do DNA analysis for personal identification of the deceased with severely burned. DNA stability against heat was therefore examined using seven kinds of human organ experimentally heated (Azumi J, DNA Polymorphism 2000, in Japanese). The DNA stability was determined by PCR efficiency and the results of genotyping of a DNA typing kit. All samples were successfully genotyped after heated for 120 min at 100 ºC. Genotype was also correctly determined from brain and lung tissues heated even for 120 min at 150 ºC. Determination of sex was applied to alcohol- or formalin-fixed fetuses by DNA typing of some loci on sex chromosomes (Azumi J et al., Res Prac Forens Med 2000, in Japanese). Since the sex determined by DNA analysis was inconsistent with those by observation of external genitalia in two out of four cases, there is a risk that the sex of the fetus in the early stage is incorrectly determined by morphological observation.

4. Forensic medicine and pathology

We also perform forensic autopsy on request from Hokkaido Prefectural government. We determine the cause of death not only by routine morphological and histological examinations (8.9) but also by immunohistochemical methods (10). In two suicide cases of paraquat ingestion, the sarcoplasmic or endoplasmic reticulum Ca^{2+} ATPase antibodies were applied for skeletal muscle fibers. A remarkable degeneration occurred in type 1 muscle fibers. This finding supported the laboratory data showing an abrupt increase of plasma CK levels.

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1) Tamaki K, Huang X-L, Mizutani M, Yamamoto Y, Katsumata R, Uchihi R, Katsumata Y, Jeffreys AJ. The potential contribution of MVR-PCR to paternity probabilities in a case lacking a mother. J Forensic Sci 44: 863-867 (1999).
2) Tamaki K, Brenner CH, Jeffreys AJ. Distinguishing minisatellite mutation from non-paternity by MVR-PCR. Forensic Sci Int 113: 56-62 (2000).
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Internal Medicine (I)

Our research field covers gastroenteroloy, hepatology, rheumatology, hematology, novel therapeutic strateges for cancer and oncology. Particularly, molecular biological and immunological approaches are extensively and effectively applied for understanding the etiology of disease, and for developing novel diagnostic and therapeutic strategies. Systemic analysis of the gene is now underway using DNA array technology in our department.

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Endoscopic diagnosis of GI tumor, Barrett's esophagus

Masaaki Adachi, M.D., Ph.D.
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Molecular medicine / clinicaoncology

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Invasion, Metastasis of GI tumor

Hiroki Takahashi, M.D., Ph. D.
Interest:
Novel therapy of rheumatoid arhtritis

Instructor
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Yoshiaki Arimura, M.D., Ph. D.
Shigeru Sasaki, M.D., Ph.D.
Hiroyuki Yamamoto, M.D., Ph. D

1. Gastroenterology

Columnar epithelium-lined esophagus (Barrett's esophagus, BE) is an acquired disorder associated with a high incidence of adenocarcinoma of the lower esophagus. We have indicated that the mucin phenotype, not of small intestinal type or gastric type mucin, but of colonic type mucin, in other words, the sulfated carbohydrate chain (sulfo-Lewis a), is associated with the malignant phenotype of BE. We have also found that the pit patterns obtained by magnifying endoscopy reflect these phenotypic expression of Barrett's mucosa.

2. Hepatology

Our efforts are aimed at the understanding of hepatocarcinogenesis. We work on two subjects; the first approach is the investigation of genetic and epigenetic alterations of hepatpcellular carcinoma (HCC) related genes, including p16, for selection of the high risk group HCC (1). Another effort is the development of novel clues using Interventional radiology for HCC and pancreatic cancer, which are combined with transarterial chemoembolization, percutaneous ethanol injection, and radio-frequency ablation. Furthemore, to detect more early stage pancreatic and hepatobiliary cancer, we analyze genetic alterations of pancreatic and bile duct cancer using pancreatic and bile juice.

3. Rhematology

Rheumatoid arthritis (RA) is a chronic inflammatory disorder in which the joints are the primary target. We are interested in glycosylation inhibiting factor (GIF), one of cytokines derived from T cell, because we found that the serum GIF levels were markedly increased in patients with RA and were significantly related with the rate of progression of joint destruction. We are investigating the role of GIF in the pathogenesis of RA and the clinical application of anti-GIF antibodies for RA treatment.

4. Clinical Hematology

Hematopoietic stem cell transplantation has become a standard therapy for many hematological diseases. In allogeneic hematopoietic stem transplantation, it is believed that graft-versus-leukemia (GVL) is the major factor leading to cure of leukemia. Therefore, the current trend is to pay attention to cell therapy and to reduce the intensity of the conditioning regimen. Therefore we can consider allogeneic stem cell transplantation as

type of immunotherapy. In the immunotherapy, antigen-presenting cells and particularly dendritic cells (DCs) are very important elements of antitumor immune responses. We investigated whether the function of Langerhans cells in tumor-bearing mice are inhibited(2). and if we can induce the CTL using DCs.

5. Novel therapeutic strategies for cancer

To control cancers, apoptosis is a desirable biological process, since this physiological occurrence causes a limited tumor lytic reaction. To date, we have demonstrated that overexpression of anti-apoptotic molecules, Bcl-2 and BAG-1 significantly inhibits apoptosis induced by various stimuli and promotes metastasis (3.4). Thus, suppression of their anti-apoptotic functions or activation of apoptotic signals may become promising therapies for cancer. In this regard, we are now establishing a novel technique which introduces pro-apoptotic signals using the adenovirus vector.

6. Oncology

The diagnostic significance of the following molecules have been studied especially from the viewpoint of predicting the malignant potential of each cancer: oncogenes, tumor suppressor genes (DCC), metastasis suppressor genes (KAI1), adhesion molecules (CAR, CEA family), protein tyrosine phosphatases (SHP-2), cell cycle regulator (14-3-3σ, p16, cyclin D1), matrix metalloproteinase (MMP), mucins (MUC1 and MUC2), and wip1 phosphatase (5.6). We have identified associations of the expression of matrilysin (MMP-7) with tumor progression and/or poor prognosis in cancers of the esophagus (7), stomach, colon (Gut 1999), liver (Gastroenterology 1998), and pancreas (8). Two major cancer phenotypes, microsatellite instability (MSI) and methylator phenotype have been analyzed extensively (9). We have recently characterized the genetic and clinical features of pancreatic cancer with MSI (Cancer Res. 2001). We have developed a quantitative DNA methylation assay by fluorescent PCR-SSCP and have revealed the distinct methylation pattern and MSI in gastric cancer. Frequent hypermethylation of the 14-3-3σ gene has been detected in gastric cancer (Cancer Res, 2000) and hepatocellular carcinoma. Based on the findings of molecular biological analyses, the following molecules are now being investigated as targets for cancer therapy: ErbB2, MUC1 mucin, matrilysin, molecules on interferon signaling pathway (10-12), and anti-idiotypic monoclonal antibody. We have identified tumor growth suppression by apoptosis induced with anti-ErbB-2 mouse-human chimeric monoclonal antibody, CH401.

List of Main Publications from 1997 to 2000

1) Iwata N, Yamamoto H, Sasaki S, Itoh F, Endo T, Imai K. Frequent hypermethylation of CpG islands and loss of expression of the 14-3-3σ gene in human hepatocellular carcinoma. Oncogene 19: 5298-5302 (2000).
2) Ishida T, Oyama T, Carbone DP, Gabrilovich DI. Defective function of Langerhans cells in tumor-bearing animals is the result of defective maturation from hemopoietic progenitors. J Immunol 161: 4842-4851 (1998).
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6) Takekawa M, Saito H. A family of stress-nducible GADD45-like proteins mediates activation of the stress-responsive MTK-1/MEKK4 MAPKKK. Cell 95: 521-530 (1998).
7) Yamamoto H, Adachi Y, Itoh F, Arimura Y, Endo T, Imai K: Association of matrilysin expression with recurrence and poor prognosis in human esophageal squamous cell carcinoma. Cancer Res 59: 3313-3316 (1999).
8) Yamamoto H, Itoh F, Sasaki S, Mukaiya M, Hirata K, Imai K. Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human pancreatic adenocarcinomas: clinicopathological and prognostic significance of matrilysin expression. J Clin Oncol 19: 1118-1127 (2001).
9) Yamamoto H, Itoh F, Imai K, Perucho M. Gastric cancers of the microsatellite mutator phenotype display characteristic genetic and clinical features. Gastroenterology 116: 1348-1357 (1999).
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11) Yamamoto H, Itoh F, Fukushima H, Sasaki S, Perucho M, Imai K: Genetic and clinical features of human pancreatic ductal adenocarcinomas with widespread microsatellite instability. Cancer Res 61: 3139-3144 (2001).
12) Naishiro Y, Yamada T, Takaoka AS, Imai K, Hirohashi S: Restroration of epithelial cell polarity in a colorectal cancer cell line by suppression of β-catenin/T cell factor 4-mediated gene transactivation. Cancer Res 61: 2751-2758 (2001).

Internal Medicine (II)

Our department has been involved in studies on cardiovascular and renal diseases using current methodology of basic, clinical and epidemiological sciences. Although studies on hypertension, myocardial ischemia, clinical cardiology and cardiovascular epidemiology are conducted by separate research groups, many of the research projects involve inter-group collaboration as well as collaboration with investigators abroad.

Professor
Kazuaki Shimamoto, M.D., Ph.D.
Interests:
Hypertension,Diabetes mellitus,
Insulin resistance, Atherosclerosis

Associate Professor
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Interests:
Insulin resistance in hypertension,
Renal kallikrein-kinin system

Tetsuji Miura, M.D., Ph.D.
Interests:
Ischemic myocardial injury,
Signal transduction in cardiomyocytes

Assistant Professor
Kazufumi Tsuchihashi, M.D., Ph.D.
Interests:
Cardiac arrhythmias,
Coronary intervention

Tomoaki Nakata, M.D., Ph.D.
Interests:
Nuclear cardiology (cardiac imaging)

Shigeyuki Saitoh, M.D., Ph.D.
Interests:
Cardiovascular epidemiology,
Diabetes mellitus

Akihito Tsuchida, M.D., Ph.D.
Interests:
Ischemic heart disease

Instructor
Katsuhiro Higashiura, M.D., Ph.D.
Akiyoshi Hashimoto, M.D., Ph.D.
Satoru Takagi, M.D., Ph.D.

1. Pathophysiology and molecular mechanisms of hypertension and insulin resistance

Over the past decade, we have characterized the contributions of blood pressure regulating systems, including renin-angiotensin systems (RAS), sympathetic nerves and the kallikrein-kinin system to the pathogenesis of essential hypertension. Since finding an association between insulin resistance and pressor mechanisms in essential hypertension (Shimamoto et al. Hypertension 1994), we have been investigating the roles of insulin resistance in hypertension. It was found that insulin resistance induces sodium retention and augmented activation of sympathetic nerves and RAS in hypertensives. Using fructose-fed rat as a model of insulin-resistant mild hypertension, we demonstrated down-regulation of a glucose transport protein, GLUT-4, and reduction in type I skeletal myocytes, an insulin-sensitive phenotype (2). Furthermore, a causal relationship between this phenotypic change in myocytes and insulin resistance was supported by attenuation of both phenomena by angiotensin-converting enzyme inhibitors and angiotensin II type 1 (AT1) receptor blockers. As an AT1 receptor-mediated mechanism of insulin resistance, we focused on tumor necrotic factor-α (TNF-α). We demonstrated that the tissue level of TNF-α is under the control of angiotensin II and is significantly elevated in skeletal muscles in insulin-resistant rats (3). Studies are under way to identify signal transduction for AT1 receptor activation to produce TNF-α and for TNF-α receptors to induce insulin resistance.

2. Signal transduction in ischemic preconditioning, an endogenous cardioprotective mechanism

Ischemic preconditioning (IPC) is defined as a phenomenon in which a transient episode of ischemia enhances myocardial tolerance against subsequent ischemic injury. Since IPC is the most potent cardioprotective intervention ever found, we are interested in its mechanism, with a view to utilizing it in clinical application. Using animal models of myocardial ischemia, we found that adenosine A1/A3 receptors (4.5), bradykinin B2 receptors and δ-opioid receptors are trigger mechanisms of IPC. A crucial step subsequent to activation of these Gi/Gq coupled receptors was found to be protein kinase C-ε activation.

Furthermore, our studies indicated that the mitochondrial ATP-sensitive K^+ (K^+_{ATP}) channel is a cytoprotective effector at the end of signal transduction in IPC (5). Interestingly, this signaling mechanism of IPC was found to be impaired by post-infarct ventricular remodeling (6). Currently, three projects on IPC are in progress: detailed analysis of protein kinase cascades in IPC, mechanism of cytoprotection by the mitochondrial K^+_{ATP} channel, and interactions between cell signaling of IPC and that of ventricular hypertrophy.

3. Clinical cardiology

A number of new findings concerning diagnosis and management of cardiovascular diseases have been obtained. Regarding arrhythmic diseases, mechanisms of hemodynamic instability associated with paroxysmal tachycardia was clarified, a new noninvasive technique for detecting right ventricular pathology in right ventricle-originated tachyarrhythmias was found (7), and diagnostic clues for arrhythmogenic right ventricular dysplasia have been characterized. Regarding coronary artery diseases, we proposed a guideline for erythropoietin supplement in renal failure complicated with coronary artery disease, demonstrated a benefit of primary coronary stenting for ventricular function in acute myocardial infarction (8), and clarified the contribution of hyper-insulinemia to coronary atherosclerosis. Imaging studies using a new fatty acid tracer demonstrated the utility of perfusion-metabolism mismatch in prediction of functional recovery after coronary intervention (9).

4. Epidemiological studies of cardiovascular diseases.

A cohort study on hypertension, coronary artery disease and cerebrovascular diseases in a Japanese population has been conducted since 1978. This study revealed that cardiovascular mortality increases almost linearly by elevation of blood pressure (10) and that multiple risk factors are clustered in the subjects who finally develop cardiovascular events (11). Interestingly, the contribution of hypercholesterolemia and diabetes to cardiovascular mortality appears minor, if any, in the Japanese population in contrast to the data reported from U.S. and Europe. Recently, we established a criteria of insulin resistance that are applicable to large population studies, and we are currently analyzing the role of insulin resistance in cardiovascular events in Japanese population.

List of Main Publications from 1997 to 2000

1) Miyazaki Y, Murakami H, Hirata A, Fukuoka M, Masuda A, Ura N, Shimamoto K: Effect of the angiotensin converting enzyme inhibitor temocapril on insulin sensitivity and its effects on renal sodium handling and the pressor system in essential hypertensive patients. Am J Hypertens 11: 962-970 (1998).
2) Higashiura K, Ura N, Takada T, Torii T, Togashi N, Takada M, Takizawa H, Shimamoto K: The effects of an angiotensin-converting enzyme inhibitor and an angiotensin II receptor antagonist on insulin resistance in fructose-fed rats. Am J Hypertens 13: 290-297 (2000).
3) Togashi N, Ura N, Higashiura K, Murakami H, Shimamoto K: The contribution of skeletal muscle tumor necrosis factor-α to insulin resistance and hypertension in fructose-fed rats. J Hypertens 18: 1605-1610 (2000).
4) Miki T, Miura T, Bunger R, Suzuki K, Sakamoto J, Shimamoto K: Ecto-5'-nucleotidase is not required for ischemic preconditioning in rabbit myocardium in situ. Am J Physiol 275: H1329-1337 (1998).
5) Miura T, Liu Y, Kita H, Ogawa T, Shimamoto K: Roles of mitochondrial ATP-sensitive K^+ channels and PKC in anti-infarct tolerance afforded by adenosine A1 receptor activation. J Am Coll Cardiol 35: 238-245 (2000).
6) Miki T, Miura T, Tsuchida A, Nakano A, Hasegawa T, Fukuma T, Shimamoto K: Cardioprotective mechanism of ischemic preconditioning is impaired by postinfarct ventricular remodeling through angiotensin II type 1 receptor activation. Circulation 102: 458-463 (2000).
7) Eguchi M, Tsuchihashi K, Nakata T, Hashimoto A, Shimamoto K: Right ventricular abnormalities assessed by myocardial single-photon emission computed tomography using technetium-99m sestamibi/tetrofosmin in right ventricular-originated ventricular tachyarrhythmias. J Am Coll Cardiol 36: 1767-1773 (2000).
8) Sasao H, Tsuchihashi K, Hase M, Nakata T, Shimamoto K: Does primary stenting preserve cardiac function in myocardial infarction? A case-control study. Heart 84: 515-521 (2000).
9) Nakata T, Hashimoto A, Kobayashi H, Miyamoto K, Tsuchihashi K, Miura T, Shimamoto K: Outcome significance of thallium-201 and iodine-123-BMIPP perfusion-metabolism mismatch in preinfarct angina. J Nucl Med 39:1492-1499 (1998).
10) Takagi S, Saitoh S, Nakano M, Hayashi Y, Obara F, Onishi H, Shimamoto K: Relationship between blood pressure level and mortality rate: an 18-year study conducted in two rural communities in Japan. J Hypertens 18: 139-144 (2000).
11) Saitoh S, Takagi S, Takahashi H, Nakano M, Hayashi Y, Obara F, Takagawa S, Fujisawa J, Shimamoto K: Epidemiology of obesity: an epidemiological study in rural communities of Hokkaido. Int Med 38: 195-197 (1999).

Internal Medicine (III)

Our department has been challenged to cure patients with refractory respiratory and allergic diseases. We have studied clinical evaluations and the pathophysiology of lung tumors, interstitial lung diseases, pulmonary emphysema, bronchial asthma, sarcoidosis and pulmonary infectious diseases by radiological, immunological, biochemical and bacteriological approaches.

Professor
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Interests:
Interstitial lung diseases,
Respiratory oncology

Associate Professor
Hiroyuki Koba, M.D., Ph.D.
Interests:
Chest image diagnosis

Hiroki Takahashi, M.D., Ph.D.
Interests:
Interstitial lung diseases
Pulmonary surfactant, host defense

Assistant Professor
Hiroshi Tanaka, M.D., Ph.D.
Interests:
Respiratory allergy,
Respiratory environment medicine

Noriharu Shijubo, M.D., Ph.D.
Interests:
Immunology, Respiratory oncology

Instructor
Michio Hirasawa, M.D., Ph.D.
Takuya Fujishima, M.D., Ph.D.
Masanori Shiratori, M.D., Ph.D.
Gen Yamada, M.D., Ph.D.
Shin Teramoto, M.D.

1. Image diagnosis of lung diseases

We have developed our ability to interpret CT images in various lung diseases by radiologic-pathologic correlative studies, including 3D reconstruction techniques (1). We quantify CT images in interstitial lung diseases (ILD) and pulmonary emphysema by densitometrical method. Densitometrical distribution in ILD with the image analyzing software “Osiris” and DICOM files of CT images are studied. The lung areas divided according to CT values were well correlated with the results of pulmonary function test (PFT) and serum surfactant proteins (SPs) in idiopathic pulmonary fibrosis. We also found a relationship between the distribution pattern of low attenuation area (LAA) on CT and PFT in emphysema patients (2). Airflow limitation and residual volume were correlated with %LAA in the lower lobes, while diffusing capacity was correlated with %LAA in the upper and middle lobes. This study suggests that pulmonary function is significantly different between predominantly upper- and lower-lung emphysema. CT densitometry is a very useful method for the quantification of morphological changes in the lung and is clinically useful for management of lung diseases. Recently we apply this method to other lung diseases, bronchial asthma (BA) and small lung tumor.

2. Biochemistry of respiratory diseases

We have studied the biochemical and pathophysiologic aspects of many diffuse lung disorders. Recently, we found that SP-A and SP-D, major glycoproteinous components of surfactant, increase in sera from patients with a specific pathophysiologic state of ILD, using assay kits originally developed with the collaboration of the Department of Biochemistry. These kits are novel tools for diagnosis and prognosis of ILD (3-5). This clinical application of the assay for SP-A and SP-D was authorized by the Ministry of Health and Welfare in 1999. We also have investigated the significance of the surfactant components as factors in host-defence situating on the opposite site of several proinflammatory cytokines in acute lung injury.

3. Respiratory allergy and environment medicine

A group of respiratory allergy and environment medicine mainly studies BA, hypersensitivity pneumonitis (HP) and chronic obstructive pulmonary disease. About 4000 asthmatic patients consult us every month in the University Hospital and its 11 branch hospitals. The research concentrates on the mechanism of airway inflammation and remodeling, and asthma therapy. The functions of airway receptors of leukotrienes, prostaglandin E2 and neuropeptide (neurokinin (NK) A, B and substance P) and the contribution of matrix metalloproteinase-9, its to airway remodeling are studied (6). We can assess airway vascularity in asthmatic patients using an originally developed high-magnification brochovideoscope. We evaluated the effects of acid fog on asthmatic patients, and found that acid fog was the most important contributor to exacerbated asthmatic symptoms (7). These

mechanisms took place through NK receptors (8). Occupational inhalation of mushroom spore, which can cause HP, asthma, cough variant asthma, and organic dust toxic syndrome, may act through CD1b and CD14 molecules and be regulated by natural-killer T cells.

4. Respiratory oncology

Lung adenocarcinoma often expresses SPs specific to the lung. We have studied the oncogenetic and clinical significance of expressions of SPs and their mRNAs in pleural effusion. We also investigated whether the immunocytochemistry (IC) of cytokeratin-18, a maker of epithelial cells, could detect micrometastasis in bone marrow (BM). To improve detection of lung adenocarcinoma cells in BM, we investigate the expressions of SPs, using IC and reverse transcriptase-polymerase chain reaction (RT-PCR) in BM, and RT-PCR for SP-A was more sensitive than IC of SP-A. RT-PCR for SP-A and SP-C in the circulation is a useful method for detecting occult metastasis in patients with lung cancer.

Tumor growth and metastasis are angiogenesis-dependent and tumor angiogenesis is a result of a complex interplay of positive and negative regulators. Vascular endothelial growth factor (VEGF) is one of the most important angiogenic factors in nonsmall cell lung cancer. Osteopontin (OPN) induces VEGF-dependent endothelial cell migration. VEGF and OPN cooperatively up-regulate tumor angiogenesis in stage I lung adenocarcinoma (9). Mast cell accumulation is related to angiogenesis in lung adenocarcinoma. Tumor associated mast cells produce VEGF. VEGF-dependent tumor angiogenesis is important to evaluate the outcome of lung cancer patients.

5. Clinical immunology of lung field

Clara cell 10 kDa protein (CC10) is the major product from non-ciliated bronchiolar epithelial cells and functions as an anti-inflammatory and immunormodulator. Several monoclonal antibodies specific to human CC10 have been established. Cigarette smoking reduces CC10 levels. CC10 expression and CC10 levels are decreased in asthmatics (10). Sarcoidosis (SAR) patients with good prognosis show significantly higher levels of CC10 than those with poor prognosis.

Expression of adhesion molecules and extracellular matrices is characteristic in SAR. The cytokine profile in SAR is dominant for type 1 (Th1) cytokines. The circulating levels of interleukin-18 (IL-18), and interferon (IFN)-γ inducing factor, are increased in SAR and tuberculosis and correlate with circulating IFN-γ levels and disease activity (11). IL-12 and IL-18 are increased and stimulate IFN-γ production showing that SAR is predominant in Th1 cytokines.

List of Main Publications from 1997 to 2000

1) Hoshino H, Koba H, Inomata S, Kurokawa K, Morita Y, Yoshida K, Akiba H, Abe S. Pulmonary alveolar microlithiasis: High–resolution CT and MR findings. J Comput Assist Tomogr 22: 245-248 (1998).
2) Saitoh T, Koba H, Shijubo N, Tanaka H, Sugaya F. Lobar distribution of emphysema in computed tomographic densitometric analysis. Invest Radiol 35: 235-243 (2000).
3) Takahashi H, Kuroki Y, Tanaka H, Saito T, Kurokawa K, Chiba H, Sagawa A, Nagae H, Abe S. Serum Levels of Surfactant proteins A and D are useful biomarkers for interstitial lung disease in patients with progressive systemic sclerosis. Am J Respir Crit Care Med 162: 258-263 (2000).
4) Takahashi H, Fujishima T, Koba H, Murakami S, Kurokawa K, Shibuya Y, Shiratori M, Kuroki Y, Abe S. Serum surfactant proteins A and D as prognostic factors in idiopathic pulmonary fibrosis and their relationship to disease extent. Am J Respir Crit Care Med 162: 1109-1114 (2000).
5) Takahashi H, Imai Y, Fujishima T, Shiratori M, Murakami S, Chiba H, Kon H, Kuroki Y, Abe S. Diagnostic significance of surfactant protein A and D in sera from patients with radiation pneumonitis. Eur Respir J 17: 481-487 (2001).
6) Tanaka H, Miyazaki N, Oashi K, Tanaka S, Ohmichi M, Abe S. Sputum matrix metalloproteinase-9: Tissue inhibitor of metallopoteinase-1 ratio in acute asthma. J Allergy Clin Immunol 105: 900-909 (2000).
7) Honma S, Tanaka H, Teramoto S, Igarashi T, Abe S. Effects of naturally-occurring acid fog on inflammatory mediators in airway and pulmonary functions in asthmatic patients. Respir Med 94:935-942 (2000).
8) Teramoto S, Tanaka H, Kaneko S, Abe S. Neurokinin-1 receptor antagonist inhibits short-acid-induced airway hyperresponsiveness in sensitized guinea pigs. Int Arch Allergy Immunol 121: 53-56 (2000).
9) Shijubo N, Uede T, Kon S, Maeda M, Segawa T, Imada A, Hirasawa M, Abe S. Vascular endothelial growth factor and osteopontin in stage I lung adenocarcinoma. Am J Respir Crit Care Med 160: 1269-1273 (1999).
10) Shijubo N, Itoh Y, Yamaguchi T, Imada A, Hirasawa M, Yamada T, Kawai T, Abe S. Clara cell protein-positive epithelial cells are reduced in small airways of asthmatics. Am J Respir Crit Care Med 160: 930-933 (1999).
11) Yamada G, Shijubo N, Shigehara K, Okamura H, Kurimoto M, Abe S. Increased levels of circulating interleukin-18 in patients with advanced tuberculosis. Am J Respir Crit Care Med 161:1786-1789 (2000).

Internal Medicine (IV)

Since the establishment of the clinical division of our cancer laboratory in 1953, our research, broadly speaking, has focused on oncology. At present, oncology, gastroenterology and hematology are the three main branches of clinical and basic research carried out in our department. Our objective is to bring about benefits for patients by achieving advances in the clinical field and resolving unanswered questions. Given the global nature of clinical research, the achievements of our department are evaluated and have clinical applications worldwide.

Professor
Yoshiro Niitsu, M.D., Ph.D.
Interests:
Oncology, Gastroenterology, Hematology

Associate Professor
Sumio Sakamaki, M.D., Ph.D.
Interests:
Hematology, Gastroenterology

Assistant Professor
Junji Kato, M.D., Ph.D.
Interests:
Hepatology, Hematology

Katsuhisa Kogawa, M.D., Ph.D.
Interests:
Oncology, Hematology

Instructor
Takeshi Terui, M.D., Ph.D.
Tetsuji Takayama, M.D., Ph.D.
Minoru Takahashi, M.D., Ph.D.
Takuya Matsunaga, M.D., Ph.D.
Tetsuro Okamoto, M.D., Ph.D.

1. Oncology

a) Apoptosis induction

We have studied TNF receptor type I (p55) gene transduction in combinantion with mutein TNF administration for pancreatic cancer (1).

b) Development of receptor mediated gene delivery method

We developed an *in vivo* tumor targeting method transferrin receptor (TfR)-DNA conjugate (2).

c) Gene therapy by tumor specific and replicable adenovirus

We have constructed a tumor specific and replicable adenovirus by expression of E1A-13S under control of tumor specific promoter and deletion of E1B-55K and are ready for clinical trial for AFP and/or CEA-producing gastrointestinal cancer.

d) Protection of hematopoietic stem cells

We succeeded in transduction of the Glutathione-S-transferase (GST-**π**) gene into human CD34+ cells utilizing fibronectin fragment to confer them resistance to cyclophosphamide *in vitro* and *in vivo* (3).

e) Anti-metastatic gene therapy

We have developed anti-metastatic gene therapy using secretory type superoxide dismutase gene (EC-SOD) (4).

f) Signal transduction of TGF-**β**

We have demonstrated a TGF-**β** mediated growth inhibitory signaling pathway through protein phosphatase-2A and p53 in epithelial cells.

g) Drug resistance

We have found that VLA4 (a4**β**1-integrin) protects acute myelogenous leukemia cells from undergoing apoptosis by chemotherapeutic drugs.

2. Gastroenterology

a) Aberrant crypt foci (ACF) as a precursor of adenomas

We succeeded in identifying human ACF by magnifying endoscopy, and demonstrated that ACF are precursors of the adenoma-carcinoma sequence (5). We also found that *ras* point mutation is frequently associated with ACF from sporadic colon adenoma or cancer, while APC mutation is a dominant genetic abnormality; p16 elevation was also detected in ACF.

b) Ulcerative colitis (UC)

Active UC patients were found to develop auto-antibody against a specific tropomyosin peptide which in turn causes colitis by the ADCC mechanism (6).

c) Infrared endoscopy

Novel infrared endoscopy and imaging processing for diagnosis of mucosal and submucosal vascular disease (varix, vascular ectasia) were developed in our department (7).

d) Carboplatin-coated biliary drainage stent

We developed a novel percutaneous transhepatic biliary drainage tube coated with carboplatin, which provides a new treatment modality for patients with unresectable biliary tract cancer (8).

e) IVT for advanced pancreatic cancer

We have developed a novel arterial infusion chemotherapy for the treatment of advanced pancreatic carcinoma after vascular supply distribution via selective embolization (9).

3. Hematology

a) prevention of GVHD

We recently succeeded in prevention of graft-versus-host disease (GVHD) by using Th1 specific inhibitor (TAK-603) in allogeneic bone marrow transplantation mouse model (10).

b) Cytokine mRNA expression of bone marrow stromal cells

We proved that bone marrow stromal cell function is regulated by various cytokines (11).

c) Regulation of megakaryopoiesis

TGF-β derived from platelets or megakaryocytes increases TPO production from bone marrow stromal cells, which increases TGF-β receptors on megakarocytes and in turn renders them susceptible to suppression by TGF-β itself (12).

d) novel hemochromatosis gene

We recently found a point mutation of iron responsive element of H-ferritin which is responsible for iron deposition in cytoplasma in a japanese family with hemochromatosis (13).

4. Rheumatology

We have developed a novel gene therapy for RA by ex vivo transfer of EC-SOD gene in a mouse model. To extend this strategy for clinical trial, we are exploring the *in vivo* gene therapy by a newly constructed viral vector expressing EC-SOD.

List of Main Publications from 1997 to 2000

1) Sato T, Yamauchi N, Sasaki H et al: An apoptosis-inducing gene therapy for pancreatic cancer with a combination of 55-kDa receptor gene transfection and mutein TNF administration. Cancer Res 58: 1677-1683 (1998).
2) Sato Y, Yamauchi N, Takahashi M et al: *In vivo* gene delivery to tumor cells by transferrin-streptavidin-DNA conjugate. FASEB J. 14: 2108-2118 (2000).
3) Matsunaga T, Sakamaki S, Kuga T et al: GST-π gene transduced hematopoietic progenitor cell transplantation overcome the bone marrow toxicity of cyclophosphamide in mice. Hum Gene Ther 11: 1671-1681 (2000).
4) Tanaka M, Kogawa K, Nakamura K et al: Anti-metastatic gene therapy utilizing subcutaneous inoculation of EC-SOD gene transduced autologous fibroblast suppressed lung metastasis of Meth-A cells and 3LL cells in mice. Gene Ther 8: 149-156 (2000).
5) Takayama T, Katsuki S, Takahashi Y et al: Aberant crypt foci of the colon as precursors of adenoma and cancer. New Eng J Med 339: 1277-1284 (1998).
6) Sakamaki S, Takayanagi N, Yoshizaki N et al: Auto-antibodies against the specific epitope of human tropomyosin(s) detected by a peptide-based enzyme immunoassay in sera of patients with ulcerative colitis show antibody dependent cell-mediated cytotoxicity against HLA-DPw9 transfected L-cells. Gut 47: 236-241 (2000).
7) Masuko E, Homma H, Ohta H et al: Rheologic analysis of gastric mucosal hemodynamics in patients with cirrhosis. Gastrointest Endosc 49: 371-379 (1999).
8) Mezawa S, Homma H, Sato T et al: A study of carboplatin coated tube for the unresectable cholangiocarcinoma. Hepatology 32: 916-923 (2000).
9) Homma H, Doi T, Mezawa S et al: A novel arterial infusion chemotherapy for treatment of advanced pancreatic cancer following vascular supply distribution via superselective embolization. Cancer 89: 303-313 (2000).
10) Lu Y, Sakamaki S,Kuroda H et al: Prevention of lethal acute graft-versus-host disease in mice by oral administration of T helper 1 inhibitor, TAK-603. Blood 97: 1123-1130 (2001).
11) Hirayama Y, Sakamaki S, Matsunaga T et al: Concentrations of thrombopoietin in bone marrow in normal subjects and in patients with idiopathic thrombocytopenic purpura, aplastic anemia, and essential thrombocythemia correlate with its mRNA expression of bone marrow stromal cells. Blood 92: 46-52 (1998).
12) Sakamaki S, Hirayama Y, Matsunaga T et al: Transforming growth factor-β1 (TGF-β1) induces thrombopoietin from bone marrow stromal cells, which stimulates the expression of TGF-β receptor on megakariocytes and, inturn, renders them susceptible to suppression by TGF-β it self with high specificity. Blood 94: 1691-1970 (1999).
13) Kato J, Fujikawa K, Kanda M et al: A mutation, in the iron-responsive element of H ferritin mRNA, causing autosomal dominant iron overload. Am J Hum Genet. 69: 191-197(2001).

Surgery (I)

Surgery is a medical science that practices the protection of human rights and respects the dignity of patients. Moreover, there are great expectations for clinical applications of surgery due to recent developments in molecular biology and engineering. We are now studying organ transplantation, artificial organs, cancer therapy using gene operations, diagnosis of cancer and clarification of the mechanism of metastasis using molecular biotechnology.

Professor
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Interests:
Gastroenterological surgery,
Hepato-biliary-pancreatic surgery,
Sugical Oncology

Assistant Professor
Mitsuhiro Mukaiya, M.D., Ph.D.
Interests:
Gastroenterological surgery,
Hepato-biliary-pancreatic surgery,
Endoscopic surgery

Tadashi Katsuramaki, M.D., Ph.D.
Interests:
Gastroenterological surgery,
Hepato-biliary-pancreatic surgery,
Organ transplantation

Takahiro Yasojima, M.D., Ph.D.
Interests:
Gastroenterological surgery

Instructor
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Tosei Ohmura, M.D., Ph.D.
Kouji Yamaguchi, M.D., Ph.D.
Toshio Honma, M.D., Ph.D.
Youjirou Okada, M.D., Ph.D.
Yasutoshi Kimura, M.D., Ph.D.

1. Basic research for treatment and clarification of the mechanisms of liver failure

Surgeons who have detailed knowledge about the pathophysiology at surgical stress should be called as intellectual surgeons. Studies on the pathogenesis of the liver, an important organ for life, under various conditions have quite been insufficient. In particular, the researches of the relationship between sinusoidal cells and postoperative cholestasis or liver failure have just begun. Our laboratory has been stereoretically studying the relationships between endotoxemia and Kupffer cells, between cholestasis and infection, and between the bile canalicular movement and expression of specific membrane receptors of hepatocytes using cultured hepatocytes (1).

2. Hepatic stem cell culture for liver regeneration and cell transplantation therapy

Recently, small hepatocytes are thought to be candidates for hepatic stem cells. Currently we are studying the culture of these cells and analyzing their functions, for the objection of liver reconstruction in vitro. If the use of these cells is possible in human, the artificial liver will be more popular and indicative because these cells produce human specific proteins and will not provoke xenoimmunoresponses. Moreover, therapy for gene-deficit diseases will be possible by transplantation of small hepatocytes with normal gene transfer isolated from the patient's liver.

3. Organ transplantation

In the study of organ transplantation, we have examined various animal models of ischemia/reperfusion injury (2-4) and also developed on estimation method for graft viability from non-heart beating donors before reperfusion using pigs. In a study of ischemia/reperfusion injury, heat-shock protein-73 protected against small intestinal warm ischemia/reperfusion injury in rat (3). On the other hand, nitric oxide (NO) from inducible NO synthase was expressed in leukocytes and Kupffer cells of the liver and caused cytotoxicty after reperfusion, and this cytotoxicty was ameliorated by control of iNOS (4). Estimation of graft viability from non-heart beating donors before reperfusion was established by measurement of purine metabolites using a microdialysis method for the first time ever.

4. Clarification of the Mechanism of cancer metastasis

Animal models of various metastasis are very important for the research of cancer, and this is one of major goals for clinical oncological research. We have performed a search of genetic and epigenetic variations of cancer- associated factors, and

identification of novel genes responsible for cancer using molecular biological procedures (5.6). The relationships between the clinicopathological factors and gene analysis results have been examined on researches by extraction of DNA and RNA from surgical specimens. Our present original hepatic or lymph node metastasis animal models have contributed significantly (5). We were the first to clarify that the VEGF family played an important role in lymph node metastasis in gastric cancer.

5. Gene therapy for cancer and tumor immunology

Recent advances in cancer immunology have provided opportunities for a variety of treatments which depend on the activation of molecular based immune responses. We have identified novel peptides for cancer antigens using the SELEX method and cDNA cloning of tumor-specific natural antigens. Our object is the clinical application of cancer gene therapy and vaccine therapy, which consists of differentiation of immunoresponsive cells stimulated by various cytokines, ex-vivo gene transfer and cancer-specific natural antigenic peptides (6-8).

6. Surgical insult and host defense

It is important for surgeons to understand the alterations and controls of host defense mechanisms following surgical insult. We have examined specific isolation of CD4+T cells in peripheral blood samples from patients who received gastrointestinal operations, and analyzed the Th1/Th2 balance by measurement of intracellular cytokines to estimate preoperative risk degrees and to establish indicators to modify postoperative immune responses appropriately.

7. Development of new surgical techniques

We have actively applied endoscopic surgery for the purpose of less invasive surgery. Furthermore, such operations have been indicated for malignancies with surgical curability. Almost most organs, including the thyroid, parathyroid, esophagus, stomach, liver, gallbladder, spleen, colon, and rectum can be indicated on by this approach. Many patients should receive much benefits in term of physiological responses.

We have also developed a new surgical procedure, so called the minimal PD, which resects the pancreas head with only the second portion of the duodenum in order to preserve the functions of digestion and absorption. Moreover, the logical methods of duodenum-preserving pancreas head resection and segmental resection of the pancreas have been presented.

8. New diagnosis of cancer and new chemotherapy

We have been studying diagnosis of cancer using new modalities. As a result of these clinical studies, we could detect various cancers, such as pancreatic cancer, in the very early stage (9). On the other hand, we have reported the effectiveness of new chemotherapy based on the synergistic antitumor activities of 5-fluorouracil and cisplatin producing biochemical modulation in solid cancers such as pancreatic cancer and colorectal cancer (10).

List of Main Publications from 1997 to 2000

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Surgery (II)

In 1958, our department was initially organized as the Department of Thoracic and Cardiovascular Surgery in Japan. Thereafter, we have made a great effort to extend our knowledge and surgical experiences in this field and to contribute to better the lives of the patients. To achieve these goals, we have been conducting basic and clinical research on surgical treatments for congenital and acquired heart diseases, thoracic and thoracoabdominal vascular diseases and another thoracic diseases as well.

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Tomio Abe, M.D., Ph.D.
Interests:
Acquired and congenital heart surgery, Vascular surgery, Organ transplantation

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Interests:
Acquired heart surgery,
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Interests:
Congenital heart surgery,
Organ transplantation, Gene therapy for cardiovascular disease

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Interests:
General thoracic surgery,
Lung transplantation

Instructor
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Nobuyuki Takagi, M.D., Ph.D.
Nobuyoshi Kawaharada, M.D., Ph.D.
Johji Fukada, M.D., Ph.D.

1. Acquired heart surgery

Minimally invasive cardiac surgery (MICS) has become a standard operation for patients of valve diseases as well as ischemic heart diseases. We have aggressively performed MICS for valvular diseases in the last 3 years, and have established some techniques including inverted-J lower partial-sternotomy for mitral valve and transverse-L upper partial-sternotomy for aortic valve. Especially in re-do AVR cases, patients may have advantages by MICS, by which a risk of dissection of adhesive tissue in front of the heart can be avoided (1). We have also started a program of off-pump CABG (OPCAB), and the early results are promising, without operative mortality and rare morbidity. We have developed a new traction-type stabilizer for OPCAB, and it has been used in clinical cases with satisfactory results. Our research activities also includes surgical early and late results after valve replacement (2), mitral valvuloplasy, MAZE operation for atrial fibrillation, aortic root remodeling and Ross' procedure.

2. Congenital heart surgery

We have performed the Fontan procedure for univentricular heart since 1981. We, therefore, evaluated heart, lung and liver function in these patients, and have shown the functional deterioration in these organs at late follow-up period (3). In addition, occurrence of atrial arrythmia is a critical late problem. Conversion of the conventional Fontan circulation to the circulation excluding right atrium by the extracardiac total cavopulmonary connection is one possible resolution for the patients with severe atrial arrythmia. The occurrence of common atrioventricular valve regurgitation is also one of late problems after the repair for univentricular heart. We described the beneficial effect of the bivalvation of common atrioventricular valve to suppress regurgitation. Thus, the number of reoperations has recently increased in the patients with complex congenital anomaly. Consequently, the surgery for adult patients with congenital heart diseases has accounted for a considerable number in our recent experiences.

3. Vascular surgery

We have developed techniques with selective organ perfusion and hypothermic circulatory arrest for aortic arch or thoracoabdominal aortic aneurysms (4). Total cases of aortic arch operation with selective cerebral perfusion have been more than 250 until now. Currently, we aim at less-invasive surgery to raise the quality of life after these operations, particularly for the patients aged over 80, and we have already performed endovascular stent grafting for two very old patients with distal arch aneurysm and aortic dissection. Our department is one of the most advanced laboratories for research on the Adamkiewicz artery, which is the key artery for spinal cord injury during the thoracoabdominal aortic aneurysm surgery (5).

4. General thoracic surgery

We have mainly been studying the effect and operative procedure of Video-assisted thoracoscopic surgery (VATS) (6). Fifty eight VATSs were performed and VATS lobectomy accounted for 55% of all surgeries for primary lung cancer last year. We believe that mediastinal lymphoadenectomy should be done even in VATS lobectomy for primary lung cancer, and we have performed lymphoadenectomy (LND2). The quality and quantity of the mediastinal lymphoadenectomy by VATS was as good as that by open thoracotomy in right side operations. The sternal turnover technique, which was initially established by our department, has been used for repair of funnel chest for more than 40 years. However, a novel minimally invasive surgical technique called Nuss procedure has recently been developed. We performed the procedure, and found that it is very effective for cases of symmetric funnel chest.

5. Extracorporeal circulation and artificial organ

Although the OPCAB has been raised as an acceptable option for certain patients of ischemic heart disease, the cardiopulmonary bypass (CPB) and ventricular assist devices (VAD) are still essential not only for many cardiovascular surgeries but also for some non-cardiovascular operations. We reported that the CPB using heparin-coated circuits under low systemic heparinization successfully supported the surgery for giant intracranical aneurysms (7). Furthermore, we experienced and reported the temporary use of left VAD, which successfully contributed to the long-term survival of patients who had severe cardiac failure after CABG.

6. Organ transplantation

Current immunosuppressive agents, such as cyclosporine and tacrolimus appear to be clinically effective to inhibit acute rejection. However, complete abrogation of the acute rejection is not yet achievable. Accordingly, recurrent rejection still occurs. To obtain an ideal method of immunosuppression, we have investigated a novel dual inhibitor of NF-κB and AP-1, which are critical transcription factors to activate T cells, and reported its potent immunosuppressive activity (8). Although heart and/or lung transplantation has become clinically acceptable options for patients in the end-stage of cardiopulmonary diseases, both coronary artery vasculopathy (CAV) and obliterative bronchiolitis (OB) following these organ transplantation still affect a significant proportion of patients, and are major causes of long-term morbidity or mortality. Currently, there are no reliable means to prevent or treat CAV or OB. We have found that FK409, a spontaneous nitric oxide releaser, attenuates experimental allograft vasculopathy following the aortic transplantation (9). In addition, blocking the CD28-B7 T-cell costimulatory pathway by CTLA4Ig reduces the development of an OB-like lesion following the experimental tracheal transplantation (10). Furthermore, we have reported that either central or peripheral tolerance experimentally induced into the recipients prevents OB.

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9) Fukada J, Schena S, Tack I, Ruiz P, Kurimoto Y, Pang M, Aitouche A, Abe T, Striker LJ, Pham S. FK409, a spontaneous nitric oxide releaser, attenuates allograft vasculopathy in a rat aortic transplant model. Circ Res 87: 66-72 (2000).
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Orthopaedic Surgery

The aim of researches of our department is to elucidate causal mechanisms of various musculoskeletal disorders such as osteoarthritis, spondylosis, tumors and sports injuries and to develop effective treatments of these disorders. Our main research fields are pathological, immunological and molecular biological studies on the malignant bone and soft tissue tumors, neurophysiologic study on the mechanoreceptors of the spine and joints, and biomechanical and anatomical studies of the joints.

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Interests:
Hand surgery,
Bone and soft tissue tumor

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Spinal surgery, Mechanoreceptors in the musculo-skeletal system

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Joint biomechanics

Instructor
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Kenji Okamura, M.D.
Takanori Murakami, M.D.
Satoshi Kawaguchi, M.D., Ph.D.
Kazuhiko Nakano, M.D.
Satoshi Isogai, M.D.

1. Neurophysiology

a) Mechanosensitive units in the spine and adjacent tissues

We are investigating the physiologic characteristics and distributions of the mechanosensitive afferent units in the lumbar spine and adjacent tissues in order to determine the sources of low back pain. Mechanosensitive units with low to high thresholds were found in the facet joint, intervertebral disc, paravertebral muscles, and posterior longitudinal ligament of the lumbar spine of the animals.

b) Joint mechanoreceptors

Joint mechanoreceptors are also investigated using electrophysiologic techniques. Nociceptors and proprioceptors have been identified in the rotator cuff and adjacent muscles of the shoulder, the lateral ligament of the ankle, and the posterior aspect of the sacroiliac joint of rabbit and cats (1-3).

2. Spinal disorders

Changes in spinal deformity and pulmonary function in Duchenne muscular dystrophy (DMD) patients were examined in a retrospective longitudinal study to investigate how age and the value of the plateau of vital capacity (VC-plateau) correlate with the severity of the progression of spinal deformity. The correlation between the patterns of progression of spinal deformity and the VC-plateau was examined using a discriminant analysis. VC-plateau may be an indicator of the severity of the progression of spinal deformity in DMD patients (4).

3. Bone and soft tissue tumors

a) Clinical study

In recent years, limb-saving surgery has become a standard procedure in the treatment of patients with bone and soft tissue malignancies. While various reconstructive techniques, including prosthetic replacement and bone grafting, have been utilized for bony and soft-tissue defects after resection of tumors, their long-term results have not been consistently successful. We have been prospectively studying the function and durability of a limb that was reconstructed with the vascularized fibula. We have reported satisfactory medium- to long-term results with this procedure in patients who had wide resection of a bone sarcoma in the proximal humerus (5), around the knee joint (6), and in the periacetabular region (7).

b) Basic study

Osteosarcoma is the most common malignant primary tumor of bone. Despite the recent progress in chemotherapy protocols that has greatly improved the survival of patients with osteosarcoma, 30-40% of patients still succumb to pulmonary metastatic disease. Therefore, it is of fundamental importance to predict pulmonary metastasis as well as to develop new therapeutic modalities that target metastasized osteosarcoma. To this end, we have conducted several basic and preclinical

studies. We have found that development of pulmonary metastasis is highly correlated with the expression of vascular endothelial growth factor (8) and p-glycoprotein (9), and loss of EerbB2 receptor. Using a rat syngeneic model system, we have demonstrated that osteosarcoma cells can serve efficiently as an antigen presenter to the host T cells and elicit anti-tumor immunity against metastatic diseases when a co-stimulatory signaling molecule, B7-1, is exogenously expressed on the cell surface. We have currently established an autologous pair of osteosarcoma cells and cytotoxic T lymphocytes (10).

4. Surgery of the upper extremity

We performed experimental studies in an effort to increase the strength of tendon repair sufficiently to allow early, active, and controlled postoperative motion. We described the clinical features of wrist disorder and their operative treatment. We described new reconstructive operative procedures and their results for hand and elbow defects (5.11). We reported the outcome of peripheral nerve resection for RSD in the extremities and investigated the pain mechanism in RSD (12).

5. Anatomy and biomechanics

We have researched the morphology of the lower extremities, especially foot, ankle, and knee to understand the pathogenesis of skeletal injury and deformity and to improve surgical procedures. We investigated cartilage degeneration of ankle and subtalar joints in order to understand how degenerative lesions extend into specific joints. Further extended research into the relation between cartilage degeneration and ligament injury is under way (13-15).

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2) Minaki Y, Yamashita T, Takebayashi T, Ishii S. Mechanosensitive afferent units in the shoulder joint and adjacent tissues. Clin Orthop Relat Res 369: 349-356 (1999).
3) Takebayashi T, Yamashita T, Minaki Y, Ishii S. Mechanosensitive afferent units in the lateral ligament of the ankle joint. J Bone Joint Surg Br 79: 490-493 (1997).
4) Yamashita T, Kanaya K, Yokogushi K, Ishikawa Y, Minami R. Correlation between spinal deformity and pulmonary function in Duchenne muscular dystrophy. J Pediatr Orthop 21: 113-116 (2001).
5) Wada T, Usui M, Isu K, Yamawakii S, Ishii S. Reconstruction and limb salvage after resection for malignant bone tumour of the proximal humerus. A sling procedure using a free vascularised fibular graft. J Bone Joint Surg Br 81: 808-813 (1999).
6) Wada T, Usui M, Nagoya S, Isu K, Yamawaki S, Ishii S. Resection arthrodesis of the knee with a vascularised fibular graft. Medium- to long-term results. J Bone Joint Surg Br 82: 489-493 (2000).
7) Nagoya S, Usui M, Wada T, Yamashita T, Ishii S. Reconstruction and limb salvage using a free vascularised fibular graft for periacetabular malignant bone tumors. J Bone Joint Surg Br 82: 1121-1124 (2000).
8) Kaya M, Wada T, Akatsuka T, Kawaguchi S, Nagoya S, Shindoh M, Higashino F, Mezawa F, Okada F, Ishii S. Vascular endothelial growth factor expression in untreated osteosarcoma is predictive of pulmonary metastasis and poor prognosis. Clin Cancer Res 6: 572-577 (2000).
9) Yamamoto O, Wada T, Takahashi M, Yamawaki S, Ishii S. Prognostic value of the P-glycoprotein expression in bone and soft tissue sarcoma. Int J Clin Oncol 5: 164-170 (2000).
10) Nabeta Y, Kawaguchi S, Sato Y, Wada T, Yamashita T, Sato N. Identification strategy and cataloguing of antigenic cancer epitopes. Mod. Asp. Immunol: 1, 17-19 (2000).
11) Wada T, Ishii S, Usui M, Miyano S. The medial approach for operative release of post-traumatic contracture of the elbow. J Bone Joint Surg Br 82: 68-73 (2000).
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13) Kura H, Harold B. Kitaoka, Zong-Ping Luo, Kai-Nan An. Measurement of surface contact area of the ankle joint. Clin Biomech 13: 365-370 (1998).
14) Kura H, Zong-Ping Luo, Harold B. Kitaoka, Kai-Nan An. Role of Medial Capsule and Transverse Metatarsal Ligament in Hallux Valgus Deformity. Clin Orthop 354: 235-240 (1998).
15) Hirose K, Murakami G, Kura H, Tokita F, Ishii S. Cartilage degeneration in talocrural and talocalcaneal joints from japanese cadaveric donors. J Orthop Sci 4: 273-285 (1999).

Neurosurgery

We have remained focused on best patient care. We have also made strong commitments to laboratory research to help us better understand the important neoplastic changes that are responsible for the development of tumors and their aggressive behavior in the central nervous system. Another important laboratory committment is to establish a method of functional recovery of any neurological deficit by transplanting neuronal stem cells taken from autogenous bone marrow cells.

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Interests:
Microneurosurgery

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Interests:
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Interests:
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Assistant Professor
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Interests:
Endoscopic neurosurgery,
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Interests:
Functional neurosurgery,
Basic neurophysiology

Instructor
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Masahiko Wanibuchi, M.D.
Ikuhide Kohama, M.D.

1. Clinical neurosurgery

A new venture in clinical neurosurgery for the past 4 years from 1997 is the introduction of intraoperative cardiac arrest under profound hypothermia to treat huge posterior circulation aneurysms, which had been regarded as inoperative with the conventional operative techniques. We have experienced 10 such cases with remarkable operative results. The modern neuronavigational tools have allowed us to perform operations more safely during recent years, and laser Doppler, intraoperative tumor staining with ALA, and endoscopic exploration in addition to conventional EEG and SEP monitoring have also contributed to improve patient outcomes (3.5). Another venture was to introduce the Stroke Care Unit at the Department of Emergency Medicine to save acute stroke patients from major neurological deficit by modern intravascular surgery such as thrombolysis (4).

2. Clinical neuroradiology

Clinical neuroradiology has made remarkable advancements in diagnosis by utilizing recent MRI technology such as diffusion-weighed image, perfusion weighted-image, surface anatomy image, 3D-MR angiography, cine MRI for CSF dynamics study, MR spectroscopy, functional mapping, in addition to the refinement of conventional T1- and T2-weighted images such as heavy T2-weighted images to improve anatomical resolution (6). This new diagnostic modality has provided very important pathophysiological information to help with evaluation of malignancy of intracerebral tumors, or to locate an epilepotogenic focus. It is also useful to make operative simulation by obtaining stereotactic brain anatomy (2).

3. Experimental neuro-oncology

Our neuro-oncology research is centered on the development of a novel immunotherapy of gliomas by producing a bunch of monoclonal antibodies directed against glioma-associated gangliosides of an individual tumor. In the chemical analysis of glioma gangllosides, we came across an interesting phenomena of ganglioside crypticity, which suggests an important physiological function of gangliosides that has so far been regarded as mysteries of the sphinx (1).

4. Experimental neurophysiology

Although it has generally been assumed that the adult brain is incapable of significant self-repair because of a lack of neurogenesis in the adult mammalian central nervous system (CNS), several studies have reported that the adult mammalian brain harbors neural stem cells that retain the potential for both neural production and differentiation in experimental animal models. These findings offer the prospect of the presence of neural precursors in the adult human brain. Our ongoing projects are to identify neural stem cells in the adult human CNS and study whether they repair the damaged CNS tissues (7.8).

Clonal neural stem cells derived from adult human brain were established in our facility (9). Single-cell clonal analysis demonstrated self-renewing and multipotential properties of neural stem cells derived from the adult human brain in vitro. The

genetically marked and engineered clonal stem cells were transplanted into several CNS disease models such as stroke, head trauma, spinal cord injury, dementia, and demyelinating diseases, in order to investigate if neural tissue reconstruction occurred following transplantation. Histological and electrophysiological examinations following transplantation revealed that the transplanted human stem cells functionally reconstructed the neural tissue in and around the damaged CNS tissues (9).

In summary, the characteristics of adult human stem cells are that they have high proliferating potential, are multipotential and their presence is seen even in the adult human CNS. They showed good adaptation in the host CNS tissue, and migrated through the normal and damaged CNS tissue subsequent to transplantation. They accumulated in and around the damaged lesion, and differentiated upon the host condition. These characteristics of adult human stem cells seem to be useful for establishing a strategy for a cell therapy for CNS diseases. They offer a potential donor source for auto-transplantation therapy.

List of Main Publications from 1997 to 2000

1) Tatewaki K, Yamaki T, Maeda Y, Tobioka H, Piao H, Yu H, Ibayashi Y, Sawada N, Hashi K. Cell density regulates crypticity of GM3 ganglioside on human glioma cells. Exp Cell Res 233:145-154 (1997).
2) Tanabe S, Uede T, Nonaka T, Ohtaki M, Hashi K. Diagnosis of cerebral arteriovenous malformations with three-dimensional CT angiography. J Clin Neuroscience 5 (Suppl.1): 33-38 (1998).
3) Ohtaki M, Uede T, Morimoto S, Nonaka T, Tanabe S, Hashi K. Intellectual functions and regional cerebral haemodynamics after extensive omental transplantation spread over both frontal lobes in childhood Moyamoya disease. Acta Neurochir (Wien) 140:1043-1053 (1998).
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6) Nakagawa T, Hashi K, Kurokawa Y, Yamamura A. Family history of subarachnoid hemorrhage and the incidence of asymptomatic, unruptured cerebral aneurysms. J Neurosurg 91: 391-395 (1999).
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9) Akiyama Y, Honmou O, Kato T, Hashi K. Transplantation of clonal neural stem cells derived from adult human brain elicits functional repair of demyelinated spinal cord axons in the adult rat. Restorative Neurology and Neuroscience 16:15 (2000).

Obstetrics and Gynecology

Our departmental goal is to provide the best healthcare for women with an advanced commitment to education and research. Our subspecialities include gynecologic oncology, reproductive endocrinology and infertility, and maternal-fetal medicine. Current research interests are cytopathological, molecular biological study of gynecologic cancer for diagnosis and treatment, clinical study of vaginal surgery, and the molecular endocrinological study of ovary.

Professor
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Interests:
Oncology and Surgery

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Interests:
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Interests:
Oncology and Surgery

Toshiaki Endo, M.D., Ph.D.
Interests:
Reproductive endocrinology

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Interests:
Oncology and Pathology

Tsuyoshi Saito, M.D., Ph.D.
Interests:
Oncology and Pathology

Masaki Takehara, M.D., Ph.D.
Interests:
Oncology and Cytology

Instructor
Akira Nishikawa, M.D., Ph.D.
Takuhiro Hayashi, M.D., Ph.D.
Shinichi Ishioka, M.D., Ph.D.
Tomoko Fujikawa, M.D.

1. Clinical research

a) Surgery

Gynecologic Surgery, especially through the vagina is also actively analyzed in our department, including total vaginal hysterectomy and radical vaginal hysterectomy (1). Clinical studies on new operative procedures for extended and radical hysterectomy with preservation of bladder function (2).

b) Combination chemotherapy for primary，advanced，or recurrent cervical adenocarcinoma (3)

2. Analysis of the invasion and metastasis mechanisms for gynecologic malignant tumors.

a) MMPs and angiogenesis

We are analyzing various gene expressions and their functions in gynecologic malignant cell lines using molecular biological techniques. We reported that specific types of MMPs, angiogenesis factor and transcription factors are important for the invasion of ovarian cancer cells. Currently, we are attempting to regulate cancer invasion by inhibiting these molecules (4).

b) Analysis of the molecules related to cellular motility

We studied the difference between human endometrial undifferentiated cancer cell lines with different motilities. In the high-motility cell line only cytokeratin 19 was observed , and the level of cytokeratin 19 mRNA was elevated. Thus, motility is suggested to be related to the induction of cytoskelton (5).

3. Analysis of cell adhesion molecules and telomerase activity in endometrial cancer

We have shown that Cx26 and Cx32 genes are expressed during the secretory phase of the menstrual cycle, and that their expression is down-regulated during the proliferative phase, suggesting a role of intercellular communication in cell growth control. In another study, we have shown that telomerase activity is detected in normal endometrium in association with proliferation and regulated during the menstrual cycle in a hormone-dependent manner (6.7).

4. Genetic diagnosis and analysis for gynecologic malignant tumor

Genetic analysis of ovarian and endometrial cancers is also another topic and several genetic abnormalities, such as beta-catenin, telomerase activity, STK11 gene, SNP of MMP-1 gene, and c-src have been examined in both malignancies (8).

5. Drug resistance and apoptosis in chemotherapy of ovarian cancer (9)

6. Reproductive endocrinology

We have studied ovarian physiology and pathology as regards reproductive endocrinology. Recently, we found some mechanisms of structural involution of corpus luteum. Using a

treated rat model, we found that MMP activation and apoptosis are two major phenomena during structural luteolysis. MMP-2 activated with MT1-MMP and MT1-MMP itself caused remodeling of extracellular matrix in corpus luteum (10.11). We have also investigated the mechanisms of ovarian hyperstimulation syndrome (OHSS). VEGF is known to be a pivotal factor of OHSS. We found that continuation of GnRHa for some days after hCG injection significantly reduced VEGF in ovaries of the rat OHSS model. The mechanism of anovulation in PCOS patients is still unknown. This experiment showed that anovulation of PCO could be caused by reduction of MMP expression and increases in lysyl oxidase, which initiates cross-link formation of the collagen and elastin.

7. Uterine cervical cancer and human papillomavirus (HPV).

Certain HPV types have been associated with uterine cervical cancer. We are investigating the mechanism of carcinogenesis by HPV and the relationship between HPV status and the clinical feature of patients with cervical cancer (12).

8. Characterization of beta-casein like protein (BCLP)

We produced a monoclonal antibody, 1C5, which reacted specifically with cervical adenocarcinoma of the uterus. Interestingly, 1C5-defined antigen exhibited immunological characteristics similar to those of bovine beta-casein. We isolated cDNA encoding the 1C5-defined antigen as BCLP in cervical adenocarcinoma cell line (13).

9. Electron microscopic examination of cytologic samples.

Using a previously reported method, we analyzed cytologic specimens over a seven-year period and investigated whether histologic origin and malignancy can be estimated from SEM and TEM findings on the cells (14).

10. Mechanism of pretern labor and premature rupture of membranes

We have analyzed vaginal bacterial cultures and clinical outcomes in 1000 pregnancies delivered at our hospital. The detection rates of some specific bacterial strains were significantly higher in preterm births with PPROM.

List of Main Publications from 1997 to 2000

1) Kudo R, Sage S, Itoh E, Hayakawa O, Koizumi M, Isioka S. A report on more than 10,000 vaginal hysterectomies performed without ligation of the paracervical ligaments. J Obstet Gynaecol Res 26:395-399 (2000).
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11) Kiya T, Endo T, Henmi H, Goto T, Kitajima Y, Manase K, Takahashi S, Kudo R. The effects of growth hormone one corpus luteum of superovualted rats. Endocr Res 25:179-193 (1999).
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13) Suzuki T, Koizumi M, Baba T, Kudo R. Clonig and characterization of a cDNA fragment coding β-casein like protein preferentially expressed in cervical adenocarcinoma cell like CAC-1. Cancer Lett 124: 165-171 (1998).
14) Ito E, Nei H, Noda M, Saito T, Koizumi M, Kudo R. Electron microscopic examination of cytologic samples. Acta Cytol 42: 1095-1103 (1998).

Pediatrics

Our department is concerned with infectious disease, blood and neoplastic disease, neurologic disease and congenital heart disease. Isolation and characterization of the infectious agents are studied to explain symptoms. Hematopoietic stem cell transplantation was included in the treatment for bone marrow reconstitution. Vagal nerve stimulation is started for patients with intractable seizures unresponsive to anticonvulsant. Interventional catheterization for congenital heart disease has been performed.

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Interests:
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Interests:
Clinical microbiology

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Interests:
Neurology

Instructor
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Shigeto Fuse, M.D., Ph.D.
Nozomi Ito, M.D., Ph.D.
Nobuo Mizue, M.D., Ph.D.

1. Viral gastroenteritis

Mexico virus (MXV) is a genogroup II human calicivirus (HuCV). In this study, we conducted an epidemiological study to determine the prevalence of MXV infection in infants and adults in Japan and Southeast Asia by enzyme-linked immunosorbent assays (ELISA). A low detection rate of MXV antigen in diarrheal stool samples was observed, whereas a high prevalence of antibody to MXV was observed, suggesting that MXV infection is common in these areas (1). Norwalk virus (NV) and Sapporo virus (SV) were approved as type species of the Genus "Norwalk-like viruses" and the Genus "Sapporo-like viruses", respectively, in the Family Caliciviridae. To clarify the importance of NV and SV as causes of gastroenteritis outbreak in infants, stool samples obtained from 36 outbreaks of non-bacterial gastroenteritis were examined for diarrhea viruses. Our data indicate that NV and SV were the most common cause of outbreaks of viral gastroenteritis in infants, and more prevalent than Rotavirus-A, in Sapporo, Japan from 1976 to 1995 (2).

2. Respiratory syncytial virus (RSV) infection

Significant activities of inflammatory cytokines such as IL-1, IL-6, IL-11, TNF-a have been observed in the respiratory tract secretions collected from infants and children during the acute phase of RSV infection. We analyzed the mechanism of IL-1 induction in RSV infected neonatal monocytes. Subsequently we explored the involvement of apoptosis in RSV-induced cell death in terms of activation of apoptosis-associated genes, such as IRF-1 and ICE (3). Finally as one of substances affecting apoptosis, the induction of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) in RSV-infected respiratory epithelial cells has been demonstrated (4). These events at the cellular level during early phase of RSV infection may contribute to the pathophysiology of RSV lower respiratory tract disease and may be involved in the development of host immune responses.

3. Feto-maternal infection

Chlamydia trachomatis infection includes neonatal conjunctivitis, infantile pneumonia, and occasional respiratory or genital infections in older children and sexually transmitted diseases in adolescents. The serovars that we identified from Japanese infants and pregnant women were similar to those reported in other studies from non-trachoma-endemic area (5).

Macrophages and natural killer cells are considered the main immunomodulating cells that control activities of infecting cells in primary and latent cytomegalovirus (CMV) infection, and these produce IFN-γ and TNF-α to express antiviral activity. It is presumed that these cytokines are involved not only in the development of persistent CMV infection and reactivation of CMV

infection but also in infectious transmission to the fetus. Numazaki et al. (6) found that serum levels of soluble IL-2 receptor and IFN-g were elevated during pregnancy in mothers who delivered babies with congenital CMV infection.

4. Hematology and oncology

Cytomegalovirus (CMV) disease is a major complication in patients with bone marrow transplantation (BMT). Recent advances in the diagnosis of CMV reactivation or infection, such as the detection of CMV antigenemia, permits prevention of the disease. CMV retinitis is a disease, which develops without detectable CMV antigenemia or CMV genome in peripheral blood mononuclear cells, as showed by PCR (7). Following allogeneic BMT, 40 to 60 percent of patients develop pneumonia. Idiopathic pneumonia syndrome (IPS) is a disease about which documented cases of infection are lacking and there is uncertainty regarding its precise etiology. A suggested cause of lung damage in patients with severe IPS includes graft-versus-host disease after transplantation from a mismatched family donor (8).

5. Neurological disease

We have analyzed ictal manifestations and simultaneous EEG findings of various seizures in our approximately 600 patients with pediatric epilepsies by means of a Video-EEG monitoring system (9.10). Based on these analyses of seizure symptoms, we try to classify the exact seizure types of each patient. According to each determined seizure type, we make diagnoses and select appropriate anti-epileptic drugs for our epilepsy patients. For patients with intractable epilepsies whose seizures are refractory to any anti-epileptic drugs now available in Japan, we try various non-pharmacological therapeutic approaches including ketogenic diet, vagal nerve stimulation therapy and epilepsy surgery in order to improve the quality of life of the patients.

6. Cardiovascular disease

Interventional catheterization has been performed for patients with congenital heart disease. We are one of pioneers of coil embolization for patent ductus arteriosus in Japan (11). Successful treatment has been performed for 70 cases of Patent ductus arteriosus; 15 cases of Coarctation of aorta; 20 cases of pulmonary stenosis and 10 cases of Aortic valve stenosis. Moreover, we have been developing an innovative transcatheter method for Atrial septal defect with our new device. The relationship between plasma thromboxane B2, CT images of the lung as a non-invasive assessment and pulmonary hypertension with congenital heart disease has been clarified (12). We have just clarified the change in size of Atrial septal defect size by means of echocardiography.

List of Main Publications from 1997 to 2000

1) Honma S, Nakata S, Kogawa K, Numata K, Yamashita T, Oseto M, Chiba S. Epidemiological study of prevalence of genogroup II human calicivirus (Mexico virus) infections in Japan and Southeast Asia as determined by enzyme-linked immunosorbent assays. J Clin Microbiol 36: 2481-2484 (1998).
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Ophthalmology

Our department had consisted of 5 major units; neuro-ophthalmology & orbital disease, glaucoma, strabismus & amblyopia, cornea, and vitreo-retinal units. Recently two new units, cataract and low-vision have been joined our clinic. These units collaborate with each other to conduct clinical practice and basic research into the visual sciences. Our goal is to build up a vision center for all patients who are suffering from visual disturbances.

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Neuro-ophthalmology, Orbital disease

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Vitreo-retinal disease

Masahiro Ohba, M.D., Ph.D.
Interests:
Strabismus & amblyopia

Masato Hashimoto, M.D., Ph.D.
Interests:
Neuro-ophthalmology

Instructor
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Shinsuke Konno, M.D., Ph.D.
Yoshinori Mitamura, M.D., Ph.D.

1.Neuro-ophthalmology & Orbital Disease

In the field of neurophysiology, we have studied the function of the superior colliculus in the control of accommodation. We have found that the area centralis of the superior colliculus is involved in the control of accommodation and vergence eye movements (1). The accommodation-related area in the rostral superior colliculus contains neurons whose axons collateralize to project both to the pretectal accommodation-related areas and the ominipause neuron area in the RIP. It is possible that neurons in the rostral superior colliculus are involved in the interaction between accommodation and active fixation (2). We have been studying the cerebellar vermis, lobules VI-VII (oculomotor vermis) in the control of eye movements. Recently we stimulated the oculomoter vermis by a transcranial magnetic stimulator in man, and showed that the stimulation of the oculomotor vermis modulates the velocity of smooth pursuit eye movements (3). Ipsiversive and contraversive pursuit were accelerated and decerelated by stimulation of one side of the oculomotor vermis, respectively. We also study the neuronal mechanisms of motion perception in the lateral suprasylvian (LS) area of the cat. The amplitude of visually evoked potentials was not altered by muscimol injection into the superior colliculus when the velocity of visual motion stimulus was $\leq 50^{\circ}$/s. On the other hand when the velocity of visual motion stimulus was $\geq 75^{\circ}$/s, the amplitude of visually evoked potentials was reduced to 62 -72% of that noted before the muscimol injection. These findings suggest that the LS area pocesses visual motion inputs reaching through the two parallel pathways, the geniculostriate pathway and the tectothalamocortical pathway, when the velocity of visual motion is $\geq 75^{\circ}$/s (4).

Our clinical reseach interests include neuroimmunology and neuroradioology of eye movement disorders. We have shown that fat-saturation enhanced magnetic resonance imaging of the orbit is useful for diagnosis of paralysis of the inferior division of the oculomotor nerve (5). And we have also studied diagnosis and surgical management of patients with orbital diseases such as thyroid associated ophthalmopathy and orbital tumors. Recently we evaluated the association of HLA types in Japanese patients with Graves ophthalmopathy (6). The frequencies of HLA-DR14 and DQ1 were significantly higher in a patient group with severe ophthalmopathy than in healthy controls, and also greater than in the patient group without ophthalmopathy. HLA-DR14 and DQ1 antigens may be genetic markers of a predisposition to the development of severe ophthalmopathy.

2. Glaucoma

We have studied retinal ganglion cells recognized by serum autoantibody in glaucoma patients, and done optic disc analysis of normal tension glaucoma and pseudoglaucoma (7-9).

3. Strabismus & Amblyopia

We have studied surgical treatment for lateral deviation of exotropia (10). We conclude that monocular recess-resect surgery gives more restriction to the rotation of the eye towards

the recessed side than that of bilateral recession in the early postoperative period. In a few cases consecutive ET persisted only in the recessed side of the monocular surgery group. Preoperative evaluation of the lateral deviation is essential to the strategy of surgery and to minimize the postoperative lateral incomitances and consecutive esotropias.

We studied A - V strabismus and exotropia by slanting muscle insertions (11.12). We conclude that the surgical technique of slanting muscle insertions for correcting exotropia of "A" pattern with superior oblique muscle overaction and "V" pattern without moderate or marked inferior oblique muscle overaction is suitable for reducing "A" and "V" patterns.

4. Vitreo-retinal Disease

We have undertaken about three hundred vitroretinal surgeries a year. In these cases, 20 consecutive cases of retinal detachment with macular hole in patients with high myopia were retrospectively studied (13). The surgical results of retinal detachment with macular hole in patients with high myopia by conventional vitrectomy were relatively poor. In those cases, additional surgery, such as scleral shortening, macular bucking, removal of inner limiting membrane may be effective.

We have developed a laser speckle flowgraphy to visualize and evaluate the human pulsatile ocular microcirculatory changes. It can measure real-time two-dimensional blood velocity of human ocular microcirculation. Using it, we could easily estimate blood flow of retinal vessels (14). Furthermore, we could estimate total retinal flow rate in normal volunteers, and the normal range was relatively small (15). Using this technique, we can evaluate the effect of blood flow changes of retinal vessels on drug or surgical treatment.

List of Main Publications from 1997 to 2000

1) Ohtsuka K, Sato A. Retinal projections to accommodation-related area in the rostral superior colliculus in the cat. Exp Brain Res 113: 169-173 (1997).
2) Ohtsuka K, Nagasaka Y. Divergent axon collaterals from the accommodation-related area of the rostral superior colliculus to the pretectal accommodation-related areas and the omnipause area in the cat. J Comp Neurol 413:68-76 (1999).
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15) Tagawa H, Sasaki N, Tashimo A, Suzuki T, Shimizu M, Fujii H: Estimation of real blood velocity measured by laser speckle flowgraphy at retinal vessels. The 71st Annual meeting of the Association for Research in Vision and Ophthalmology. Invest Ophthalmol Vis Sci 1999, 40 (Suppl): 369 (1999).

Dermatology

Our department has been engaged in basic research and clinical treatment of a variety of cutaneous disorders. We are particularly interested in the physiological and pathological mechanisms of melanin biosynthesis with the aim of finding new cures for relevant skin diseases (i.e. malignant melanoma, vitiligo etc.). We have employed a lot of updated technologies such as gene transduction using recombinant adenoviruses in our research projects.

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Dermatological oncology,
Dermatological immunology,
Dermatological physiology

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Photobiology, Immunology, Dermatology

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Interests:
DNA diagnosis and gene therapy of melanoma

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Interests:
Immuno (Gene) therapy of elanoma and lymphoma

Ichiro Ono, M.D., Ph.D.
Interests:
Cytokine modulation of wound healing and laser therapy on pigment ski lesions

Instructor
Yasushi Minamitsuji, M.D., Ph.D.
Yasuhiro Yamada, M.D.

1. Melanogenesis and related disorders

We have recently been focusing on projects related to melanocyte biology, the mechanism of melanin pigmentation, and the pathogenesis of various pigmentary disorders (1). Furthermore, we have a research focus on the better management of skin cancers such as malignant melanoma. Specifically, the latter approach is based upon the utilization of the specific metabolic pathway of melanocytes and melanoma cells, i.e., melanin biosynthesis (2). Melanocyte biology studies include gene transfection of melanin forming enzymes (tyrosinase and tyrosinase related proteins) in order to identify biogenesis of melanosomes as well as to develop targeted chemotherapy and immunotherapy (3).

2. Pathological physiology of skin diseases

We have studied the distribution of anionic sites in normal and pathological skin using cationic colloidal gold (4). We found that extramammary Paget's disease, and eczema- or fungus infection-like carcinoma of the genital area, express anionic sites found in apocrine sweat glands. Patients with atopic dermatitis show many physiological abnormalities. Our study showed that the deactivation system of cholinergic sweat secretion is impaired in atopic dermatitis although there is no abnormality in cholinergic sweat secretion (5). Immunohistochemistry using a confocal laser scanning microscope showed that EGF in sweat stimulates EGF receptors in the nuclei of sweat ducts and secretory cells in paracrine and intracrine manner respectively.

3. Immunological dermatology

Skin is the largest organ, covering the entire body surface. Keratinocytes (KC) are its major component. The KC, by making keratin protein, function as a protective barrier against exogenous stimuli. As KC have been demonstrated to produce various kinds of cytokines, skin plays an important role in immunologic and inflammatory responses of the body. Cytokines affect other cells and organs, mediating cellular growth and differentiation as well as inflammation and immune reactions (6). Thus, cytokines maintain cellular and intercellular homeostasis. Dysregulation and abnormal production of cytokines are detected in various skin diseases. Evidence is accumulating to show the significant contribution of cytokines to the pathogenesis or severity of certain diseases. We have found that ultraviolet light affects KC's production of a variety of cytokines (7), and suggested that they are involved in various pathophysiologic conditions observed in

the skin exposed to sunlight, including erythema formation, immune suppression, carcinogenesis and photoaging.

4. DNA diagnosis and gene therapy of malignant melanoma

There is no sensitive tumor marker which can detect or reflect circulating melanoma cells. In order to establish DNA diagnosis of malignant melanoma, we have developed the RT-PCR method, which amplifies melanoma-specific mRNAs, tyrosinase and its related protein (TRP-1), from peripheral bloods of melanoma patients. Our method can detect as few as ten melanoma cells in 6 ml of blood. By using this RT-PCR, we have analyzed >50 blood samples from melanoma patients and found that our RT-PCR is more sensitive than serum 5-S-CD value (8). We have been studying the molecular basis of p53-mediated apoptosis in cancer cells (9). Recombinant adenoviruses expressing p53 or its related p51A and p73β were constructed and their apoptotic activities compared. We found that p51A induces apoptosis in cancer cells, including melanoma cells, more efficiently than p53 or p73β. Construction of recombinant adenoviruses which express apoptosis-inducing or growth-inhibitory proteins driven by tyrosinase promoter is under way.

5. Immuno (gene) therapy of melanoma

It has been shown that conventional chemotherapy for advanced melanoma with organ metastasis is almost useless. We have been trying to find new therapeutic methods in the light of our knowledge of immunological aspects. To begin with, we plan to soon start vaccination therapy using synthesized peptides of tyrosinase, gp100 and TRP-2. Moreover, sufficient experience of gene manipulation (10.11) has promoted us to take up the challenge of gene therapy by transduction of melanoma cells with several cytokine genes in murine models.

6. Cytokine modulation of wound healing process

Research on the clinical application of cytokines has focused mainly on the treatment of chronic ulcers, and not much research has been done on the effects of cytokines such as EGF or bFGF on the quality of scars or the breaking strength of sutured wounds especially with rather long follow up periods. We think this cytokine therapy may be highly effective clinically not only chronic wounds but also sutured wound. This may be especially true for patients whose wound healing process tends to be delayed such as aged patients or when high breaking strength is recommended as well as to refine the wounds quality, preventing scar formation, even for patients with normal wound healing potential (12.13).

List of Main Publications from 1997 to 2000

1) Jimbow K, Park JS, Kato F, Hirosaki K, Toyofuku, Hua C, Yamashita T: Assembly, target-signaling and intracellular transport of tyrosinase gene family proteins in the initial stage of melanosome biogenesis. Pigment Cell Res. 13: 222-229 (2000).
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6) Kondo S, Kooshesh F, Sauder DN: Basement membrane is not a barrier for cytokine permeability. J Cell Physiol. 171: 190-195 (1997).
7) Kondo S, Jimbow K: Dose-dependent induction of IL-12 but not IL-10 from human keratinocytes after exposure to UVA. J Cell Physiol. 177: 493-498 (1998).
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Urology

Medical staff in the department have dedicated themselves to better care for patients with urological diseases. We provide various strategies for treatment of the diseases, with a view to patient satisfaction. These include function-preserved radical surgeries for cancer and minimally invasive treatment such as laparoscopic surgery. We are also enthusiastic about studying the basic science of urology that will lead to a future innovative treatments. Integration of humanity, art and science is our final goal.

Professor
Taiji Tsukamoto, M.D., D.Med.Sci.
Interests:
Oncology, Urinary tract infection, Andrology, BPH

Associate Professor
Naoki Itoh, M.D., Ph.D.
Interests:
Male infertility, Physiology of prostate, Laparoscopic surgery

Assistant Professor
Yoshikazu Sato, M.D., Ph.D.
Interests:
Erectile dysfunction, Physiology of lower urinary tract, Female urology

Instructor
Atsushi Takahashi, M.D., Ph.D.
Masahiro Yanase, M.D., Ph.D.
Masanori Matsukawa, M.D., Ph.D.
Hideki Adachi, M.D.
Yasuharu Kunishima, M.D.
Satoshi Takahashi, M.D., Ph.D.

1. Urinary tract infection (UTI) and sexually transmitted disease (STD)

Our projects in this field include “pathogenesis and local immunity of UTI”, “experimental chemotherapy”, “STD” and “nosocomial infection” (1-4).

2. Andrology

a) Male infertility

Vasoreconstruction including vasovasostomy, vasoepididymostomy and transurethral resection of ejaculatory duct obstruction, for obstructive azoospermia and microscopic testicular sperm extraction (TESE) for non-obstructive azoospermia has been performed to provide the chance of pregnancy for these patients. Our research has focused on apoptosis as one of causes of hypospermatogenesis in male infertility and aged males.

b) Sexual function

b-1) Central mechanism of penile erection

We established a unique animal model to investigate the physiology of penile erection (5). With this animal model, we have been studying the roles of central neurotransmitters in modulation of sexual behavior and penile erection (6). Furthermore, hormonal replacement restored these neurotransmissions diminished by aging (7).

3. Urologic oncology

a) Renal cell carcinoma (RCC)

We have been interested in the mechanism of invasion and metastasis in RCC (8). Recently, we have focused on angiogenesis, which is one of the biological characteristics of RCC. We have demonstrated that VEGF-VEGF receptors pathway plays a major role in angiogenesis in RCC (9).

b) Bladder cancer

Detection of LOH (Loss of heterozygosity) on chromosome 9 from urine samples was revealed to be a more sensitivity diagnostic modality than cytological examination (10).

c) Prostate cancer

Better care of patients with prostate cancer in the clinical setting has been our end goal. In particular, hormone-refractory prostate cancer has been a target for our clinical and experimental research (11.12).

4. BPH and voiding function.

a) BPH

We revealed the epidemiology and natural history of BPH in men of the Hokkaido area and participated in an international study on BPH (13). In addition, we have studied the regulation of

prostate growth, in which we found that TGF-beta was one of the factors responsible for the interaction between epithelial and stromal cells (14).

b) Voiding function.

The autonomic nervous system in the pelvis was studied by the whole mount staining method, and the right-left communicating nerves between bilateral pelvic plexus were identified (15).

5. Urinary reconstruction and laparoscopic surgery.

a) Urinary reconstruction

For a better QOL for patients receiving radical surgery for prostate or bladder cancer, we have improved the surgical procedures for these diseases (16).

b) Laparoscopic surgery

Laparoscopic adrenalectomy, simple nephrectomy, pyeloplasty and pelvic lymphadenectomy have been routinely performed. Hand-assisted radical nephrectomy and nephroureterectomy are also indicated for patients with early stage renal cell carcinoma and upper urinary tract carcinoma. Laparoscopic surgery accounts for 15% of all the operations in this department annually.

List of Main Publications from1997 to 2000

1) Yokoo A, Hirose T, Matsukawa M, Hotta H, Kunishima Y, Takahashi S. Expression of intercellular adhesion molecule-a in mice with Pseudomonas induced pyelonephritis. J Urol 160: 592-596 (1998).
2) Takahashi S, Sano M, Nishimura M, Matsukawa M, Hirose T, Tsukamoto T. Bactericidal effects of levofloxacin on strains with equal susceptibility in an in vitro urinary bladder model. Chemotherapy 44: 337-342 (1998).
3) Takahashi S, Hagiwara T, Shiga S, Hirose T, Tsukamoto T. In vitro Analysis of Chlamydia trachomatis under exposure to sub-MIC Levofloxacin for a therapeutic term. Chemotherapy 46: 402-407 (2000).
4) Takahashi S, Hirose T, Matsukawa M, Shimizu T, Kunishima Y, Takeyama K, Yokoo A, Hotta H, Mikami M, Tsukamoto T. Analysis of cross infection using genomic fingerprinting in nosocomial urinary tract infection caused by Enterococcus faecalis. J Infect Chemother 5: 46-48 (1999).
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6) Sato Y, Christ JG, Horita H, Adachi H, Suzuki N, Tsukamoto T. The effects of alterations in nitric oxide levels in the paraventricular nucleus on copulatory behavior and reflexive erections in male rats. J Urol 162: 2182-2185 (1999).
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14) Itho N, Patel U, Cupp AS, Skinner MK. Developmental and hormonal regulation of transforming growth factor beta-1, -2 and -3 gene expression in isolated prostatic epithelial and stromal cells: epidermal growth factors and transforming growth factor beta interactions. Endocrinology 139: 1378-1388 (1998).
15) Taguchi K, Tsukamoto T, Murakami G. Anatomical studies of the autonomic nervous system in the human pelvis by the whole-mount staining method: Left-right communicating nerves between bilateral pelvic plexuses. J Urol 161: 320-325 (1999).
16) Kitamura H, Miyao N, Yanase M, Masumori N, Matsukawa M, Takahashi A, Itoh N, Tsukamoto T. Quality of life in patients having an leal conduit, continent reservoir or orthotopic neobladder after cystectomy for bladder cancer. Int J Urol 6: 393-399 (1999).

Otolaryngology

Our department has been dealing with a variety of diseases in the field of otolaryngology, such as sensorineural hearing loss, otitis media, head and neck tumor, sleep apnea syndrome, tonsillar focal infection, and so on. We are confident that our department has been at the leading edge of research concerning tonsil and adenoid. We are also getting noteworthy data about the pathogenesis of many other diseases by studies in immunology, microbiology, biochemistry and molecular biology.

Professor
Tetsuo Himi, M.D., Ph.D.
Interests:
Otology, Defense mechanism of upper respiratory tract

Assistant Professor
Hiroshi Tsubota, M.D., Ph.D.
Interests:
Immune system of upper respiratory tract, Head and neck tumor

Hideaki Shirasaki, M.D., Ph.D.
Interests:
Nasal allergy

Instructor
Tomoko Shintani, M.D., Ph.D.
Motoyasu Hirao, M.D., Ph.D.
Masami Kamimura, M.D., Ph.D.
Hisao Murakata, M.D., Ph.D.
Masato Hata, M.D.
Hiroaki Mitsuzawa, M.D.

1. Tonsil and Immune system of upper respiratory tract

This field is one of the most important parts of research in our department. To assess the mechanisms of tonsillar focal infection, immunological, bacteriological and biochemical approaches to basic tonsillar functions have been applied. We have shown the specific character of tonsillar lymphocytes, bacterial flora of tonsillar cryptes, the roles of cell adhesion molecules on tonsillar tissues, and recently we have proved the unique cytokine production by adenoidal and tonsillar epithelium (1), and tonsillar lymphocytes stimulated with alpha-streptococci, which add new aspects to the thesis of the cause of tonsillar focal infection (2).

We have also analyzed the treatment results of the tonsillar infection cases statistically.

2. Nasal Allergy

Using animal models and human specimens, several projects are going on simultaneously in our department.

First, to clarify the mechanisms of Th2 cell homing, ovalbmin-specific T cell receptor-transgenic mice are used with nasal drip of antigen. Second, to know the role of local steroid application for improvement of nasal polyp with eosinophil infiltration, we are studying the mechanisms of steroid-induced apoptosis of eosinophils by means of molecular biology (3). Localization and expression of various chemical mediators, neuropeptides, and regulation of mucin-gene expression are also being studied using human nasal mucosa (4).

3. Otology

Two subjects in this field are main stream in our department. One is the study of the mechanisms of otitis media with effusion (OME) and the other is clinical evaluation of cochlear implant (CI).

To clarify the pathogenesis of OME, we are carrying out several projects. First, from the microbiological point of view, we have attempted to understand the role of *Alloiococcus otitidis* and have showed that the bacteria is involved in the pathogenesis of OME (5). At the present time, we are determining the specific antigenic structure of *A otitidis.* Another project concerns the Eustachian tube. Two different approaches are being pursued in our department. Immunologically, the MALT system around Eustachian tube has been characterized (6). And biochemically, the distribution and contribution of surfactant Sp-A and Sp-D to the tubal system are being studied.

In our department, CI surgery is actively performed for many patients with severe sensorineural hearing loss under a variety of conditions (7). Nowadays, young patients are beginning to constitute the majority of CI cases. We have established a rehabilitation unit including doctors and co-medical staff. We are also studying high dimensional cerebral function after CI using SPECT on the basis of evaluations of speech recognition in

patients with CI (8).

4. Obstructive sleep apnea syndrome

We have summarized the characteristics of a number of patients suffering from obstructive sleep apnea syndrome (OSAS). To establish the indications for each treatment, we have applied a series of examinations. Then we published the data about our morphological analysis and the value of tonsillectomy and adenoidectomy in children with OSAS (9).

At the present time, we are demonstrating that dynamic MRI is very useful to diagnose the site of obstruction. We are also applying auto-CPAP for diagnostic measures.

5. Head and Neck tumor

To establish diagnostic and therapeutic strategies, clinical and immunological research into head and neck tumors is carried out in our department.

First of all, a clinical evaluation of non-Hodgkin lymphoma of Waldeyel's ring based upon our analysis about prognostic factor has been published (10).

Secondly, as a molecular biological approach, the expression of IAP, especially Survivin, has been measured at mRNA level in fresh tumor tissue by way of RT-PCR to see if it becomes a good marker of prognosis. Now we are accumulating data about many types of head and neck tumors. We are also studying the specific tumor antigen of head and neck tumor cells, and inducing the tumor specific T-cell line in collaboration with the department of pathology section1 of our university. We are preparing publications about these projects.

List of Main Publications from 1997 to 2000

1) Ishikawa T, Ohguro S, Yoshioka I, Harabuchi Y, Himi T, Kataura A. In vivo cytokine production of adenoidal and tonsillar epithelial cells. In: Tos M, Thomsen J, Balle V (eds). Otitis Media Today. pp273-277. Kugler. Hague. Netherland (1999).
2) Murakata H, Harabuchi Y, Kataura A: Increased interleukin-6, interferon-gamma and tumor necrosis factor-alpha production by tonsillar mononuclear cells stimulated with alpha-streptococci in patients with pustulosis palmaris et plantaris. Acta Otolaryngol 119 (3): 384-391 (1999).
3) Shirasaki H, Watanabe K, Kanaizumi E, Kataura A: Analysis of apoptotic cells in human nose. Rhinol suppl 15: 113-114 (1999).
4) Shirasaki H, Asakura K, Narita S, Kataura A: Expression of substance P (NK1) receptor mRNA in human nose. Acta Otolaryngol 118: 717-722 (1998).
5) Himi T, Kita H, Mitsuzawa H, Harimaya A, Tarkkaren J, Hendolin P, Ylikoski J, Fujii N: Effect of alloiococcus otitidis and three pathogens of otitis media in production of interleukin-12 by human monocyte cell line. FEMS Immunol Med Microbiol 29 (2): 101-106 (2000).
6) Kamimura M, Balaban CD, Sando I, Ganbo T, Suzuki C: Cellular distribution of mucosa-associated lymphoid tissue with otitis media in children. Ann Otol Rhinol Laryngol 109: 467-472 (2000).
7) Himi T, Harabuchi Y, Shintani T, Yamaguchi T, Yoshioka I, Kataura A: Surgical strategy of cochlear implant in patients with chronic middle ear disease. Audiol Neurootol 2: 410-417 (1997).
8) Shintani T, Himi T, Yamaguchi T, Kataura A: Longitudial analysis of hearing ability and electrodes status in post-lingual deaf adults with cochlear implantation. Adv Otorhinolaryngol 52: 229-232 (1997).
9) Shintani T, Asakura K, Kataura A: The effect of adenotonsillectomy in children with OSAS. Int J Pediatr Otorhinolaryngol 44: 51-58 (1998).
10) Harabuchi Y, Tsubota H, Ohguro S, Himi T, Asakura K, Kataura A, Ohuchi A, Hareyama M: Prognostic factors and tratment outcome in non-Hodgkin's lymphoma of Waldeyel's ring. Acta Oncol 36: 413-420 (1997).

Neuropsychiatry

The scope of our research activities covers a broad range of topics from psychosocial psychiatry to biological psychiatry, including the brain mechanism of mental disorders such as alcohol and drug dependence, mood disorders, schizophrenia, senile dementia as well as research on the theory and practice of clinical psychology, including group psychotherapy and self help systems for mental patients.

Professor
Toshikazu Saito, M.D., Ph.D.
Interests:
Alcohol and drug dependence

Associate Professor
Hiroki Ozawa, M.D., Ph.D.
Interests:
Mood disorder and schizophrenia

Assistant Professor
Takeshi Ashizawa, M.D., Ph.D.
Interests:
Group psychotherapy for chronic pain and palliative patients

Norihito Nakano, M.D., Ph.D.
Interests:
Senile dementia

Instructor
Sadamu Toki, M.D., Ph.D.
Eri Hashimoto, M.D., Ph.D.
Satoshi Saito M.D.
Hiroki Ishikawa M.D.
Yorihide Hayashi M.D.

1. Neurochemistry and molecular biology on alcohol dependence, mood disorder and schizophrenia

Research in this department focuses on the molecular mechanisms of information processing in neuronal cells. In particular, we are studying the function of G-proteins-mediated signal transduction in neuropsychiatric disorders.

Altered intracellular signal transduction in alcoholic brain has been proven by many neurochemical investigations in the last 20 years. Ethanol acts specifically on certain G proteins in the synaptic cell membrane and altered cAMP signaling was observed in ethanol-treated animals and alcoholics. The cAMP response element binding protein (CREB) is a transcription factor of particular interest as a downstream target in the cAMP signaling pathway. CREB is involved in regulation of genes essential for neuronal function and production of important proteins, such as brain-derived neurotrophic factor (BDNF). Increased adenylyl cyclase (AC) activity via activation of stimulatory G protein (Gs) is induced by acute exposure to ethanol, whereas chronic exposure to ethanol attenuates AC activity through inactivation of Gs in cells and tissues. Previously, our studies with post-mortem human brains reported decreased amount and functions of the G proteins in the cerebral cortex of alcoholics. Moreover, a reduced level of the type I adenylyl cyclase (AC-I) has been found in the temporal cortex of alcoholics. These findings suggest a disturbance of the cAMP signal transduction mediated by dysfunctional G proteins and impaired particular AC isoform in alcoholic and heroin addicted brain (1-4).

We have established a cooperation with Prof. P. Riederer in the university of Wuerzburg (Germany) and with the German-Austro Brain Bank, which is one of the biggest European brain Banks, and performed postmortem studies on brain signal transduction and its relation to the pathophysiology of psychiatric disorders since 1992. Our previous works have demonstrated an imbalance between Gs and Gi functions and decreased cAMP production in cortices of depressed patients, suggesting that antidepressants improve this imbalance. We have found a decreased cAMP production in the frontal cortex of depressive subjects, an increased cAMP production in the same brain region from schizophrenics, and an increased 5-HT stimulated-inositol phosphates production in the frontal cortex in both disorders (5.6). Moreover, we have reported that the levels of G proteins, which are the important brain signal transduction-related molecules, increase by 200-500% between 0-5 years of age and decrease to the levels at birth between 5-10 years of age in human frontal cortex (7). Recently we discovered novel antidepressant target genes (8).

2. Clinical cognitive science

It has been reported that Alzheimer's disease (AD) patients show drawing and visuospatial impairments from early clinical stage. We found that AD patients show eye-head coordination abnormalities, which consist of a reduction of gaze accuracy, head share and prolonged latency of saccade (9). AD patients had

reduced rCBF in the parieto-occipital lobe. Donepezil (AchE inhibitor) treatment increased the rCBF of the parietal and frontal lobe in the same AD patients (10).

3. Neuropathology

Advanced glycation end products (AGEs) have been implicated in the chronic complications of diabetes mellitus and have been reported to play a role in the pathogenesis of AD. We examined the immunohistochemical localization of AGEs, Aβ, and apolipoprrptein E (ApoE) in senile plaques, neurofibrillary tangles (NFTs) and cerebral amyloid angiopathy (CAA) in AD brain. In most senile plaques and CAA from AD brain, AGE and ApoE were observed together. However, approximately 5% of plaques were AGE positive but Aβ negative, and the vessels without CAA often showed AGE immunoreactivity. In AD, AGEs were mainly present in intracellular NFTs, whereas ApoE was mainly present in extracellular NFTs. These results suggest that AGE may contribute to eventual neuronal dysfunction and death as an important factor in the progression of AD (11). In addition we examined signal tranduction systems in AD brains and platelets (12-14).

4.Clinical psychotherapy

This section is involved in research on the theory and practice of group psychotherapy using Morita therapy and self-help systems for chronic pain, palliative care, chronic schizophrenia, and eating disorders (15).

List of Main Publications from 1997 to 2000

1) Sohma H, Hashimoto E, Shirasaka T, Tsunematsu R, Ozawa H, Boissl K.W, Boening J, Riederer P, Saito T. Quantitative reduction of type I adenylyl cyclase in human alcoholics. Biochim Biophys Acta 1454: 11-18 (1999).
2) Hashimoto E, Froelich L, Ozawa H, Saito T, Maurer K, Boning J, Takahata N, Riederer P. Reduced immunoreactivity of type Ⅰ adenylyl cyclase in the postmortem brains of alcoholics. Alcohol Clin Exp Res 22: 88S-92S (1998).
3) Ikeda H, Hatta S, Ozawa H, Ohshika H. Saito T. Effect of chronic ethanol treatment of Ca^{2+}-inhibited adenylyl cyclase in mouse striatum. Alcohol Clin Exp Res 23:7S-10S (1999).
4) Shichinohe S, Ozawa H, Saito T, Hashimoto E, Riedere P, Takahata N. Differential alteration of adenylyl cyclase subtypes Ⅰ, Ⅱ and V/VI in postmortem human brain of heroin addicts. Alcohol Clin Exp Res 22: 84S-87S (1998).
5) Ozawa H, Takahata N. The role of G proteins in pathophysiology and treatment of affective disorders In: Ozawa H, Saito T, Takahata N, (eds) Signal Transduction in Affective Disorders. Springer-Verlag. Tokyo. 49-65 (1998).
6) Toki S, Donati RJ, Rasenick MM. Treatment of C6 glioma cells and rats with antidepressant drugs increases the detergent extraction of G (s alpha) from plasma membrane. J Neurochem 73: 1114-1120 (1999).
7) Ozawa H, Ukai W,Kornhuber J,Yamaguchi T,Froelich L, Ikeda H, Saito T, Riederer P. Postnatal ontogeny of GTP binding protein in the human frontal cortex. Life Sci 65: 2315-2323 (1999).
8) Yamada M, Yamada M, Yamazaki S, Takahashi K, Nishioka G, Kudo K, Ozawa H, Yamada S, Kiuchi Y, Kamijima K, Higuchi T, Momose K. Identification of a novel gene with RING-H2 finger motif induced after chronic antidepressant treatment in rat brain. Biochem Biophys Res Commun 11: 278:150-157 (2000).
9) Nakano N, Hatakeyama Y, Fukatsu R, Hayashi S, Fujii M, Fujimori K, Takahata N. Eye-head coordination abnormalities and regional cerebral blood flow in Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 23: 1053-1062 (1999).
10) Nakano N, Fukatsu R, Fujii M, Murakami S, Hayashi S, Saito S, Hayashi Y, Fujimori K, Takahata N. Effects of cholinesterase inhibitor, E2020, on the regional blood flow in Alzheimer's disease as measured by ^{99m}Tc-ECD SPECT. Neurobiol Aging 19: S178 (1998).
11) Sasaki N, Toki S, Chowei H, Saito T, Nakano N, Hayashi Y, Takeuchi M, Makita Z. Immunohistochemical distribution of the receptor for advanced glycation end products in neurons and astrocytes in Alzheimer's disease. Brain Res 12: 888: 256-262 (2001).
12) Yamamoto M, Gotz ME, Ozawa H, Luckhaus C, Saito T, Rosler M, Riederer P.Hippocampal level of neural specific adenylyl cyclase type I is decreased in Alzheimer's disease. Biochim Biophys Acta 15: 1535: 60-68 (2000).
13) Ishikawa H, Ozawa H, Saito T, Takahata N, Takemura H. Calcium mobilization evoked by amyloid β-protein involves inositol 1,4,5- trisphosphate production in human platelets. Life Sci 62: 705-713 (1998).
14) Hayashi Y, Fukatsu R, Tsuzuki K, Yoshida T, Sasaki N, Kimura K, Yamaguchi H, St George-Hyslop PH, Fujii N, Takahata N. Evidence for presenilin-1 involvement in amyloid angiopathy in the Alzheimer's disease-affected brain. Brain Res 13, 789: 307-314 (1998).
15) Ashizawa T, Anazawa T, Honma M. Morita terapeutic approach to chronic pain in combination with group psychotherapy. J. MORITA THERAPY. Brain Res 11: 233-235 (2000).

Radiology

Our department consists of 4 major divisions, namely Radiation Oncology, Diagnostic Imaging, Intervention Radiology, and Nuclear Medicine. There are 9 senior staff, 9 junior staff and 3 residents. We try to work very hard under these guidelines: 1) Radiotherapy for patients with a high quality of life 2) Diagnostic imaging for patients without suffering 3) Education about radiology for the general public 4) Research into radiation oncology.

Professor
Masato Hareyama, M.D., Ph.D.
Interests: Radiation oncology

Associate Professor
Koh-ichi Sakata, M.D., Ph.D.
Interests: Radiation oncology

Assistant Professor
Kazumitsu Koito, M.D., Ph.D.
Interests: Imaging of the liver, biliary tract, and pancreas, Interventional radiology of the abdomen

Kenji Fujimori, M.D., Ph.D.
Interests: General nuclear medicine, Multicompartment model and computer simulation of pharmacodinamics

Instructor
Hidenari Akiba, M.D.
Atsushi Oouchi, M.D.
Mitsuharu Tamakawa, M.D.
Hisayasu Nagakura, M.D.
Hideki Hyodo, M.D., Ph.D.

1. Radiation oncology

a). Clinical radiation oncology

The goal of radiation oncology for malignant tumors is to obtain tumor control with the preservation of organ, function and aesthesia. We have tried to elucidate the prognostic factors of patients treated with radiotherapy for survival and complications by MRI and immunohistochemistry (1.2).

b). Immunologic study on the effect of irradiation

We have investigated the enhancing effect of small doses of irradiation on the cell surface expression of tumor-associated antigen, MHC-class I antigen, c-erb B2 and ICAM-1 in human gastric and colon adenocarcinoma. Enhancement of expression of these antigens would help cytotoxic killer cells recognize the tumor cells (3). We have also investigated several possible factors for determining apoptosis to irradiation.

c). Expression of genes involved in repair of DNA double-strand breaks in tumors

We immunohistochemically investigated the expression of DNA-PKcs, Ku 70, Ku85, XRCC4 and NBS1 in 134 specimens from various normal or tumor tissues with different radiosensitivities. Immunopositivity to Ku70, Ku85, DNA-PKcs, XRCC4 and NBS1 was found in all tumor tissues examined. No apparent differences in nuclear staining intensity were detected in the expression of these five proteins among tumor tissues with different radiosensitivities (4).

2. Diagnostic radiology

Ninety-five percent of clinical MR images and 61.4% of CT images have been evaluated by diagnostic radiologists. We have several clinical conferences (neurology, surgery section2, urology, orthopedic surgery, and gynecology, etc.). We have discussed future research works to the benefits of diagnostic imaging for patients.

We have evaluated MR imaging for histopathological study in the field of the liver (5) and breast (6). We have also studied cancer recurrence using enhanced MR imaging of the head and neck (7) and local invasion or location of prostate cancer by the endorectal coil. We are now evaluating the efficacy of the Adamkiewicz arterial MR angiograms compared with the operative records and cardiac MR perfusion imaging assess cardiac nuclear medicine imaging compared to US. We reported that MR cholangiopancreatography (MRCP) is feasible for follow-up study after surgical resection of IPMT (8).

3. Interventional Radiology

Differentiation of the inflammatory pancreatic mass from ductal cancer is clinically important because their treatments are extremely different. We evaluated the diagnostic efficacy of enhanced ultrasonography (US) by using arterial injection of CO2 microbubbles, and revealed that enhanced US shows higher diagnostic ability of differentiating them compared with DSA (9). An important issue of hepatic interventional radiology is precise

differentiation of hepatocellular carcinoma (HCC) from adenomatous hyperplasia (AH). We compared power Doppler US with color Doppler US to differentiate HCC from AH. Our study revealed that HCC shows pulsatile flows in the nodules only with power Doppler US (10). Power Doppler US is also useful for evaluation of interventional treatments (TAE, PEIT, and PMCT) for unresected HCC (11). Intraductal papillary mucinous tumor of the pancreas (IPMT) shows comparatively better prognosis than ductal cancer of the pancreas. However, there is a problem with recurrence after surgery.

The metallic stent (graft) has established a new field of endovascular therapy. We have produced the Nitinol stent for vascular repair and the stentgraft for aneurysm (co-research with surgery section 2 and The University of Texas MD. Anderson Cancer Center).

4. Nuclear medicine

We have been focusing on the biodistribution and pharmacodynamics of radiopharmaceuticals, particularly 99mTc-GSA (galacotsyl human serum albumin), an analog of asiaologlycoprotein (AGP) which binds to AGP receptors only expressed on the hepatocyte surface membrane. Collaboration with the 1 st. Department of Surgery resulted in the non-linear compartment model to estimate binding capacity of the liver being developed and validated (12). This parameter is meant to be a critical index for hepatectomy candidates with severe liver dysfunction.

In collaboration with the Dept. of Psychiatry, we have started statistical analysis of the brain SPECT images of Alzheimer's disease and other psychiatric diseases to characterize brain perfusion abnormalities and to monitor drug therapy effectiveness.

List of Main Publications from 1997 to 2000

1) Sakata K, Hareyama M, Tamakawa M, Oouchi A, Sido M, Nagakura H, Akiba H, Koito K, Himi T, Asakura K. Prognostic factors of nasopharynx tumors investigated by MR imaging and the value of MR imaging in the newly published TNM staging. Int J Radiat Oncol Biol Phys 43: 273-278 (1999).
2) Sakata K, Oouchi A, Nagakura H, Akiba H, Tamakawa M, Koito K, Hareyama M, Asakura K, Satoh M, Ohtani S. Accelerated radiotherapy for T1, 2 glottic carcinoma: analysis of results with KI-67 index. Int J Radiat Oncol Biol Phys 47: 81-88 (2000).
3) Hareyama M, Imai K, Oouchi A, Takahashi H, Hinoda Y, Tsujisaki M, Adachi M, Shonai T, Sakata K, Morita K. The effect of radiation on the expression of intercellular adhesion molecule-1 of human adenocarcinoma cells. Int J Radiat Oncol Biol Phys 40: 691-696 (1998).
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6) Tamakawa M, Akiba H, Yoshida S, Takeda M, Yama N, Hareyama M. MRI appearance of histological subtype of breast cancer. Radiology 213: 505p (1999).
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8) Koito K, Namieno T, Ichimura T, Yama N, Hareyama M, Morita K, Nishi M Mucin-producing pancreatic tumors: comparison of MR cholangiopancreatography with endoscopic retrograde cholangiopancreatography. Radiology 208: 231-237 (1998).
9) Koito K, Namieno T, Nagakawa T, Morita K. Inflammatory pancreatic mass: differentiation from ductal carcinomas with contrast-enhanced sonography using carbon dioxide microbubbles. Am J Roentgenol 169: 1263-1267 (1997).
10) Koito K, Namieno T, Morita K. Differential diagnosis of small hepatocellular carcinoma and adenomatous hyperplasia with power Doppler sonography. Am J Roentgenol 170: 157-161 (1998).
11) Koito K, Namieno T, Ichimura T, Hirokawa N, Syonai T, Hareyama M, Katsuramaki T, Hirata K, Nishi M. Power Doppler sonography: evaluation of hepatocellular carcinoma after treatment with transarterial embolization or percutaneous ethanol injection therapy. Am J Roentgenol 174: 337-341 (2000).
12) Fujimori K, Ichimura K, Hirokawa N, Takeda M, Yama N, Yoshida S, Shounai T, Koito K, Hareyama M. Segmental Decreased Uptake of 99mTc-labeld Galactosyl-Neoglycoalbumin in Non-tumorous Liver Tissue: Sensitivity Analysis of the Factors Influencing Liver Uptake with Non-Linear Compartment Model. J Nucl Med 41: 319p (2000).

Anesthesiology

Our department has been investigating the basic mechanisms of anesthetics, pain, sepsis, and cardioprotection. These studies are aimed at improving the safety of clinical anesthesia, pain management, and intensive care. In order to achieve our goal, we employ a variety of advanced electrophysiological, biochemical, and biomolecular techniques. We are also engaged in improving perioperative systems of monitoring the safety and QOL of surgical patients.

Professor
Akiyoshi Namiki, M.D., Ph.D.
Interests:
Pain, Neuroscience

Associate Professor
Hiroaki Watanabe, M.D., Ph.D.
Interests:
Circulation, Pain

Keiichi Omote, M.D., Ph.D.
Interests:
Pain, Neuroscience

Assistant Professor
Maki Matsumoto, M.D., Ph.D.
Interests:
Pain, Education

Noriaki Kanaya, M.D., Ph.D.
Interests:
Circulation, Volatile anesthetics

Mikito Kawamata, M.D., Ph.D.
Interests:
Pain, Neuroscience

Michiaki Yamakage, M.D., Ph.D.
Interests:
Respiration, Volatile anesthetics,
Blood coagulation

Instructor
Naoyuki Fujimura, M.D., Ph.D.
Tomoyuki Kawamata, M.D., Ph.D.

1. Experimental circulation

Anesthetics are known to have myocardial depressant effects. Despite their prevalent use, the direct effects of anesthetics on cardiac contractility are poorly understood and controversial. We made use of cardiomyocytes to examine the direct effects of volatile and intravenous anesthetics on excitation-contraction coupling processes in cardiac muscle. We have found that volatile anesthetics (halothane, isoflurane and sevoflurane) decreased amplitude and beating rate in cultured rat ventricular myocytes. Bay K 8644 significantly prevented the anesthetic-depressed amplitude. These results and a receptor binding study indicate that volatile anesthetics have a direct cardiodepressant effect on cardiac excitation-contraction coupling in the immature heart, which is mediated by an interaction with the sarcolemmal L-type Ca^{2+} channel. In adult hearts, intravenous anesthetics (propofol, ketamine, thiopental, midazolam, diazepam, morphine and fentanyl) had direct effects on contractility by altering intracellular Ca^{2+} and myofilament Ca^{2+} sensitivity (1-3).

2. Anesthetics and environmental changes on airway smooth muscle

During the last few years, we have investigated the effects of some perioperatively administered drugs on the airway smooth muscle. First, we investigated the inhibitory effect of a novel Ca^{2+} antagonist, RWJ-22108, on voltage-dependent Ca^{2+} channel activity in porcine tracheal smooth muscle cells using the patch clamp technique. Second, we have demonstrated the inhibitory effects of benzodiazepines on Ca^{2+} and K^{+} channels in canine tracheal smooth muscle cells (4). We also investigated the interaction between the environmental changes [hypothermia and hypoxia (5)] and anesthetic actions on airway smooth muscle using patch clamp and fluorescence techniques. Using the fluorescence technique, we have clarified the mechanisms both of volatile anesthetics on intracellular Ca^{2+} stores (6) and of heparin and protamine, usually used in cardiac surgery.

3. Spinal and peripheral mechanisms of pathophysiological pain state

We have investigated the spinal and peripheral mechanisms of persistent pain state, using neurochemical and electrophysiological techniques. In particular we have focused on the interaction between glutamate and nitric oxide (NO). Peripheral tissue inflammation induces both glutamate and NO release within the inflamed area (7.8). NO was released from peripheral nerves, through the activation of peripheral, ionotrophic glutamate receptor, NMDA receptor within the inflamed area (9).

In spinal cord, the activation of spinal NMDA receptors stimulated a nitric oxide/cGMP/glutamate release cascade, modulating spinal nociceptive transmission (9). Now we are investigating the mechanisms of postoperative pain using a rat incisional pain model.

4. Experimental critical care

Diaphragmatic dysfunction plays an important role in acute respiratory failure during sepsis. We have found that intra-abdominal sepsis is closely associated with diaphragmatic dysfunction (10). Diaphragmatic dysfunction begins to develop from an early phase of sepsis. Polyethylene glycol-adsorbed superoxide dismutase (scavengers of superoxide ions), polyethylene glycol-adsorbed catalase (scavengers of hydrogen peroxide) and dimethyl sulfoxide (scavengers of hydroxyl radicals) significantly improved diaphragmatic dysfunction and prevented oxygen-derived free radical-mediated lipid peroxidation during intra-abdominal sepsis. These results indicated that several types of oxygen-derived free radicals play a role in diaphragmatic dysfunction during intra-abdominal sepsis.

In recent years, much attention has been paid to pharmacological agents useful in the treatment of respiratory muscle dysfunction. We found that isoproterenol improved diaphragmatic dysfunction during intra-abdominal sepsis (11). The force potenciating effect of isoproterenol on intra-abdominal septic rat diaphragm was via activation of the adenylate cyclase system. We have been investigating pharmacological agents useful in the treatment of diaphragmatic dysfunction during sepsis.

List of Main Publications from 1997 to 2000

1) Kanaya N, Murray PA, Damron DS. Propofol and ketamine only inhibit intracellular Ca^{2+} transients and contraction in rat ventricular myocytes at supraclinical concentrations. Anesthesiology 88: 781-791 (1998).
2) Kanaya N, Zakhary DR, Murray PA, Damron DS. Thiopental alters contraction, intracellular Ca^{2+} and pH in rat ventricular myocytes. Anesthesiology 89: 202-214 (1998).
3) Kanaya N, Zakhary DR, Murray PA, Damron DS, Differential effects of fentanyl and morphine on intracellular Ca^{2+} transients and contraction in rat ventricular myocytes. Anesthesiology 89: 1532-1542 (1998).
4) Yamakage M, Matsuzaki T, Tsujiguchi N, Honma Y, Namiki A. Inhibitory effects of benzodiazepines diazepam and midazolam on Ca^{2+} and K^{+} channels in canine tracheal smooth muscle cells. Anesthesiology 90: 197-207 (1999).
5) Yamakage M, Tsujiguchi N, Hattori J, Kamada Y, Namiki A. Low-temperature modification of the inhibitory effects of volatile anesthetics on airway smooth muscle contraction in dogs. Anesthesiology 93: 179-188 (2000).
6) Yamakage M, Kohro S, Matsuzaki T, Tsuchida H, Namiki A. Role of intracellular Ca^{2+} stores in the inhibitory effect of halothane on airway smooth muscle contraction. Anesthesiology 89: 165-173 (1998).
7) Omote K, Kawamata T, Kawamata M, Namiki A. Formalin-induced release of excitatory amino acids in the skin of the rat hindpaw. Brain Res 787:161-164 (1998).
8) Omote K, Kawamata T, Kawamata M, Namiki A. Activation of peripheral NMDA-nitric oxide cascade in formalin test. Anesthesiology 93: 173-178 (2000).
9) Kawamata T, Omote K. The activation of spinal NMDA receptors stimulates a nitric oxide/cyclic GMP/glutamate release cascade in nociceptive signaling. Anesthesiology 91: 1415-1424 (1999).
10) Fujimura N, Sumita S, Aimono M, Masuda Y, Shichinohe Y, Narimatsu E, Namiki A. Effect of free radical scavengers on diaphragmatic contractility in septic peritonitis. Am J Respir Crit Care Med 162: 2159-2165 (2000).
11) Fujimura N, Sumita S, Narimatsu E, Nakayama Y, Shichinohe Y, Namiki A. Effects of isoproterenol on diaphragmatic contractility in septic peritonitis. Am J Respir Crit Care Med 161: 440-446 (2000).

Community and General Medicine

Our Department was established in 1999. Its mission is to make a significant contribution to community medical care in Hokkaido. The Department has two primary goals: one is to produce primary care physicians through sound, systematic, undergraduate and graduate medical education; the other is to promote research on community medical care, general medicine/practice, clinical epidemiology and holistic medicine.

Professor

Wari Yamamoto, M.D., Ph.D. FJSIM

Interests:

Clinical epidemiology, Cost-effectiveness analysis, Medical decision making, General medicine, Diabetes mellitus

Instructor

Hidenobu Kawabata, M.D.

Yasushi Miyata, M.D., FJSIM

Shinji Kimura, M.D., M.S.

1. Medical student education

As we become more and more actively involved in undergraduate medical education, not only do we find it vital in terms of generating patient-oriented physicians, but also important in terms of research since the methodology of medical education lags behind time and remains to be improved. We are currently planning to conduct the following research.

a) Qualitative analysis of student education in medical interviewing.
b) Qualitative analysis of student education in physical examination
c) Research on the role of generalists in medical student education

2. Common diseases/medical problems in primary care

We as generalists encounter common medical problems in our daily practice which are somewhat different from those of the usual population, as our clinic is located in a tertiary care setting. We intend to focus on these common problems and plan on conducting prospective research into their epidemiology, diagnosis, treatment, and natural history. Currently projects on the following are under protocol development.

1) Headache
2) Dizziness
3) Insomnia

3. Physical diagnosis

We have a special interest in the characteristics of physical findings in medical diagnosis, i.e., their specificity, sensitivity, positive/negative predictive values, and likelihood ratios. We plan to select important clinical findings encountered in primary care and study their sensitivity and specificity in comparison with the gold standard in diagnosis.

4. Cost-effectiveness analysis

Another of our interests is to study cost-effectiveness in health care, thereby understanding the logic behind important health policy decisions and also possibly contributing to such decision making.

5. Cultural aspects of medicine and medical care

The patient develops illnesses, and the physician and the surgeon diagnose and treat diseases. What is also important when working as a doctor is to understand the patients' background and culture and not just the disease the patient has. "Narrative-based medicine" is a new and old way of looking at medical care from the patient's perspective through the story that the patient gives. It departs from the traditional, biological model, and introduces sociological and anthropological methods into medicine. Our goal is to conduct quantitative research in this arena, e.g., on patients' behavior, their understanding of illnesses, and their compliance.

List of Main Publications from 1999 to 2000

1) Yamamoto W, Maekawa M, Fukui T, et al. What aspects of patient affect medical students?; A qualitative analysis of the experience of medical students at general medical ward and clinic. Medical Education (Japan) 31: 429-434 (2000).
2) Yamamoto W. Clinical epidemiology in primary care. Sapporo Med J 69(Suppl.): 11-13 (2000).
3) Yamamoto W, Fukui T, Shimbo T, et al. Combining data from subjects' histories for the prediction of imminent atrial fibrillation is useful for the elderly male population. Gen Med 1: 3-8 (2000).

4) Yamamoto W, Fukui T, Maekawa M, et al. Predictive factors for cancer in patients with abdominal pain. Gen Med 1: 9-16 (2000).

Clinical Laboratory Medicine

Our department has been attempting to produce a high quality of laboratory data to make the diagnosis, including molecular and genetic diagnoses. The mainstay to achieve our purposes is, nowadays, the development of new methods and markers for biochemical, immunological, molecular and genetic diagnosis for various cancers, infectious diseases and hereditary diseases.

Professor
Naoki Watanabe, M.D., Ph.D.
Interests:
Laboratory medicine, Oncology, Hematology,
Gastroenterology, Molecular biology

Associate Professor
Tsutomu Michibayashi, M.D., Ph.D.
Interests:
Laboratory medicine, Nephrology

Assistant Professor
Atsuhito Yagihashi, M.D., Ph.D.
Interests:
Laboratory medicine, Oncology, Surgery
Molecular biology, Immunology, Transplantation

Instructor
Daisuke Kobayashi, M.D., Ph.D.

1. Establishment and clinical application of highly sensitive detection method for very small amounts of various substances *In Vivo*

Advances in molecular biology permit the detection of very small amounts of DNA or RNA from biologic molecules such as cytokines and viral constituents. However, the quantity of DNA or RNA does not necessarily reflect the quantity of the corresponding proteins. We therefore have attempted to lower the detection limit of the standard ELISA and have developed immuno-PCR assays for TNF-alpha, IL-18, Osteoclast inhibitory factor and angiotensinogen (1.2). In our immuno-PCR assays, biotinylated DNA amplified by PCR was used instead of enzyme activity as the signal to detect antigen-antibody complexes, and the detection limit could approximately 10^4 to10^5 times lower than that of the conventional ELISAs.

By using immuno-PCR assay for TNF-alpha, we found the following evidence: 1) The detection limit could 5 x 10^5 times lower than that of the conventional ELISA, 2) Mean level of serum TNF-alpha in healthy individuals, which could not be measured by a conventional ELISA or any other methods until establishment of Immuno-PCR assay, was 0.027pg/ml (ranging from 0.004 to 0.118pg/ml), 3) Levels of serum TNF-alpha in all patients with Duchenne muscular dystrophy, Crohn's disease and Ulcerative colitis were measurable and their mean levels were 27.8, 208.4 and 30.3 pg/ml respectively (3).

2. Host-responses against various stresses

Based on evidence that cells producing anti-tumor cytokine such as TNF-alpha never suffer damage by anti-tumor cytokine itself, we investigated whether endogenous (en) TNF-alpha acts as a resistance factor to various stresses. We found the following evidence: 1) enTNF-alpha acts as a resistance factor to various apoptotic stimuli such as anticancer drugs, heat and exogenous TNF, via enhancing MnSOD activity and expression of heat-shock protein (HSP), 2) enTNF-alpha induces HSP72 expression by enhancing the binding capacity of heat shock factor-1 to heat shock element (4), 3) By measurement of enTNF-alpha levels in patients with lymphoma and leukemia, sensitivity to antitumor drug could be predicted (5).

Survivin, a recently described member of the inhibitor of apoptosis protein (IAP) family, is expressed in most cancer cells. Like other IAP-family members, survivin binds specifically to the terminal effector cell death proteases, i.e. caspase-3 and -7, and substantially reduces caspase activity and cell death exposed to diverse apoptotic stimuli. We therefore investigated the relationship between survivin mRNA expression and radioresistance in pancreatic cancer cells. We found that survivin acts as a radioresistance factor in pancreatic cancer cells by reduction of caspase-3 activity via augmentation of survivin mRNA expression after exposure to sublethal dose of X-irradiation (6).

3. Molecular diagnosis for cancer.

In previous reports, survivin expression has been studied by various immunostaining techniques, immunoblotting, in situ hybridisation, and RT-PCR. The diversity of methods makes comparisons between studies difficult. Therefore, we quantitatively measured survivin mRNA expression in breast cancer, gastric cancer and leukemia using TaqMan-reverse-transcription (RT)-PCR. We found the following evidence: 1) Survivin mRNA expression was detected in 85.7 % of gastric cancer patients, 67.5 % of breast cancer patients, and 76.2 % of leukemia patients, but not detected in noncancerous cells, 2) Quantitation of survivin mRNA expression could be useful as both a diagnostic tumor marker and an index of resistance acquired during treatment with antineoplastic drug (7).

4. Cell senescence and immotalization, and carcinogenesis

Telomerase is an enzyme that replaces repetitive $(TTAGGG)_n$ sequences on ends of chromosomes that otherwise would be lost with successive cell divisions. Accordingly, telomerase activity is linked closely to attainment of cellular immortality, a step in carcinogenesis, while lack of such activity contributes to cellular senescence. Based on these previous reports, we investigated telomerase activity in gastric mucosal tissues. Telomerase activity is highest in cancer, followed by intestinal metaplasia, chronic gastritis, and normal mucosa. In patients with intestinal type gastric cancer, telomerase activity in intestinal metaplasia with *H. pylori* infection was higher than without infection. Our results suggest that *H. pylori* infection may influence telomerase activity in cancer and noncancerous tissue (8).

Recently, genes encoding three major components of human telomerase and two human telomeric-repeat binding factor proteins (TRFs) have been cloned. However, details of the mechanisms regulating telomerase activity still are poorly understood, and specific components or binding proteins that might represent suitable targets for cancer gene therapy have not been identified. We therefore established quantitative assays using TaqMan RT-PCR for mRNAs encoding the telomerase components hTR, hTERT, and TEP1 as well as for those encoding TRF1 and TRF2 (9). By using our quantitative assays, we found the following evidence: 1) Expression of TRF1and TRF2 mRNA was greater in the normal cells than in human malignant hematopoietic cell lines and in patients with acute leukemia, 2) Of the three telomerase components, only hTERT mRNA expression showed changes paralleling telomerase activity, becoming undetectable with HL60 cell differentiation, 3) In contrast, initially low expression of TRF1 and TRF2 mRNA increased during differentiation. Our results suggest that not only hTERT but also TRF1 and 2 are important regulators of telomerase activity (10).

List of Main Publications from 1997 to 2000.

1) Saito K, Kobayashi D, Sasaki M, Araake H, Kida T, Yagihashi A, Yajima T, Kameshima H, Watanabe N. Detection of human serum tumor necrosis factor-alpha in healthy donors using a highly sensitive immuno-PCR assay. Clin Chim 45: 665-669 (1999).
2) Furuya D, Yagihashi A, Yajima T, Kobayashi D, Orita K, Kurimoto M, Watanabe N. An immuno-polymerase chain reaction assay for human interleukin-18. J Immunol Methods 238: 173-180 (2000).
3) Saito K, Kobayashi D, Komatsu M, Yajima T, Yagihashi A, Ishikawa Y, Minamai R, Watanabe N. A sensitive assay of tumor necrosis factor-alpha in sera from Duchenne muscular dystrophy patients. Clin Chim 46: 1703-1704 (2000).
4) Watanabe N, Tsuji N, Akiyama S, Sasaki H, Okamoto T, Kobayashi D, Sato T, Hagino T, Yamauchi N, Niitsu Y, Nakai A, Nagata K. Induction of heat shock protein (HSP) 72 synthesis by endogenous tumor necrosis factor via enhancement of the heat shock element-binding activity of heat shock factor 1. Eur J Immunol 27: 2830-2834 (1997).
5) Kobayashi D, Watanabe N, Yamauchi N, Tsuji N, Sato T, Niitsu Y. Endogenous tumor necrosis factor as a predictor of doxorubicin sensitivity in leukemic patients. Blood 89: 2472-2479 (1997).
6) Asanuma K, Moriai R, Yajima T, Yagihashi A, Yamada M, Kobayashi D, Watanabe N. Survivin as a radioresistance factor in pancreatic cancer cells. Jpn J Cancer Res 91: 1204-1209 (2000).
7) Moriai R, Asanuma K, Kobayashi D, Yajima T, Yagihashi A, Yamada M, Watanabe N. Quantitative analysis of the anti-apoptotic gene survivin expression in malignant heamatopoietic cells. Anticancer Res 21: 595-600 (2001).
8) Kameshima H, Yagihashi A, Yajima T, Kobayashi D, Denno R, Hirata K, Watanabe N. Helicobacter pylori infection augments telomerase activity in gastric cancer and noncancerous tissue. World J Surg 24: 1243-1249 (2000).
9) Yajima T, Yagihashi A, Kameshima H, Kobayashi D, Furuya D, Hirata K, Watanabe N. Quantitative reverse transcription-polymerase chain reaction assay of the RNA component of human telomerase using the TaqMan fluorogenic detection system. Clin Chim 44: 241-244 (1998).
10) Yamada K, Yajima T, Yagihashi A, kobayashi D, Koyanagi Y, Asanuma K, Yamada M, Moriai R, Kameshima H, Watanabe N. Role of human telomerase reverse transcriptase and telomeric-repeat binding factor protein 1 and 2 in human hematopoietic cells. Jpn J Cancer Res 91: 1278-1284 (2000).

Oral Surgery

Our department deals with a variety of oral diseases such as tumor, deformity, fracture, periodontitis, and dental caries. In order to maintain or reconstruct oral function including occlusion, in addition to achieving long-term survival, we have made an effort to improve the operation method for these diseases through consideration of the results of the basic sciences which are related to the diseases.

Professor
Geniku Kohama, DDS, Ph.D.
Interests:
Oral tumor, Cleft lip and palate

Associate professor
Nobuyuki Tanaka, DDS, Ph.D.
Interests:
Oral tumor, Jaw deformity

Assistant professor
Itaru Nagai, DDS, Ph.D.
Interests:
Temporomandibular joint, Jaw deformity

Makoto Noguchi, DDS, Ph.D.
Interests:
Oral tumor, Cleft lip and palate

Instructor
Shizuyo Itoh, MA. Ph.D.
Tatsuru Suyama, DDS
Takashi Ide, DDS, Ph.D.
Akihiro Miyazaki, DDS, Ph.D.
Hisanori Kinjo, DDS, Ph.D.
Toshiaki Nakano, DDS

1. Oral oncology

In order to preserve postoperative aesthetics and function, minimally invasive surgery has been applied for some cases with oral squamous cell carcinoma. It is very important to determine which cases are applicable for this surgery and so we have investigated the biological behavior of oral carcinoma by immunohistochemistry with various cancer-suppressor proteins, and nuclear DNA content, in addition to conventional histopathology. Chemotherapy has an important role to play in multidisciplinary treatment of head and neck cancer. However, so far, studies on the biological aspects of preoperative chemotherapy have been insufficient. To elucidate the clinical significance of preoperative chemotherapy on cell kinetics, the changes in nuclear DNA content and mitotic index before and after chemotharpy were examined by image analysis. It appeared that image analysis of nuclear DNA content in combination with mitotic index is useful for assessment of the effects of preoperative chemotherapy on cancer cell kinetics (Sapporo Medical Journal 69,45-54, 2000). We investigated the expression of pRb2/p130 protein by immunohistochemical staining in oral squamous cell carcinomas. Positive staining for pRb2/p130 was observed in well-differentiated carcinomas more than in poorly differentiated carcinomas and the expression of pRb2/p130 was related to the degree of tumor differentiation, thus it is suggested that pRb2/p130 gene may be associated with the development of a wide variety of human malignancies rather than the progression (1). The records of 136 patients with N1-3 oral squamous cell carcinoma treated by surgery were investigated and four independent factors significantly influenced survival in the following order: pN stage, T stage, histopathological grade, and N stage. Multivariate analysis of 80 patients with pN1-3 oral squamous cell carcinoma showed that T stage and existence of extra capsular spread to metastatic lymph nodes were the prognostic factors for survival and independent factors (2). On the other hand, the mechanism of tumor suppression by p53 gene was investigated. In combined infection with Ad-p21 and Ad-p53, it was found that introduction of exogenous p53 in RERF-LC-OK, BT549 and ZR-75-1 cells overcame p21-mediated cell cycle arrest at G1 and induced apoptosis, and it was suggested that this affect is a general event among human cancer cell lines. And a significantly greater amount of Bax protein was present in cell lines undergoing apoptosis than in cells with arrested growth, suggesting Bax might be an important component of the p53-mediated apoptosis of cancer cells (3).

Furthermore we have examined the usefulness of preoperative chemotherapy (4) and the excisional biopsy. For oral and maxillofacial tumor in children, surgery is the treatment of choice, however, first of all, minimally invasive treatment should be performed on the basis of the results of clinical investigation (5). For oral malignant melanoma which tends to invade or metastasize and has poor prognoses as compared with cutaneous malignant melanoma, a relatively small resection might

lead to a better prognosis (6.7), however its biological behavior remains controversial because the number of case of oral malignant melanoma is few and accumulation of more data is needed.

Recently we examine the methylation of cancer suppressor genes in oral cancer cell lines and tissues and gene therapy with modified adenovirus vector for oral malignant tumor in vivo and in vitro.

2. Clinical and basic studies of oral deformities

In our department, palatoplasty with supraperiosteal mucosal flap (Kohama Supraperiosteal flap technique(SP technique)) was established and we found that palatal sensibility of patients operated on by the SP technique is superior to that resulting from operation by the Mucoperiosteal flap technique (MP technique). Immunohistological examination revealed that preservation of periosteum and connective tissue in the palate resulted in a rich nerve growth in the regenerated mucosal tissue, while the nerve growth in the developing submucosal scar tissue was poor when the periosteum was not preserved (Sapporo Medical Journal 69, 65-73, 75-86 2000). For jaw deformities, various operation have been performed and the occlusal status was measured by dental scaler. The occlusal status (occulusal contact area and bite force) of patients with mandibular prognathism decreased 1 month after the operation, however, 12 months after it increased as compared with the preoperative status. Occlusal pressure reached its maximum value 1 month after the operation and 12 months after it was close to the value for controls.

3. Clinical and basic studies of reconstruction cases by dental implant

Dental implant is useful for reconstruction of occlusion and widely accepted. There are many studies of the tissue surrounding the implants. We investigated the tissue response associated with dental titanium implant in beagle dogs. Scanning electron microscopic observation showed titanium particles on the implant-bone interface, and investigation by microanalyzer revealed titanium not only on the implant-bone interface but also in the bone tissue. Transmission electron microscopic observation and investigation by electron diffraction showed titanium in the bone matrix and cells other than macrophages (8). Ultrastructural and biophysical investigation of the human bone tissue surrounding a hydroxyapatite-coated implant was performed. Calcified material was recognized by scanning electron and transmission electron microscopys, X-ray microanalyzer. X-ray diffraction analysis showed that the relatively high-density area of the calcified material was hydroxyapatite, whereas the lower density area was bone. Titanium was not found in the tissue investigated (9).

List of Main Publications from 1997 to 2000

1) Tanaka N, Odajima T, Nakano T, Kimijima Y, Yamada S, Ogi K, Kohama G. Immunohistochemical investigation of new suppressor oncogene p130 in oral squamous cell carcinoma. Eur J Cancer 35: 321-325 (1999).
2) Noguchi M, Kido Y, Kubota H, Kinjo H, Kohama G. Prognostic factors and relative risk for survival in N1-3 oral Squamous cell carcinoma: a mutivariate analysis using Cox's hazard model. Br J Oral Maxillofac Surg 37: 433-437 (1999).
3) Nakaya U, Yamashita T, Odajima T, Shindou M, Tokino T, Fujinaga K, Kohama G. Wild-type p53 expression Overcoms p21-mediated G1 arrest and induces apoptosis in cancer cells expressing Bax. Tumor Res 34: 69-81 (1999).
4) Noguchi M, Sekiguchi T, Kido Y, Kinjo H, Ide T, Suda Y, Kohama G. Prognostic value of response to preoperative chemotherapy in patients with oral squamous cell carcinoma. Asian J Oral Maxillofac Surg 11: 47-53 (1999).
5) Tanaka N, Murata A, Yamaguchi A, Kohama G. Clinical features and management of oral and maxillofacial tumors in children. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 88: 11-15 (1999).
6) Tanaka N, Nagai I, Hiratsuka H, Kohama G. Oral malignant melanoma: long-term follow up in three patients. Int J Oral Maxillofac Surg 27: 111-114 (1998).
7) Tanaka N, Kohama G. Melanoma of the tongue: Case report. J Oral Maxillofac Surg 55: 1469-1471 (1997).
8) Tanaka N, Ichinose S, Kimijima Y, Mimura M. Investigation of titanium leak to bone tissue surrounding dental titanium implant: electron microscopic findings and analysis by electron diffraction. Med Electron Microsc 33: 96-101 (2000).
9) Tanaka N, Ichinose S, Suyama T, Kohama G Ultrastructural and biophysical studies of the bone tissue surrounding a hydroxyapatite-coated implant: Case report. J Oral Maxillofac Surg 58: 685-690 (2000).

Neurology

We have been taking care of many patients with numerous neuromuscular disorders, enabling us to carry out research which will produce a better quality of life for the patients. Our main interests are in the fields of neurophysiology (motor evoked potentials, transcranial magnetic stimulation and diaphragmatic action potentials), neuroimmuno-logy (Guillain-Barre/Fischer syndromes and polymyalgia rheumatica), and non-invasive ventilation for various neurodegenerative diseases.

Professor
Hiroyuki Matsumoto, M.D., Ph.D.
Interests:
Clinical neurology

Associate Professor
Susumu Chiba, M.D., Ph.D.
Interests:
Neuroimmunology, Neurochemistry

Assistant Professor
Tomihiro Imai, M.D., Ph.D.
Interests:
Neurophysiology, Muscle histology

Instructor
Motoi Kashiwagi, M.D., Ph.D.

1. Clinical neurophysiology

Spinal muscular atrophy (SMA) patients showed decreased amplitudes of the M and F waves, decreased frequency of the F waves and an increase of the F/M ratio. The F wave frequency and the amplitudes of M and F waves, which showed a significant linear correlation with each other, became lower in accordance with the decrease of grip power. The properties of M and F waves strongly correlated with the number of surviving motor neurons which would be fewer in those severely affected by SMA (1).

We recorded masseter reflex potentials to examine the correlation between the masseter reflex and the stretch reflexes of limb muscles in 19 patients with olivo-ponto-cerebellar atrophy (OPCA). The patients were subdivided into hyper-, normo- and hyporeflexia groups. The latency of the potentials in the hyporeflexia group was significantly longer than in the other groups, while the amplitude of those in the hyperreflexia group was significantly higher than in the other groups. These results suggest that OPCA patients may have a common representation of the muscle stretch reflexes both in the masseter and the limb muscles (2).

Changes of MEPs from the agonist and antagonist forearm muscles were investigated in 13 patients with Parkinson's disease, in whom transcranial magnetic stimulation (TCMS) was delivered to the cortical hand motor area immediately before voluntary wrist flexion. MEPs recorded from the agonist muscles were gradually facilitated in accordance with a shortening of the interval between TCMS and wrist flexion in both groups. In contrast, MEPs recorded from the antagonist muscles were gradually facilitated only in parkinsonian patients as the intervals were shortened. The reciprocal facilitation of the antagonist MEPs was statistically significant when TCMS was delivered within 80 msec before the voluntary movements, suggesting that the presence of the same underlying mechanism of symptomatic cocontraction observed in patients with Parkinson's disease (3).

We recorded the motor evoked potentials (MEPs) from the affected thenar muscles during neck flexion in 4 patients in the early progressive phase of Hirayama disease. The MEP size significantly increased during neck flexion in patients compared with normal controls. In 2 patients, who were treated with a neck collar, the intrinsic muscle atrophy gradually recovered together with increased grip power, and the time course of changes in MEP during neck flexion became normal after treatment with a neck collar for 7-16 months (4). Diaphragmatic action potentials (DAPs) were mapped on the thorax bilaterally in 16 neurologically normal infants and 8 boys aged 1 to 4 years during artificial ventilation after thoracic surgery. Transcutaneous stimulation was used to activate the phrenic nerve at the supraclavicular fossa at the end of an artificial inspiration. The DAPs were of positive polarity and were recorded on the ipsilateral anterolateral

chest wall over the sixth to the eighth intercostal spaces with a maximal peak at the seventh intercostal space. The DAP latencies gradually decreased from 6-8 ms at birth to about 5 ms at the age of 1 year despite an increase in conduction distance. Statistical analyses revealed that DAP amplitude did not correlate with age. The latencies and amplitudes of the DAPs displayed little interside variation (5).

2. Neuroimmunology

We examined the antibodies against *Helicobacter pylori* (*H. pylori*) proteins in the cerebrospinal fluid (CSF) of seven patients with Guillain-Barre syndrome (GBS). Crude *H. pylori* antigens, fractionated heat shock protein (HSP) and urease B (UB) from *H. pylori* antigens were separated by SDS-PAGE. With Western blot analysis, 4 of 7 CSF samples had several IgG antibodies against *H. pylori* proteins including HSP60 family and UB. No cross-reactivity against *Campylobacter jejuni* was observed. These antibodies may be involved in the immune responses of patients with GBS (6). We also examined the antibody against recombinant vacuolating cytotoxin (r-Vac-A) of *H. pylori* in the CSF of patients with GBS and Fisher syndrome (FS). Western blot analyses were carried out to examine the samples of CSF of 10 GBS and 3 FS patients. Six of these samples (4 GBS and 2 FS) were confirmed to have a specific IgG antibody against r-Vac-A. This antibody may be involved in the physiological malfunctions of the peripheral nerves of patients with GBS and FS (7). On the other hand, we tried to detect the antibodies against *H. pylori* proteins in the CSF of eight patients with peripheral neuropathy including chronic inflammatory demyelinating polyneuropathy (CIDP). Crude *H. pylori* antigens, fractionated heat shock protein (HSP) and UB from *H. pylori* antigens were separated by SDS-PAGE. With Western blot analysis, two of five patients with CIDP and one of two patients with polyneuropathy had several IgG antibodies against *H. pylori* proteins, including HSP60 family and UB in their CSF (8).

HSP is thought to be one of the most important molecular chaperoning proteins which have a role to rescue damaged cells. Our preliminary data disclosed that some patients with amyotrophic lateral sclerosis (ALS) have a specific IgG antibody against HSP of *H. pylori* in their CSFs. With Western blot analysis using the recombinant HSP and its fragment as antigens, the evaluation of the CSF antibody against HSP in patients with ALS has been carried out.

3. Neuroimaging

We have shown the usefulness of the MR imagings to identify affected muscles and for monitoring treatment in patients with focal myositis (9). We found that MR imaging and synovial scintigraphy were useful not only to evaluate the pathophysiology of PMR but also to distinguish polymyalgia rheumatica from RA-negative chondroarthropathies or myositis.

4. Respiratory care in the neurodegenerative disorders

We have been accumulating clinical data to evaluate the usefulness of non-invasive positive pressure ventilation in advanced stages of ALS and Shy-Drager syndrome. We found that volume-cycled ventilators were useful if obstruction of the mid-pharynx was not found by the lateral view of the pharynx in supine position.

List of Main Publications from 1997 to 2000

1) Imai T, Saito M, Matsumoto H, et al. Correlation between the M and F wave characteristics and the innervated muscle strength in spinal muscular atrophy. Brain Dev 20: 44-46 (1998).
2) Imai T, Matsumoto H, Ohmoto H, et al. Masseter reflex potentials in olivo-ponto-cerebellar atrophy. Electromyogr clin Neurophysiol 38: 147-151 (1998).
3) Imai T, Yamamoto T, Ohkubo Y, et al. Reciprocal facilitation of motor evoked potentials immediately before voluntary movements in Parkinson's disease. Electromyogr clin Neurophysiol 39: 201-206 (1999).
4) Imai T, Shizukawa H, Nakanishi K, et al. Hyperexcitability of cervical motor neurons during neck flexion in patients with Hirayama disease. Electromyogr clin Neurophysiol 40: 11-15 (2000).
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Plastic and Reconstructive Surgery

Plastic surgery encompasses both cosmetic and reconstructive surgery. Cosmetic surgery is performed to reshape normal structures of the body in order to improve the patient's appearance and self-esteem. Reconstructive surgery is performed on abnormal structures of the body, caused by congenital defects, developmental abnormalities, trauma, burn injury, infection, tumors or disease. It is generally performed to improve functions, but may also be done to approximate a normal appearance.

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Kowichi Jimbow, M.D., Ph.D.
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Dermatology

Assistant Professor
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Interests:
Prefabricated flap, Microvascular surgery,
Microangiography, Microcirculation, Cosmetic surgery,
Blepharoplasty, Osmidrosis

Tatsuya Fujita, M.D., Ph.D.
Interests:
Artificial skin, Skin preservation, Microvascular Surgery,
Wound healing, Maxillofacial injury, Severe burn injury

Instructor
Kyori Ezoe, M.D., Ph.D.

1. Prefabricated flap (1)

Tissue neovascularized by implanting a vascular pedicle can be transferred as a "prefabricated flap" based on the blood flow through the implanted pedicle. This technique potentially allows any defined tissue volume to be transferred to any specified recipient site, greatly expanding the armamentarium of reconstructive options. Prefabricated flaps allow the transfer of moderate-sized units of thin tissue to recipient sites throughout the body. They have been particularly useful in patients recovering from extensive burn injury on whom thin donor sites are limited. We have investigated the microcirculation of prefabricated flaps using tissue expanders and have applied this unique flap to clinical cases.

2. Anatomical study of the fasciocutaneous flaps

We studied the use of the postero-medial thigh fasciocutaneous flap for the treatment of ischial pressure sores. The source of circulation to this flap was the suprafascial vascular plexus and the musculocutaneous perforator. The dominant pedicle was the musculocutaneous perforator from either the adductor magnus muscle or the gracilis muscle. It is the nearest fasciocutaneous flap to the ischial region, easy to raise and it causes no donor site morbidity. Eleven ischial pressure sores in ten paraplegic patients were closed using these postero-medial thigh fasciocutaneous flaps.

3. Cosmetic surgery (2)**, surgical treatment of osmidrosis** (3)

In surgery for axillary osmidrosis or hyperhidrosis, excision of subcutaneous tissue with scissors is time consuming and hard work for the operator. We have utilized a disposable plastic razor. The subcutaneous tissue is shaved like shaving bears. After shaving with a razor, not only apocrine and eccrine glands but also hair follicles are completely removed. We experienced no hematoma, no local infection and no skin necrosis. Young women who desired hair-removal of the axilla are very satisfied with this method.

4. Establishment of a new method for allo-skin preservation and clinical trial in severe burn cases (4)

Vitrification technique was applied to preservation of human skin. Viability of vitrified and cryopreserved skin was evaluated by trypan blue dye exclusion test, MTT colorimetric assay and culture test of the keratinocytes from vitrified skin. The viability of vitrified skins revealed no significant difference when they were compared with fresh skin. We used these vitrified skin allografts for flame burn and electric burn patients and they took well in both cases. We conclude that the vitrification method for skin preservation is very simple and reliable and this method would contribute to skin banking.

5. Skin allograft and topical immunosuppression using FK506 ointment (5)

We studied the effect of FK506 ointment on rat skin allograft survival. The Lewis rat (RT1l) skin allografts were histologically evaluated on day 7 after the graft transplantation to the recipient ACI (RT1a) rats. We set histological gradings for rejection as follows; grade 1, intraepidermal blister formation; grade 2, partial epidermal separation from dermis; and grade 3, complete epidermal separation from epidermis. According to these histological criteria, the immunosuppressive effect of FK506 ointment was assessed. Our data indicated that all rats without FK506 ointment showed grade 3 on day 7 after transplantation. However, the allografts treated with FK506 ointment showed grade 1 or 2, and the rejection of 80 % of grafts was prolonged until 14 days after transplantation. The present study suggests that FK506 on skin allografts may be useful and effective in the suppression of skin allograft rejection.

6. Topical immunosuppression using IL-16-cDNA transfected human squamous cell line (6)

It is well known that it is difficult to induce an immunotolerance with allogeneic skin transplantation. We attempted to find out the immunosuppressive protocol for prolonging skin allograft rejection by using interluikin-16. Our data indicated that IL-16 clearly inhibited human MLR, and that IL-16 increased synergistically the immunosuppressive effect of anti-CD4 mAb. We also used IL-16 transfectant and this produced more than 50 ng/ml of IL-16 in the supernatant that resulting in significant inhibition of human MLR. These results indicated that the IL-16-producing allogeneic skin graft might have a local immunosuppressive action that would prolong graft survival.

7. Development of a new immunosuppressive agent using sea urchin. Its immunosuppressive effect has been investigated in-vitro and in-vivo (7)

It is known that SGDG (sulfolipids) is isolated from sea urchin as well as plants, and this reagent has an antiproliferative effect on tumor cells. We determined if several synthetic analogues of sulfolipids could act as the immunosuppressive reagent in the T-cell proliferation assay (mixed lymphocyte reaction, MLR) and rat allogeneic skin graft. Our data indicated that b-SQDG(18:0) had a strong immunosuppressive effect in human MLR. b-SQDG(18:0) prolonged rat skin allograft rejection in vivo. These results suggested that b-SQDG(18:0) might be a new class of immunosuppressive reagent.

8. Studies on mechanisms of keloid and hyperplastiic scar

To investigate the mechanisms of keloid and hyperplastic scar, the expression and function of cell adhesion molecules (integrin family, CD44 family) expressed on cultured fibroblasts derived from keloid and hyperplastiic scar have been examined.

9. Studies on mechanisms of cell-cycle, apoptosis (8-10)

List of Main Publications from 1997 to 2000

1) Homma K, Sugihara T, Igawa HH, Ezoe K, Fujita T. Technique for selective microangiography of skin flaps with high contrast and fine detail. J Reconstr Microsurg 16(5): 367-370 (2000).
2) Homma K, Mutou Y, Mutou H, Ezoe K, Fujita T. Intradermal stitch blepharoplasty for Orientals: Does it disappear? Aesthetic Plast Surg 24 (4): 289-291 (2000).
3) Homma K, Maeda K, Ezoe K, Fujita T, Mutou Y. Razor assisted treatment of axillary osmidrosis Plast Reconstr Surg 105(3): 1031-1033 (2000).
4) Fujita T, Takami Y, Ezoe K, Saito T, Sato K, Takeda T, Matsumoto Y, Homma K, Jimbow K, Sato N. Successful preservation of human skin by vitrification, J Burn Care Rehabil 21(4): 304-309 (2000).
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7) Matsumoto Y, Sahara H, Fujita T, Hanashima S, Yamazaki T, Takahashi S, Sugawara F, Mizushina Y, Ohta K, Takahashi N, Jimbow K, Sakaguchi K, Sato N. A novel immunosuppressive agent, SQDG, derived from sea urchin. Transplant Proc 32: 2051-2053 (2000).
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Traumatology and Critical Care Medicine

Our department covers various aspects of severe, emergency and critical patients in a tertiary setting, including the patient transport system. The mainstay consists of resuscitation using cardiopulmonary bypass, brain hypothermia therapy for severe head trauma and postresuscitation encepholopathy, treatment of septic shock and organ dysfunction, and disaster medicine. We also prioritize the limb salvage for trauma patients and immunonutrition for critical patients.

Professor and Director
Yasufumi Asai, M.D., Ph.D.
Interests:
Emergency and critical care medicine,
Disaster medicine,
Air emergency service,
Medical care for remote rural area

Associate Professor
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Interests:
Pathophysiology of sepsis,
Cannabinoids, Blood purification

Assistant Professor
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Interests:
Host-Defense system,
Emergency surgery, Gastroenterology

Instructor
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Eichi Narimatsu, M.D., Ph.D.
Masashi Yoshida, M.D., Ph.D.
Yoshihiko Tsuchida, M.D.
Yoshihiko Kurimoto, M.D., Ph.D.
Morihito Sato, M.D.
Mamoru Hase, M.D.
Hideaki Yoshida, M.D., Ph.D.
Kazuhisa Mori, M.D.
Eiri Ezoe, M.D., Ph.D.
Hidetoshi Inomata, M.D.
Yasuyuki Yonemasu, M.D.

1. Cardiopulmonary cerebral resuscitation

We have been investigating the usefulness of cardiopulmonary bypass (CPB) for the out-of-hospital cardiac arrest patients for more than 10 years. Patients with cardiac arrest who are refractory to conventional advanced cardiac life support benefit from CPB (1). In some cases of cardiopulmonary arrest caused by acute myocardial infarction or refractory arrhythmia, CPB plays a major role during treatment of underlying disease and is followed by brain hypothermia therapy.

We found that the serum level of neuron specific enolase (NSE) correlates with neurological outcome in patients with postresuscitation encephalopathy (2).

2. Brain hypothermia

The introduction of brain hypothermia therapy in 1993 has remarkably changed the neurological outcome for patients with severe head trauma and postresuscitation encephalopathy (3). Brain hypothermia reduces the secondary damage of neurons, but it can cause adverse effects on other organ systems. Early induction, temperature control, monitoring of brain and vital organs are critical for better outcome. We have investigated the effective induction (4-6), problems and countermeasures (7), and complication (8) on experimental and clinical bases. Multivariate analysis of prognostic factors revealed the indications for brain hypothermia (9).

3. Pathogenesis and treatment of sepsis

Serum level of inflammatory cytokines increase in septic shock. Direct hemoperfusion (DHP) using polymixin-B immobilized fiber (PMX-F) decreased them (10) and was clinically effective (11.12), though the endotoxin level did not change. It has recently been reported that the endogenous cannabinoids induced hypotension and tachycardia in sepsis. In our study, the serum levels of anandamide and 2-arachidonyl glycerol (2-AG) in septic patients were significantly higher than those in normal subjects, and they decreased after DHP with PMX-F (13.14). These results suggests that PMX-F therapy removes anandamide instead of endotoxin (13.14).

In patients with septic shock, early jejunal feeding improved organ dysfunction and reduced mortality in critically ill patients (15).

4. Disaster medicine

We routinely make the best use of air transport, especially a helicopter, for severe patients from remote rural hospitals. When a disaster arises, we dispatch triage doctors and transport the selected patients to our institute for further treatment (16). We participate in disaster relief not only domestically but also

internationally (17). The treatment of serious burns in disaster was reported (18).

List of Main Publications from 1997 to 2000

1) Itoh Y, Kaneko M, Imaizumi H, Yoshida M, Mori K, Nara S, Sato M, Asai Y. Cardiopulmonary Bypass (CPB) for the Resuscitation of Out-of-hospital Cardiac Arrest Patients, 2000 Annual Meeting Issue, Academic Emergency Medicine 7 (5) (2000).
2) Imaizumi H, Schichinohe Y, Sato M, Masuda Y, Kita A, Asai Y. Serum neuron specific enolase (NSE) as a prognostic maker for hypoxic brain injury after cardiac arrest in man. Critical Care and Shock 3 (4), 49 (2000.11. Supple 1).
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10) Imaizumi H, Yoshida M, Satoh M, Shichinohe Y, Honda R, Hatamoto K, Ura N, Kaneko M: Change of inflammatory cytokines before and after new endotoxin elimination therapy using polymyxin B immobilized fiber in patients with septic shock. Crit Care Med 25 (1, Supple): A182 (1997).
11) Imaizumi H, Yoshida M, Sato M, Shichinohe Y, Kaneko M: New anti-endotoxin therapy using polymyxin B immobilized fiber in patients with septic shock: A comparison between intraabdominal and intrathoracic infection. 7th World Congress of Intensive & Critical Care Medicine, Ottawa, Canada (1997).
12) Tani T, Kodama M, Imaizumi H, Yonekawa M, Saitoh M, Koga N, Fujita N, Critical Care Network Group: Endotoxin adsorption improves APACHE II and Goris's score in critical ill patients. Shock 7 (Suppl), A162 (1997).
13) Imaizumi H, Masuda Y, Ezoe E, Sato M, Kita A, Shichinohe Y, Aimono M, Takahashi N, Yoshida H, Asai, Y: Polymixin B immobilized fiber improves hemodynamic state and mortality in MRSA septic patients. Crit Care Med 28 (12 Supple): A172 (2000).
14) Imaizumi H, Masuda Y, Ezoe E, Sato M, Kita A, Shichinohe Y, Takahashi N, Yoshida H, Asai, Y, Maruyama I: Absorption of anandamide using polymixin B immobilized fiber improves hemodynamic state in septic patients. Crit Care Med 28 (12 Supple): A142 (2000).
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16) Asai Y, Itoh Y, Tanno K, Narimatsu E, Yoshida M, Mori K, Nara S: Mount Usu Volcanic Eruption in 2000 and Transportation of Severely Injured Patients by Helicopter. Prehospital and Disaster Medicine, Vancouver, British Columbia, Canada (2000).
17) Asai Y, Kaneko M, Tanno K, Nara S, Mori K, Sakano S, Yoshida M, Itoh Y, Yamamoto Y, Ohta M: The role of Japan to improve international disaster relief activities. 4th Asian Pacific Disaster Medicine, Sapporo (1998).
18) Imaizumi H: Treatment of patients with serious burns caused by disasters. Asian Med J 43(6): 260-268 (2000).

Diagnostic Ultrasound and Medical Electronics

We have been engaged in studying clinical electrophysiology, electrocardiology and real time medical imaging by means of the various electronic devices, ultrasonography, and endoscopy. We are conducting research on computer aided diagnosis systems for medical images, and a virtualized endoscopy system by digital three dimensional image processing. Medical informatics, including telemedicine for the support of clinics in isolated islands and in rural districts, is also a theme of our research.

Professor and Director
Hiroshi Natori, M.D., Ph.D.
Interests:
Internal medicine, Respiratory medicine, Diagnostic ultrasound, Bronchoscopy, Medical imaging, Computer aided diagnosis of medical images, Telemedicine, Medical informatics

Assistant Professor
Ken-ichiro Hirata, M.D., Ph.D.
Interests:
Diagnostic ultrasound, Endoscopic ultrasonography,

Instructor
Kikuya Uno, M.D., Ph.D.

1. Cardiac Electrophysiology and Electrocardiology

We have been approaching three themes within these fields; (i) Brugada syndrome (1), (ii) atrial fibrillation (2), (iii) cardiac mapping (3.4). Brugada-type ST shift in electrocardiology was investigated in normal subjects and patients who had ECG recorded in the university hospital. We obtained results showing the prevalence of this syndrome in Japanese subjects. Furthermore, we also obtained results from a loading test of sodium channel blocking agent which was studied by 87-point body surface mapping and high resolution electrocardiography. Studies of autonomic nerve activities in arrhythmia, atrial fibrillation, and clinical electrophysiology are still under investigation, and will be goals to be achieved in the division of cardiology of this department.

2. Diagnostic Ultrasound

a) Diagnostic Ultrasound of the Abdominal Region

Study on ultrasonographic non-invasive real time imaging of the abdominal organs with blood flow image was one of the themes of our research. Our department was involved in the early phase of the development of endoscopic ultrasonography. The research projects are focused on the assessment of depth of invasion of malignancies of the stomach and the colon. Endoscopic ultrasonography of submucosal tumors were also studied (5-7).

b) Study on Diagnostic Ultrasound for Chest Diseases.

Indication and efficacy of diagnostic ultrasound for the lung and the mediastinum were studied. Reseach forcused on ultrasonographic evaluation of pleural invasion of lung cancer, and transesophageal endoscopic ultrasonography for the mediastinum. Ultrasonic guidance is useful for needle biopsy of the pleural and pericardial spaces, and lesion in atelectatic lung, to enhance diagnostic accuracy and to maintain safety. Research on "Ultrasonography of the diseases of the chest and clinical application of digital medical images" was commended by the Hokkaido Medical Association, and the Governor of Hokkaido in 1998 (8.9).

c) Study on Echocardiography

Intracardiac echocardiography (ICE) is a promising modality in the new field of ultrasound cardiology. ICE essentially provides morphological information about the heart. We have been studying the clinical usefulness of ICE technology for evaluating endocardial structure of the right atrium in cardiac catheterization. A presentation on the usefulness of ICE technology during cardiac electrophysiological study and/or catheter ablation was presented by Dr.Uno, the winner of the Young Investigators Award at the annual meeting of the Japan Society of Ultrasonics in Medicine, 2000.

3. Computer Aided Diagnosis of Medical Images.

a) Computer aided detection of pulmonary nodules

A directional contrast filter for nodule and vessel was developed for computer aided detection of pulmonary nodules in chest radiogram and CT. A diagnostic system, and an educational system were developed.

b) Virtualized Endoscopy (10-12)

Three dimensional images of the tracheobronchial air space were extracted from helical CT data for virtualized

bronchoscopy. Virtualized bronchoscopy was developed by joint research with the faculty members of the Department of Information Engineering, the School of Engineering at Nagoya University. This system was used not only for clinical study, but also to develop a curriculum on Bedside Learning. Clinical Professor, Dr. Mori et al. won an award at the Japan Society of Bronchology in 2000 for a presentation based on our report entitled, "Development of the function of the virtualized bronchoscopy".

4. Medical Informatics and Telemedicine

In 1993 a telemedicine system was introduced to support the town hospitals in Okushiri island, and Rishiri island, and the prefectural hospital in Esashi. Our department and 6 rural hospitals are connected with ISDN. Telemedicine was introduced into the curriculum of Bedside Learning. Five hundred medical students have experienced case tele-conference. Recent advances in medical informatics research projects in our department were made by Dr. Mitani, Assistant Professor (13.14).

List of Main Publications from 1997 to 2000

1) Uno K, Shimoshige S, Miyamoto K, Tsuchihashi K, Shimamoto K. Dynamic electrocardiographycal enhancement of depolarization phases by the sodium channel blockade in asymptomatic Brugada type ST-elevation. In the22nd Congress of the European Society of Cardiology: 2000 Aug 26-30, Amsterdam, Eur Heart J 21:449 (2000).
2) Uno K, Miyamoto K, Shimoshige S, Tsuchihashi K. Basket catheter left atrial mapping of atrial fibrillation in mitral stenosis. In: The 12th International Congress of the Cardiostim: the Working Group on Cardiac Pacing, Arrhythmias, and Cardiac Cellular Electrophysiology of the European Society of Cardiology: 2000 June 14-17, Nice, Europace Suppl I: D180 (2000).
3) Uno K, Kumagai K, Khrestian CM, Waldo AL. New insights regarding the atrial flutter reentrant circuit: studies in the canine sterile pericarditis model. Circulation 100: 1354-1360 (1999).
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6) Nishimori H, Sasaki K, Hirata K, Natori H. The value of endoscopic ultrasonography in preoperative evaluation of rectal cancer. Int Surg 83: 157-160 (1998).
7) Natori H, Hirata K., Shiramatsu F., Teramoto J., Sun X., Mitani M., Uno K, Inomata H., Tatsuguchi H Interpretation experiment of endoscopic ultrasonograms of the myogenic submucosal tumors of the stomach. In: The 9th Congress of World Federation for Ultrasound in Medicine and Biology: Florence, Italy: Ultrasound Med Biol 26, Suppl 2: A170 (2000).
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12) Takabatake H, Mori M, Natori H, Mori K, Toriwaki J. Virtual bronchoscopy system: A teaching tool for medical students. The 86th Scientific Assembly and Annual Meeting of the Radiological Society of North America, Chicago, Radiology 217suppl: 697 (2000).
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Rehabilitation Medicine

The Division of Rehabilitation at Sapporo Medical University provides acute phase rehabilitation in each department in the hospital and assists patients in making a comeback to normal life in Hokkaido. Because of the heavy snowfalls in this northern region, we carry out research into walking on snowy, icy roads in winter, and the development of trans-femoral prosthesis and wheelchairs suitable for icy roads. Furthermore, we have established a medical support system for athletes and offer preventive and medical treatment for sports injury.

Professor and Director (Affiliated)
Seiichi Ishii, M.D., Ph.D.
Interests:
Rehabilitation of hand dysfunction

Associate Professor
Kazutoshi Yokogushi, M.D., Ph.D.
Interests:
Rehabilitation of spinal cord Injury

Assistant Professor
Hiroshi Narita, M.D., Ph.D.
Interests:
Rehabilitation of amputee, Sports medicine

1. Amputation and orthopaedic rehabilitation

Many trauma patients are transported to the hospital of Sapporo Medical University from other hospitals in Hokkaido island. For the cases which need emergency amputation, we are able to do immediate rigid dressing after the operation. This method is useful for the amputees' early rehabilitation. Additionally there are many cases of requests for amputation form the cardiovascular department and in the department of orthopaedic surgery there are many cases of limb saving operations to malignant tumors of the extremities and other hand surgery, following which splint or brace therapy is popular. And various rehabilitation techniques are employed for problems of the upper extremities, for example congenital anomaly and postoperative hand (9).

2. Walking and motion analysis

We use the force plate and motion analyzer for analysis of walking and motion. This system is useful for the whole range of rehabilitation, from handicapped person to athlete (10).

3. Prosthetics and orthotics

Hokkaido island is a subarctic snow zone. We have researched the walking ability of amputees on snow roads and our analysis has helped us to develop our understanding of the function of prosthesis, especially for knee joint (6). As a result we decided that in cases of above knee amputation the best prosthesis is the ischial ramal containment socket (1-3) and for below the knee amputation, the total surface bearing socket with silicone sleeve is the best (4). In these cases, we evaluate the fitting of the socket by using a motion analyzer, X-ray and cineradiography.

4. Spinal cord injury and myelopathy

We do rehabilitation for spinal cord injury in the early phase and we evaluated myelopathy or ataxia patients by using a walking analyzer. It is popular for spinal cord injury patients to plan to return to home and redesign it to suit their disability (8).

5. Sports medicine

We do medical checks of athletes in Hokkaido and we analyze sports performance and measure muscle strength using the motion analyzer and an isokinetic machine (5). This system is useful for rehabilitation following sports injury or after an operation. We have developed a prosthesis for sprinting, with good results.

6. Others

We have designed powered wheelchairs with six wheels, useful in snowfall conditions. Other research activities include pain control, the respiratory problems of Duchenne muscular dystrophy and post-polio syndrome (7).

List of Main Publications from 1997 to 2000

1) Narita H, Yokogushi K, Ishii S, Kakizawa M, Nosaka T. Suspension effect and dynamic evaluation of the total surface bearing (TSB) trans-tibial prosthesis: a comparison with the patellar tendon bearing (PTB) trans-tibial prosthesis. Prosthet

Orthot Int 21: 175-178 (1997).

2) Narita H, Yokogushi K, Ishii S, Kakizawa M, Nosaka T, Nakamura Y. Suspension effect of the TSB BK prosthesis: A comparison with the PTB BK prosthesis. Jpn Journal Rehabil Med 34:418-428 (1997).
3) Narita H, Yokogushi K, Ishii S, Kakizawa M, Nosaka T. Suspension effect and dynamic evaluation of the TSB BK prosthesis: a comparison with the PTB BK prosthesis. In: Ueda S, Nakamura R. Ishigami. (eds) Proceedings of the 8th World Congress of the International Rehabilitation Medicine Association. Aug. 31-Sep. 4, Bologna, Monduzzi Editore, 665-668 (1998).
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5) Narita H, Yokogushi K, Kura H, Ishii S, Mutoh Y, Oota M. Conservative Treatment of Fatigue Fracture in Young Athletes. In: 5th Japan-Korea Joint Meeting of Orthopaedic Sports Medicine. Kyonju. Korea. Abstract (2000).
6) Narita H, Yokogushi K, Ishii S. Development of transfemoral prosthesis which is suitable for walking on freezing road: New Millennium Asian Symposium on Rehabilitation Medicine. Tokyo. Japan. Abstract (2001).
7) Uchiyama E, Sasaki T, Takahashi T, Yokogushi K, Kawamura B. Retrospective study on the longitudinal overgrowth after limb lengthening in polio-myelitis. 20th Societe Internationale de Chirurugie Orthopedique et de Traumatologie. Amsterdam. Netherlands. Abstract (1997).
8) Yamashita T, Sakamoto N, Sekine M, Takebayashi T. Yokogushi Y. An electro-physiological study on the mechanoreceptors in the lumbar posterior longitudinal ligament. 26th International Society of the Study of the Lumbar Spine. Cona. USA. Abstract (1999).
9) Tsubota S, Kato M, Aoki M, Kitamura M, Wada T, Yokogushi K, Ogino T. Hand Therapy for restoring function of upper extremities in a case of arthrogryposis. 5th International Symposium on Congenital differences of the Upper Limb. Kyoto. Japan. Abstract (2000).
10) Yokogushi K. Analysis of posture and gait. 2nd International Symposium of Electro-Kinesiology. Sapporo. Japan. Abstract (2000).

Clinical Pathology

The Department of Clinical Pathology has three main duties: histopathology by microscopic observation of biopsied and operated specimens to identify the disease, cytopathology to judge the malignancy, and autopsy to clarify the cause of the disease and examine the appropiateness of the clinical diagnosis and treatment. Information is exchanged with various clinical departments by means of pre- and post-operational meetings to seek a higher level of diagnosis and treatment.

Professor and Director (Affiliated)
Noriyuki Sato, M.D., Ph.D.
Interests:
Tumor immunity, Molecular pathology

Associate Professor
Masaaki Satoh, M.D., Ph.D.
Interests:
Pulmonary pathology, Hepatology

Assistant Professor
Tatsuru Ikeda, M.D., Ph.D.
Interests:
Surgical pathology, Cell biology

Instructor
Yasuaki Tamura, M.D., Ph.D.

1. Basic pathology

a) Cell biology

The role of intracellular organelles, especially cytoskeltons and tight junctions in the process of carcinogenesis, cancer progression, metastasis and other diseases were investigated (1). We found that the organization of microtubules was necessary on the expression of the liver specific functions in the primary cultured hepatocytes (2).

b) Tumor suppressor genes

p73, a novel member of the p53 family, at chromosome 1p36.3, at which locus frequent defects were seen in many tumors (3). We are investigating p73 status in many tumors including neuroblastoma, malignant lymphoma and thymoma using molecular biological and immunohistochemical tequniques.

c) Tumor immunity

Immunotherapy of mice with preexisting cancers with heat shock protein preparations derived from autologous cancer resulted in retarded progression of the primary cancer, a reduce metastatic load, and prolongation of life-span. These results support the efficacy of autologous cancer-derived heat shock protein-peptide complexes in immunotherapy of cancers without the need to identify specific tumor antigenic epitopes (4).

2. Surgical pathology

Histopathologic and clinicopathologic studies using electron microscopy, immunohistochemistry and molecular biological tequniques are being investigated in many organ systems in collaboration with other clinicial departments.

a) Pulmonary pathology

Clinicopathologic studies on lung cancers and interstitial lung diseases were carried out with special reference to surfactant apo-proteins. We investigated the histological and molecular characteristics of pulmonary alveolar proteinosis in infants (5).

b) Gastrointestinal pathology

We have collected gastrointestinal stromal tumors (GISTs), and investigated 16 cases of GISTs immunohistochemically and ultrastructurally. On the basis of the immunohistochemical anaysis, GISTs were classified as myogenic type, Schwann cell type, Cajal cell type and mixed cell type. This classfication revealed good correlation to the ultrastructural findings (6.7).

c) Hepatology

Hepatocarcinogenesis has been studied with experimental animals and human hepatocellular carcinomas(HCCs). We found that hypermethylation of the 14-3-3 **σ** gene (8) and cytoplasmic cyclin D1 expression (9) were important in the development and progression of HCCs.

d) Ophthalmic pathology

Clinicopathologic and genetic studies of MALT lymphoma of the conjunctiva (10), and second primary sarcoma in patients with bilateral retinoblastoma (11) were carried out.

e) Cytopathology

In search of a qualitatively superior, higher level diagnosis, cytopathologic studies have been done in many organ systems (12.13).

f) Ultrastructural pathology (14-16)

List of Main Publications from 1997 to 2000

1) Mori M, Sawada N, Kokai Y, Satoh M. Role of tight junctions in the occurrence of cancer invasion and metastasis. Med Electron Microsc 32: 204-208 (1999).
2) Ikeda T, Sawada N, Satoh M, Mori M: Induction of tyrosine aminotransferase of primary cultured rat hepatocytes depends on the organization of microtubules. J Cell Physiol 175: 41-49 (1998).
3) Ichimiya S, Nakagawara A., Sakuma Y, Kimura S, Ikeda T, Satoh M, Takahashi N, Sato N, Mori M. p73: Structure and function. Pathol Int 50: 589-593 (2000).
4) Tamura Y, Peng P, Liu K, Daou M, Srivastava PK. Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. Science 278: 117-120 (1997).
5) Sakai Y, Abo W, Yoshimura H, Sano H, Kuroki Y, Satoh M, Kaimori M. Pulmonary alveolar proteinosis in infants. Eur J Pediatr 158: 424-426 (1999).
6) Yamaguchi J, Sawada N, Tobioka H, Takakuwa R, Goto T, Sakuma Y, Ikeda T, Satoh M, Mori M: Electron microscopic and immunohistochemical studies of gastrointestinal stromal tumors. Med Electron Microsc 32: 213-220 (1999).
7) Ikeda T, Yoshida Y, Satoh, M, Sawada N, Mori M. Oesophageal sarcoma with CD34 positivity. Histopathology 33: 284-286 (1998).
8) Iwata N, Yamamoto H, Sasaki S, Itoh H, Suzuki H, Kikuchi T, Kaneto H, Iku S, Ozeki I, Karino Y, Satoh T, Toyota J, Satoh M, Endo T, Imai K. Frequent hypermethylation of CpG islands and loss of expression of the 14-3-3 sigma gene in human hepatocellular carcinoma. Oncogene 46: 5298-5302 (2000).
9) Sato Y, Itoh F, Hareyama M, Satoh M, Hinoda Y, Seto M, Ueda R, Imai K. Association of cycline D1 expression with factors correlated with tumor progression in human hepatocellular carcinoma. J Gastroenterol 34: 486-493 (1999).
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11) Hasegawa T, Matsuno Y, Niki T, Hirohashi S, Shimoda T, Takayama J, Watanabe C, Kaneko A, Sano T, Satoh M, Suzuki J. Second primary rhabdomyosarcomas in patients with bilateral retinoblastoma: A clinicopathologic and immunohistochemical study. Am J Surg Pathol 22: 1351-1360 (1998).
12) Ohta Y, Ikeda T, Satoh M, Takahashi S, Konishi Y, Kimura S, Gotoh T, Azuma K, Asanuma H, Kondoh K, Mori M. Cytology of malignant endocrine tumor of the pancreas: a case report. Tumor Res 32: 49-55 (1997).
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15) Tanaka N, Kimijima Y, Mimura M, Yamaguchi A, Ichinose S, Kohama G, Satoh M. Ultrastructure of lentigo maligna in the oral cavity. Med Electron Microsc 31: 156-161 (1998).
16) Ikeda T, Satoh M, Azuma K, Sawada T, Mori M. Medullary thyroid carcinoma with a paraganglioma -like pattern and melanin production: a case with ultrastructural and immunohistochemical studies. Arch Pathol Lab Med 122: 555-558 (1998).

Hospital Pharmacy

Drug information service, therapeutic drug monitoring service and bedside pharmaceutical care service are our main daily activities in the field. On the other hand, as a research theme, we have been taking an interest in polymorphisms in drug metabolizing enzymes in some inpatients who have complained about their medication recently. Under the disadvantageous conditions which exist, young pharmacists are doing their best. So our next goal is to create research positions.

Professor and Director
Koichi Itaya, M.P., Ph.D.
Interests:
Clinical pharmacology, Cell receptorology

1. Drug information service

Our experience in the drug information service led us to the conclusion that information obtained from a single compendium or handbook is often not satisfactory. Therefore, we have compiled our own catalog of more than 1500 commonly-used drugs based on eight standard references and observations of these drugs' actions. The catalog includes indices for cross-reference to the names of diseases, symptoms and side effects. Then we officially established a Drug Information Service office in our hospital pharmacy in 1989. This office has handled almost three hundred and fifty requests for information per month recently, about 80% or more of which are from doctors.

Recent university graduates are insufficiently educated about the effects of the agents they are to use and need a reliable and speedy source of information. They often use the service when standard references fail to provide the information they need. Experienced physicians also regularly use the service to verify trade names, ingredients, recommended dosages and availability of drugs.

Education is very important in any field, so about ten years ago I edited a textbook on drug information entitled "A GUIDE TO DRUG INFORMATION". The 3rd edition was published last year (Nanzando. Tokyo. 1999, in Japanese).

2. Therapeutic drug monitoring (TDM) service

In 1985, we set up a service to provide data on therapeutic drug monitoring. The service is open three days a week (every other day) for use by doctors who wish to verify the dosages of the drugs they prescribe. The doctor provides a sample of blood with the time and means of administration of the drug and the time of sampling. The TDM service tests the sample and provides the doctor with both the total plasma concentration of the drug and a graph showing the simulated time course of plasma concentration for that individual. The graph is derived numerically by an analysis based on population pharmacokinetics. We determine only total drug concentration, because it is technically more difficult to measure only free active drug in plasma in routine clinical practice. This service has been used for about 300 cases a month lately; about 60% of requests concern antiepileptics such as phenytoin. Half of the rest are about theophylline. Lately these also have been many requests for information about special drugs such as methotrexate, cyclosporin or vancomycin. With particular reference to MRSA, the monitoring of vancomysin has been much appreciated by young doctors.

3. Bedside pharmaceutical care service

As a result of our experience in the two kinds of services mentioned above in section 1 and 2, we started educating inpatients about medication as the 3rd service in 1996. Patients are given information about medication during hospitalization at several departments of the clinical divisions, including the Department of Orthopedic Surgery, Internal Medicine (II), Ophthalmology, Urology, Otolaryngology, and some others. For this education session, not only are patients encouraged to ask questions, but also patients are queried as to the methods of administration, dosage and the taste of drugs, interactions with drinks or meals, and other things such as adverse reactions. The effects of drug consultation with pharmacists on medication compliance in inpatients have been studied. Due to differences in their past disease history, the objective knowledge regarding medication and the drug action varies widely among patients. However, the analysis of any kind of problems concerning medication contributes to improving the patients' quality of life during their hospitalization.

4. Joint research projects

Unfortunately, there are no teaching staff members in our hospital pharmacy except myself. This is a common problem in public or private colleges or university hospital pharmacies in Japan. National teaching hospitals have many teaching staff even in the pharmacy section. Lately, however, the number of graduate students who want to be hospital pharmacists has increased slightly. We have a few pharmacists now who have graduated from a graduate school of pharmacy, but unfortunately, their terms of employment do not allow them to perform research toward getting a Ph.D. degree during the eight-hour working day. Those who wish to pursue further studies must do so in other departments outside working hours. Three of our staff with Master's degrees are now continuing their studies in the Department of Public Health and Legal Medicine in Sapporo Medical University or in the Department of Clinical Pharmacy in Hokkaido Institute of Pharmaceutical Sciences. In the Department of Public Health, they have been studying the effects of rare metals, especially the relation between aluminum and Alzheimer's disease. This is an appropriate topic for pharmacists because aluminum is used widely in medicines. They study poisoning in the Department of Legal Medicine with a heavy emphasis on qualitative and quantitative analysis. The TDM service described above is helped by the Department of Clinical Pharmacy in Hokkaido Institute of Pharmaceutical Sciences. Fortunately, two of the three staff mentioned above obtained an M.D. degree last year (1.2). Our next goal is to create positions in which young pharmacists can do research on more basic subjects of their own choosing.

On the other hand, as a research theme, we have been taking an interest in polymorphisms in drug metabolizing enzymes recently. Polymorphisms in the enzymes are postulated to contribute to interindividual variation in drug disposition and its effects. Under the disadvantages conditions in which we operate, such as shortage of teaching staff, young pharmacists in our hospital pharmacy have been doing their best, and come up with data showing the presence of the variant alleles encoding UDP-glucuronosyltransferease 2B7(UGT2B7)H and UGT2B7Y from an analysis of genomic DNA from more than 130 unrelated Japanese, and the existence of polymorphisms in cytochrome P-450s (CYP2C9, CYP2C19, CYP2D6) in some inpatients who had complained about their medication.

List of Main Publications from 1997 to 2000

1) Watanabe T, Morita M. Asphyxia due to oxygen deficiency by gaseous substances. Forensic Sci Int 96: 47-59 (1998).
2) Sugawara N, Katakura M, Sugawara C. Preventive effect of zince compound, polaprezine and zince acetate against the onset of hepatitis in Long-Evans Cinnamon rat. Res Commun Chem Pathol Pharmacol 103: 167-176 (1999).

Biochemistry

~Cancer Research Institute~

Protein-tyrosine kinases are important as early signal transducers of external signals to cells. We have been trying to find the cell-biological functions of a protein-tyrosine kinase CAKβ/PYK-2 and an adaptor protein Efs, both of which we identified in 1995. In the search for proteins interacting with CAKβ, we identified Hic-5 and other proteins. We are studying these proteins in cultured cells by using recombinant DNA technology, protein chemistry, immunocytochemistry, and GFP-aided cell-imaging.

Professor
Terukatsu Sasaki, M.D., Ph.D.
Interests:
Regulation of cell activities by protein-tyrosine kinases

Assistant Professor
Hiroko Sasaki, M.P., Ph.D.
Interests:
Signaling pathways transduced by CAKβ/PYK2.

Masaho Ishino, M.Env.Sci., Ph.D.
Interests:
Protein-protein interactions,
Signal transduction by tyrosine phosphorylation

Instructor
Hiroshi Aoto, M.Sci., Ph.D.

1. Studies on cell adhesion kinase β (CAKβ/PYK2), the second protein-tyrosine kinase of the focal adhesion kinase (FAK) subfamily.

The activation of protein-tyrosine kinases (PTKs) is one of the most common signal transduction mechanisms directly coupled to receptor activation by external signals. In 1995 we published the cDNA cloning of CAKβ (J Biol Chem, (1995) 270: 21206-21219), which is now known also as PYK2, RAFTK, and CADTK. CAKβ is a nonreceptor PTK of the focal adhesion kinase (FAK) family. Although the two proteins have similar domain structures and amino acid sequences (about 46% identical), CAKβ and FAK exhibit different intracellular localization, different cell and tissue expression, and different upstream signals for activation (9). CAKβ has an autophosphorylation site at tyrosine 402 and this phosphorylated residue provides a docking site for the SH2 domains of c-Src and Fyn. A cascade of tyrosine-phosphorylation follows the binding and activation of c-Src/Fyn (2).

The C-terminal region of CAKβ has two proline-rich sequences, PPPKP^{717}SRP and PPQKP^{859}PRL, that contain the PXXP motif and are the binding sites of SH3 domains. In our studies on the signaling pathways regulated by CAKβ, we found that CAKβ associates with Cas and Graf by binding their SH3 domains to its proline-rich sequences (4.5). To study the cell-biological importance of the signal transduction initiated by the binding of these and, possibly, other proteins to the proline-rich sequences of CAKβ, P717A and P859A mutants of CAKβ were expressed in cultured cells by the use of expression plasmids and recombinant adenoviruses. In marked contrast to our previous finding that wild-type CAKβ localized at the cytoplasm and the surface membrane, P859A-CAKβ exclusively localized in the cell nucleus. The wild-type CAKβ also accumulated in the nucleus under some conditions. Moreover, several proteins known to associate with CAKβ were found to co-localize with the mutant CAKβ in the nucleus. These results indicate that CAKβ shuttles between the cytoplasm and the nucleus and imply that CAKβ may regulate nuclear processes such as transcription, particularly because Hic-5, a CAKβ-binding protein, was recently shown to be a coactivator of nuclear receptors.

CAKβ is less evenly expressed than FAK. We found that axons of the central nervous system, ciliated epithelial cells of the epididymis and bronchus, and microvilli of intestinal and urinary tubular epithelia are rich in CAKβ (8). CAKβ is highly expressed in hematopoietic cells and in macrophage. Using immunocytochemistry and immunoelectron microscopy, we showed that CAKβ is colocalized with microtubules and microfilaments in some cell lines and in a fibroblast line transfected with CAKβ cDNA (1). Studies on the regulation of cell spreading and migration by CAKβ are now under way.

2. Studies on Hic-5, a CAKβ-binding protein found at focal adhesions and translocated to the cell nucleus.

In a study to elucidate the upstream and downstream signaling pathways of CAKβ, we used an expression cloning technique to identify binding partners for CAKβ. A cDNA for a CAKβ-binding protein thus identified encodes the human homologue of Hic-5 (6). We found that Hic-5 localized at focal adhesions in a rat fibroblast line WFB. The amino acid sequence of Hic-5 is highly similar to that of paxillin in the four LD motifs as well as in the four contiguous LIM domains, although Hic-5 has unique sequences in its N-domain important for signal transduction.

CAKβ was coimmunoprecipitated with Hic-5 from the WFB cell lysate. One of the LD motifs directly mediates the association of Hic-5 with the extreme C-terminal region of CAKβ. The Hic-5 that coimmunoprecipitated with CAKβ was selectively tyrosine-phosphorylated in WFB cells exposed to hypertonic osmotic-stress. We showed that Hic-5 was phosphorylated at tyrosine 60 by CAKβ and also by Fyn (3). This phosphorylation on tyrosine 60 created in Hic-5 a binding site for the SH2 domain of Csk. Specific phosphorylation of Hic-5 by CAKβ and Fyn may activate a signaling pathway mediated by Hic-5. We recently cloned, by yeast two-hybrid screening, a new CAKβ-binding protein with an activity of transcription regulator.

3. Studies on Efs, a docking protein transducing signals from protein-tyrosine kinases.

We identified a cDNA of an Fyn-SH3 binding protein, Efs (Embryonl Fyn-assoicated Substrate), by expression cloning of mouse embryonic cDNA library (Oncogene (1995) 11, 2331-2338). Efs is characterized by two consecutive proline-rich motifs, a YXXP cluster, and an SH3 domain; these are the motifs and domain of signaling molecules for protein-protein interaction. The overall structure of Efs is closely related to that of $p130^{Cas}$ (Cas). Efs seems, however, different in many functional properties from Cas. Efs is highly expressed in embryo and cartilage. Unlike Cas, Efs does not localize at focal adhesions and is not phosphorylated in response to signalings from integrins or growth factor receptors. We have cloned two human homologues of Efs cDNA; hEfs1 and hEfs2 (10). hEfs1 is a human homologue of Efs. hEfs2 is an hEfs1's variant that does not have the SH3 domain. Identification of the ligand protein(s) binding to Efs-SH3 is a critical step in the study of Efs function. We compared the binding activity of SH3 domains of hEfs1 and Efs-related proteins. We found that Efs-SH3 bound only weakly to FAK and CAKβ, two known ligands of Cas. The result suggests the presence of so far unknown ligand(s) unique to Efs-SH3 (4).

Peptide-mass fingerprinting, a method combining peptide fingerprinting, mass spectrometry and computer-based data base searching, is a powerful method for identifying simultaneously a series of proteins at the subpicomol level. Because natural association of proteins within cells is best reproduced by experimental systems that can utilize the cellular environment for the detection of binding, this method is also suitable for studying protein-protein interactions. By using this method, we are now searching for proteins that are pulled down with recombinant Fyn-SH3, Efs-SH3 and CAKβ from cell and tissue lysates (Tumor Res 35: 57-62(2000)).

List of Main Publications from 1997 to 2000

1) Aoto H, Mitaka T, Sasaki H, Ishino M, Mochizuki Y, Sasaki T. Association of cell adhesion kinase β (CAKβ/PYK2) with cytoskeleton. Tumor Res 35: 35-47 (2000).
2) Katagiri T, Takahashi T, Sasaki T, Nakamura S, Hattori S. Protein-tyrosine kinase Pyk2 is involved in interleukin-2 production by Jurkat T cells via its tyrosine 402. J Biol Chem 275: 19645-19652 (2000).
3) Ishino M, Aoto H, Sasaki H, Suzuki R, Sasaki T. Phosphorylation of Hic-5 at tyrosine 60 by CAKβ and Fyn. FEBS Lett 474: 179-183 (2000).
4) Ohba T, Ishino M., Aoto H, Sasaki T. Dot far-Western blot analysis of relative binding affinities of the Src homology 3 domains of Efs and its related proteins. Anal Biochem 262: 185-192 (1998).
5) Ohba T, Ishino M, Aoto H, Sasaki T. Interaction of two proline-rich sequences of cell adhesion kinase β with SH3 domains of $p130^{Cas}$-related proteins and a GTPase-activating protein Graf. Biochem J 330: 1249-1254 (1998).
6) Matsuya M, Sasaki H, Aoto H, Mitaka T, Nagura K, Ohba T, Ishino M, Takahashi S, Suzuki R, Sasaki T. Cell adhesion kinase β forms a complex with a new member, Hic-5, of proteins localized at focal adhesions. J Biol Chem 273: 1003-1014 (1998).
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8) Mitaka T, Shindoh M, Mochizuki Y, Sasaki H, Ishino M, Matsuya M, Ninomiya T, Sasaki T. Restricted expression of cell adhesion kinase-β in rat tissues. Am J Pathol 150: 267-281 (1997).
9) Schaller M, Sasaki T Differential signaling by the focal adhesion kinase and cell adhesion kinase β. J Biol Chem 272: 25319-25325 (1997).
10) Ishino M., Ohba T, Inazawa J, Sasaki H, Ariyama Y, Sasaki T Identification of an Efs isoform that lacks the SH3 domain and chromosomal mapping of human Efs. Oncogene 15: 1741-1746 (1997).

Molecular Biology

~ Cancer Research Institute ~

Our department has been attempting to isolate and characterize genes associated with carcinogenesis and those causing or predisposing to human cancer. One of the major goals is to identify genes predisposing to diseases, and to develop novel diagnostic and therapeutic tools. By means of research technologies applicable to molecular biology, we have contributed to the identification of a number of biologically and medically interesting genes.

Professor
Takashi Tokino, Ph.D.
Interests:
Molecular biology of human cancer, Human cancer genetics, Human genome analysis

Instructor
Yasushi Sasaki, M.D.
Minoru Toyota, M.D., Ph.D.
Hiroyuki Nishimori, M.D., Ph.D.

1. Identification and functional analysis of p53-target genes

The p53 tumor suppressor gene is involved in cell cycle control, apoptosis, genome stability, and angiogenesis, through the transactivation of p53-target genes. Mutations in the p53 gene are the most common mutations detected in human cancers. Inactivation of p53 is involved not only in carcinogenesis but also in conferring resistance of cancer cells to chemotherapy.

We have been attemping to identify biologically important genes that are transcriptionally regulated by p53 by two approaches. The first of these strategies involved subtractive hybridization and differential screening. These techniques can successfully detect p53-target genes that are expressed in relative abundance. By this strategy, we previously isolated two novel p53-inducible genes, TP53TG1 and TP53TG3. The other approach is a yeast enhancer trap system that allows direct cloning of p53 binding sequences (p53-tagged sites) from human genomic DNA. We isolated cosmid clones containing "functional" p53 binding sites and screened candidate genes in the vicinity of those sites. In this manner, we have isolated several p53 target genes, such as GML, P2XM, BAI1, CSR, and p53AIP1.

2. Function of p53 family genes

The p53 family genes, p73 and p51/p63, have been isolated recently and encode proteins that share considerable structural and functional homology with p53, suggesting that the function of these genes may be similar to that of p53. Similar to p53, these related genes induce apoptosis. To evaluate the anti-cancer activity of p73 and p51/p63 in comparison with that of p53, we sought to analyze growth arrest and apoptosis induced by the p53 family genes in a panel of human colorectal cancer cell lines. We found that the apoptotic response to the p53 family genes was uniform in some cell lines and heterogenous in others. Our results suggest that adenovirus-mediated p51/p63 and p73 gene transfer is a potential novel approach for the treatment of colon cancers, particularly for tumors that are resistant to wild-type p53 gene therapy.

3. Molecular mechanism of oncogenesis by EWS-Ets fusion genes in Ewing tumors

Ewing tumors, including Ewing sarcoma and perpheral neuroectodermal tumor, are well characterized at the molecular level by a unique chromosomal rearrangement which makes the fusion gene between the EWS gene and one of the Ets family genes. Ets family genes are comprised by a group of transcription factors and EWS-Ets fusion genes are supposed to have the ability of transactivation and/or repression to the target gene(s). To elucidate the molecular mechanism or signal network regulated or deregulated by EWS-ETS(s) fusion gene, we generated several cell lines constitutively expressing EWS-ETS fusion protein. To identify the direct and/or indirect target gene(s), we performed cDNA array hybridization, representative difference analysis (RDA) or mRNA differential display method using mRNA extracted from these cell lines. Our goal is identification of main target gene(s) or signal transduction pathway(s) regulated or deregulated by EWS-Ets fusion gene, and to eventually contribute to finding some diagnostic and/or therapeutic clue(s) in Ewing tumors patients.

4. DNA methylation profile of colorectal and gastric cancers

Transcriptional silencing of selected genes by DNA methylation plays a crucial role in the development and progression of human gastrointestinal malignancies. To date,

genes involved in regulation of the cell cycle, DNA repair, angiogenesis and apoptosis have all been shown to be inactivated by hypermethylation of respective 5' CpG islands.

We have developed a novel technique, methylated CpG island amplification (MCA), for detecting changes in DNA methylation and determined the methylation profile of colorectal and gastric cancers. One key process involved in aberrant methylation is related to aging, and because it affects a large number of CpG islands, age-related methylation may be a primary cause of the increased incidence of cancer seen among older individuals. Other patterns of methylation are cancer-specific and are detected only in a subset of tumors exhibiting the CpG island methylator phenotype (CIMP). In that regard, the majority of sporadic colorectal cancers exhibiting microsatellite instability (MSI) appear to be associated with CIMP, which leads to aberrant methylation of hMLH1 and the loss of its expression. We anticipate that as the various components of the molecular machinery involved in aberrant DNA methylation become better understood, they will prove to be useful targets, serving as the basis for the development of new methods for the diagnosis and treatment of gastrointestinal malignancies.

List of Main Publications from 1997 to 2000

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Pathology

~ Cancer Research Institute ~

Our department has been studying the regulation of growth and maturation of hepatocytes. We established a primary culture system for rat hepatocytes to investigate the mechanisms of their proliferation and differentiation. Recently, we found that small hepatocytes, which are hepatic stem cells that were first found by us, could form the hepatic organoid interacting with hepatic nonparenchymal cells *in vitro*. Our aim is to make transplantable hepatic tissues and an artificial liver.

Professor
Yohichi Mochizuki, M.D., Ph.D.
Interests:
Hepatocyte culture

Associate Professor
Toshihiro Mitaka, M.D., Ph.D.
Interests:
Hepatic stem cells, growth and maturation of hepatocytes

1. Our main research projects

a) Studies on rat hepatic progenitor cells, 'small hepatocytes'

Small hepatocytes have been identified as proliferating cells with hepatic characteristics. We first found a remarkable increase in small mononucleate cells within primary hepatocytes cultured in medium supplemented with 10 mM nicotinamide and EGF. A small hepatocyte can proliferate and form a colony. The growth speed is slow and 5 to 6 divisions occur within 10 days. The population of small hepatocytes in the adult rat liver is estimated to be 1.5-2.0% of hepatocytes, and the number of the cells decreases with age. The cells can also be isolated from the human liver and their clonal expansion has been demonstrated in culture. Although the cells can continue growing without losing hepatic characteristics for several months, the immortalization of the cells is so difficult that cell lines have not yet been established. As specific antibodies to small hepatocytes have not been found, the precise origin or location within the liver can not be defined either. Small hepatocytes *in vitro* can maintain the ability of rapid proliferation unless hepatic nonparenchymal cells (NPCs) such as stellate cells grow and attach to them. When NPCs rapidly proliferate and invade under colonies of proliferating small hepatocytes, maturation of small hepatocytes is stimulated with accumulation of an extracellular matrix (ECM). The cells enlarge their cytoplasm, which is rich in mitochondria, rough endoplasmic reticulum, peroxisomes and glycogen. Some cells possess two nuclei. Thereafter, alteration of the cellular morphology is attributed to the reconstruction of hepatic tissues, which may mimic hepatic plate formation. Between the cells comprising plates, bile canaliculi are formed and these actively contract. On the other hand, proliferating small hepatocytes express hepatocyte nuclear factor 4 (HNF4), whereas the mature large hepatocytes express CCAAT/enhancer binding protein α and HNF6 in their nuclei as well as HNF4. Changes of cell shape may result in the sequential expression of liver-enriched transcription factors. Although it is unclear which ECMs can induce the maturation, these results also fuel speculation that, in pre- and post-natal periods, the arrangement of plates in liver lobules may be performed by the invasion of stellate and sinusoidal endothelial cells. Plates several cells thick become 1 or 2 cells thick accompanying the maturation of hepatic cells. When NPCs invade and secrete ECMs while forming sinusoids, hepatic cells are becoming mature (1.3.6-8).

b) Studies on Growth and Differentiation of Cultured Hepatocytes

Primary hepatocytes represent an excellent system to study the regulation of gene expression, as well as its relation to cellular differentiation and growth control. In reproducing hepatic functions in vitro similar to those in vivo, although many researchers have been using primary hepatocytes, it has been very difficult to maintain the differentiated functions of the hepatocytes, especially the levels of tryptophan 2,3-dioxygenase (TO), serine dehydratase (SDH), connexin 32 (Cx32) and 26 (Cx26). When primary rat hepatocytes were cultured in L-15 (amino acid-rich) medium supplemented with 20 mM $NaHCO_3$ and 10 ng/ml EGF, and 2% dimethylsulfoxide was added to the medium from day 4, we could reinduce both mRNAs and proteins of TO, SDH, and Cxs and maintain the levels of expression for about one month. We also examined the changes in the expression of liver-enriched transcription factors in the transition between growth and differentiation of the cultured hepatocytes.

For hepatic differentiation, not only inhibition of DNA synthesis but also induction of appropriate transcription factors should be required. Thus, expression of HNF3γ, C/EBPα, and C/EBPβ may be necessary for hepatocytes to acquire highly differentiated functions in addition to coexpression of certain amounts of transcripts of HNF1α, HNF1β, HNF3α, HNF3β, and HNF4, as well as suppression of C/EBPδ (7.9).

(c) The roles of cytoskeleton and cell-cell junctions in hepatocytes

Gap junctional intercellular communication is thought to play a crucial role in the maintenance of homeostasis, morphogenesis, cell differentiation and growth control in multicellular organisms. Gap junctions are composed of proteins called `connexins'. Hepatocytes have two homologous connexin molecules, Cx32 and Cx26. Moreover, as epithelial cells differentiate, tight junction strands establish cell polarity, and small gap junction plaques appear within the tight junction web. We are now investigating the roles of such junctional proteins in connexin-knockout mice and primary hepatocytes (4.5.10.11).

(d) Artificial Liver

To develop an effective hybrid bioartificial liver device, the material of the scaffold is very important to support hepatocytes that have both growth ability and hepatic differentiated functions. We found that primary hepatocytes cultured on water-insoluble paper could grow and survive for a long time while maintaining differentiated functions. Woven cellulose fibers may be a good bed for hepatocytes to form a three-dimensional structure similar to the in vivo hepatic microenvironment. (2; in collaboration with the Department of System Design Engineering, Keio University).

2. Collaboration with other laboratories

a) Isolation and culture of human hepatocytes:

We have been trying to isolate human hepatocytes that can proliferate and be maintained for a long time. In addition, we are looking for hepatic progenitor cells, ‘small hepatocytes,’ which are often observed within rat hepatocytes (collaboration with Department of Gastroenterological Surgery, Kyoto University).

b) The mechanisms of bile canalicular contraction in the reconstructed hepatic organoid:

We are investigating the mechanism of bile canalicular contraction in the hepatic organoid reconstructed by small hepatocytes and hepatic nonparenchymal cells (collaboration with First Department of Physiology, Sapporo Medical University).

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Molecular Medicine

～Biomedical Research, Education and Instrumentation Center～

Our research interests are directed at the elucidation of the molecular mechanisms underlying disease and its application for the better treatment of patients. The ongoing research fields are: 1) basic and preclinical studies on gene therapy for cancer, immune/inflammatory disorders, and vascular diseases; 2) basic stem cell biology as well as preclinical studies in regenerative medicine.

Professor
Hirofumi Hamada, M.D., Ph.D.
Interests:
Gene therapy, Regenerative medicine, Molecular biology

Instructor
Yoshinori Ito, M.D.
Katsunori Sasaki, Ph.D.
Masayoshi Kobune, M.D., Ph.D.
Sachie Hirai

1. Gene therapy: basic biotechnology

a) Generation of fiber-mutant recombinant adenoviruses in gene therapy for malignant glioma (Yoshida, Y. et al. *Human Gene Therapy* 9(17): 2503-2515, 1998) Recombinant adenovirus (Adv)-mediated gene transduction is a powerful technology for cancer gene therapy. In this paper, we report the generation of a fiber-mutant Adv vector, using the Adv genomic DNA-terminal protein complex (DNA-TPC) cotransfection method. By this procedure, we have generated a fiber mutant, F/K20, which has a linker and a stretch of 20 lysine residues added at the C-terminus of the fiber. Using Adv for a reporter LacZ gene (AxCAZ2) with either F/K20 or wild-type fiber (F/wt), we examined the transduction efficiency of F/K20-Adv. The F/K20-Adv showed a remarkably enhanced efficiency in genetic transduction of human glioma cells. In all four human glioma lines tested, the multiplicities of infection (MOIs) for transduction of 50% of the population (ED_{50}) were decreased with the F/K20-Adv compared with the F/wt-Adv: by 7-fold for T98G, 14-fold for U251, 9-fold for U373, and 42-fold for U87 cells. Therefore, we attempted to apply the F/K20-Adv in gene therapy for malignant glioma. Glioma cells infected with the F/K20-Adv for interleukin-2 or interleukin-12 produced a high level of each cytokine at a much smaller MOI than that with the F/wt-Adv. Infection with the F/K20-Adv for wild-type p53 tumor suppressor gene resulted in an enhanced level of the p53 protein expression and an increased apoptotic cell death of glioma cells. These data demonstrated a definite advantage of F/K20-Adv in transduction efficiency for malignant glioma, providing promising tools for gene therapy.

2. Gene therapy for cancer

a) Highly augmented cytopathic effect of a fiber-mutant E1B-defective adenovirus for gene therapy of glioma. (Shinoura et al. *Cancer Res* 59(14): 3411-3416, 1999) An E1B 55-kD gene-defective adenovirus has been reported to be a highly useful replication-competent adenovirus (Adv) which shows a cytopathic effect on cancers with abnormality of p53 gene without damaging normal tissues. In this study, we combined this adenovirus (Adv-E1AdB) with a fiber mutation, F/K20 (Adv-E1AdB-F/K20), which has a stretch of 20 lysine residues added at the C-terminus of the fiber and shows high transduction efficiency to gliomas (Yoshida et al). In U-373MG glioma cells, Adv-F/K20 for lacZ showed 9 times higher transduction efficiency than that of Adv with wild-type fiber (Adv-F/wt) for lacZ. At the multiplicity of infection (MOI) of 30, Adv-E1AdB-F/K20 had 11 times higher replication efficiency compared with that of Adv-E1AdB with wild-type fiber (Adv-E1AdB-F/wt). The *in vitro* cytopathic effect of Adv-E1AdB-F/K20 on U-373MG cells increased the ED_{50} value to 32 times stronger than that of Adv-E1AdB-F/wt. The injection of Adv-E1AdB-F/K20 suppressed the *in vivo* growth of tumors; The anti-tumoral effect was remarkably stronger than that of Adv-E1AdB-F/wt. Gene therapy utilizing Adv-E1AdB-F/K20, which drastically augmented the anti-tumoral effect of Adv-E1AdB, would be a promising therapeutic approach for gliomas.

b) Adenovirus-mediated transfer of p33ING1 with p53 drastically augments apoptosis in gliomas (*Cancer Res* 59(21): 5521-5528, 1999). The p53 tumor suppressor gene is an important target for gene therapy for cancers, and clinical trials targeting this gene have been conducted. Some cancers, however, are refractory to p53 gene therapy. Therefore, it has been combined with other therapies, including chemotherapy and radiotherapy, to enhance the cytopathic effect of p53 induction.

The p33ING1 gene cooperates with p53 to block cell proliferation. In this study, we investigated whether adenovirus (Adv)-mediated coinduction of p33ING1 and p53 enhances apoptosis in glioma cells (U251 and U-373 MG), which showed no genetic alterations but low expression levels of p33ING1. Although the single infection of Adv for p33ING1 (Adv-p33) at a multiplicity of infection (MOI) of 100, or Adv for p53 controlled by myelin basic protein (MBP) promoter (Adv-MBP-p53), a glioma-specific promoter, at a MOI of 50, did not induce apoptosis in U251 or U-373 MG glioma cells; coinfection of Adv-p33 and Adv-MBP-p53 at the same MOIs induced drastically enhanced apoptosis in both cell lines. Apoptosis was not induced in NGF-treated PC-12 cells infected with a high MOI (300) of Adv-p33 nor in those coinfected with Adv-p33 (100) and Adv-MBP-p53 (50). Coinfection of Adv-p33 and Adv-MBP-p53 demonstrated morphological mitochondrial damage during the initial stage of apoptosis, which likely led to apoptotic cell death. Our results indicate that this coinfection approach can be used as a modality in gene therapy for gliomas, sparing damage to normal tissues.

3. Regenerative medicine.

a) *Stem cell biology:* Long-term lymphohematopoietic reconstitution by a single $CD34^{low/-}$ hematopoietic stem cell (Osawa et al. *Science* 273: 242-245, 1996). Hematopoietic stem cells (HSCs) supply all blood cell throughout life, by making use of their self-renewal and multilineage differentiation capabilities. A monoclonal antibody (mAb) was raised to the mouse homologue of CD34 (mCD34), and was used to purify mouse HSCs to near homogeneity. Unlike human, primitive adult mouse bone marrow (BM) HSCs were found in the mCD34 low to negative fraction. Injection of a single $mCD34^{low/-}$, $c\text{-}Kit^{+}$, $Sca\text{-}1^{+}$, lineage markers negative (Lin^{-}) cell resulted in long-term reconstitution of the lymphohematopoietic system in 21% of recipients. Thus, the purified HSC population will enable analysis of the self-renewal and multilineage differentiation of individual HSCs.

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Cell and Tissue Engineering

～Biomedical Research, Education and Instrumentation Center～

Our department has two different facilities, one is for pathological, immunocyto – histological, and cell biological works, and the other for electron microscopic studies. These two facilities are open for all researches in Sapporo Medical University. It is also possible to analyze and study genetic characteristics by using very sophisticated laser confocal micrography, laser microdissecting apparatus and very new electron microscopes and scanning electron microscope.

Professor and Director (Affiliated)
Noriyuki Sato, M.D., Ph.D.
Interests:
Pathology, Basic Immunology & Tumor Immunology

One of the main functions of our department is to facilitate histological and electron microscopic research as well as cell biological studies. Each year this department has contributed to innumerable research efforts, namely more than one thousand and two hundreds / year in the cell & tissue research section and electron microscope section, respectively. Our other work involves basic study into the injury and regeneration mechanism of electric shock in skeletal muscle. We have established a histological model characteristic of electric injury, muscle degeneration & regeneration.

List of Main Publications from 1997 to 2000

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Kamiguchi K, Tamura Y, Matsuura A, Takahashi S, Torigoe T. Human CD8 and CD4 T cell epitope of epithelial cancer antigens. Cancer Chemother Phamacol 46: 86-90 (2000).

11) Maeda A, Ohguro H, Maeda T, Wada I, Sato N, Kuroki Y, Nakagawa T. Aberrant expression of photoreceptor specific calcium binding protein (recoverin) in cancer cell lines. Cancer Res 60: 1914-1920 (2000).
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17) Asanuma H, Takahashi S, Ishikawa M, Kamiguchi K, Sato N, Poppema S, Fujimoto J, Kikuchi K. A monoclonal antibody, 3G12, reacts with a novel surface molecule, Hal-1, with high expression in CD30-positive an aplastic large cell lymphomas. Brit J Hematol 106: 55-63 (1999).
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19) Ishikura H, Aikawa M, Itakura H. Pathology and Differential Diagnosis of Internationalized Zoonoses Summering Discution of the Organizers In: H. Ishikura (Editor-in-Chief) Aikawa M, Itakura K, Kikuchi K. (eds) Springer-Verlag. Tokyo. 1-8 (1998).
20) Shigehara K, Shujubo N, Ohmichi M, Takahashi R, Kon S, Okamura H, Kurimoto M, Hiraga Y, Tatsuno T, Abe S, Sato N. IL-12 and IL-18 are increased in sarcoid lungs and stimulate IFN-É¡production. J Immunol 166: 642-649 (2001).

Laboratory of Radioisotope Research

~ Biomedical Research, Education and Instrumentation Center ~

Laboratory of Radioisotope Research is open 24-hours a day and used by 36 departments belonging to the Faculty of Medicine. We support all experiments with use of radioisotopes and run a course on radiation safety for all scientists.

Professor and Director (Affiliated)
Naoki Watanabe, M.D., Ph.D.
Interests:
Radiology, Oncology,
Hematology, and Laboratory Medicine

1. Organization

The laboratory of Radioisotope Research (formerly-Radioisotope Research Institute) was established in December 1959 for the purpose of radiation safety control of a radioisotope research laboratory. Two radioisotope research laboratories belonging to Biomedical Research Center are located in the Radioisotope Research Institute and in the basic science buildings (9 to 13 F). The former was newly constructed in 1974, and the later was built in 1999. Twenty-nine kinds of radioisotopes for biochemical experiment and eight for animal experiment are permitted for use. The Laboratory is open 24-hours a day and entrance to and departure from the management zone is controlled by ID card. The Laboratory is used by 36 departments belonging to the Faculty of Medicine. A total of approximately 10,000 scientists/year use the Laboratory. Staff in the Laboratory are composed of a professor, four radiation technologists, and a research assistant.

2. Teaching activities

All scientists registered as users of the Laboratories of Radioisotope Research must take a course in radiation safety including radiation hazards to man, basic handling of radioisotope and the rules of the facilities. The Laboratory is in charge of the running of this course.

3. Lists of radioisotope and equipment

The following radioisotope and equipment are available in the Institute.

1) Radioisotope for Biochemical Experiment

105-Ag 195-Au 14-C 45-Ca 109-Cd 36-Cl 57-Co 58-Co 60-Co 51-Cr 64-Cu 55-Fe 59-Fe 3-H 203-Hg 125-I 131-I 42-K 99-Mo 22-Na 24-Na 63-Ni 32-P 33-P 86-Rb 35-S 75-Se 99m-Tc 65-Zn

2) Radioisotope for Animal Experiments

14-C 51-Cr 59-Fe 3-H 125-I 32-P 35-S 99m-Tc

3) Equipment

Liquid Scintillation Counter	Beckman x 4
Automated Gamma Counter	LKB-Wallac x 3
Automated Gamma Counter	Aloka x 1
Bio Imaging Analyzer	Fuji Film x 1
Topcount	Packard x 1
Hand-Foot-Clothes Monitoring System	Aloka x 6
Room Gas Monitor	Aloka x 8
Poket Dose Monitor	Aloka x 6
Geiger-Muller Survey Meter	Aloka x 13
Scintillation Survey Meter	Aloka x 4
Ionization Chamber Survey Meter	Aloka x 2
Ultracentrifuge (Desktop model)	Beckman x 1
Centrifuge (Desktop model)	Beckman x 1
Centrifuge (Floor model)	Kubota x 1
Automatic Enviromental Speedvac	Savant x 1
PCR System	Applid Biosystems x 1
CO2 Incubator	Revco x 3
Cell Distruptor	Branson x 1
Ultrasonic Cleaner	Branson x 1
Safty Cabinet	Dalton x 6
Autoclave	Tomy x 2
Electronic Balance	Mettler x 1
Ultraputure Water System	Barnstead x 1
Enviromental Control Hood for Animal	Japan Clea x 1
Clifornia Type Hood	Dalton x 17
OakRidge Type Hood	Dalton x 1
Dry-Distillation of Experimental Animal Waste	Sinsei x 1
Incinerator of Liquid Scinti-Cocktail Waste	Sinsei x 1

Philosophy and Ethics

The history of European scientific philosophy since the 19th century is our main subject of research. The research is basic and necessary to attain a philosophy which views and understands our civilized world rightly. Other subjects are the history and philosophy of medicine, and bioethics or medical ethics. Both subjects are, in our research, related to each other and we intend to establish our own philosophical and ethical point of view about medicine.

Professor
Michio Imai, M.A.
Interests:
Western Philosophy

1. The history of European scientific philosophy since the 19th century

German positivistic or scientific philosophy and its background are studied.

We are particularly interested in the philosophy of Ernst Mach and have found one of its origins in the mystical tradition of mode of thought (1). We have also studied Sigmund Freud and his relation to Mach from the point of view of intellectual history (2).

2. The history and philosophy of medicine

The history and philosophy of medicine is another subject of research in our department. We have investigated the thought of a German doctor, Ernst Schweninger (1850-1924) about the task of the doctor, which gives us an idea of medicine as art (Imai M: The Task of the Doctor – On Schweninger's "The Doctor"– (1). J. Lib. Arts & Sci. Sapporo Med. Univ. 38: 11-16 (1997) (in Japanese), Imai M: The Task of the Doctor – On Schweninger's "The Doctor"– (2). J. Lib. Arts & Sci. Sapporo Med. Univ. 39: 9- 13 (1998) (in Japanese)).

3. Bioethics or medical ethics

We are also studying bioethics or medical ethics. We have examined "terminal care" from a philosophical and ethical perspective and discussed the conception of death and dying (Imai M: Terminal care in the perspective of philosophy and ethics. Kataura A, Satohisa E eds. In Search for the better Life and Death: 49-56. Nanzando, Tokyo (1999) (in Japanese)).

We have published a textbook of bioethics, which is now used as an introductory and standard text for medical students in Japan (Imai M: Introduction to Bioethics. 199 pp. Sangyo-tosho, Tokyo (1999) (in Japanese)).

List of Main Publications from 1997 to 2000

1) Imai M: Die mystische Denkform bei Ernst Mach. Papers of the 22nd International Wittgenstein Symposium. **VII** (1): 266-271 (1999).
2) Imai M: Mach and Freud-A Probable Confrontation. 3rd International History of Philosophy of Science Conference Abstracts, 53 (2000).

Psychology

The leading aim of our Department is to explore the psychophysiological mechanisms underlying human stress reaction, by adopting the current methodology of cardiovascular psychophysiology. Our basic research, especially on developing the non-invasive new measures of cardiovascular hemodynamics and autonomic regulation, has stimulated application studies oriented to the human mind-body interaction and health promotion.

Professor
Yukihiro Sawada, Ph.D.
Interests:
Cardiovascular psyhophysiology
Clinical psychophysiology

Associate Professor
Gohichi Tanaka，Ph.D.
Interest:
Cardiovascular psyhophysiology
Health psychology

Instructor
Yuichiro Nagano，M.A.

1. Introducing a new analytical method for assessing the beat-by-beat variations of cardiovascular responses

We introduced a new technique for time series analysis, which is a combination of the maximum entropy method (MEM) for spectral analysis and the non-linear least squares (LSM) method for fitting analysis. The theoretical and practical standpoints of the MEM plus LSM technique were discussed, compared with a traditional autoregressive modeling (1). To validate the MEM plus LSM technique, heart rate variability was analyzed during resting and mental arithmetic task (MA) under pharmacological interventions (phenylephrine:PE and trinitroglycerine:NG). Results on the low- and high-frequency powers (LF and HF) indicated that PE increased both the LF and HF, and that the MA decreased both the LF and HF under PE and NG injections. Therefore, LF could be interpreted as a marker of both the vagal and sympathetic activities, whereas HF seemed to be solely associated with vagal activity (1.2).

2. Developing new measures of sympathetically mediated finger vascular tone

The pulsatile component (PV) of finger photoplethysmogram (FPG) has been used in a diversity of psychophysiological studies as an alpha-adrenergic sympathetic activity to the finger tip. However, what PV denotes hemodynamically has not been strictly clarified. We have advocated new quantitative measures of finger vascular tone as superior alternatives to PV, i.e., normalized pulse volume (NPV) and double-normalized pulse volume (DNPV), by applying Lambert-Beer's Law to the human finger. We demonstrated a high beat-by-beat correlation between cutaneous vascular resistance and DNPV under contralateral hand immersions into cold and warm water (3). Subsequently, we compared the correlation of cutaneous vascular resistance in the finger tip to NPV with that to PV during mental stress (mental arithmetic and mirror drawing), and demonstrated an even higher correlation in the former. NPV revealed larger and more consistent reductions than PV and DNPV during mental stress (4).

3. Experimental study of human stress reactions to the mental stress test

According to the reactivity hypothesis, people who are hyper-reactive to stressful stimuli may be at increased risk of cardiovascular disease. One of the important underlying assumptions is that hyper-reactivity is consistent from one type of stressor to another, i.e., inter-task consistency. Experimental study demonstrated that prolonged exposure to the digit-scan test yielded a shift of the hemodynamic pressor mechanism from increase in cardiac output to that in peripheral vascular resistance. The hemodynamic shift seemed to heighten the intertask consistency between digit scan and cold pressor test (5). Subsequently, to examine whether one of the psychological factors, i.e., threatening the performance of the task, can modify the hemodynamic pattern of stress reactions, time limited and unlimited versions of tracking figure stress were compared. Results indicated that the pattern of responses was clearly differentiated by two types of tasks (indexed by heart rate, blood pressure, skin blood flow, respiration rate and amplitude, cardiac pre-ejection period, and baroreflex sensitivity) (6).

4. The construction of a general model for understanding psychophysiological cardiovascular reactions

The attention-affect model postulates that cognitive appraisal of a psychosocial pressor stimulus primarily results in an unpleasant affect or in an attention to the stimulus and

subsequently that the hemodynamic reaction pattern-I or –II is produced according to the respective cognitive process. In addition, it is assumed that often an attention to performance or sometimes a pleasant affect could secondarily contribute to the respective pattern formation. In this connection, the coping response to a psyhosocial pressor stimulus is more overwhelmingly cognitive than somatic in our modernized society (e.g., mental workload) and does not necessarily correspond to its hemodynamic reaction pattern. According to this new model, the convergence of superficially contrastive with active vs. passive coping nature to the same hemodynamic reaction pattern can be fully explained (7).

5. Experimental and theoretical examination for behavioral stress reduction intervention

Paced respiration, especially at slow rate (e.g., 6-8 breaths per minute), can have therapeutic effects, for example on anxiety, through its influence on the cardiorespiratory system. A study was undertaken to establish a subjectively comfortable respiration pattern, using self-paced respiration (SR). The results of experiment indicated that SR was most comfortable when a respiration pattern was attained at half the rate and near twice the tidal volume of the trainee's resting respiration, and that a decrease in cardiac vagal activity (indexed by respiratory sinus arrhythmia and heart rate) and end-tidal PCO_2 were suggestive of a reduction in anxiety (8). Subsequently, to initiate synthesis of the inconsistent findings on stress reduction interventions from a standpoint of cardiovascular hemodynamics, the effects of a kind of mindfulness meditation during an exposure to stressful stimuli were examined. The results were interpreted in line with the attention-affect model described above, and a concept of the specificity of stress reduction intervention was advocated (7).

6. Evaluating a clinical applicability of new measures of human vascular tone: pre-operative anxiety and sedation

Confronting a surgical operation is likely to provoke anxiety and physiological reactions in the clinical situation. Comparisons of sensitivities of the FPG-related measures were carried out during a resting condition a few days before a surgical operation and during pre-anaesthetized and anaesthetized conditions by intravenously injected propofol at the outset of the operation. NPV appeared to be the best indicator of heightened arteriolar vascular tone. A partial correlation, after controlling for patients' body mass index, was observed between the change in NPV and the dose of propofol; a larger reduction in NPV before anaesthesia compared with the control condition was accompanied by the need for a greater infusion of propofol for sedation.

List of Main Publications from 1997 to 2000

1) Sawada Y, Ohtomo N, Tanaka Y, Tanaka G, Yamakoshi K, Terachi S, Shimamoto K, Nakagawa M, Satoh S, Kuroda S, Iimura O. A new technique of time series analysis by combining maximum entropy method and nonlinear least sqares method and its application for an assessment of cardiac autonomic tones. Med Biol Eng Comput 35: 318-322 (1997).
2) Sawada Y, Tanaka G. Application of a new method of time series analysis to the data on short-term variability in inter-beat interval and a trial toward an integration of different interpretations of the power related indices. Jpn Psychol Res 40: 1-9 (1998).
3) Tanaka G, Sawada Y, Yamakoshi K. Beat-by-beat double normalized pulse volume derived photoplethysmo-graphically as a new quantitative index of finger vascular tone in humans. Eur J Appl Physiol 81: 148-154 (2000).
4) Sawada Y, Tanaka G, Yamakoshi K. Normalized pulse volume derived photo-plethysmographically as a more valid measure of the finger vascular tone. Int J Psychophysiol 41: 1-10 (2001).
5) Sawada Y. Intertask consistency of blood pressure responses to laboratory stressors may increase with prolonged exposure. Jpn Psychol Res 41: 112-120 (1999).
6) Nagano Y, Obata A, Kodama M: The variation of autonomic nervous system in tracking task: What is the key factor of differentiation in stress response? AAPB 31st Annual Meeting. Denver, Colorado. Proceedings, 76-79 (2000).
7) Sawada Y. Is meditation effective as stress reduction intervention? An approach from cardiovascular hemodynamics. In: KT Kaku (ed) Meditation as health promotion: A lifestyle modification approach. 132-151(2000).
8) Sawada Y. A preliminary study of the confort of paced respiration. Jpn Psychol Res 42: 123-127 (2000).

Jurisprudence and Sociology

Teaching and studying bioethics, medical ethics, medical law, jurisprudence, and other approaches to medical ethics from the perspective of the social sciences are being studied.

Associate Professor
Toshihiko Hatate, J.D.
Interests:
Legal and political theory, Bioethics, Medical ethics

1.General Theory of Jurisprudence

I am studying the general theory of jurisprudence. The main themes are legal reasoning, - theory of rights, theory of Justice, and their philosophical foundations. I also committed to the study of legal education and the training system for lawyers, both of which are lively topics in Japan today.

2. The Relation Between Legal Theory and Political Theory

In the field of political theory, liberalism has been dominant. But in recent years, we have witnessed a lively debate between liberalism and its critics. I pointed out that the theory of rights has been largely based on liberalism, meaning liberalists must reconstruct the political theory of liberalism. I am now trying to reconstruct liberal political theory from the point of view of citizenship and empowerment. Concerning this issue, I published the paper "Law's Empire and Participatory Democracy, From Liberal Legalism to Deliberative Democracy as a form of Postliberalism in *The Law at a Critical Pont [1] Legal Thinking Reoriented* (Tokyo University Press 1999, in Japanese). I am a member of the Japan Society of Legal Philosophy, and this is the main arena for me to study the above fields of 1 and 2.

3. Bioethics and Medical ethics

Bioethics is a suprainterdisciplinary science, and I approach it from the standpoint of legal theory with the emphasis on patient's rights, and patient participation. Medical ethics has been an applied field of bioethics for many years, but today it is not only an applied field of bioethics, but also a new method of clinical decision making. I've got several guidelines from WHO and other organizations and I am trying to introduce them into the Institutional Review Board and Hospital Ethics Committee at this university. In this field, the main publication is *Iryourinnri no Yoake (Shobunsha, 2000)* which is a Japanese translation *of Strangers at the Bedside, A History of How Law and Bioethics Transformed Medical Decision Making (by David Rothman, Basic Books, 1991).*

4. Medical Law

Medical sciences are developing so rapidly that many acts are constantly being enacted and revised in the field of medial law. I published a paper about Japan's transplantation act of 1997, and reported the outline and issues of the fourth revision of health care act at the annual conference of Japan Association for Bioethics in 2000. And I also published "Comments on the Transplantation Act in Japan From Viewpoints of Ethical Principles and of Public Policy in *Bioethics* (Journal of Japan Association for Bioethics) vol.8, no.1, at 100-104 (1998, in Japanese)

Concerning3 and 4 above, I am a member of Japan Association for Bioethics and International Association of Bioethics. I attended the forth world congress in Tokyo in 1998, and fifth world congress in London in 2000.

Information Sciences

Our laboratory has developed the eye-ball model for computer simulation, and has studied permeability in the blood-retinal barrier. We are also interested in analyzing digital images by computer, so we have undertaken studies to measure bone density and bone mineral contents in rat with soft X-ray images. Our next project will be to study the security of digital medical data appearing on the internet.

Assistant Professor
Mitsuru Kojima, M.T., Ph.D.
Interests :
Computer simulation method, Analysis of digital Images

1. Computer simulations and analyses.

a). We have made a computer analysis of permeability in the blood-retinal barrier (BRB) to human eyes. It is suggested that our simulation method in conjunction with vitreous fluorophotometry can effectively estimate permeability of BRB in human subjects.

b). Dynamics of local cerebral blood flow in rat was studied by autoradiographic diffusible tracer (14C-iodoantipyrine) technique. We have discussed the effect of hyperglycemia on ischemic brain damage using this technique.

c). We developed an experimental on-line system for analyzing Pracido's disk images projected by the Maloney surgical keratometer(Keratoring), and tested its usefulness. It is concluded that this image processing system provides acceptably accurate measurement for the radius of corneal curvature.

2. Measurement of bone density in rats with the soft X-ray images.

We have developed an experimental system for analyzing soft X-ray digital images of bones in male rats and tested its usefulness. The objects measured were dry bones of male Wistar rats bred in various conditions.

The results were as follows:

a) The induces of bone mass and density in rats with high calcium(Ca) intake were higher than those in the low Ca group.

b) The relation between image tone of each step in an the aluminum step wedge and thickness followed the so-called S-curve. It is concluded that this image processing system provides acceptably accurate measurements (1).

List of Main Publications from 1997 to 2000

1) Okano G, Suzuki M, Kojima M, Sato Y, Lee SJ, Okamura K, Noriyasu S, Doi T, Shimomura Y, Fushiki T, Shimizu S: Effect of timing of meal intake after squat exercise training on bone formation in the rat hindlimb. J Nutr Sci Vitaminol 45: 543-552 (1999).

Mathematics

Our department is concerned with mathematical sciences including statistics. In recent years, some optimization problems on generalized networks have been investigated. In particular, we are interested in dualities such as the relation between max-flow and min-cut problems on a continuous medium, which is regarded as an approximation of a complex network.

Associate Professor
Ryohei Nozawa, M.S., Ph.D.
Interest:
Optimization theory

1. Classification for infinite dimensional max-flow min-cut dual optimization problems

A definitive classification is established for all conceivable and permissible duality states of two slightly different versions (Strang and Iri) of infinite dimensional max-flow min-cut dual optimization problems. The approach builds on three elements: (a) previous work on duality gaps in max-flow problems under appropriate choices of function spaces, (b) construction of a class of perturbations in infinite convex optimizations germane to max-flow, and (c) a literature on convex classification theory for linear spaces having Hahn Banach extension property (1).

2. Relaxation for infinite dimensional min-cut problems

Strang introduced optimization problems on an Euclidean domain which are closely related with problems in mechanics and noted that the problems are regarded as continuous versions of famous max-flow min-cut problems. We generalized the problems and called the generalized problems max-flow and min-cut problems of Strang's type in a former work. Here we formulate a relaxed version of the min-cut problem of Strang's type and prove the existence of optimal solutions under some suitable conditions. The conditions are essential. In fact, there is an example of the relaxed version which has no optimal solutions if the conditions are not fulfilled (2).

3. Gale's feasibility theorem on a generalized networks

Gale's feasibility theorem was originally formulated on a discrete network in 1957. It is known as the Supply - Demand Theorem in a special case and gives a necessary and sufficient condition for an existence of feasible flows. Our problem is formulated in a framework of a continuous network introduced by Iri, 1979 and Strang,1983. In contrast with discrete cases, our continuous version is essentially related with the boundedness of constraints of flows. However, we can deal with a certain special case with unbounded constraints (3.4).

List of Main Publications from 1997 to 2000

1) Nozawa, R, Kortanek, KO: A perturbation-based duality classification for max-flow min-cut problems of Strang and Iri. In: Fiacco, AV, (ed.) Mathematical programming with data perturbations. Marcel Dekker, Inc, New York-Basel-Hong Kong, 285 - 303 (1998).
2) Nozawa, R: A relaxation of a min-cut problem in an anisotropic continuous network. Applied Math. Optimization 40: 1 – 18 (1999).
3) Nozawa, R: Gale's feasibility theorem and max-flow problems in a continuous network. RIMS Kokyuroku 1031: 29 – 41 (1998).
4) Nozawa, R: Gale's feasibility theorem and max-flow problems in a continuous network, Proceedings of the International Conference on Nonlinear Analysis and Convex Analysis. Takahashi, W and Tanaka, T eds, World Scientific, 297-304 (1999).

Physics

Our department has been investigating the microscopic structure of assembly of lipids and biological molecules embedded in lipid membrane by means of x-ray diffraction and electron microscope. Assembly of lipids shows polymorphism and phase separation. The x-ray beam used in x-ray diffraction study was synchrotron orbital radiation. In addition, studies relating to the methodology of teaching have been performed.

Professor
Morio Akiyama, B.S., Ph.D.
Interests:
Microscopic structure of membrane, Concept of time

Assistant Professor
Sinzi Matuoka, M.S., Ph.D.
Interests:
Microscopic structure of membrane, Phase transition

Instructor
Hirokuni Yamada, M.S., Ph.D.

1. Phase transition and physical property of lipids

a) The relationship between biological functions and physical properties in model membranes consisting of glicolipid and phospholipid has been investigated. In particular, we focused on ganglioside GM3, one of the glycosphingolipids, known to be involved in cell growth regulation and differentiation. It is also known that a large amount of GM3 is expressed in murine melanoma and antibody M2590 against this melanoma recognizes GM3. The reactivity of M2590 depends greatly on the GM3 content in liposome; it abruptly increases at a threshold point. To explain this, we investigated phase behavior and phase separation of DPPC multilamellar vesicles containing GM3 using x-ray diffraction. Consequently, we observed the coexistence of two lamellar structures in the range of 4 mol % to 7 mol% GM3 content. These peaks correspond to the GM3-rich region and the GM3-poor region and the former is present above 4 mol%. Thus we concluded that the formation of the GM3-rich region by phase separation was the explanation for the threshold in the reactivity of antibody M2590 (1).

Physical properties of DPPC multilamellar vesicles containing 3-*O*-acetylated GM3 were studied by x-ray diffraction (2). Also in this system we observed a relationship between phase separation and reactivity of antibody M2590 (3).

b) The physical properties of dipalmitoylphosphatidyl-choline(DPPC) were studied by synchrotron x-ray diffraction. It is well known that the multilamellar vesicles of DPPC show phase transitions in the sequence of the L_c phase, the $L_{\beta'}$ phase, the $P_{\beta'}$ phase and the L_α phase from low to high temperature. The bilayer separation in water and hydrocarbon chain packing of DPPC bilayer membrane varies in different phases and there may be a correlation between them. We observed changes in lamellar repeat distance of DPPC during temperature jump from the $P_{\beta'}$ phase to the $L_{\beta'}$ phase by small-angle x-ray diffraction. Consequently, it was found that lamellar repeat distance temporally increased until 20 s after the temperature jump and then decreased. This decrease of lamellar repeat distance consisted of a faster process with a time constant of 100 – 120 s and a slower one with one of 900-1200 s. A change in hydrocarbon chain packing during the temperature jump from the $P_{\beta'}$ to the $L_{\beta'}$ was observed by wide angle x-ray diffraction. We found that the tilt angle of hydrocarbon chains with respect to the bilayer normal increased in correspondence to the slower process in the decrease of the lamellar repeat distance (4).

2. Structure and location of membrane-binding proteins and the method their analysis by electron microscopy

Isolated neuro-toxin molecule (Botulinum Type C_1, 142kDa) is not distinguished easily from the noise of mixed impurities and backgrounds with the electron microscope in an acidic solution. Vagueness by piling up of the molecules occur when it is made into a three-dimensional crystal.

In the first stage of the crystallization of the toxin molecule, in which the observation of an individual molecule is possible, the molecule connects to another molecule like string. Strings of the molecule gather further, eventually forming into a needle crystal. The interval between strings of the molecule narrows from 17nm to 15nm while formation of the three-dimensional crystal, takes

place but, it does not narrow up to the molecular interval (12.5nm) on the string, and the binding force between strings is relatively weaker than the molecular interval on the string. The existence of more than 10 molecules in a string cannot be definitively confirmed, which means that it is difficult to prove that it is a toxin molecule that has a center hole. The molecular set becoming the string like, it is thought to be caused by a twisting between the molecules, although the binding position is invariable. The binding force between strings becomes weak for this twist. It seems that it becomes a loose spiral state when molecules of one string in the crystal are traced because it becomes three dimensional with this twist held.

It is necessary to create a spiral model for the analysis of a string toxin molecular set. The composition protein of the tobacco mosaic virus arranges itself spirally. When the shape of this protein is simplified to be spheroidal, it becomes a basic structural model of spiral arrangement. When the Fourier transformation of this model is done on the computer, the diffraction image is made. It was possible to know the difference in the diffraction image corresponding to an unlike array of the protein, and the first stage of the analysis of a toxin molecular set was thus done.

By the freeze-fracturing and etching method used to understand the stereoscopic shape of the small molecules, biomolecules (protein molecules of the TMV) were observed. In the deposited part where the shadowing angle was small, and the platinum was shadowed in the direction in which the surface molecular particles were arranged, the array of the rule of spiral pitch of the protein molecules of about 2nm was observable. Thus it will be possible to observe the toxin molecule by this shadowing method in future (Yamada H: J Lib Arts & Sci Sapporo Med Univ Sch Med 38, 1997, 39, 1998, 40, 1999, in Japanese).

3. Others

Follow up study of students' evaluation of lectures was done. Moreover, we have studied how to teach the concept of time to students. The concept of time is a fundamental but difficult problem (Akiyama M:J Lib Arts &Sci Sapporo Med Univ Sch Med 40(2000) in Japanese).

List of Main Publications from 1997 to 2000

1) Matuoka S, Akiyama M, Tsuchihashi K and Gasa S. Phase separation in binary mixtures of GM3 and DPPC. Photon Factory Activity Report 16: 268 (1998).
2) Matuoka S, Akiyama M, Tsuchihashi K and Gasa S. Phase separation in binary mixtures of 3-O-acetylated GM3 and DPPC. Photon Factory Activity Report 17: 267 (1999).
3) Akiyama M, Matuoka S, Tsuchihashi K. and Gasa S: Phase behavior of GM3/DPPC and 3-O-AcGM3/DPPC multilamellar vesicle. The 13th International Biophysics Congress. New Delhi, India. J Biosciences 24, S1: 118 (1999).
4) Matuoka S and Kato S: Temperature jump experiment from the $P_{\beta'}$ phase to the $L_{\beta'}$ phase in fully hydrated DPPC. Photon Factory Activity Report 15: 267 (1997).

Chemistry

This department has been analyzing the structure of newly modified glycolipid isolated from normal and diseased central nervous tissue or other normal tissues using NMR, mass spectrometry etc. The stereo-structure of Ca-binding protein in a solution state has also been examined using X-ray scattering method. As to the former project, we are continuing to further clarify the modification mechanism in the disease.

Professor
Shinsei Gasa, Ph.D.
Interests:
Biochemistry, Organic chemistry

Associate Professor
Hidenori Yoshino, Ph.D.
Interests:
Biochemistry, Physical chemistry

Assistant Professor
Keiko tsuchihashi, Ph.D.
Interests:
Biochemistry, Organic chemistry

Instructor
Youichi Yachida, Ph.D.

1. Structural analysis of glycolipid

Di-O-Ac GM3 from equine erythrocytes was identified in a mixture of the different acetylated positions, one contained 4,9-di-O-Ac-N-glycolyl neuraminic acid residue (4,9-di-O-Ac GM3) and another, 9-O-Ac N-glycolyl neuraminic acid with 6-O-Ac Gal moiety in the GM3 structure, by means of NMR and mass-spectrometry after chemical modification of this method (1). To identify the O-acetylated position in glycolipid, we established a new blocking method for the hydroxyl group, including acetalization reaction (2). The sialic acid species of the ganglioseds in mammalian central nervous system was exclusively N-acetyl form, but not N-glycolyl form. We examined the species of gangliosides in equine brain, and found that the lipid had significant N-glycolyl form, and that the content was characteristic for equine brain (3). From equine brain, we isolated a further three glydolipids, O-fatty acylated 3-sulfogalactosylceramide at C-6-O (4), two plasmalo glycolipids, one was 4,6-O-fatty acetalgalactosylceramide (plasmaloGalCer) (5) and another was 3-O-(4',6'-O-fatty acetalgalactosyl)-1-O-alkylglycerol (6). The orientation of the fatty acetal chain of these plasmalo glycolipids was clearly determined as "endo" type with the spectra in the Nuclear Overhouser Effect (NOE).

2. Biological activity of glycolipids

The glycolipid isolated from sea urchin intestine, whose structure was identified to be 3'-sulfonoquinovosyl 1'-monoacylglycerol by NMR and mass-spectrometry, was found to have anti-tumor activity in vivo (7). The sulfonoglycolipid and the isomers were chemically synthesized, and these glycolipids acted as a potent inhibitor of the activities of calf and rat DNA polymerases (8). A glioma-related glycolipid, O-Acetylated GM3 having 3-O-Ac ceramide, which was isolated previously, was examined for its immunological activity. The antibody M2590, the recognized sugar moiety of GM3 structure, was found to be affected for the recognition of sugar moiety by lipid residue, since the activity with 3-O-AcGM3 was lesser than that with non-acetylated GM3 (9).

3. Ceramide-related research

We found that sphinganine, a biosynthetic precursor of ceramide, reacted non-enzymatically with AcCoA to produce N-AcShinganine (C_2H_2-ceramide) in a solution containing buffer or organic solvents (10). The C_2H_2-ceramide should be regarded as a biosynthetic precursor for C_2-ceramide, which is routinely employed as a substance for apoptosis research, however, the former was not converted to the latter in an experiment to obtain substrate specficity with the ceramide synthetase (H_2-ceramide desaturase) from rat fetal skin (11). An assay method for the activity of ceramide synthetase was newly established using the above tissue, and some enzymatic properties were examined, demonstrating that the enzyme required NADH for the reaction and desaturated from C_{12} - to C_{16} -H_2-ceramide as substrates (11). Concerning research into

apoptosis, the actin-regulatory protein, gelsolin, was found to have an inhibitory effect on apoptosis of cells (12).

4. Structural studies of proteins in solution

Solution X-ray scattering using synchrotron radiation as an X-ray source has been used to study the solution structures of Ca^{2+}-binding proteins, calmodulin (CaM) and calcineurin (CaN). CaM appears in all equkaryotic cells as a ubiquitous Ca^{2+}-sensor, and regulates over thirty kinds of different enzymes. Our data suggest that the minimum structural requirement recognized by CaM as a domain structure in the targets proteins would be of a basic-amphiphilic nature, but not a basic-amphiphilic-helical structure of the domain (13.14). CaN is Ca^{2+}/CaM dependent protein phosphatase, known as the target of immunosuppressant drugs cyclosporin A and FK506. Scattering data indicated that CaN without Ca^{2+}/CaM has the oval structure of one-domain, while CaN with Ca^{2+} or Ca^{2+}/CaM has a two-domain structure resembling the crystal structure. This is the first evidence showing the solution structure of CaN. Our findings suggest that Ca^{2+} or Ca^{2+}/CaM binding induces a large structural change in CaN from closed to open-up structure, exposing the active site to solvent (15).

List of Main Publications from 1997 to 2000

1) Yachida Y, Tsuchihashi K, Gasa S. Novel di-O-acetylated GM3s from equine erythrocytes, one containing 4,9-di-O-acetyl-N-glycolylneuraminic acid and another containing 4-O-acetyl- N-glycolylneuraminic acid and 6-O-acetyl-D-galactose. Carbohydr Res 298: 201-212 (1997).
2) Yachida Y, Tsuchihashi K, Gasa S, Kashiwagi M, Daino T, Mikami T, Akino T. New blocking method for the hydroxyl group on carbohydrate. Determination of the O-acylated position of the modified glycolipid. J Chromatogr A 813: 325-332 (1998).
3) Mikami T, Kashiwagi M, Tsuchihashi K, Daino T, Akino T, Gasa S. Further Characterization of Equine Brain Gangliosides: The Presence of GM3 having *N*- Glycolyl Neuraminic Acid in the Central Nervous System. J Biochem 123: 487-491 (1998).
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5) Yachida Y, Kashiwagi M, Mikami T, Tsuchihashi K, Daino T, Akino T, Gasa S. Stereochemical structures of synthesized and natural plasmalogalactosylceramides from equine brain. J Lipid Res 39: 1039-1045 (1998).
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7) Sahara H, Ishikawa M, Takahashi N, Ohtani S, Sato N, Gasa S, Akino T, Kikuchi K. In vivo anti-tumour effect of 3'-sulphonoquinovosyl 1'-monoacylglyceride isolated from sea urchin (Strongylocentrotus intermedius) intestine. Br J Cancer 75: 324-332 (1997).
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10) Kashiwagi M, Mikami T, Chiba M, Chiba S, Matsumoto H, Akino T, Gasa S. Occurrence of nonenzymatic N-acetylation of sphinganine with acetyl coenzyme A producing C2-H2-ceramide and its inconvertibility to apoptotic C2-ceramide. Biochem Mol Biol Int 42: 1071-1080 (1997).
11) Mikami T, Kashiwagi M, Tsuchihashi K, Akino T, Gasa S. Substrate specificity and some other enzymatic properties of dihydroceramide desaturase (ceramide synthase) in fetal rat skin. J Biochem (Tokyo) 123: 906-911 (1998).
12) Ohtsu M, Sakai N, Fujita H, Kashiwagi M, Gasa S, Shimizu S, Eguchi Y, Tsujimoto Y, Sakiyama Y, Kobayashi K, Kuzumaki N. Inhibition of apoptosis by the actin-regulatory protein gelsolin. EMBO J 16: 4650-4656 (1997).
13) Izumi Y, Kuwamoto S, Kondo H, Hayasaka T, Honma T, Yoshino H and Sato M. Solution X-ray Scattering Data Reveal Sequence Motifs and Need Central Helix of Calmodurlin for its Recognition. Photon Factory Activity Report #16 (part B) 240 (1998).
14) Izumi Y, Yokouchi T, Nogami H, Yoshino H, Nakashima K and Yazawa M. Solution X-ray Scattering Data Show Structural Differeces of Chimeras ofYeast and Chicken Calmodulin; Implication for Structure/Function. Photon Factory Activity Report #16 (part B) 241 (1998).
15) Izumi Y, Kuwamoto S, Hayasaka T, Sakakibara A, Shishido K,Yoshino H and Sato M: Solution X-ray Scattering Data Reveal Sequence Motifs and Need Central Helix of Calmodulin for Its Recognition. The 11th. International Symposium on Calcium-Binding Proteins and Calcium Function in Health and Disease; Kisarazu, Japan. Abstract, V-6 (1999).

Biology

The Department has been actively engaging in modern biological research. Four talented faculty members perform research on fundamental problems in Biology. Areas of interests include regulation of transcription, molecular pathogenesis of sarcoma, physiological systems for animal behavior and color changes, and taxonomy and ecology of nematodes. The Department offers a graduate program in Molecular Cell Biology, leading to the Doctor of Philosophy degree.

Professor
Koichi Yoshida, M.S., Ph.D.
Interests:
Molecular biology, Molecular oncology

Associate Professor
Tsuneo Moriya, B.S., Ph.D.
Interests:
Animal ethology and physiology

Assistant Professor
Yoko Miyashita, M.S., Ph.D.
Interests:
Animal ethology and physiology

Instructor
Kenji Kito, M.S., Ph.D.

1. ETS transcription factors in invasion and metastasis of cancer cells

Invasion and metastasis, major obstacles to an effective cancer therapy, are complex multi-step processes. We found that an ETS transcription factor, E1AF, positively regulated transcription of the invasion-associated matrix metallo-protease (MMP) genes. Inversely, transfection with the anti-sense E1AF gene restrained cancer cell invasion by reducing MMP activities (1). Both E1AF and MMP genes were up-regulated in oral cancer cells stimulated with Hepatocyte growth factor (HGF). In contrast, no obvious activation of MMPs was observed in cells expressing the anti-sense E1AF gene, suggesting some roles of E1AF in HGF-mediated invasion (2). We also found that the increased E1AF expression in fibrosarcoma cells contributed to invasive phenotypes, including expression of membrane-type-1 MMP, but was not sufficient to exhibit highly metastatic activity in vivo (3). Urokinase plasminogen activator (uPA) has been associated with invasion and metastasis in breast cancer. We showed the ability of Ets-1 and Ets-2 to activate the uPA promoter in response to epidermal growth factor (EGF) (4). ETS transcription factors may provide the link between EGF and uPA expression, contributing to highly invasive phenotyes in ErbB2 receptor-overexpressing breast cancer cells.

2. Molecular pathogenesis of Ewing`s sarcoma and peripheral neuro-ectodermal tumor (ES/PNET)

ES/PNET is a primitive mesenchymal tumor composed of small round cells showing limited neural differentiation, arising within bone or soft tissue in a child or adolescent. ES/PNETs contain specific chromosomal translocation resulting in the fusion of the EWS gene and the ETS family gene of transcription factor. We previously identified a fusion of the EWS and E1AF gene by t (17;22)(q12;q12) chromosomal translocation in an undifferentiated sarcoma of infancy. Molecular analysis provided structural characteristics of the EWS-E1AF gene and an insight into the mechanism of chromosomal translocation (5). ES/PNET-specific fusion proteins act as an oncogenic transcription factor. To understand better the molecular mechanism of sarcoma-genesis, we are currently investigating the biological significance of the fusion oncogene in mice by a novel transgenic technique that allows cell type-specific expression.

3. Physiological systems for animal behavior and color changes

a) Expression of opsin molecule in pigment cell

Pigment cells in animal skin, which are usually exposed to sunshine, are likely to be one of the non-visual photoreceptive systems. We demonstrated the expression of rhodopsin in the tail fin of Xenopus tadpole, in which photosensitive melanophores exist (6). The presence of opsin molecules in pigment cells of lower vertebrates raises the possibility that pigment cells in animal skin function as photo-sensors generally. We tried to detect photoreception molecules in mammalian melanocyte. RT-PCR and Western blotting showed the expression and the existence of

opsin in murine and human cultured melanocytes. The role of the opsin molecule in melanocyte is not known at present, but this will provide additional insight into photoreception systems in animal skin.

b) Detection of temperature preference for various animals using an apparatus with temperature gradient

A thermal gradient apparatus was designed for determination of temperature preference of various animals including insects, mollusca, annelida, amphibia and reptilia. Most of the test animals came to rest in areas within a narrow range of temperature, indicating their preference for a specific temperature. Larvae of fry, *Delia antiqua* altered their preference for temperature during their development. An isopod of *Porcellio scaber* showed seasonal change in their preference for temperature.

c) Physiological and morphological adaptation of *Xenopus laevis* with long term starvation

Xenopus laevis survived more than 300days with no food-cultivation. During this cultivation, degree of haematocrit decreased but the blood suger content didn't change. The weights of the ovary and liver decreased remarkably and various morphorogical changes were observed. The height of rugae of the gastric epithelium lowered and fused. This flattened structural change returned to the normal gastric epithelium structutre immediately after feeding.

d) Catadromous migration of salmon

In order to understand the mechanism of catadromous migration, the swimming behavior of salmon fingerling (7), *Oncorhynchus keta*, was observed using a circular experimental tank which had a current. They swam with the current in the experimental tank throughout the observation time after hatching. On the other hand, if the fingerlings were kept in still water for some periods, they soon started to swim with, and faster than the current. and after their swimming behavior to the water current changed, the monoamine compounds in the brain were analyzed.

4. Taxonomy and ecology of nematodes

Free-living nematodes, which are found in nearly every conceivable niche of the biosphere, have been taxonomically and ecologically studied. The main objective is to clarify marine nematode fauna in the Pacific and taxonomic study has been carried out on the coast of Japan (8) and Thailand (9). Antarctic terrestrial nematodes have also been studied for assessment of the human impact on terrestrial invertebrates in the maritime and continental Antarctica.

List of Main Publications from 1997 to 2000

1) Hida K, Shindoh M, Yasuda M, Hanzawa M, Funaoka K, Kohgo T, Amemiya A, Totsuka Y, Yoshida, K, Fujinaga K. Antisense E1AF transfection restrains oral cancer invasion by reducing MMPs activities. Am J Pathol 150: 2125-2132 (1997).
2) Hanzawa M, Shindoh M, Higashino F, Yasuda M, Inoue N, Hida K, Ono M, Kohgo T, Nakamura M, Notani K, Fukuda H, Totsuka Y, Yoshida K, Fujinaga K. Hepatocyte growth factor upregulatees E1AF that induces oral cell carcinoma cell invasion by activating matrix metalloprotenase genes. Carcinogenesis 21: 1079-1085 (2000).
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4) Watabe T, Yoshida K, Shindoh M, Kaya M, Fujikawa K, Sato H, Seiki M, Ishii S, Fujinaga K. The Ets-1 and Ets-2 transcription factors activates the promoters for invasion-associated urokinase and collagenase genes in response to epidermal growth factor. Int J Cancer 77: 128-137 (1998).
5) Ishida S, Yoshida K, Kaneko Y, Tanaka Y, Sasaki Y, Urano F, Umezawa A, Hata J-i, Fujinaga K. The genomic breakpoint and chimeric transcripts in the EWS-E1AF gene fusion in Ewing sarcoma. Cytogenet Cell Genet 82 (3-4): 278-283 (1998).
6) Miyashita Y, Moriya T, Yamada K, Kubota T, Shirakawa S, Fujii N, Asami K: The photoreceptor molecule in melanophores of Xenopus tadpole. Comp Biochem Physiol, Fifth International Congress of Comparative Physiology and Biochemistry: Calgary Canada, Supple, 124A (1999).
7) Moriya T, Miyashita Y. Catadromous migration of salmon smolt observed in an aquarium with a current. Journal of Liberal Arts and Sciences Sapporo Medical University, School of Medicine. 41: 9-16 (2000).
8) Kito K, Hope WD. Leptosomatides brevicaudatus n. sp. and a redescription of Leptosomatides marinae Platonova, 1967 (Enoplida: Leptosomatidae). J Nematol 31: 460-474 (1999).
9) Kito K, Aryuthaka C. Free-living marine nematodes of shrimp culture ponds in Thailand. I. New species of the genera Diplolaimella and Thalassomonhystera (Monhysteridae) and Theristus (Xyalidae). Hydrobiol 379: 123-133 (1998).

English

Our department has been occupied with a variety of themes involving English which cover a wide range of specialist fields. These are comprised of: modern literary criticism with particular reference to Victorian authors; linguistic analysis dealing with linguistic form/structure and its deeper semantic significance; and some pedagogical problems in language teaching methodology.

Professor
Shin Morioka, M.A.
Interests:
Victorian literature, Modern literary criticism

Assistant Professor
Kazuhiko Yamaguchi, M.A.
Interests:
Syntax, Grammar, Discourse, Typology

English Instructor
Robert Holmes, B.A.
Interests:
Dialectics, The social structure, Language and class relations.

1. Literary criticism

We have explored the imaginative and literary dimension in some Victorian writers from the viewpoint of their rhetorics about gender and sexuality. Our study also examines the broader scope allowed by present-day Feminism for our reading of nineteenth century English literature.

2. Cognitive linguistics

We make a cognitive approach to the description of language and consider the relation between language and thought. Current topics under investigation are as follows: various aspects of marked word order constructions in English, the description of emphatic *do*, shape and number in language and thought, the relations between various finite clause constructions, the problem of complement selection.

3. Teaching methodology

The application of the communicative approach in T.E.F.L. has been reviewed in an attempt to establish a teaching methodology which is capable of improving the communicative ability of Japanese students of English.

List of Main Publications from 1997 to 2000

1) Morioka S. A Reading of Ruskin's "King of the Golden River" J.Lib.Arts.and Sci.Sapporo Med.Univ. 41: 17-23 (2000).
2) Yamaguchi K. Contents of emphatic *do*. Kasai S. (ed): English Linguistics and Current Linguistic Theory. Hokkaido University Publishing Co.236-252 (1999).
3) Yamaguchi K. Some speculations on shape and number in language. J.Lib.Arts.and Sci.Sapporo Med.Univ. 40: 33-45 (1999).
4) Yamaguchi K. Access types and finite clause constructions. J.Lib.Arts.and Sci.Sapporo Med.Univ. 41:25-37. (2000).
5) Yamaguchi K. Complements and layers: *that*-clauses vs. equi-*to*-infinitives. J.of Hokkaido Linguistics.1:70-82 (2000).
6) Okano, Holmes et al.: Low Prevalence of Disordered eating patterns and Menstrual Irregularities in Chinese female Athletes. Japanese Journal of Physical Fitness and Sport Medicine. Vol 47 no.3, 271-278. (1998).
7) R.Holmes. The Communicative Approach to Teaching English as a Foreign Language and its importance for Japanese Students. J.Lib.Arts.and Sci.Sapporo Med.Univ. 40: 47-51 (1999).

Exercise Science

Our laboratory has investigated the relationship between nutrition and physical training in promoting physical fitness, improving body composition, increasing bone formation and preventing chronic disease related to lifestyle. Recently, epidemiological studies started to clarify the health behavior and lifestyle factors contributing to good health in middle aged and older adults as well as the prevalence of and reasons for disordered eating of female athletes.

Associate Professor
Goroh Okano, M.S., Ph.D.
Interests:
Exercise physiology, Exercise epidemiology

1. Physiology in exercise and nutrition

a) Food intake timing for obtaining better body composition

Our co-researcher recently demonstrated that the early provision of nutrients after exercise more effectively enhanced protein synthesis in skeletal muscle than nutrients administered later. Then, we investigated in rats whether early ingestion of meal vs. later one results in muscle atrophy or not. As a result, muscle volume in leg was greater in the rats fed right after exercise than those fed later (1). In this experiment, we also found that early ingestion of meal not only decreased abdominal fat content but increased bone accretion in the leg (1.2). Meal intake timing after exercise is very important to obtain better body composition and bone formation.

b) Endurance performance and diet

It has been demonstrated that elevated blood FFA level by heparin or caffeine administration enhances endurance performance due to increment of FA oxidation and resultant sparing of glycogen. We investigated the efficacy of elevated blood FFA induced by a single high fat diet ingestion on endurance. A single dose of high fat diet resulted in a modest elevation of blood FFA and an enhanced oxidation of FA, but this effect was weak and didn't contribute to an enhanced performance (3).

2. Exercise epidemiology

a) Athletes

So far, we have surveyed the nutritional status and eating behavior of young elite Japanese and Chinese athletes. Our previous study reported that the Japanese athletes obtained less nutrients and energy than their Chinese counterparts, and a number of the Japanese female athletes suffered from disordered eating and resultant amenorrhea. Prevalence of disordered eating and amenorrhea, however, was much lower in the Chinese female athletes, as compared with the Japanese (4). This difference was partly explained by the difference between Chinese and Japanese athletes in socioeconomic factors and/or weight ideation for improving performance.

b) Middle aged and older adults

Recently, new epidemiologic studies have started to evaluate the importance of physical activity for good health status. This study is on-going and seems to be indicating that physical activity is one of the most effective factors contributing to good health status in middle aged and older adults (Hokkaido JPH, 2000. 14, in Japanese).

List of Main Publications from 1997 to 2000

1) Suzuki M, Doi T, Lee SJ, Okamura K, Shimizu S, Okano G, Sato Y, Shimomura Y, Fushiki T. Effect of meal timing after resistance exercise on hindlimb muscle mass and fat accumulation in trained rats. J Nutr Sci Vitaminol. 45: 401-409 (1999).
2) Okano G, Suzuki M, Kojima M, Sato Y, Lee SJ, Okamura K, Noriyasu S, Doi T, Shimomura Y, Fushiki T, Shimizu S. Effect of timing of meal intake after squat exercise training on bone formation in the rat hindlimb. J Nutr Sci Vitaminol. 45: 543-552 (1999).
3) Okano G, Sato Y, Murata Y. Effect of elevated blood FFA levels on endurance performance after a single fat meal ingestion. Med Sci Sports Exerc. 30: 763-768 (1998).
4) Okano G, Mu Z, Holmes R, Lin Z, Sato Y, Suzuki M, Nakai Y. Low prevalence of disordered eating patterns and menstrual irregularities in Chinese female athletes. Jpn J Phys Fitness Sports Med. 47: 271-278 (1998).

Animal Research Center

The Mechanisms of Infectious diseases are the main focus of our studies. The diseases and microorganisms studied are gastritis induced by *Helicobacter pylori*, Lyme disease by various speciese of *Borrelia*, Leptospirosis, entero haemorrhagic *Escherichia coli* and periodontal disease by *Porphyromonas*.

Gene targetting as well as transgenic animals are under investigation in our research center.

Professor and Director (Affiliated)
Kohzoh Imai, M.D., Ph.D.
Interests:
Gastroenterology, Immunology

Associate Professor
Hiroshi Isogai, DVM, Ph.D.,
Interests:
Infectious disease, Microbiology.

1. Prof. Imai's research and publication list appears on page 36-37.

2. *H. pylori* and disease

The relation between *Helicobacter pylori* and other bacterial flora has been investigated using experimental mice inoculated with *H. pylori*. *H. pylori* can grow in the stomach when there is enough time for colonization. Bacterial growth was easier in the stomach of germ free mice than in that of microbiologically non-controlled mice. *H. pylori* has been able to colonize in other tissues or organs when these exist in a microbiologically free environment. These findings indicated that *H. pylori* has an ability to colonize on the epithelial surface. On the other hand, bacterial flora on the surface of the epithelium is effective for colonization of *H. pylori*. The relation of Heat Shock Proteins (HSP) to tissue damage caused by *H. pylori* has been studied. The study demonstrated that sera from patients with gastritis or gastric ulcer showed high titer of antibody to HSP. The results indicated that HSP and anti-HSP antibodies was associated with tissue destruction in the stomach of patients infected with this organism.

3. Lyme disease

The pathogenesis and Epidemiological status of Lyme disease have been studied. The studies demonstrated that several cytokins and other biological factors affected the pathogenesis of Lyme disease. The effects of TNF was evaluated by antagonist to that factor in experimental animal studies. Our studies also investigated the association of other factors including Interleukin-1, interleukin-6, to the pathogenesis of Lyme disease. Serological studies demonstrated the incidence of Lyme disease in Hokkaido and the relation of Lyme disease *Borrelia* to patients with neural symptoms. About 1000 serum and cerebro-spinal fluid samples from patients clinically diagnosed with Lyme disease have been accumulated. These samples were examined for antibodies and nucleic acid from *Borrelia* by dot blot methods and PCR methods. The results helped clinicians to diagnose this disease in patients.

4. Leptospirosis

The component of Leptospiral lipopolysaccharide associated with antigen determination has not been clarified. Our study demonstrated that a repeating structure including mannos was the component which determined antigenisity of lipopolysaccharide from *Leptospira*. Furthermore, our study showed that the structure was distributed widely among many microorganisms, especially fungi. It is possible that the structure can be used for vaccination against leptospirosis.

5. *E. coli* O157

The lethal factors of enterohemohhagic *Escherichia coli* O157; H7 (EHEC) have been studied. Our study showed that gnotobiotic mice infected with EHEC could be a useful animal model for the disease. The studies demonstrated that TNF released from intestinal tissues after infection was significantly related to damage of the tissues. Furthermore, this infection and tissue damages could be inhibited by pre-inoculation of catechin from Japanese green tea to the mice. The results indicated that the pre-inoculation or pre-treatment of catechin was applicable to human. Because catechin can inhibit bacterial growth in intestine, antibiotic treatment can be effective when EHEC infection occurs.

6. *Porphyromonas*

Black-pigmented *Porphyromonas* originating from oral cavity has been studied. *Porphyromonas* from animal was different from that from human. In our study, many black-pigmented *Porphyromonas* were isolated from plaque of dogs and cats.

These were examined for their biological characteristics. It was not possible to isolate human type *P. gingivalis* from animal. Our study demonstrated that some strains isolated from animal were new species.

List of Main Publications from 1997 to 2000

1) Isogai E, Hirose H, Kimura K, Hayashi S, Kubota T, Fujii N, Isogai H. Role of platelet-activating-factor (PAF) on cellular responses after stimulation with leptospire lipopolysaccharide. Micorbiol Immunol 41(3): 271-275 (1997).
2) Isogai H, Isogai E, Hayashi S, Kimura K, Kubota T, Fujii N, Oguma K. Experimental *Helicobactor pyroli* infection in association with other bacteria. Microbiol Immunol 41(4): 361-365 (1997).
3) Isogai H, Kimura K, Hayashi S, Kubota T, Fujii N, Nishikawa T, Kotake S, Isogai E. Levels of endogenous interleukin-1, interleukin-6 and tumor necrosis factor in congenic mice infected with *Borrelia garinii*. Microbiol Immunol 41(5): 427-430 (1997).
4) Isogai E, Isogai H, Hirose K, Miura H, Kimura K, Hayashi S, Hayashi M, Fujii N, Benno Y. Platelet responses after stimulation lipopolysaccharide from *Porphyromonas gingivalis*. Bioscience and Microflora. 16 (2): 73-76 (1997).
5) Isogai E, Isogai H, Kimura K, Hayasahi S, Kubota T, Fujii N, Takeshi K. Role of tumor necrosis factor (TNF)-alpha in gnotobiotic mice infected with an *Escherichia coli* O157: H7 strain. Infect Immun 68 (1): 197-202 (1998).
6) Isogai E, Isogai H, Takeshi K, Nishikawa K. Protective effect of Japanese green tea extract on gnotobiotic mice infected with an *Escherichia coli* O157: H7. Microbiol Immunol 42 (2): 125-128 (1998).
7) Kubota T, Yonekura N, Hariya Y, Isogai E, Isogai H, Amano Ken-ichi, Fujii N. Gene arrangement in the upstream region of *Clostridium boturinum* type E and *Clostridium butyricum* BL6340 progenitor toxin genes is different from that of other types. FEMS Microbiol Lett 158: 215-221 (1998).
8) Kaneda K, Masuzawa T, Simon MM, Isogai E, Isogai H, Yasugai K, Suzuki T, Suzuki Y, Yanagihara Y. Infectivity and arthritis-induction of *Borrelia japonica* on SCID mice and immune competent mice: possible role of galactosylceramide binding activity on initiation of infection. Microbiol Immunol 42(3): 171-175 (1998).
9) Kimura K, Kimura H, Yokosawa N, Isogai H, Isogai E, Kozaki S, Miyamoto A, Nishikawa T, Ohshika H, Kubota K, Fujii N. Negative chronotropic effect of botulinum toxin on neonatal rat cardiac myocytes. BBRC 244: 275-279 (1998).
10) Kimura K, Hayashi S, Isogai H, Isogai E, Sugiyama T, Asaka M, Anbo T, Imamura A, Nishikawa T, Kubota K, Fujii N. Restriction fragment length polymorphism in *Helicobacter pylori* associated with gastric cancer and duodenal ulcer. Biosci Microflora 17 (1): 61-64 (1998).
11) Hayashi S, Sugiyama T, Yokota K, Isogai H, Isogai E, Oguma K, Asaka M, Fujii N, Hirai Y. Analysis of immunoglobulin A antibodies to *Helicobacter pylori* in serum and gastric juice in relation to mucosal inflammation. Clin Diagn Lab Immunol 5 (5): 617-621 (1998).
12) Kimura K, Kubota T, Ohishi I, Isogai E, Isogai H, Fujii N. The gene for component-II of botulinum C2 toxin. Vet Microbiol 62: 27-34 (1998).
13) Hayashi S, Sugiyama T, Amano K, Isogai H, Isogai E, Aihara M, Kikuchi M, Asaka M, Yokota K, Oguma K, Fujii N, Hirai Y. Effect of rebamipide, a novel antiulcer agent, on *Helicobacter pylori* adhesion to gastric epithelial cells. Antimicrobial Agents Chemocer 42 (8): 1895-1899 (1998).
14) Isogai E, Isogai H, Kubota T, Fujii N, Hayashi S, Indoh T, Takagi S, Miura H, Kimura K. Apoptosis of lymphocytes in mice administered lipopolysaccharide from *Leptospira interrogans*. J Vet Med B45: 529-537 (1998).
15) Isogai H, Kosako Y, Benno Y, Isogai E. Ecology of Genus *Porphyromonas* in canine periodontal disease. J Vet Med B 46: 467-473 (1999).
16) Isogai E, Isogai H, Yokota S, Amano K.-I., Hayashi S, Hirose K, Oguma K, Hirai Y, Sugiyama T. Effect of *Helicobacter pylori* lipopolysaccharides on human polymorphonuclear neutrophils and endothelial cells. Biosci. Microflora 18 (2): 119-124 (1999).
17) Hang'ombe BM, Isogai E, Lungu J, Mubita C, Nambota A, Kirisawa R, Kimura K, Isogai H. Detection and characterization of *Clostridium* species in soil of Zambia. Comp Immunol Microbiol Infect Dis 23: 277-284 (2000).
18) Isogai E, Isogai H, Hayashi S, Kubota T, Kimura K, Fujii N, Ohtani T, Sato K. Effect of antibiotics, levofloxacin and fosfomycin, in the mouse model with *Escherichia coli* O157 infection. Microbiol Immunol 44 (2): 89-95 (2000).
19) Hayashi S, Sugiyama T, Yokota K, Isogai H, Isogai E, Shimomura H, Oguma K, Asaka M, Hirai Y, Combined effect of rebamipide and ecabet sodium on *Helicobacter pylori* adhesion to gastric epithelial cells. Microbiol Immunol 44 (7): 557-562 (2000).

Marine Biomedical Institute

Human life appears to have begun in the forest, but, if there is no ocean nearby, a green area will turn in to desert. The sea created and maintained life. All life is associated with it. Though studying the mechanism by which life is maintained in the sea, we will better understand human life and will be able to obtain hints about suppressing disease. As a result, we report here that some natural substances from the sea are useful for prevention of and chemotherapy for cancer.

Professor and Director (Affiliated)
Kowichi Jimbow, M.D., Ph.D.
Interests:
Melanocyte biology,
Diagnosis and treatment of melanocytic tumors

Associate Professor
Nobuaki Takahashi, Ph.D.
Interests:
Cancer chemotherapy,Reproduction of marine organisms

Instructor
Hiroeki Sahara, M.D.

1. Bioactive substances for cancer chemo- and immunotherapy

Usually, marine organisms show poor development of the immune system. Their body protection depends mostly upon a chemical defense. We isolated the sea urchin's defensive chemical materials from its intestine. One molecule that possessed cytotoxity and anti-tumor activity was detected and determined to be 3'-sulphonoquinovosyl-1'-monoacylglyceride (SQG)(1.2). Recently, analogues of SQG were synthesized, and, one molecule among its chemicals was also shown to suppress the immune system. Recently, a cytotoxic peptide was isolated from the scallop midgut. Its partial primary molecular structure is Trp-Asp-Ser-Met-Glu-His-Trp-Ile-Gly-Try---(?) from the N-terminal.

In general, a patient's immune system can respond against his or her own neoplastic cells. There is a possibility that the elucidation of human tumor antigens may directly lead to a drastic improvement in tumor immunotherapy and establish a new modality of cancer therapy. Meanwhile, attempts to determine the tumor antigenic peptides in mainly gastric cancer were carried out (3-5).

2. Bioactive substances for cancer prevention

One of the ultimate objectives of cancer research is to acquire various methods of chemical cancer prevention (chemoprevention). Chemoprevention methods in response to developmental stages of cancer must be produced (6). We isolated fucoidan, which induces flat-form reversion of cancer cells, from the thallus in brown algae. On the other hand, from the rhizoid, L-tryptophan was isolated as a cell division-suppressing material for MCF-7 breast cancers (7). Trigonelline, which suppress angiogenesis and inversion by cancer cells, was extracted from the sea urchin intestine. In addition, ovotiol-B with anti-oxidation activity also was isolated from sea urchin ovaries.

3. Anti-bioactive substances for enviromental endocrine disruptors

Endocrine-disrupting chemicals can be either synthetic or naturally occuring (8). They may be characterized as estrogen or androgen (thereby producing similar responses to them) or they may block the activities of estrogen or androgen (i.e., be anti-estrogens or anti-androgens). Therefore, we began to isolate bioactive substances possessing the activities of anti-estrogens or anti-androgens from foods (9).

4. Chemical signals between marine organisms, with special reference to predator avoidance

Many marine creatures have a large variety of traits that have evolved expressly to deter predators. Some marine animals avoid predators by means of crypsis, deceit, and avoidance responses. We studied the avoidance response-inducing substance of the sea urchin *Strongylocentrotus nudus* from the starfish *Plazaster boreials*. As a result, the material was determined to be $C_{27}H_{44}O_9S_2Na_2$ (10).

5. Reproduction and the biological rhythm in marine organisms

We studied external and internal chemical communication mechanisms occurring during reproduction of marine organisms. Among those signals we paid special attention to one signal of the spawning-inducing substance, owing to the appearance of

biological rhythm, of the sea urchin. The rhythm was analyzed by using a computer program called "MemCalc", which is a linearized version of the nonlinear least squares method for fitting analysis in the time domain, combined with the maximum entropy method (MEM) for spectral analysis. Our results led to a hypothesis of dual nervous regulation during sea urchin spawning (11).

List of Main Publications from 1997 to 2000

1) Sahara H, Ishikawa M, Takahashi N, Ohtani S, Sato N, Gasa S, Akino T, Kikuchi K. In vivo antitumor effect of 3'-sulfonoquinovosyl 1'-monoacyl-glyceride, isolated from sea urchin (*Strongylocentrotus intermedius*) intestine. Br J Cancer 75: 324-332 (1997).
2) Mizushima Y, Watanabe I, Ohta K, Takemura M, Sahara H, Takahashi N, Gasa S, Sugawara F, Matukage A, Yoshida S, Sakaguchi K. Studies on inhibitors of mammalian DNA plymerase a and b. Biochem Pharmacol 55: 537-541 (1998).
3) Suzuki K, Sahara H, Okada Y, Yasoshima T, Hirohashi Y, Nabeta H, Hirai I, Torigoe T, Takahashi S, Matuura A, Takahashi N, Sasaki A, Suzuki M, Hamuro J, Ikeda H, Wada Y, Hirata K, Kikuchi K, Sato N. Identification of natural antigenic peptides of a human gastric signet ring cell carcinoma recognized by HLA-A31-restricted cytotoxic T lymphocytes. J Immunol 163: 2783-2791 (1999).
4) Sahara H, Nabeta Y, Sato N. Identification of natural antigenic peptide and gene cloning of tumor antigen on stomach cancer for immunotherapy. Hematology and Oncology 39: 1-7 (1999).
5) Nabeta Y, Sahara H, Suzuki K, Kondo H, Nagata M, Hirohashi Y, Wada Y, Sato T, Wada T, Kikuchi K, Sato N. Induction of CTL from peripheral blood of HLA-A31(+) gastric cancer patients by in vitro stimulation with antigenic peptide of signet ring cell carcinoma. Jpn J Cancer Res 91: 616-621 (2000).
6) Ichimiya S, Nakagawara A, Sakuma Y, Kimura S, Ikeda T, Sato M, Takahashi N, Sato N, Mori M. p73: structure and function. Pathol Int 50: 589-593 (2000).
7) Takahashi N, Ojika M, Dogasaki C, Nishizawa M, Fukuoka H, Sahara H, Sato N, Mori M, Kikuchi K. Substance isolated from the kelp rhizoid identified as L-tryptophan has a high degree of inhibition toward breast cancer. Jpn J Cancer Chemotherapy 27(2): 251-255 (2000).
8) Ogi H, Takahashi N, Nakata S, Ito S, Matsushita Y, Shibata Y. Egg-shape variation of the black-tailed gull (*Larus crassirostris*) on Rishiri Island, norther Japan. Bull Fac Fish Hokkaido Univ. 50: 1-10 (1999).
9) Takahashi N, Ogi H. Inhibitory effects of several amino acids on proliferation of breast cancer cell line MCF-7. Bull Marine Biomed Inst, Sapporo Med Univ. 4: 85-89 (1999).
10) Takahashi N, Ojika M, Ejima D. Isolation and identification of a trihydroxysteroid disulfate from the starfish *Plazaster borealis* which induces avoidance reaction-inducing substance in the sea urchin *Strongylocentrotus nudus*. Fis Sci 66: 412-413 (2000).
11) Takahashi N, Ohtomo N, Sahara H, Sato N and Mori M. Gonad response to ATP in the sea urchin. Bull Marine Biomed Inst, Sapporo Med Univ. 4: 51-54 (1999).

B SCHOOL OF HEALTH SCIENCES
1 Nursing

Medical and Behavioral Subjects

Four of our section's staff are in charge of teaching health science, social medicine, and basic and clinical medicine to students of nursing, physical therapy, and occupational therapy.

Research activities of staff members of this section are epidemilogic and virologic studies of infectious diseases (T.U), tumor immunology and molecular pathology (K.K), metabolic disorders and alcoholic pancreatic disorders(A.K), cancer research and climical research (R.D).

Professor
Tomoko Urasawa, M.D., Ph.D.
Interests:
Epidemiology, Public health

Akira Kihara, M.D., Ph.D.
Interests:
Internal medicine

Ryuichi Denno, M.D., Ph.D.
Interests:
Gastroenterological surgery

Associate Professor
Kiyoshi Kasai, M.D., Ph.D.
Interests:
Pathology

1. Epidemilogic and virologic studies of infectious diseases

The prevalence of rotaviruses and characterization of virus isolates were investigated by ELISA, PCR and uncleotide sequence analysis using stool samples collected from human and animals in various countries (1-3). The prevalence of antibodies to rubella and toxoplasma gondii, both inducing congenital abnormalities in humans, among nursing students has been investigated consecutively from 1983.

The development of a heat-stable live oral poliovaccine has been pursued (4).

2. Tumor immunology and molecular pathology

Lymphoepithelioma-like carcinoma (LELC) has been proposed for histologically unique neoplasms characterized by an undifferentiated carcinoma and a dense lymphoid infiltrate. However, the immunophenotype of tumor infiltrating T-lymphocytes (TITL) in LELC is not yet fully documented. We thus examined the immunophenotype of TITL of 6 LELCs including 2 LELCs of the lung and 4 of the stomach immunohistochemically. The tumors of all the cases were shown to be positive for Epstein-Barr virus (EBV) by in situ hybridization. Most TITL of the 6 cases were labeled with both CD8 and TIA-1 but not with Granzyme B, indicating the TITL to be cytotoxic T-lymphocytes (CTL) in the resting state and local inhibition of EBV-specific CTL responses to occur in the tumors (5.7).

Recently, we have developed a new protocol of in situ RT-PCR to detect cytokine mRNA and viral RNA inside cells. Positive signals obtained were distributed in the cytoplasm in accordance with RNA localization. Thus, this new protocol has improved the sensitivity of the technique compared with conventional methods of in situ hybridization (6-9).

3. Metabolic disorders

a) Diabetes mellitus

Clinical studies have been performed for micro- and macro-angiopathy observed in this disease (Effect of alpha-glucosidases inhibitor for NIDDM patients with hyperlipidemia after meal. J. Japan Diab. Soc., 41, 731. 1998. in Japanese. Insulin resistance and pancreatic diabetes mellitus (3rd Report). J. Japan Diab. Soc., 41, 734. 1998. in Japanese).

b) Hyperlipidemia

Clinical studies have been carried out for new antilipidemic drugs (A case of fungal ophthalmitis for a 35-year old patient with diabetes mellitus. J. Japan Diab. Soc., 41, 725. 1998. in Japanese.Insulin resistance and pancreatic diabetes mellitus (2nd Report). J. Japan Diab. Soc., 40, 310. 1998. in Japanese).

4. Alcoholic pancreatic disorders

In order to investigate acute and chronic alcoholic pancreatitis, changes of serum pancreatic phospholipase A2 (PLA-2) and pancreatic secretory trypsin inhibitor (PST1) have been studied in chronic alcoholics(A study for a correlation of alcoholic chronic pancreatitis and diabetes mellitus in serum phospholipase A2. J. Japan Diab. Soc., 40, 322. 1997. in Japanese).

5. Cancer research

Peritoneal dissemination is most frequently observed in human gastric cancer and is one of most important causes of cancer deaths in Japan. Some trials with gastric cancer patients have been done, including chemotherapy (10). I think that the establishment of relevant animal models of metastasis is extremely important for the development of new therapeutic modalities for gastric cancer. We established a highly liver metastatic cell line, AZ-H5c derived from a human gastric cancer cell line, AZ-521(11.12.14) and a new cell line, AZ-P7a, with high peritoneal-metastatic potential in nude mice (13).

6. Clinical research

Postgastrectomy complications include reflux esophagitis, dumping syndrome, and malnutrition. To prevent or minimized such sequelae, proximal gastrectomy with an interposed jejunal pouch has been advocated as an organ-preserving surgical strategy to improve quality of life for patients. We performed proximal gastrectomy in 44 patients with tumors in the upper third of the stomach. The jejunal pouch procedure was effective for treating early cancers of the upper part of the stomach. This operation improved patients' postoperative quality of life.

These works were supported in part by a grant (10671202) from The Japan Society for the Promotion of Science (JSPS).

List of Main Publications from 1997 to 2000

1) Ahmed MU, Alam MM, Chowdhury NS, Haque NS, Shahid N, Kobayashi N, Taniguchi K, Urasawa T, Urasawa S. Analysis of human rotavirus G serotype in Bangladesch by enzyme-linked immunosorbent assay and polymerase chain reaction. J Diarrhoeal Dis Res 17: 22-27 (1999).
2) Okada J, Urasawa T, Kobayashi N, Taniguchi K, Hasegawa A, Mise K, Urasawa S. New serotype of group A human rotavirus closely related to that of a porcine rotavirus. J Med Virol 60: 63-69 (2000).
3) Kojima K, Taniguchi K, Kobayashi N, Urasawa T, Urasawa S: An analysis of the reversion generated during RT-PCR procedure to amplify concatemerized NSP5 gene of rotavirus. The 9th Int. Congress Infect. Dis. Abstracts, 199 (2000).
4) Urasawa T, Shiomi H, Kobayashi N, Urasawa S, Horie H, Abe S, Doi Y: Heat stabilization of Sabin poliovaccine. The 9th Int. Congress Infect. Dis. Abstracts, 15 (2000).
5) Kasai K, Kon S, Sato N. Immunohistochemical examination of tumor infiltrating T-lymphocytes in lymphoepithelioma-like carcinoma. Pathol Int 50, Suppl: 184 (2000).
6) Mattsuura A, Kinebuchi A, Katabami A, Hong-Zhi C, Kasai K et al. Comparative analysis of the structure and chromosome assignment of CD1: an evidence for different evolutionary histories between classic CD1 and CD1D class genes. J Exp Anim Sci 41: 87-90 (2000).
7) Kasai K, Kon S, Sato N, Muraishi K, et al. Case report of lymphoepithelioma-like carcinoma of the lung-lymphoid population consisting of cytotoxic T cells in resting state. Pathol Res Pract 195: 773-779 (1999).
8) Kon S, Sasamori T, Kasai K, Yamano Y, Endo T, Kon H, Kikuchi K. Ongoing somatic mutations of the immunoglobulin gene in MALT lymphoma with widespread MLP-type polypoid lesions. Leukemia 12: 1495-1497 (1998).
9) Kasai K, Kon S, Kikuchi K, Sato Y, Kameya T. Expression of carbohydrate antigens, $p80^{NPM/ALK}$, cytotoxic cell antigens and EB virus gene products in Anaplastic Large Cell Lymphomas. Pathol Int 48: 171-178 (1998).
10) Hirata K, Honrikoshi N, Aiba K, Okazaki M, Denno R, Sasaki K, Nakano Y, Isizuka H, Yamada Y, Uno S, Taguchi T, Sirasaka T. Pharmacokinetic study of S-1, a novel oral fluorouracil antitumor drug. Clin Cancer Res 5: 2000-2005 (1999).
11) Kameshima H, Yagihashi A, Yajima T, Kobayashi D, Denno R, Hirata K, Watanabe N. Helicobacter pylori infection: augmentation of telomerase activity in cancer and noncancerous tissues. World J Surg 24: 1243-1249 (2000).
12) Nishimori H, Yasoshima T, Denno R, Shishido T, Hata F, Okada Y, Ura H, Yamaguchi K, Isomura H, Sato N, Hirata K. A novel experimental mouse model of peritoneal dissemination of human gastric cancer cells: different mechanisms in peritoneal dissemination and hematogenous metastasis. Jpn J Cancer Res 91: 715-722 (2000).
13) Yamaguchi K, Ura H, Yasoshima T, Shishido T, Denno R, Hirata K. Establishment and characterization of a human gastric carcinoma cell line that is highly metastatic to lymph nodes. J Exp Clin Cancer Res 19: 113-120 (2000).
14) Shishido T, Yasoshima T, Hirata K, Denno R, Mukaiya M, Ura H, Yamaguchi K, Kawaguchi S, Sato N. Establishment and characterization of human pancreatic carcinoma lines with a high metastatic potential in the liver of nude mice. Surg Today 29: 519-525 (1999).

Fundamental and Adult Nursing

This department is composed of fundamental nursing and adult nursing. Research projects in our department involve both basic and clinical investigations. Basic research deals with nursing education, which is focused on nursing technology, nursing ethics and health education whereas clinical research deals with cancer and stress care nursing. Some of our projects are collaborative studies with researchers at other departments and/or colleges.

Professor
Yoshie Inaba, R.N., M.S.
Interests:
Nursing ethics, Nursing education,
Habits and health of female

Associate Professor
Tomoko Minagawa, R.N., M.S.
Interests:
Terminal nursing care, Economics in nursing

Masako Momma, R.N., M.S.
Interests:
Critical nursing care, Stress and nursing

Assistant Professor
Terumi Ohinata, R.N., M.S.
Interests:
Nursing technology, Nursing education
Habits and health of female

Masami Horiguchi, R.N., M.N.
Interests: Habits and health, Nursing ethics, Nursing education

Instructor
Sachiko Kiguchi, R.N.
Chieko Itaki, R.N.
Hidemi Sakai, R.N.

1. Education in nursing technologies

With the aim of establishing effective teaching methods for nursing technologies, we are attempting to clarify the definition of nursing skills, analyze the process for attaining technologies and examine teaching methods. In clarifying the definition of nursing technologies (Exploring Instructional Sciences, 17 • 2000, in Japanese), the composition of nursing practice is analyzed in order to establish a basic theory for the teaching of skills. Concerning specific teaching methods, study of such nursing skills as body mechanics and assistance in dietary habit is being carried out (Bulletin of School of Health Sciences Sapporo Medical University, 2 • 1999 and 3 • 2000). The study examines an experimental program formulated with the students' body and life experience as material and with a teaching theory based in a scientific process of recognition.

A study is being conducted, with a Japanese Education Ministry's research grant, on the development of materials for a CAI simulation program for ethical education in nursing and of a linkage model to facilitate educational collaboration between nursing and clinical teaching staff. The first stage of the study is clarifying the definition of nursing ethics and the content of the education (Exploring Instructional Sciences, 18, 2001, in Japanese).

2. Health education

In order to establish a health education program for women in Hokkaido, a study was conducted to investigate the drinking and smoking behavior of 40 to 50 year old women in the three cities of Sapporo, Sendai and Nagoya from a socio-cultural perspective (The 6th Research Aid Report in Social Health Science of Meiji Life Foundation of Health and Welfare, 6 • 2000, in Japanese). The result showed that the frequency of drinking and amount consumed were higher in Sapporo than in the other two cities. It also showed a tendency for women to go drinking at such places as Japanese style pubs with their coworkers. Furthermore it was found that smoking was more prevalent in women in Sapporo. It is suggested that the results are related to the social, cultural and historical background of Hokkaido.

Aiming to probe into health education for students, a study was done to investigate the dietary habits of nursing students and comparisons were made of results for physical features, blood condition and eating habits between this study and a national nutrition survey. The results indicated that it is necessary to

improve students' eating habits from the perspective of health care (Japanese Journal of School Health, 42 • 2000, in Japanese). Another study dealt with stress of nursing students during clinical practice, investigating a change in activation of NK cell and emotion prior to and following practice. The findings showed that there was an individual difference in the decline of immunity, however, it was also found that NK cell activation is low in students with negative feeling (Bulletin of School of Health Sciences Sapporo Medical University. 3 • 2000, in Japanese).

3. Clinical nursing for adult.

A study on how to acquire basic knowledge and skills in emergency nursing is being conducted on nurses and nursing students. In addition, a survey was carried out on the actual conditions of admission of foreign patients that often prevail owing to Hokkaido's geographical position and on legal matters in medical practice with regard to foreign patients. The result showed that it is necessary to establish medical facilities and standards for accepting foreign patients and to set up a fund and a support system for solving language problems (Bulletin of School of Health Science Sapporo Medical University, 1, 1997, in Japanese).

A study on terminal care nursing emphasizes the significance of terminal care from an economic perspective. The health care cost for terminally ill patients has been increasing in proportion with that for total health care. Today's nurse executives are faced with the challenge of balancing the quality and cost of care in health care organizations. The current concerns about the costs of health care are giving many opportunities for nursing (Health Care Economics in Terminally ill Patients, 1999, in Japanese).

Maternal and Child Nursing

Our nursing section is composed of maternal-child nursing and nursing administration. Our section's goal is to make a strong bridge between nursing theory and nursing practice in order to improve the quality of nursing care. The health care provided by nurses must be constantly evaluated and improved based on new information. Our research project concerns respite care for mothers who are taking care of their children with chronic illness.

Professor and Chair
Tomoko Maruyama, R.N., C.N.M. Ph.D.
Interests:
Maternal nursing

Yuriko Ishizuka, R.N., M.S.
Interests:
Child nursing

Choko Sumiyoshi, R.N., M.S.
Interests:
Nursing administration

Associate Professor
Kiyoko Kihara, R.N.
Interests:
Child nursing

Assistant Professor
Atsuko Sugiyama, R.N., C.N.M., M.S.
Interests:
Maternal nursing

Miki Konno, R.N., D.S.N.
Interests:
Child nursing

Instructor
Yasuko Yoshida, R.N., C.N.M., M.S.

1. Nursing interventions to family and children with chronic illnesses

We are studying the problems of mothers who have children with chronically illnesses with a Grant-in Aid for Scientific research from the JSPS since 1995.

We have established a volunteer group which is trying to help the mothers. And we would like to develop a theory of nursing care for these kinds of problem families (Report of Grant-in Aid for Scientific Research of JSPS, 5,1998, in Japanese).

We are studying from the point of view of a family having chronically ill children. The effects of diabetic patients' self-care activities on family members were studied. Our attempts to provide nursing care for children with diabetes and their family from the early onset led us to conclude that nursing interventions were effective in helping patients and parents to undertake effective self-care decision-making. We have also started a study on life skills among adolescent with chronic illnesses. These studies are attempting to elucidate how nursing intervention can offer support for parents and children in problem families (1). These studies were reported in the following journals (Journal of Japan Academy of Diabetes Education and Nursing, 2・1998， Journal of Chiba Academy of Nursing Science, 5・1999， Journal of Japan Academy of Diabetes Education and Nursing, 4・2000, in Japanese).

2. Psychosocial aspects of women during child rearing

The birthrate is on the decline and the number of nuclear families is on the rise nowadays. Although women's contributions to society has come to be better recognized, married women are still often expected to raise their children without much support from their husband/partner or extended family. Thus, we used a cross-sectional study to assess the psychosocial aspects of women from pregnancy to two years after delivery. In this study we used the Maternal Concerns Questionnaire (MCQ), Edinburgh Postnatal Depression Scale (EPDS), and Self-esteem (SE). Professor Maruyama developed a MCQ consisting of 29-items and composed of eight categories. Women with high EPDS scores showed remarkably higher scores on the MCQ score also. In the MCQ, the categories that scored higher were Support of Husband, Body Image, and Concerns for the Infant. In addition, we support those women by telephone, letter and small group meetings. We have reported our work in the following journals (Jpn J Psychosom Med, 39・1999, Hokkaido Journal of public Health, 12・1999, Journal of Japan Academy of Midwifery, 13・2001, in Japanese).

We are now starting a study on Domestic violence, which is a current social problem.

3. Well children and family

We are studying mainly the relationship between parents and children, children at play and their development. Additionally, practical guidance on childcare and group play therapy and family members' practice of child minding were studied (Journal of Child Reserch, 76・1997, in Japanese). We also studied experiences of mothers of children with Down's syndrome (The Japanese Journal of Child Nursing, 22, 1999, in Japanese).

4. Nursing management

Allocation of human resources is an important subject in nursing administration. Staffing requires a balance between the quantity of staff available and the numbers needed to provide effective quality nursing care while keeping cost down. Patient Classification Systems were developed approximately 25 years ago in the USA, were put in place to help determining nursing staff numbers and levels. A research study of patient classification system was conducted at 250-bed hospital in Tokyo. The purpose of this research was to evaluate: (1) to match patient care needs with available resources, (2) to measure efficiency of care delivery, (3) to assess quality and quantity of care delivery (4) to ensure cost-effectiveness (Igaku-Shoin, 2000, Japanese Journal of Nursing Administration, 10・ 2000, in Japanese).

List of Main Publications from 1997 to 2000

1) Minagawa M: Sibling Relationship of Japanese Children With Diabetes. Journal of Pediatric Nursing 12: 311-316 (1997).

Community Health, Gerontological and Psychiatric Nursing

This Division consists of three Nursing specialities; Community Health Nursing, Gerontological, and Psychiatric Nursing. Our major goal is to develop nursing assessment skills, intervention programs and evaluation methods in response to the changing roles of nurses and changing health care needs of our society. Both Quantitative and Qualitative nursing research methods are used. The research data contributes to the development of Nursing theory and the refinement of educational methods.

Professor
Rita Weingourt, R.N., C.S., Ph.D.
Interests:
Domestic violence,
Nurse patient relationship

Ariko Noji, R.N., P.H.N., Ph.D.
Interests:
Health promotion & health education, Community health nursing administration

Associate Professor
Kazuko Saeki, R.N., P.H.N., M.S.
Interests:
Community health nursing Community nursing assessment

Keiko Fukazawa, R.N., M.S.
Interests:
Gerontological nursing

Assistant Professor
Kinko Kato, R.N., P.H.N.
Interests:
Maternal and child health in community, Mental health nursing in community

Noriko Hirano, R.N., P.H.N., M.S.W.
Interests:
Home health nursing,
Gerontological nursing in community

Junichi Yoshino, R.N., M.S.W.
Interests:
Psychiatric & mental health nursing,
Social welfare programs for mental disorders

Instructor
Izumi Sawada, R.N., P.H.N., M.N.
Hisako Izumi, R.N., P.H.N., M.S.N.
Masumi Hasegawa, R.N., M.N.

1. Community health nursing

Our goal is to develop high quality methods of practice and education in community health nursing. We are exploring the effect of community health nursing interventions on our clients (2). Also we are evaluating the level and quality of critical thinking in nursing education (3), evaluating programs in which faculty cooperate with public heath nurses using the telnet communication system, and developing a community nursing assessment tool that is useful in an educational setting (4).

We are interested in community health promotion and empowerment to enhance health and quality of life. Our focus is on women's health (5.6), elderly health (7), (Saeki K, et al.: Elderly thoughts for home care: Qualitative data analysis after empowerment education program for home care. Jpn J Gerontology 22: 375-384, 2000, in Japanese), and mental health (Kato K: Team Approach of Psychiatric Rehabilitation Activities based on Community health-In case of Public Hearth Nurses. Jpn J Psychiatric Rehabilitation 3:102-105,1999, in Japanese). It is Important to identify and understand the health needs of the elderly and their families so that nurses can design appropriate supportive interventions (Noji A, et al.: A Study on the Development of a Counseling Tool for Visiting Nurses, Journal of Japan Academy of Gerontological Nursing, 5 (1), 40-50,2000 in Japanese). We have studied elderly home care issues from various perspectives (Izumi H, et al.: The importance of interest in health education: Development of a caring attitude of the elderly for home care. Hokkaido J Public Health 11:171-177,1997, in Japanese). Including, long-term care system (8), male caregivers, spouse as caregiver, old people with dementia (Hirano N, et al.: Wives' perception of husbands suffering from Alzheimer-type dementia. Bulletin of school of health sciences Sapporo medical university 3: 37-43, 2000, in Japanese), (Kato K, et al.: Family Care Burden and its Correlates for Frail Elderly at Home. Hokkaido j public Health 12: 176-184,1998,in Japanese).

2. Gerontological nursing

The Gerontological nursing area has been doing research on communications with the aged, terminal care support systems, and care of elderly the patient with delirium.

a) The purpose of this study was to identify patient needs for the establishment of a terminal care system for the elderly who were going to die at home or in a community setting. Families whose elder family member lived in the towns in Hokkaido were interviewed. All of the elder people who had passed away were able to die at home where they preferred to be, in cooperation with the family and the hospital. However, the results suggest that, in the future, reconstruction of the relationship between the health care system and the welfare system is needed because the family's power to provide care will decrease (Fukazawa.K :The terminal stage of elderly living at home －T town and B town－, Hokkai-Gakuen University Deportment of Economics research annual report, 1-15, 2001, in Japanese).

b) Risk factors for the development of delirium in elderly patients were identified as: bed rest, number of tubes, activities of daily living score, level of anxiety, stubborn or expressive patient personality. Observation and interview identified the progress of delirium with delirious patients. The results suggest it is important for nursing assessment to identify patients who may have a maladaptive response to acute care hospitalization (Hasegawa M: An Analysis of the Risk Factors in the Development of Delirium among Elderly Parsons in an Acute Medical Setting, Journal of Japan Academy of Gerontological Nursing, 4(1), 36-46,1999 in Japanese).

3. Psychiatric and mental health nursing

a) Families and mental health

We are exploring mental health nursing for families with problems. We are particularly interested in studying domestic violence, child abuse, and suicide because they are increasing and becoming more serious in Japan. We identified an association between domestic violence and women's mental health (10.11.13).

b) Therapeutic communication

Therapeutic communication is a basic and complicated skill for any kind of nursing. However, in the field of psychiatric and mental health nursing, therapeutic communication is absolutely essential to good nursing care. We studied the effects and methods of psychotherapeutic communication skills on individuals and groups from the viewpoint of nursing (9).

c) Nursing theory and mental health

Psychiatric nursing care is based on the five steps of the nursing process. Nurses need an understanding of theories so that they can formulate meaningful nursing diagnosis from their assessment data. The future of nursing education in Japan will include study and application of theoretical frameworks for nursing practice (8). We demonstrated the value of using nursing theory in psychiatric nursing practice (12).

List of Main Publications from 1997 to 2000

1) Clark MJ, Curran C & Noji A: The Effects of community health nurse monitoring on hypertension identification and control. Public Health Nursing 17(6): 452-459 (2000).
2) Clark MJ, Noji A, Curran C. Evidence-based Community Health Nursing Practice. The Sapporo Medical Journal 69 (Suppl.) 14-21 (2000).
3) Noji A, Oda M, Yamase H, Kushiro W, Yuki M: Evaluation of Critical Thinking in Nursing Education. The 3rd International Nursing Research Conference. Tokyo. Japan, 115 (1998).
4) Saeki K, Izumi H, Ohyanagi T, Sugiyama A, Sawada I, Suzuki K, Hatazawa M, Ohuch M: Distance education for health centre staff in rural Japan. J Telemedicine and Telecare 6(Suppl 2): 67-69 (2000).
5) Noji A: Quality of life for changing health: perimenopause among Japanese in Japan and the USA. In: Aso T, Yanaihara T, Fujimoto S, (eds) The Menopause at the MILLENNIUM. The Proceedings of the 9th International Menopause Society World Congress on the Menopause. Parthenon Publishing. 127-132 (2000).
6) Noji A: Quality of life for changing health in perimenopause Japanese women, The 11th International Congress on Women's Health Issues. San Francisco. USA. Abstracts, 192 (2000).
7) Saeki K, Izumi H, Sawada I: Evaluation of the empowerment program for elderly home care in community. The 3rd International Nursing Research Conference. Tokyo. Japan, Abstracts, 268 (1998).
8) Noji A: Care management in public long-term care insurance -Japan's system-. Aging & Independence Services Long Term Care Integration Project Planning Committee. San Diego. USA, 1-7 (2000).
9) Weingourt R: Human to human contact: the future of nursing education Japan. Propositions for nursing in Next Decades of the New Millennium. Shizuoka University, 4: 78-80 (2000).
10) Weingourt R, Maruyama T, Yoshino J, Sawada I: Domestic violence and women's mental health in Japan. Annual Research Report. Meij Insurance Company. 3 (2000).
11) Weingourt R: Wife rape in a sample of psychiatric patients. Journal of Psychiatric Consultation and Liaison Nursing 3(3): 269-272 (1999).
12) Weingourt R: The application of Margaret Newman's Theory of Health to psychiatric nursing interventions with elderly nursing home residents. Perspectives Psychiatric Care 34(3): 25-30 (1998).
13) Weingourt R: A comparison of heterosexual and homosexual long-term sexual relationships. Archives Psychiatric Nursing 12(2): 114-118 (1998).

Physical Therapy

Our department has been challenged to establish therapeutic science and evidence-based physical therapy. Analyses from physiological, kinesiological, cardiopulmonary and immunologic points of view have been achieved with regard to specific movements, movements of daily activities and movements during sports performances. This research has been carried out in laboratories, in clinical facilities and at athletic sites.

-Physical-Therapeutic Sciences-

Professor

Seiji Noriyasu, Ph.D.
Interests:
Morphological study on Hokkaido men of old, Multivariate allometry on schoolchildren growth

Hidekatsu Takeda, Ph.D.
Interests:
Immnologic study on stress
Sports, Temporomandibular / oral system

Associate Professor

Nobuya Hashimoto, R.P.T., M.A.
Interests:
Community-based rehabilitation,
Welfare services system

Toshiaki Tanaka, R.P.T., Ph.D.
Interests:
Rehabilitation engineering of aid devices, Virtual reality systems, Balance control for the elderly and stroke patients

Mitsuhiro Aoki, M.D.,Ph.D.
Interests:
Surgical tendon reconstruction,
Mechanical and histiological studies on tendon restoration

Assistant Professor

Kiyomi Takayanagi, R.P.T., M.A.
Interests:
Orthotic treatment for ACL, Histiological studies on ACL repair, Neuro-muscular and cardio-adaptation with exercise

Instructor

Satoru Kojima, R.P.T.,M.A.

-Applied Physical Therapy-

Professor and Chair

Shigenori Miyamoto, R.P.T. (J. & C.), Ph.D.
Interests:
Musculoskeletal physical therapy,
Manual therapy, Proprioception

Kimiharu Inui, R.P.T., Ph.D.
Interests:
Orthotics and prosthetics,
Proprioceptive neuromuscular facilitation,
Muscle physiology

David J. Magee, R.P.T. (Canada), Ph.D.
Interests:
Musculoskeletal physical therapy, Sports physical therapy, Manual therapy

Associate Professor

Masaharu Yoshio, R.P.T.
Interests:
Neurological physical therapy,
Functional anatomy, QOL

Naoki Kozuka, R.P.T., Ph.D.
Interests:
Pediatric physical therapy, Kinesiological analysis of C.P. children, Molecular studies of neuromuscular disorders

Assistant Professor

Akira Ishikawa, R.P.T., Ph.D.
Interests:
Chest physical therapy, Home oxygen therapy, ADL

Instructor

Masaki Katayose, R.P.T., M.Sc.

1. Basic studies

a) Analyses of morphological characteristics of the skeletal construction of the shoulder joint by using tri-dimensional measurement, archaeological differences in extremity bones, and multivariate allometry on the growth of school childen in Hokkaido (Bull Rural Education Institute. 53・1999, in Japanese).

b) Fundamental analysis of effect of plasma β-endorphin and NK cell activity by administrating Acanthopanax Senticosus Hams (ASH) for stressed rats (1).

c) Basic studies on community-based rehabilitation projects, technical aids and housing for physically handicapped persons and the coordinate system of welfare services, health and medical care for the elderly (2).

d) Rehabilitation engineering study on the new aid devices and virtual reality systems for disabled people (3).

e) Mechanical and histiological evaluation using animal

models. Restoration of collagen fibers at the tendon insertion to bone (4).

f) Basic research on conservative treatment for the anterior cruciate ligament and also neuro-muscular and cardio-adaptation in exercise (5).

2. Clinical Studies

a) Neurophysiological and kinesiological studies in musculoskeletal physical therapy and manual therapy (6.7).

b) Studies on prosthetics and orthotics, therapeutic exercise and muscle physiology (8).

c) Systematic reference review and functional outcome measures in sports physical therapy (9).

d) Studies on kinematics and physiological study on functional assessment for stroke patients and morphological study on the functions of shoulder and hip muscles (10).

e) Studies on kinematics and physiological analyses on childhood cerebral palsy and also gene analysis of hereditary motor and sensory neuropathy including Charcot-Marie-Tooth Disease type 1A, 1B and Dejerine-Sottas Syndrome (11).

f) Studies on chest physical therapy for acute and chronic stages of respiratory failure at institutions and at home (12).

List of Main Publications from 1997 to 2000

1) Hibasami T, Fujikawa T, Takeda H, Nishibe S, Satou T, Fujisawa T, Nakasima K. Induction of apotosis by Acanthopanax senticosus HARMS and its component, sesamin in human stomach cancer KATO III cells. Oncol Rep 7: 1213-1216 (2000).
2) Kojima S, Tanaka T, Hashimoto N, Takeda H: The influence of initial foot position and trunk flexion on sit-to-stand movement. 13 th intematinal congress WCPT. Proceedings, 421 (1999).
3) Tanaka T, Izumi T, Ino S, Ifukube T. Effects on the location of the center of gravity and foot pressure contribution to standing balance associated with aging. Ergonomics 42(7): 997-1010 (1999).
4) Aoki M, Takayanagi K, Miyamoto S, Ishii S, Uchiyama E, Saino H. Early active motion and weight bearing after cross-stitch achilles tendon repair. Am J Sports Med 26: 794-800 (1998).
5) Maejima H, Yoshimura O, Takayanagi, K et al. Modification of postural response caused by footwear conditions. J Japan Phys Ther Assoc 3(1): 21-25 (2000).
6) Miyamoto S, Aoki M: Accurcy of finger and nose pointing after passive rotation of the head. 13 th International congress WCPT. Proceedings, 270 (1999).
7) Araki H, Sugaya R, Miyamoto S: The effect of systematic approach for patients with subacute low back pain. 13th International congress. WCPT. Proceedings, 186 (1999).
8) Inui K, Narita H, Yokogushi K, Ishikawa A, Nosaka T et al: A/K prostathesis gait analysis on the simulated frozen surfice. 13th International congress. WCPT. Proceedings, 544 (1999).
9) Magee DJ, Oborn-Barrett E, Turner S, Fenning N. A systematic overview of the effectiveness of physical therapy intervention on soft tissue neck Injury following trauma. Physiother Can 52: 111-130 (2000).
10) Yoshio M, Nishimura Y, Sato S, Nara Y, Matsuda J, Noriyasu S: Approach to the subscapularis, teres major and triceps brachii as factors that limit the range of motion of the shoulder joint. 13 th International Congress. WCPT. Proceedings, 439 (1999).
11) Tachi N, Kozuka N, Ohya K, Chiba S, Yamashita S. A small direct tandem duplication of the myelin protein zero gene in a patient with Dejerine-Sottas disease phenotype. J Neurol Sci 156: 167-171 (1998).
12) Ishikawa A, Miyagawa T: The ability of stepping up and down stairs of people receiving home oxygentherapy. 13th International congress. WCPT. Proceedings, 320 (1999).

Occupational Therapy

Research activity in our department consists of basic studies and clinical studies for occupational therapy. In basic studies, we have been attempting to understand visual cognition and the function of parietal cognition in the human brain, and have analyzed the function of wrist and hand activities in daily living. In clinical studies, we have been investigating occupational therapy in health care facilities for the elderly, and occupational therapy for children with developmental disorders.

-Occupational-Therapeutic Sciences-
Professor and Chair
Mitsuo Ishizawa, M.D., Ph.D.
Interests: Gastrointestinal physiology and pharmacology, Physiological function of elderly.

Noboru Matsushita, Ph.D., O.T.R.
Interests: Functional electrical stimulation of paralyzed wrist and hand

Associate Professors
Nobutada Tachi, M.D., Ph.D.
Interests: Gene analysis of hereditary neuro-muscular disorder

Satoshi Senda, M.A., O.T.R.
Interests: Occupational therapy of community rehabilitation

Assistant Professor
Mariko Nakamura, M.S., O.T.R.
Interests: Anatomy and function of fingers and hand in man

Instructor
Mari Sakaue, M.A., O.T.R.
Minako Goto,O.T.R.

-Applied Occupational Therapy-
Professor
Dean, School of Health Sciences
Tsuyoshi Sato, Ph.D., O.T.R.
Interests: Research for occupational science, Occupational therapy of sensory integrative dysfunction among learning disabled children.

Professor
Sinji Murakami, M.D., Ph.D.
Interests: Visual-spatial cognitive dysfunction in Alzheimer's disease.

Professor
Ruth Zemke, Ph.D., O.T.R.
Interests: Occupational science

Associate Professor
Hiroshi Aoyama, Ph.D., O.T.R.
Interests: Psycho-behavioral characteristics of patients with chronic pain

Assistant Professor
Yasuhito Sengoku, M.A., O.T.R.
Interests: Occupational therapy for children with developmental disorders

Nozomu Ikeda, M.A., O.T.R.
Interests: Psycho-social occupational therapy

Instructor
Sonomi Nakajima, M.A., O.T.R.

1.Basic studies

a) Visual-spatial cognitive dysfunction in Alzheimer's disease

The prevalence of Alzheimer's disease (AD) has increased in recent years and diagnosis in the early stage is both important and difficult. We have reported that visual-spatial cognitive function was disturbed even in the early stage, and we have investigated this visual-spatial cognitive dysfunction to record the eye movements and gazing in patients with AD (1).

b) Visual cognition of three dimension spaces and function of parietal lobe in human brain

Visual proceeding streams originating in the occipital lobe have two main paths, one of the temporal lobe and other to the parietal lobe of the two streams, the latter is related to visual-spatial processing. The neural activities of the parietal association cortex were studied in humans through the use of magneto encephalo gram (MEG). The magnetic field responses were recorded in the parietal association cortex following visual stimulation in space. The estimated current dipole sources of the magnetic field responses were found in the intraparietal sulcus. A change in the magnetic field responses as the eye position was found in the amplitude (2.3).

c) Analysis of wrist and hand functions for application of occupational therapy

Research interests focused on the analysis of the wrist and hand movements and functions in normal adults and physically handicapped. A three dimensional motion analyzer and electoromyogram have been used to clarify the movements of wrist and each finger joint, In order to apply the results to clinical occupational therapy for paralyzed hands by spinal cord injury and stroke, the muscle activities of wrist and hand in grasping a cup were examined. Moreover, the relationship of the three finger joints between simple finger movements and hand tasks in daily activities was analyzed. From the results, it is suggested that deliberate activities of the finger and sophisticated joint

movements provided delicate adjustments to fit the fingers to the size of the object (4.5).

d)Study on clinico-pathological significance of the immuno-staining of myosin heavy chain isoforms in pathological muscle with type I spinal muscular atrophy and cerebral palsy.

2. Clinical Studies

a) Occupational science

Occupational science is the new evolving science in occupational therapy, with a focus on the form, function and meaning of occupation (daily human activities), such as study on the use of time by outpatient schizophrenic patients or well-elderly people in the community (8-10). The research has mainly been conducted in collaboration between faculty of the school of Health Science and faculty members of Occupational Science and Occupational Therapy at the University of Southern California.

b) Effect of rehabilitation in the care facilities for the elderly are expected with new care insurance

The current status of occupational therapy in the health care facilities was surveyed to clarify the roles of occupational therapists, and differences in the priorities of the therapists posted in the facilities with regard to rehabilitation services using the questionnaires and case-study method. The results showed that facilities, which had occupational therapists and physical therapists, provided more assistant device services. And the effect of postural behavior on the activities of elderly person needing care was investigated to establish more effective therapeutic interventions in two different environments, at home and in the health care facilities. The subjects, who were using short stay services, were compared for their frequency of posture changes at home with the facilities. This study indicated that the environment in the facilities provided more possibilities of posture changes than at home for the elderly person using wheel chairs.

c) Occupational therapy for children wlth developmental disorders

We have been analyzing the effectiveness of occupational therapy in terms of the process of interaction between therapists and children with mild developmental disorder and children's behavior changes through family-centered group occupational therapy. And we have also been analyzing sensory integration dysfunction and occupational therapy for learning disabilities, and mentally retarded and autistic children (6).

d) Group therapy for persons with mental disorders

Group therapy is effective intervention in the psycho-social occupational therapy for persons with disabilities. The effectiveness of group therapy for patients with schizophrenia, chronic pain and dementia was investigated based on behavioral sciences and neuroscience. The results suggested that group therapy facilitated empowerment in schizophrenia, decreased pain behavior and increased life satisfaction for patients with chronic pain, and that non-verbal reminiscence activities in group therapy could facilitate social interaction in dementia by stimulation of procedural memory (7).

List of Main Publications from 1997 to 2000

1) Murakami S. et al: The eye movement in Alzheimer's disease. The proceedings, the international symposium on medical information and fuzzy technology. MIF'99. Abstracts, 222-223 (1999).
2) Murakami S. et al: Co-ordinate binocular eye movement during binocular and monocular viewing.10th ECEM. Abstracts, 127-128 (1999).
3) Murakami S. et al: Eye positional effect to visual response in parietal cortex recording by MEG. Society for Neuroscience 27th Ann. Meeting. Abstracts, 1305 (1997).
4) Nakamura M, Matsushita N, Miyawaki C, Yagi R, Handa Y. Analysis of voluntary finger movements during hand tasks by a motion analyzer. J Electromyogr Kinesiol 8: 295-303 (1998).
5) Sawada Y, Nakamura M, Sekito Y, Aoki M, Lewis M. Contribution of peripheral input to length discrimination during use of a precision grip. J Hand Ther 12: 291-297 (1999).
6) Sengoku Y, Nakamura M, Senda S, Ishizawa M, Sato T, Matsushita N: Effectiveness of a family-centered occupational therapy for social skill deficient learning disabled children.12th International congress of the World of Occupational therapists Abstracts, 201 (1998).
7) Ikeda N, Sakaue M, Osaka M, Sato T: The value of non-verbal activities in the reminiscence and life review group work for the elders with dementia. The Second Asia-Pacific Occupational Therapy Congress. Abstracts, 71 (1999).
8) Clark F, Azen S, Hay J, Carlson M, Mandel D, LaBree L, Hay J, Zemke R, Jackson J, Lipson L. Embedding health-promoting changes into the daily lives of independent-living oldeer adults: Long-term follow-up of occupational therapy intervention. J Gerontol B Psychol Sci Soc Sci 56(1): 60-63 (2001).
9) Jackson J, Kennedy BL, Mandel D, Carlson M, Cherry B, Fanchiang S-P, Ding L, Zemke R, Azen SP, LaBree L, Clark F. Derivation and pilot assessment of a health promotion program for Mandarin-speaking Chinese older adults. Int J Aging Hum Dev 50(2): 127-149 (2000).
10) Jackson J, Carlson M, Mandel D, Zemke R, Clark F. Occupation in life-style redesign: The USC well elderly study occupational therapy program. Am J Occup Ther 52(5): 326-336 (1998).
11) Zemke R. Cultural issues in occupational science and occupational therapy. Japanese Journal of Occupational Therapy 31: 962-967 (1997).

Liberal Arts and Sciences

The members of the Dept. of Liberal Arts and Sciences have been working in various fields to contribute to the development of the Health Sciences. These fields include biochemistry, bioinformatics, biophysics, and telehealth in life science. In addition, utterance Interpretation, area studies on the U.K and U.S.A., and emotional and cognitive development studies are also inlcuded. The main focus for all the members is elucidation of the methodlogy of teaching within their respective fields.

Physics

Professor and Chair
Norio Matsushima, Ph. D.
Interests:
Biophysics, Bioinfomatics, Biomechanics

1. Tandem repeats within proteins

We are addressing the general phenomenon of tandem repeats in protein structure. Leucine-rich repeats (LRRs) are present in over two hundreds different proteins in organisms that range from bacteria to man. We found that a family of nine small proteoglycans has super-repeats of LRRs (1.2). Mammalian prion proteins contain five PHGGGWGQ repeats. NMR studies of the synthetic peptides showed that His (i) is in close proximity to Trp (i+4) (3). US11 protein that binds to RNA and DNA contains 24 repeats of PRX. We developed a model for PRA complexed with DNA (Ngasaki Igakukkai Zasshi, Vol. 75, pp. 301-308, 2000, in Japanese).

2. Three-dimensional structure of proteins

P26olf from olfactory tissue of frog is a Ca^{2+}-binding protein with 217 amino acids. We constructed a three-dimensional model of the p26olf molecule based on the structures of dimeric S100B($\beta\beta$) (5). Small-angle X-ray scattering (SAXS) was used to investigate the conformational changes of calmodulin (CaM) upon binding to TFP, W-7, or a peptide from CAP-23/NAP-22 (6-8). Amelogenins that play a regulatory role in the process of enamel crystal formation, are rich in Pro, Gln, Leu, and His. We developed an elongated bundle structure by a combination of SAXS and computer-aided molecular modeling (4).

3. Biomechanics and Physics education

Time-resolved, SAXD measurements were performed on bovine Achilles tendon collagen under creep in order to understand the molecular mechanism of relaxation phenomena in collagenous tissue (9). Bone properties were discussed from a point of view of material science and biomechanics (Bull. Sch. Health Sci. Sapporo Med. Univ. Vol.3, pp. 1-9, 2000, in Japanese). In order to provide materials for physics education in the future, I described some experiences in this field (J. Appl. Phys. Education Vol. 24, pp. 71-76, 2000, in Japanese).

List of Main Publications from 1997 to 2001

1) Matsushima N, Ohyanagi T, Tanaka T, Kretsinger RH. Super-motifs and evolution of super-motifs of tandem leuine-rich repeats within small proteoglycans - biglycan, decorin, lumican, fibromodulin, PRELP, keratocan, epiphycan, and osteoglycin. Proteins 38: 210-225 (2000).
2) Matsushima N, Kamiya M, Suzuki N, Tanaka T. Super-motifs of leucine-rich repeats (LRRs) proteins. Genome informatics 11: 343-345 (2000).
3) Yoshida H, Matsushima N, Kumaki Y, Nakata M, Hikichi K. NMR studies of model peptides of PHGGGWGQ repeats within the N-terminus of prion proteins: a loop conformation with histidine and Tryptophan in close proximity. J Biochem (Tokyo) 128: 271-281 (2000).
4) Matsushima N, Izumi Y, Aoba T. Small-angle X-ray scattering and computer-aided molecular modeling studies of 20kDa fragment of porcine amelogenin: does amelogenin adopt an elongated bundle structure? J Biochem (Tokyo) 123: 150-156 (1998).
5) Tanaka T, Miwa N, Kawamura S, Sohma H, Nitta K, Matsushima N. Molecular modeling of single polypeptide chain of calcium-binding protein p26olf from dimeric S100($\beta\beta$). Protein Eng 12: 395-405 (1999).
6) Osawa M, Kuwamoto S, Izumi Y, Yap K. L, Ikura M, Shibanuma H, Yokokura H, Hidaka H, Matsushima N. Evidenvce for calmodulin inter-domain campaction in solution induced by W-7 binding. FEBS Lett 442: 173-177 (1999).
7) Matsushima N, Hayashi N, Taniguchi H, Jinbo Y, Izumi Y. Calcium-bound calmodulin forms compact globular structure upon the binding of four trifluoroperazine molecules in solution. Biochem J 47: 211-215 (2000).
8) Hayashi N, Izumi Y, Titani K, Matsushima N. The binding of myristoylated N-terminal nonapeptide from neuron-specific protein CAP-23/NAP-22 to calmodulin induces a ‘relaxed’

globular structure different from calmodulin-non-myristoylated peptide complex. Protein Sci 9: 1905-1913 (2000).
9) Sasaki N, Shukunami N, Matsushima N, Izumi Y. Time-resolved X-ray diffraction from tendon collagen during creep using synchrotron radiation. J Biomechanics 32: 285-292 (1999).

Chemistry

Professor

Hirotada Fujii, Ph. D.

Interests:

In vivo detection of free radicals, Biological function of free radicals

1. In vivo detection and imaging of bioradicals

The challenge of developing both spin-imaging and in vivo electron spin resonance (ESR) in living systems requires a large number of changes in classical x-band ESR spectroscopy. These changes were necessary in x-band ESR system for two reasons; (1) the large water content of living tissue, and (2) the large volume of the sample itself. In order to obtain free radical information from the biological system including small animals and cultured cell systems, we have been developing in vivo ESR spectroscopy at L-band microwave frequency (300-1200 MHz). ESR imaging instrumentation, enabling the performance of three-dimensional spectral-spatial images of free radicals, has been developed to study spatially defined differences in tissue metabolism and oxygenation. Using L-band in vivo ESR spectroscopy, we succeeded in detecting three bioradicals generated in mice or rats; bioradicals generated both from the metabolism of nitroso-compounds and a prescription drug for patients with hypertension, and nitric oxide (NO) generated in septic-shock animals.

2. MRI as a new tool to visualize free radicals

We developed a new approach to use the NMR/MRI method combined with the spin-trapping method to visualize bioradicals generated in small animals. Free radicals captured by a spin-trapping agent, if their stability is long enough, can be used as contrast agents in MRI, and spatial localization of free radicals might then be visualized by MRI. We did a feasibility study showing that a new methodology called “MRI spin-trapping method” can visualize NO distribution in septic-shock rats. Our ultimate goal is to develop new approaches that couple the strengths of spin trapping with methodologies that promise to overcome some of the problems, in particular that of radical adduct decomposition. Besides the MRI spin trapping method, new complementary techniques include: 1) NMR spin trapping, which monitors new NMR lines resulting from diamagnetic products of radical spin adduct degradation and reduction, and 2) oxygen mapping by ESR imaging/MRI methodology using oxygen-sensitive paramagnetic materials. Although some of these approaches are in their infancy, they are promising and versatile techniques to measure and possibly image oxidative stress in living systems.

List of Main Publications from 1997 to 2000

1) Fujii H, Belriner LJ, Yoshikawa K. In vivo Imaging of spin-trapped nitric oxide in rats with septic shock: MRI Spin Trapping. Magn Reson Med 42: 235-239 (1999).
2) Fujii H, Koscielniak J, Berliner LJ. Determination and characterization of nitric oxide generation in mice by in vivo L-band EPR spectroscopy. Magn Reson Med 38: 565-568 (1997).
3) Fujii H, Ichimori, K, Nakazawa H. Nitric oxide inactivates NADPH oxidase in neutrophils by inhibiting its assembling process. J Biol Chem 272: 32773-32778 (1997).

English

Professor

Makoto Nemoto, M.A.

Interests:

Utterance interpretation, Area studies on the U.K. and U.S.A., Methods in teaching English as a foreign language

1. Utterance interpretation

We have explored some theoretical issues in discourse analysis. Special emphasis has been placed on the processes of utterance interpretations whose theoretical basis is the Theory of Relevance, originally proposed by Sperber and Wilson in1986. The Theory of Relevance is an attempt to describe the cognitive process of utterance interpretation in terms of a listener. The theory is still being developed and refined through reviews and the research of various students of linguistics. The processes of interpretation and the factors involved in their processes still remain unspecified. However, the theory is expected to contribute to a description of the epistemological stages of utterance interpretations.

2. Area studies

In view of the educational aspects of teaching English, the social and cultural knowledge about the countries where English is spoken provides students of English with informative and instructive background information. Currently, I am interested in an area of study dealing with the U. K. in general and Scotland in particular (“Scotland under Devolution and Its Speech Community” Bulletin of School of Health Sciences Sapporo Med. Univ., Vol. 2, pp. 51-55, 1999, in Japanese; “Scots as a Symbol of the Scottish Identity” Bulletin of School of Health Sciences,

Sapporo Med. Univ., Vol. 3, pp. 19-25, 2000, in Japanese). As well as revealing useful information about the U.K. and Scotland, these studies also provide interesting facts about the U.S.A. and a valuable insight into its history.

The different regional communities in Scotland present very complicated socio-linguistic phenomena including particular historical heritages such as Scots, Gaelic, and standard British English. The Optimality Theory proposed by Prince and Smolensky in 1993 may be a useful tool to describe the linguistic variations in Scottish speech communities where language contact has existed for centuries. Scottish Gaelic is a Celtic language, which has been undergoing a revival in these communities. The language could provide a good model for a more general comparison of different language structures and their functions. A typological comparison can be made, for example, among the languages of Japanese, English, and Gaelic, each of which represents a major language structure.

3. Methods in teaching English as a foreign language

A part of our research activities involves the methodology of teaching English as a foreign language. This is an indispensable part of our studies as it is closely geared to our educational objective of expanding the nature of our instruction and making it more effective.

One of our current interests lies in facilitating students' abilities to express themselves through learning the functions of basic English vocabulary ("On the Usage of <Basic Verb Plus Noun Constructions> – for the Improvement of Students' Communicative Skills in English" Bulletin of School of Health Sciences, Sapporo Med. Univ., Vol. 1, pp. 51-57, 1997, in Japanese). It is hoped that by studying the semantic structure of English and how its basic verbs function, our students will be able to establish a firm foundation for their conversational skills.

List of Main Publications from 1997 to 2000

1) Nemoto M. Steps to be Taken within the Principle of Relevance. J. Lib. Arts & Sci. Sapporo Med. Coll. 38: 65-72 (1997).

Biology

Associate Professor

Keiko Yamada, M.A., Ph.D.

Interests:

Biological function of DGK, Nutrition of vitamin B_{12}, Dietary habits in young people

1. Physiological function of diacylglycerol kinase (DGK) isozymes and study of calcium-induced conformational change of EF hand motifs within DGK

We measured the expression of DGKα and DGKγ mRNA by RT-PCR and protein levels by Western blot analysis in the process of macrophagic differentiation by TPA and granulocytic differentiation induced by DMSO to examine whether DGK isozymes are involved in nuclear events (Sapporo Med. Univ. Foundation for Medical Science Report, pp. 94-102, 1999, in Japanese). We found that DGKα mRNA increased less than twofold and that DGKγ significantly decreased at 24h in TPA-treated cells. We showed that type I DGK isoforms (α, β and γ) possess EF-hand structures with intrinsic properties different from each other with respect to affinities for Ca^{2+} and Ca^{2+}-induced conformational changes (1). Moreover, using CD analysis and tryptophan fluorescence we showed Ca^{2+} induced conformational changes in EF hand motifs (Seikagaku Vol. 72, Abstract p740, 2000, in Japanese). I published reviews entitled "Diversity of DGK isozymes and their biological roles" (Vitamins Vol. 71, pp. 343-355, 1997, in Japanese), "Diacylglycerol kinase" (Neuro-Science Lectures Vol. 6, pp. 74-81, Hirokawa Publishing Company, Tokyo, Japan, 1997, in Japanese) and "Physiological function of diacylglycerol kinase (DGK) isozymes –Role of EF-hand motifs within diacylglycerol kinase", Bulletin of School of Health Sciences, Sapporo Med. Univ., in press, in Japanese). Studies on the mechanism of DGK activity and expression of DGKδ are in progress.

2. Cobalamin (vitamin B_{12}) contents in foods and bioavailability of seaweeds as a source of cobalamin

We reported the bioavailability of dried nori as a source of cobalamin (2, 3 and Bulletin of School of Health Sciences, Sapporo Med. Univ., Vol. 3, pp. 11-17, 2000, in Japanese). To develop a cheap, rapid, simple method for determination of cobalamin, glycerol dehydrase, which requires 5'-deoxyadenosy cobalamin as a coenzyme, was used (4). We determined cobalamin contents in feces of rats and mice and found them to contain more cobalamins than previously reported. Cobalamin accumulation in asakusanori by suspending powdered rat feces in seawater may be a pormising way to put the cobalamin in animal feces to good use (5). Moreover, we determined vitamin B_{12} contents in various beverages and solid dietry supplements and found that the vitamin B_{12} contents of some beverages were less than stated on the label and decreased in a time-dependent manner (6).

3. Studies on body image and dietary habits in adolescent males and females

We examined whether body image and strategies to reduce weight in adolescent males and females desiring weight loss would vary according to the reason for desired weight loss (Hokkaido Food Science Technology Promotion Foundation. Report. Vol. 6, pp. 32-41, 2001, in Japanese). Futher study about the relationship between physique and dietary habits is in

progress.

List of Main Publications from 1997 to 2000

1) Yamada K, Sakane F, Matsushima N, Kanoh H. EF-hand motifs of α-, β- and γ-isoforms of diacylglycerol kinase bind calcium with different affinities and conformational changes. Biochem J 321: 59-64 (1997).
2) Yamada K, Yamada Y: Bioavailability of dried seaweeeds as a source of vitamin B_{12}. 16th Intenational Congress of Nutrition (ICN), Abstracts, 127 (1997).
3) Yamada K, Yamada Y, Fukuda M, Yamada S. Bioavailability of dried asakusanori (*Porphyra tenera*) as a source of cobalamin (vitamin B_{12}). Int J Vitam Nutr Res 69: 412-418 (1999).
4) Yamada S, Bessho R, Yamada K: Determination of cobalamin using the enzyme glycerol dehydrase. 16th ICN Abstracts, 124 (1997).
5) Yamada S, Nishijima M, Umemoto K, Yamada K: Cobalamins in feces of rats and mice. 17th ICN Abstracts, (2001).
6) Yamada K, Miyazawa M, Chida S, Kawashima S, Yamada S: Vitamin B_{12} contents in various beverages and solid dietary supplements. 17th ICN Abstracts, (2001).
7) Miyashita Y, Moriya T, Yamada K, Kubota T, Shirakawa S, Fujii N, Asami K. The photoreceptor molecules in Xenopus tadpole tail fin, in which melanophores exist. Zool Sci 18 (5), (2001).

Information Science

Associate Professor

Toshio Ohyanagi, Dr. of Engineering

Interests:

Telehealth system, Multimedia information and Communication technologies

1. Telehealth System

Telehealth is a means of sharing health information and providing health care services and professional education using interactive video, audio, and other multimedia technologies. Telehealth covers not only medicine but also other health sciences such as nursing and rehabilitation sciences. The so-called "Telemedicine" might be involved in Telehealth. We have studied the uses of Telehealth in supporting health-care professionals living in a rural community, and then we investigated what kind of Telehealth system should be developed in Hokkaido (Bulletin of School of Health Sciences, Sapporo Med. Univ., Vol 2, pp57-61, 1999, in Japanese) (1-3). As a result, we realized that Telehealth has a great potential to provide high quality healthcare services equally to all rural communities in Hokkaido.

We have been developing new Telehealth software, termed *MediaCollaborator*, which allows multimedia data to be shared through the IP network. With MediaCollaborator two or more people in remote sites can work collaboratively. It was tested during the proof of concept projects; sports physical therapy and Teleultrasound. We tested MediaCollaborator over ATM, LAN, ISDN and POTS. As a result, it was confirmed that MediaCollaborator can be used over both a high bandwidth and lower bandwidth network (4.5). We also investigated the potential of the latest video telephone as the basic component of the Telehealth system of the next generation (6.7).

2. Bio-informatics

We did sequence analysis of tandem leucine-rich repeats (LRRs) within nine small proteoglycans of biglycan, decorin, lumican, fibromodulin, PRELP, keratocan, osteoadherin, epiphycan, and osteoglycin. As a result, it was revealed that these proteoglycans form three subfamilies and have two types of LRRs (8).

List of Main Publications from 1997 to 2000

1) Jin C, Ishikawa A, Sengoku Y, Ohyanagi T. A telehealth project for supporting an isolated physical therapist in a rural community of Hokkaido, J Telemedicine and Telecare 6 (Suppl. 2), 35-37 (2000).
2) Saeki K, Izumi H, Ohyanagi T, Sugiyama A, Sawada I, Suzuki K, Hatazawa M, Ohuchi M. Distance education for health centre staff in rural Japan, J Telemedicine and Telecare 6 (Suppl. 2), 67-69 (2000).
3) Sawada I, Sugiyama A, Ishikawa A, Ohyanagi T, Saeki K, Izumi H, Kawase S, Matsukura S. Upgrading rural Japanese nurses' respiratory rehabilitation skills through videoconferencing, J Telemedicine and Telecare 6 (Suppl. 2), 69-72 (2000).
4) Ohyanagi T, Mitani M, Sora Y, Katayose M, Miyagishi O, Miyazaki M: Telehealth software for sharing multimedia data through an IP network, 86th Scientific Assembly and Annual Meeting of Radiological Society of North America, Radiology 217 (Suppl.), 9508NT-I (2000).
5) Ohyanagi T, Miyazaki M, Sutphen S, Katayose M, Mitani M, Miyagishi O, Yamamoto T: Tele-sports medicine project between Japan and Canada, The 5th International Conference on the Medical Aspect of Telemedicine, 126 (2000).
6) Ohyanagi T, Miyazaki M, Mitani M: The potential of video-telephone for Telehealth, The 5th International Conference on the Medical Aspect of Telemedicine, 81 (2000).
7) Miyazaki M, Ohyanagi T, Sutphen S, Reinbold D, Lamoureax

W, Knoll C, Wally A, Pau K: Interconnectivity challenges associated with telehealth, The 5th International Conference on the Medical Aspect of Telemedicine, 124 (2000).
8) Matsushima N, Ohyanagi T, Tanaka T, Kretsinger RH. Super-motifs and evolution of super-motifs of tandem leucine-rich repeats within small proteoglycans – biglycan, decorin, lumican, fibromodulin, PRELP, keratocan, osteoadherin, epiphycan, and osteoglycin, Proteins: Structure, Function and Genetics, 38: 210-225 (2000).

Psychology

Associate Professor
Yoshinobu Takahashi, M. A.
Interests:
Emotional development, Cognitive development

1. Control of emotional expressions in 3-year-olds

Spontaneous control of negative emotional expression was examined in three-year-olds. The disappointing procedure was utilized. After a series of tasks, the examiner announced the child would get a prize. Children were shown 5 potential prizes. Each child rank-ordered the prizes by picking the best prize, the second best, and so on until all 5 were ranked. The fifth-ranked prize was given to the child. The responses of the child when given the disappointing prize were analyzed. The results indicated that half of the children controlled negative emotional expression and the girls did so more than the boys (“Control of emotional expression in 3-year-olds” Bulletin of School of Health Sciences. Sapporo Med. Univ. Vol. 1, pp. 33-37. 1997, in Japanese).

2. Three-year-olds' difficulty with false belief

Many investigators in cognitive development assume that 3-year-olds either lack the notion of belief entirely, or fail to realize that beliefs can misrepresent the world. I reexamined the main technique used to show a basic inability in 3-year-olds to make judgments about a person’s thoughts when that person’s knowledge happens to be false. Children were shown the real, unexpected contents of a candy box and required to answer what a friend (or their mother) would think was in it and what their own previous expectations had been. Children were divided into two groups. One group answered verbally like in previous studies, the other answered by selecting one from five choices.

Answers in this task were compared between the 2 groups. It was found, in contrast to previous findings, that most 3-year-olds who answered by selecting attributed false beliefs to others. This result did not support the previous explanation of 3-year-olds’ failure in the false belief task. This result suggested that 3-year-olds’ difficulty with false belief resulted from a procedural bias of previous studies (“Three-year-olds' difficulty with false belief” Bulletin of School of Health Sciences. Sapporo Med. Univ. Vol. 2, pp. 33-38, 1999, in Japanese).

List of Main Publications from 1997 to 2000

1) Takahashi Y, Kusanagi E, Hoshi N. Masking of negative emotional expression in 3-year-olds. Annual Report 1996-1997 Research and Clinical Center for Child Development. No. 20: 43-46 (1998).
2) Hoshi N, Kusangi E, Chen S, Takahashi Y. The relation between emotionality and empathy-related responses in Japanese young children. Annual Report 1996-1997 Research and Clinical Center for Child Development. No. 20: 31-42 (1998).
3) Kusanagi E, Hoshi N, Takahashi Y. The relations of children's narratives about hypothetical situations to the contemporaneous empathy-related responses and the future moral development. Annual Report 1996-1997 Research and Clinical Center for Child Development. No. 20: 63-74 (1998).

Information Center of Computer Communication

Our department has been conducting border research activities in computer science and bio-medical fields. We are attempting to widen the applications of information technology to medicine and healthcare to improve the total infrastructure related to digital education resources, high performance network environment, post genome applications, multi-parallel network computing, medical imaging, telemedicine, and 3D reconstruction, on the basis of our staff's excellent research.

Professor and Director (Affiliated)
Haruyuki Tatsumi, M.D., Ph.D.
Interests:
Computerized anatomy, Applied information technology,
Applied Internet technology

Instructor
Fumio Aoki, Ph.D.

1. VHP viewer for anatomy education

VHP (Visible Human Project) is a worldwide project began in 1986 by the NLM (National Library of Medicine, USA), which plans to build a digital image library of volumetric data representing a complete, normal adult male and female. The 1st Department of Anatomy, Sapporo Medical University School of Medicine and the Information Center have been involved in VHP for several years as a project of G7 Information Society (GHAP: Global Healthcare Application Project, and GIBN: Global Interoperability for Broadband Network)(1). Our main work concerns the efficient use of the image data for collaborative (3) educational work on anatomical issues (1.2) using NGI (Next Generation Internet), especially in morphological visualization and identification. For the real-time retrieval of dissections along arbitrary directions from the VHP image data, we propose a three-layer architecture. The implementation has excellent portability based on OpenGL rendering libraries, and generates dissection images not only orthogonal with coordinate axes but also in arbitrary directions. A parallel processing solution is used to create full-size dissection images from original VHP data sets. The application provides better visibility based on 3D rendering algorithm and better maneuverability using both keyboard and pointing device (4).

2. Multi-parallel network computing using NGI

To use the high resolution medical image sets provided by VHP for anatomy education, we need powerful computers to handle the large data sets of up to 15-40GB. For this purpose, we propose a distributed computing approach implemented on low--cost PCs connected with a high performance network, instead of expensive super computers. The implementation is based on a three-layer architecture consisting of an OpenGL image viewer available on many different OS platforms, distributed data processors, each of which handles a subset of VHP image data on memory and works independently and a central controller which receives requests from the viewer, controls the distributed processors, collects the results generated by the processors and forwards the high resolution image to the viewer. Experiments of distributed computing on 35 PCs proved that the proposed solution can response in about 2 seconds to a request to retrieve a 12MB image from about 15GB VHP image data, this is over 1000 times faster than a straightforward method on one PC (5.6). This system also proved to be useful between Japan and USA via NGI using Satellite link or APAN (Asia Pacific Advanced Network) (7.8).

Future objectives include: (a) real-time morphology segmentation by distributed processing, (b) 3D visualization based on volume rendering technology, (c) performance improvement for large numbers of processing nodes, (d) auto-generation of new control procedures should be done. Also, we have designed a parallel computer with 50 processors, running Linux OS on a low-cost PC core, which can do the above work more efficiently.

3. Post genome applications

To retrieve a particular sequence and to deduce a biological meaning from the monotonous arrangement of nucleotides, we need a powerful computer and a huge storage device. One of the solutions is a multi-parallel network computing system, which we devised to get an arbitrary section of the human body using the VHP data set. We improved the system for the purpose of retrieving a particular DNA sequence and investigating through the human genome sequence data, instead of using expensive super

computers. The application is implemented to work for (a) p53 protein binding site prediction by multi-step searching, (b) cDNA matching onto genomic DNA by reverse splicing, (c) translating the DNA sequence into an amino acid one. The background engine is powered by the multi-parallel computing clusters connected with a TCP/IP network. The engine has a head computer, which issues control commands to data processing nodes for various tasks such as retrieving particular patterns, generating images, loading sequence from the genome database, and so on. By using this flexible client/server structure over a high performance network, we can efficiently modify our system to accept the increasing of sequence data and various new investigation algorithms, by slightly changing the control procedures and increasing the number of the processor nodes (9).

We are developing a Web site for public service of our post genome applications, including more investigation algorithms for genetic research. We are now improving the control procedure to accept the increasing of parallel processing nodes and genome sequence database. The significant detection method based on artificial intelligence will also be focused on next.

4. Regional medical network integration

We have developed an experimental medical network system and tried DICOM (Digital Imaging and Communication in Medicine) transmission and experimental interactive teleconference via the Internet with IPsec security. These experiments were performed successfully from the technical and medical point of view. In order to get a robust network in Hokkaido (10), we have performed regional Internet exchange (IX) experiments between OCN (NTT Communications) and NORTH with BGP4 routing protocol, which was based on the same network redundancy architecture as in the G7 Information Society GIBN project mentioned above. This experiment confirmed that the BGP peering worked well in artificial disconnection and reconnection of a physical link and the regional IX shortened the pathway to the destination. We concluded that the system was low cost, highly confidential and stable and was likely to be very effective in improving the level of medical provision in rural areas of the Hokkaido Prefecture (8).

We are now planning further experiments with NGI techniques (metropolitan gigabit ether network, wireless router and 1.5Mbps access line) connected with many hospitals. The project includes a lot of advanced medical applications taking advantage of the Internet, including a network-based genetic analyzing system, tele-pathology, tele-radiology, tele-dermatology, tele-ophthalmology based on teleconference and VoIP (Voice over IP).

List of Main Publications from 1997 to 2000

1) Tatsumi H, Nakamura M, et al.: Medical education at a distance - a future vision for the way to study medicine. Program and Book of Abstracts, 85 The 3rd International Conference on the Medical Aspects of Telemedicine (1997).
2) Nogawa H, Tatsumi H, et al.: An Application of End-User-Computing Environment for VHP. The Second Visible Human Project Conference. 9-100 (1998).
3) Tatsumi H, Thomas G, Gill M, Ackerman MJ. Visible Human Anatomical Co-laboratory. At The Workshop: Bridging the Gap from Network Technology to Applications, NASA Ames Research Center. (1999).
4) Aoki F, Tatsumi H, Nogawa H, Akashi H, et al. A Parallel Approach for VHP Image Viewer. International Workshop 2000, Proceedings on Medical Session-I, 209-214 (2000).
5) Aoki F, Tatsumi H, Nogawa H, Akashi H, Nakahashi N: Distributed Processing for Large Medical Image Database. JAMIT Annual Meeting 2000, Proceedings, 58-59 (2000).
6) Aoki F, Nogawa H, Tatsumi H, Akashi H, et al, Maeda T: Distributed Computing Approach for High Resolution Medical Images. 16th World Computer Congress 2000, Proceedings on Software: Theory and Practice, 611-618 (2000).
7) Nogawa H, Aoki F, Akashi H, Nakahashi N, Tatsumi H. Network Design and Management for Medical Institutes: a Case in Sapporo Medical University. International Workshop 2000, Proceedings on Medical Session-II, 221-226 (2000).
8) Akashi H, Nakahashi N, Aoki F, Goudge M, Nakamura M, Kobayashi S, Nakayama M, Nishikage K, Imai K, Hareyama M, Tatsumi H: Development and Implementation of an Experimental Medical Network System in Hokkaido, Taking Advantage of the Results of NGI Project. International Workshop 2001, Proceedings on Bio-Medical Applications, 19-22 (2001).
9) Aoki F, Akashi H, Goudge M, Toyota M, Sasaki Y, Guo X, Li SJ, Tokino T, Tatsumi H: Post-Genome Applications Based on Multi-Parallel Computing over High Performance Network. ibid. 61-67 (2001).
10) Nogawa H, Tatsumi H, et al. Configurations of the Internet Server with Multi-Home Environment for E-mail Robustness. 1999 Internet Workshop (ISBN 0-7803-5925-9) by IEEE, Inc. 61-68 (1999).

III INTERNATIONAL EXCHANGES

INTERNATIONAL EXCHANGES

NORTHERN REGION MEDICAL EXCHANGE PROGRAM

Sapporo Medical University actively promotes exchange activities with northern region countries whose climates and living conditions are similar to those of Hokkaido to improve the health and welfare of people living in these regions.

Since 1977, the university has established medical exchange programs with universities in Finland, Canada, China and the U.S.A.

In 1999, the university initiated a study abroad program such as a language training at University of Alberta, a clinical training at University of Calgary and a medical subinternship at University of Massachusetts.

EXCHANGE PROGRAMS	
FINLAND	Paulo Foundation (1977-) University of Helsinki, University of Turku, University of Oulu, University of Tampere, University of Kuopio
CANADA	University of Alberta (1983-) University of Calgary (1984-)
CHINA	China Medical University (1984-)
USA	University of Massachusetts (1994-)

EXCHANGE SCIENTISTS

FINLAND → SAPPORO MEDICAL UNIVERSITY

NAME & TITLE	HOST DEPARTMENT	PERIOD
Seppo Autio Lecturer, Dept. of Child Neurology, University of Helsinki	Dept. of Pediatrics	1978.1.25 -1978.3.24
Seppo Takki Instructor, Dept. of Anesthesiology University of Helsinki	Dept. of Anesthesiology	1979.8.31 -1979.11.11
Per Rosenberg Associate Professor Dept. of Anesthesiology University of Helsinki	Dept. of Anesthesiology	1980.12.5 -1981.1.24
Reijo Punnonen Senior Lecturer (Docent) Dept. of Gynecology & Obstetrics University of Turku	Dept. of Obstetrics & Gynecology	1982.2.4 -1982.3.31
Seppo Tunonen Senior Lecturer Consultant Pediatric Surgeon University of Oulu	Dept. of Surgery (II)	1983.1.4 -1983.3.3
Timo Nevalainen Professor Dept. of Pathology University of Turku	Dept. of Pathology (II)	1984.1.21 -1984.3.20
Ranan Hilel Rimon Professor Dept. of Psychiatry University of Helsinki	Dept. of Neuropsychiatry	1985.2.1 -1985.3.30
Simo Vilkki Docent Dept. of Orthopedic Surgery University of Turku	Dept. of Orthopedic Surgery	1986.1.27 -1986.3.25
Seppo Santavirta Associate Professor Dept. of Orthopedic Surgery University of Helsinki	Dept. of Orthopedic Surgery	1986.10.2 -1986.11.27
Olli Ruuskanen Docent Dept. of Pediatrics University of Turku	Dept. of Pediatrics	1988.1.19 -1988.3.15
Olof Selroos Associate Professor Dept. of Chest Medicine University of Helsinki	Dept. of Internal Medicine (III)	1989.3.4 -1989.4.27
Mikko Hallman Professor Dept. of Obstetrics & Gynecology University of Helsinki	Dept. of Biochemistry (I)	1990.3.3 -1990.3.31
Ervo Vesterinen Docent Dept. of Obstetrics & Gynecology University of Helsinki	Dept. of Obstetrics & Gynecology	1991.1.15 -1991.3.23
Jorma Paavonen Associate Professor Dept. of Obstetrics & Gynecology University of Helsinki	Dept. of Obstetrics & Gynecology	1992.2.2 -1992.3.31
Pekka-J. Klemi Associate Professor & Senior Lecturer Dept. of Pathology University of Turku	Dept. of Pathology (II)	1993.1.31 -1993.3.31
Martti Vastamaki Associate Professor Dept. of Orthopedic Surgery University of Helsinki	Dept. of Orthopedic Surgery	1993.12.22 -1994.1.16
Jussi Kant Professor Dept. of Anesthesiology University of Turku	Dept. of Anesthesiology	1994.6.8 -1994.7.31
Kimmo T. Kyosola Associate Professor Dept. of Thoracic & Cardiovascular Surgery University of Helsinki	Dept. of Surgery (II)	1995.8.21 -1995.10.17
Sylvi Kassinen Associate Professor Dept. of Medical Microbiology University of Oulu	Dept. of Pathology (I)	1996.6.27 -1996.9.8
Tarja H. Ruuska Docent Dept. of Pediatrics University of Tampere	Dept. of Pediatrics	1998. 3.14 -1998. 4.10
Pentti JA. Kiilholma Docent Dept. of Obsterics & Gynecology Univetrsity of Turku	Dept. of Obsterics & Gynecology	1999.1.31 -1999.2.27
Markus EP. Rautiainen Docent Dept. of Otorhinolaryngology University of Tampere	Dept. of Otolaryngology	2000. 1. 20 -2000. 3. 3
Kari Punnonen Assistant Professor Dept. of Clinical Chemistry & Hematology University of Kuopio	Dept. of Internal Medicine (IV)	2001. 2. 4 -2001. 3. 21

SAPPORO MEDICAL UNIVERSITY → FINLAND

NAME & TITLE	HOST DEPARTMENT	PERIOD
Mayumi Takasaki Assistant Professor Dept. of Anesthesiology	Dept. of Anesthesiology University of Helsinki	1978.12.27 -1979.3.24
Kowichi Jimbow Associate Professor Dept. of Dermatology	Dept. of Dermatology University of Helsinki	1980.2.17 -1980.3.26
Motoi Ogata Associate Professor Dept. of Psychiatry	Dept. of Psychiatry University of Helsinki	1981.2.25 - 1981.4.10
Takeo Takahashi Professor Dept. of Anesthesiology	Dept. of Anesthesiology University of Helsinki	1981.11.4 -1981.12.10
Ryuichi Kudo Associate Professor Dept. of Gynecology & Obstetrics	Dept. of Gynecology & Obstetrics University of Turku	1983.1.14 -1983.3.3
Teruhisa Kazui Assistant Professor Dept. of Surgery (II)	Dept. of Surgery University of Helsinki	1983.12.25 -1984.2.22
Hiroyuki Matsumoto Associate Professor Dept. of Internal Medicine (I)	Dept. of Neurology University of Helsinki	1984.10.29 -1984.12.25
Toyoaki Akino Professor Dept. of Biochemistry (I)	Dept. of Pediatrics University of Helsinki	1985.10.23 -1985.11.21
Yutaka Yoshida Assistant Professor Dept. of Pathology (II)	Dept. of Pathology University of Turku	1986.8.10 -1986.10.25
Masamichi Usui Associate Professor Dept. of Orthopedic Surgery	Dept. of Orthopedic Surgery University of Helsinki University of Tampere	1987.11.2 -1987.12.31
Hiroshi Ajiki Associate Professor Dept. of Surgery (II)	Dept. of Pediatrics University of Helsinki University of Oulu	1988.8.1 -1988.10.2
Mamoru Aoki Professor Dept. of Physiology (II)	Dept. of Physiology University of Helsinki University of Tampere University of Oulu	1989.1.20 -1989.7.22
Nobuo Maeda Professor Dept. of Sociology & Economics	Dept. of Internal Medicine(II) University of Helsinki University of Tampere University of Turku	1990.7.30 -1990.9.3
Hiroaki Watanabe Associate Professor Dept. of Anesthesiology	Dept. of Anesthesiology University of Helsinki	1992.1.2 -1992.3.10
Minoru Okazaki Assistant Professor Dept. of Surgery (I)	Dept. of Surgery University of Helsinki	1993.1.12 -1993.2.14
Shigeo Yoshida Associate Professor Dept. of Diagnostic Ultrasound & Medical Electronics	Dept. of Internal Medicine (I) University of Helsinki	1993.8.24 -1993.10.3
Reiko Kishi Assistant Professor Dept. of Public Health	Dept. of Geriatrics University of Helsinki	1994.9.24 -1994.11.20
Kazuo Hashi Professor Dept. of Neurosurgery	Dept. of Neurosurgery University of Helsinki	1995.8.7 -1995.8.17
Tetsuo Himi Associate Professor Dept. of Otolaryngology	Dept. of Otorhinolaryngology University of Helsinki	1996.10.14 -1996.12.23
Takashi Nakagawa Professor Dept. of Ophthalmology	Dept. of Ophthalmology University of Helsinki	1997. 11. 4 -1997. 11. 26
Shuji Nakata Assistant Professor Dept. of Pediatrics	Dept. of Pediatrics University of Tampere	1998. 9. 13 -1998. 10. 4
Tetsuo Himi Proffesor Dept. of Otolaryngology	Dept. of Otorhinolaryngology University of Tampere	1999. 11. 17 -1999. 12. 2
Hideaki Shirasaki Assistant Professor Dept. of Otolaryngology	Dept. of Otorhinolaryngology University of Tampere	2001. 3. 11 -2001. 4. 1

CHINA MEDICAL UNIVERSITY

→ SAPPORO MEDICAL UNIVERSITY

NAME & TITLE	HOST DEPARTMENT	PERIOD
Li Yy-quan Professor Dept. of Internal Medicine	Dept. of Internal Medicine (I)	1982.6.12 -1982.7.11
Tan Pu-quan Associate Professor Dept. of Internal Medicine	Dept. of Internal Medicine (II)	1983.12.23 -1984.2.22
Xie Yu-dong Associate Professor Dept. of Internal Medicine	Dept. of Internal Medicine (III)	1983.12.23 -1984.2.22
Ba Jing-yang Associate Professor Dept. of Obstetrics & Gynecology	Dept. of Obstetrics & Gynecology	1984.9.9 -1984.11.8
Xia Zhen-long Associate Professor Dept. of Surgery	Dept. of Surgery (I)	1984.9.9 -1984.11.8
Li Guang-ying Associate Professor Dept. of Cardiac Surgery	Dept. of Surgery (II)	1984.10.9 -1984.12.8
Piao Ying-ai Assistant Professor Dept. of Pediatrics	Dept. of Pediatrics	1984.10.9 -1984.12.8
Zhang Bing-jun Associate Professor Dept. of Anesthesiology	Dept. of Anesthesiology	1985.11.8 -1986.1.6
Sun Zhen-sheng Associate Professor Dept. of Ophthalmology	Dept. of Ophthalmology	1985.11.8 -1986.1.6
Liang Key-l Associate Professor Dept. of Otolaryngology	Dept. of Otolaryngology	1985.11.8 -1986.1.6
Zhou Yong-de Lecturer Dept. of Pediatric Orthopedics	Dept. of Orthopedic Surgery	1985.11.8 -1986.1.6
Zhao Nai-cai Professor Dept. of Pharmacology	Dept. of Pharmacology	1986.10.3 -1986.12.1
Chen Li-ying Associate Professor Dept. of Radiology	Dept. of Radiology	1986.10.3 -1986.12.1
Zhao Zi-liang Associate Professor Dept. of Urogental Surgery	Dept. of Urology	1986.10.3 -1986.12.1
Wang Shi-chi Associate Professor Dept. of Oral Surgery	Dept. of Oral Surgery	1986.10.3 -1986.12.1

NAME & TITLE	HOST DEPARTMENT	PERIOD
Gao Ji-yuan Professor Dept. of Pathology	Dept. of Pathology (I)	1987.11.10 -1988.1.8
Li Yong-chang Professor Dept. of Pediatrics	Dept. of Pediatrics	1987.11.10 -1988.1.8
Gao Peng-yuan Professor Dept. of Internal Medicine	Dept. of Internal Medicine (I)	1987.11.10 -1988.1.8
Zhou Jian-ying Professor Dept. of Surgery	Dept. of Surgery (I)	1987.11.10 -1988.1.8
Yijing Yao Professor Dept of Surgery	Dept. of Surgery (I)	1988.9.8 -1988.11.6
Liu Zong-Han Professor Dept. of Ophthalmology	Dept. of Ophthalmology	1988.9.8 -1988.11.6
Chun-zheng Wang Professor Dept. of Internal Medicine	Dept. of Internal Medicine (I)	1988.9.8 -1988.11.6
Shi Guirong Associate Professor Dept. of Epidemiology	Dept. of Hygiene	1988.9.8 -1988.11.6
Li Ji Professor Dept. of Anatomy	Dept. of Anatomy (I)	1989.12.8 -1990.2.5
Xia Ying-Kui Professor Dept. of Dermatology	Dept of Dermatology	1989.12.8 -1990.2.5
Yu Yun Associate Professor Dept. of Anesthesiology	Dept. of Surgery (I)	1989.12.8 -1989.2.5
Qin Zhen-Yuan Associate Professor Dept. of Surgery	Dept. of Anesthesiology	1989.12.8 -1990.2.5
Han Naiying Associate Professor Dept. of Internal Medicine	Dept. of Internal Medicine (IV)	1990.11.30 -1991.1.28
Zhang Hui Associate Professor Dept. of Pediatrics	Dept. of Pediatrics	1990.11.30 -1992.1.28
Yu Qianyi Associate Professor Dept. of Preventive Medicine	Dept. of Public Health	1990.11.30 -1991.1.28

NAME & TITLE	HOST DEPARTMENT	PERIOD
Xu Fungtong Associate Professor Dept. of Surgery	Dept. of Surgery (I)	1990.11.30 -1991.1.28
Lu Yun-shi Professor Dept. of Obstetrics & Gynecology	Dept. of Gynecology & Obstetrics	1991.11.20 -1992.1.18
Wang Bao-hua Professor Dept. of Otolaryngology	Dept. of Otolaryngology	1991.11.20 -1992.1.18
Wang De-wen Associate Professor Dept. of Pathological Laboratory	Dept. of Legal Medicine	1991.11.20 -1992.1.18
Ma Zong-sheng Associate Professor Dept. of Internal Medicine	Dept. of Internal Medicine (III)	1991.11.20 -1992.1.18
Li Xin-yuan Associate Professor Dept. of Pediatrics Surgery	Dept. of Surgery (I)	1993.1.31 -1993.3.31
Tao Jing Associate Professor Dept. of Pediatrics	Dept. of Pediatrics	1993.1.31 -1993.3.31
Liu Ying min Professor Dept. of Internal Medicine	Dept. of Internal Medicine (II)	1993.1.31 -1993.3.31
Wang Yan-feng Vice Director Technician Division of Laboratory Diagnosis	Division of Laboratory Diagnosis	1993.1.31 -1993.3.31
Shi Yu Xiu Professor Dept. of Histology & Embryology	Dept. of Anatomy (I)	1994.6.22 -1994.8.20
Sun Xin Xiang Associate Professor Dept. of Internal Medicine	Dept. of Internal Medicine (IV)	1994.6.22 -1994.8.20
Wang Tie Assistant Professor Dept. of Otolaryngology	Dept. of Otolaryngology	1995.1.16 -1995.3.31
Wang Tie Associate Professor Dept. of Otolaryngology	Dept. of Otolaryngology	1996.2.14 -1996.4.13
Wang Yunjie Assistant Professor Dept. of Neurosurgery	Dept. of Neurosurgery	1996.2.14 -1996.4.13
Zhang Lin Associate Professor Dept. of Surgery	Dept. of Surgery (II)	1997.3.1 -1997.3.31

NAME & TITLE	HOST DEPARTMENT	PERIOD
Song Li Chwen Assistant Professor Dept. of Neurology	Dept. of Neurosurgery	1997.3.1 -1997.3.31
Hang Ping Associate Professor Dept. of Internal Medicine	Dept. of Internal Medicine (II)	1998. 1. 8 -1998. 3. 5
Wang Zhenyu Assistant Professor Dept. of Anatomy	Dept. of Physiology (II)	1998. 1. 8 -1998. 3. 5
Lu Yongli Professor Dept. of Anatomy	Dept. of Anatomy (I)	1998. 11. 29 -1999. 2. 21
Min-Jie WEI Associate Professor Dept. of Pharmacology	Dept. of Pharmacology	1998. 11. 29 -1998. 12. 27
Kong Lingfei Associate Professor Dept. of Internal Medicine	Dept. of Internal Medicine (III)	2000. 1. 23 -2000. 4. 30
Xie Hui Fnag Professor Dept. of Internal Medicine	Dept. of Internal Medicine (III)	2000. 6. 21 -2000. 6. 30
Li Shengjun Instructor International Exchange Center	Information Center of Computer Communication	2000. 12. 17 -2001. 3. 31

SAPPORO MEDICAL UNIVERSITY

→ CHINA MEDICAL UNIVERSITY

NAME & TITLE	HOST DEPARTMENT	PERIOD
Morimichi Fukuda Associate Professor Dept. of Internal Medicine (IV)	Dept of Internal Medicine	1983.10.31 -1983.11.12
Shoichi Tanaka Assistant Professor Dept. of Gynecology & Obstetrics	Dept. of Gynecology & Obstetrics	1983.10.31 -1983.11.12
Yutaka Kohgo Assistant Professor Dept. of Internal Medicine (IV)	Dept. of Internal Medicine	1983.10.31 -1983.11.12
Tsuyoshi Yabana Assistant Professor Dept. of Internal Medicine (I)	Dept. of Internal Medicine	1983.11.7 -1983.11.20
Shuichi Maeda Instructor Dept. of Internal Medicine (I)	Dept. of Internal Medicine	1983.11.7 -1983.11.20
Sakuzo Komatsu Professor Dept. of Surgery (II)	Dept. of Cardiac Surgery	1984.5.18 -1984.5.30
Tomio Abe Associate Professor Dept. of Surgery (II)	Dept. of Cardiac Surgery	1984.5.18 -1984.5.30
Akira Yachi Professor Dept. of Internal Medicine (I)	Dept. of Internal Medicine	1984.5.29 -1984.6.10
Takeo Wada President Sapporo Medical College	China Medical University	1984.5.26 -1984.6.5
Yoshikazu Narasaki Instructor Dept. of Internal Medicine (I)	Dept. of Internal Medicine	1984.5.26 -1984.6.10
Takeshi Miki Professor Dept. of Sociology & Economics	School of Public Health	1984.5.26 -1984.6.10 1984.9.23 -1984.9.24
Tohru Nakao Professor Dept. of Pediatrics	Dept. of Internal Medicine	1984.9.23 -1984.9.24
Takeo Takahashi Professor Dept. of Anesthesiology	Dept. of Anesthesiology	1985.8.3 -1985.8.17
Sadatsugu Tagawa Professor Dept. of Ophthalmology	Dept. of Ophthalmology	1985.8.3 -1985.8.17
Osamu Iimura Professor Dept. of Internal Medicine (II)	Dept. of Internal Medicine	1985.8.3 -1985.8.17
Akira Suzuki Professor Dept. of Internal Medicine (III)	Dept. of Internal Medicine	1985.8.3 -1985.8.17
Kokichi Kikuchi Professor Dept. of Pathology (I)	Dept. of Pathology	1986.4.13 -1986.4.23
Kei Fujinaga Professor Dept. of Molecular Biology Cancer Research Institute	China Medical University	1986.4.13 -1986.4.23
Kazuo Morita Professor Dept. of Radiology	Dept. of Radiology	1986.9.7 -1986.9.21
Masayoshi Hashimoto Professor Dept. of Obstetrics & Gynecology	Dept. of Obstetrics & Gynecology	1986.9.7 -1986.9.21
Shuzo Chiba Professor Dept. of Pediatrics	Dept. of Pediatrics	1987.10.17 -1987.10.24
Akikatsu Kataura Professor Dept. of Otolaryngology	Dept. of Otolaryngology	1987.10.17 -1987.10.24
Morimichi Fukuda Professor Division of Ultrasound & Medical Electronics	China Medical University	1987.10.24 -1987.10.30
Hideyo Ohshika Professor Dept. of Pharmacology	Dept. of Pharmacology	1988.9.28 -1988.10.10
Kazuaki Asaishi Assistant Professor Dept. of Surgery (I)	Dept. of Surgery	1988.9.28 -1988.10.10
Kei Fujinaga Professor Dept. of Molecular Biology Cancer Research Institute	China Medical University	1988.11.15 -1988.11.23
Yukiharu Sawada Associate Professor Cancer Research Institute	China Medical University	1988.11.15 -1988.11.23
Hiroaki Watanabe Assistant Professor Dept. of Anesthesiology	Dept. of Anesthesiology	1990.2.25 -1990.3.12
Masahiko Kida Instructor Dept. of Anatomy (II)	Dept. of Anatomy	1990.2.25 -1990.3.12
Kohzoh Imai Assistant Professor Dept. of Internal Medicine (I)	China Medical University	1990.5.21 -1990.5.26

NAME & TITLE	HOST DEPARTMENT	PERIOD
Kokichi Kikuchi President Sapporo Medical University	China Medical University	1990.10.14 -1990.10.20
Naoki Sugawara Assistant Professor Dept. of Public Health	Dept. of Preventive Medicine	1991.2.28 -1991.3.13
Yoshiro Niitsu Professor Dept. of Internal Medicine (IV)	Dept. of Internal Medicine	1991.3.17 -1991.3.24
Hideyo Yabu Professor Dept. of Physiology (I)	Dept. of Pharmacology	1991.10.10 -1991.10.19
Kazuaki Shimamoto Associate Professor Dept. of Internal Medicine (II)	Dept. of Internal Medicine	1992.2.29 -1992.3.8
Kazuo Hashi Professor Dept. of Neurological Surgery	Dept. of Surgery	1993.1.9 -1993.1.16
Haruo Takemura Instructor Dept. of Pharmacology	Dept. of Pharmacology	1992.11.8 -1992.11.20
Yoshiaki Kumamoto Professor Dept. of Urology	Dept. of Urology	1994.3.31 -1994.4.6
Yoshihito Ujike Assistant Professor Division of Traumatology & Critical Care Medicine	Dept. of Anesthesiology	1994.3.21 -1994.3.28
Ichiro Kurokawa Professor Division of Laboratory Diagnosis	China Medical University	1995.3.22 -1995.3.29
Sumiyoshi Tanabe Associate Professor Dept. of Neurological Surgery	Dept. of Neurological Surgery	1995.3.27 -1995.3.31
Tohru Kudo Associate Professor Dept. of Pediatrics	Dept. of Pediatrics The second affiliated hospital	1995.9.18 -1995.9.28
Takafumi Ninomiya Assistant Professor Dept. of Anatomy (I)	Dept. of Histology & Embryology	1995.9.18 -1995.9.28
Seiichi Ishii Professor Dept. of Orthopedic Surgery	Dept. of Orthopedic Surgery	1997.3.6 -1997.3.12
Teiji Uede Associate Professor Dept. of Neurological Surgery	Dept. of Neurological Surgery	1997.3.24 -1997.3.30

NAME & TITLE	HOST DEPARTMENT	PERIOD
Mamoru Aoki Professor Dept. of Physiology (II)	Dept. of Physiology	1997. 10. 18 -1997. 10. 24
Ryuichi Kudo Professor Dept. of Obstetrics & Gynecology	Dept. of Obstetrics & Gynecology	1997. 8. 31 -1997. 9. 7
Ryuichi Denno Associate Professor Dept. of Surgery (I)	Dept. of Surgery	1998. 8. 24 -1998. 9. 6
Yukihiro Ibayashi Assistant professor Dept. of Neurosurgery	Dept. of Neurosurgery	1998. 9. 12 -1998. 9. 19
Tomio Abe Professor Dept. of Surgery (II)	Dept. of Surgery	1999. 10. 31 -1999. 11. 7
Nobuyuki Ura Associate Professor Dept. of Internal Medicine (II)	Dept. of Internal Medicine	1999. 9. 26 -1999. 10. 10
Fumio Aoki Instructor Information Center of Computer Communication	International Exchange Center	2000. 8. 6 -2000. 8. 27
Hiroyuki Matsumoto Professor Dept. of Neurology	Dept. of Neurosurgery	2001. 3. 13 - 2001. 3.18

CANADA

UNIVERSITY OF ALBERTA

→ SAPPORO MEDICAL UNIVERSITY

NAME & TITLE	HOST DEPARTMENT	PERIOD
Robert S. Fraser Acting Dean, Professor	Dept. of Surgery (II)	1984.3.10 -1984.3.17
Thomas A. McPherson Assistant Dean, Professor Dept. of Pathology	Dept. of Pathology Cancer Research Institute	1984.3.10 -1984.3.17
Ronald H. Wensel Professor Dept. of Gastroenterology	Dept. of Internal Medicine (I)	1984.3.10 -1984.3.23
Alan J. Lupin Associate Clinical Professor Division of Otolaryngology	Dept. of Otolaryngology	1985.3.11 -1985.3.23
Bryan M. Longenecker Professor Dept. of Immunology	Dept. of Pathology (I)	1985.3.3 -1985.3.30
Wanda M. Wenman Associate Professor Dept. of Pediatrics	Dept. of Pediatrics	1985.9.29 -1985.10.12
Neil N. Finer Professor Dept. of Pediatrics	Dept. of Pediatrics	1986.10.5 -1986.10.17
Edgar G. King Professor & Chairman Dept. of Medicine	Dept. of Emergency and Critical Care Medicine	1987.3.24 -1987.3.31
George B. Frank Professor Dept. of Pharmacology	Dept. of Physiology (I)	1988.1.10 -1988.2.18
Donald R. Mclean Professor Division of Neurology	Dept. of Internal Medicine (I)	1988.3.11 -1988.3.25
Bill Johnston Assistant Professor Division of Orthopedic surgery	Dept. of Orthopedic Surgery	1988.9.8 -1988.9.12
Peter M. Olley Professor Dept. of Pediatrics	Dept. of Pediatrics	1989.1.22 -1989.2.4
Teresa M. Allen Professor Dept. of Pharmacology	Dept. of Pharmacology	1990.1.14 -1990.2.23 1990.3.11 -1990.4.12
Terrence J. Montague Professor Dept. of Internal Medicine	Dept. of Internal Medicine (II)	1990.1.23 -1990.2.5
Roderick A. Morgan Professor Dept. of Ophthalmology	Dept. of Ophthalmology	1991.3.14 -1991.3.24
Colin L. Soskolne Associate Professor Dept. of Epidemiology	Dept. of Public Health	1991.3.18 -1991.3.31
Sibrand Poppema Professor Dept. of Pathology	College Hospital Laboratory Diagnosis	1992.2.14 -1992.2.27
S. F. Paul Man Professor Dept. of Medicine	Dept of Physiology (I)	1992.2.4 -1992.3.11
Dennis L. Modry Associate Professor Dept. of Surgery	Dept of Surgery (II)	1993.2.21 -1993.2.27
Stewart M. Hamilton Professor Dept. of Surgery	Division of Traumatology & Critical Care Medicine	1993.12.4 -1993.12.16
Peter N. Mccracken Professor Dept. of Geriatric Medicine	Dept. of Internal Medicine (II)	1994.2.12 -1994.2.26
Henry F. Pabst Professor Dept. of Pediatrics	Dept. of Pediatrics	1995.1.16 -1995.1.31
Malcolm C. Paterson Professor Dept. of Medicine Cross Cancer Institute	Dept. of Internal Medicine (I)	1995.3.14 -1995.3.26
Janice Lander Professor Dept. of Psychology	Dept. of Nursing School of Health Sciences	1996.3.2 -1996.3.17
James C. Russell Professor Dept. of Surgery	Dept. of Internal Medicine (II)	1996.11.13 -1996.11.27
Richard Schulz Assistant Professor Dept. of Pediatrics & Pharmacology	Dept. of Pharmacology	1997.1.9 -1997.1.22
Paul W. Armstrong Professor Dept. of Medicine	Sapporo Medical University	2000. 6. 23 -2000. 6. 26

CANADA

UNIVERSITY OF CALGARY

→ SAPPORO MEDICAL UNIVERSITY

NAME & TITLE	HOST DEPARTMENT	PERIOD
Norman S. Schachar Associate Professor Dept. of Orthopedic Surgery	Dept. of Orthopedic Surgery	1985.11.19 -1986.2.8
Thomas P. Hicks Assistant Professor Dept. of Medical Physiology	Dept. of Pharmacology	1986.3.1 -1986.3.14
Eldon R. Smith Professor & Head Dept. of Medicine	Dept. of Internal Medicine (II)	1987.1.26 -1987.2.6
Eldon A. Shaffer Head Division of Gastroenterology Dept. of Medicine	Dept. of Internal Medicine (IV)	1987.10.12 -1987.10.25
John E. Remmers Professor Dept. of Internal Medicine	Dept. of Internal Medicine (III)	1989.1.11 -1989.2.10
D. Grant Gall Professor Dept. of Pediatrics	Dept. of Pediatrics	1989.9.3 -1989.9.10
Jerry H-C. Wang Professor Dept. of Medical Biochemistry	Dept. of Biochemistry (I)	1991.7.17 -1991.7.22
Brian A. MacVicar Associate Professor Dept. of Medical Physiology	Dept. of Physiology (II)	1991.2.14 -1991.3.24
Nady, el-Guebaly Professor & Head Dept. of Psychiatry	Dept. of Neuropsychiatry	1992.8.21 -1992.9.5
Taiki Tamaoki Professor Dept. of Medical Biochemistry	Dept. of Molecular Biology Cancer Research Institute	1992.9.2 -1992.10.13
Richard S. Hannah Professor Dept. of Anatomy	Dept. of Anatomy (I)	1993.10.30 -1993.12.10
Norman C. Wong Professor Dept. of Medical Biochemistry	Dept. of Biochemistry (II)	1994.3.6 -1994.3.17
Clarence A. Guenter Professor Emeritus	Dept. of Internal Medicine (III)	1994.4.20 -1994.4.27
Sheldon H. Roth Professor Dept. of Pharmacology & Therapeutics & Anaesthesia	Dept. of Pharmacology	1994.5.20 -1994.6.3
Randal N. Johnston Professor & Director Southern Alberta Cancer Research Center	Dept. of Molecular Biology Cancer Research Institute	1996.2.2 -1996.2.20

NAME & TITLE	HOST DEPARTMENT	PERIOD
Samuel Song-Gu Lee Associate Professor Dept. of Medicine	Dept. of Internal Medicine (I)	1996.3.12 -1996.3.24
Norman S. Schachar Professor Dept. of Surgery	Dept. of Orthopedic Surgery	1996.7.8 -1996.7.22
Andrew G.M. Bulloch Professor Dept. Medical Physiology	Dept. of Physiology (II)	1996.9.21 -1996.10.12
Pamera A. Socol Professor Dept. of Microbiology & Infectious Diseases	Dept. of Urology	1998. 1. 22 -1998. 1. 29
Donald E. Woods Professor Dept. of Microbiology & Infectious Diseases	Dept. of Urology	1998. 1. 22 -1998. 1. 29
David L. Severson Professor Dept. of Pharmacology & Therapeutics	Dept. of Internal Medicine (II)	1999. 2. 1 -1999. 2. 21
Peter J. Forsyth Associate Professor Dept. of Clinical Neurosciences, & Medicine	Dept. of Neurosurgery	1999. 3. 21 -1999. 4. 3
Jaques Belik Professor Dept. of Pediatrics	Dept. of Pediatrics	2000. 2. 6 -2000. 2. 29
Norman C. Wong Professor Dept. of Medicine & Medical Biochemistry	Dept. of Biochemistry (I)	2000. 3. 4 -2000. 3. 31
Deborah L. Tamlyn Professor and Dean Faculty of Nursing	Dept. of Nursing	2000. 6. 20 -2000. 6. 26
Johan H. van de Sande Professor & Vice Dean Faculty of Medicine	Dept. of Molecular Biology Cancer Research Institute	2000. 6. 22 -2000. 6. 26

SAPPORO MEDICAL UNIVERSITY → CANADA

NAME & TITLE	HOST DEPARTMENT	PERIOD
Kohzoh Imai Assistant Professor Dept. of Internal Medicine (I)	Dept. of Internal Medicine University of Alberta	1984.2.16 -1984.4.11
Noboru Yamanaka Assistant Professor Dept. of Otolaryngology	Dept. of Otolaryngology University of Alberta	1984.2.15 -1984.4.13
Hideyuki Tsukada Professor Dept. of Pathology Cancer Research Institute	University of Alberta University of Calgary	1984.9.16 -1984.10.3
Katsuyuki Kusajima Assistant Professor Dept. of Surgery (II)	Division of Pulmonary Diseases University of Alberta	1985.1.25 -1985.3.27
Kowichi Jimbow Associate Professor Dept. of Dermatology	Dept. of Dermatology University of Alberta University of Calgary	1985.9.29 -1985.10.6
Yoshikazu Akahonai Associate Professor Dept. of Internal Medicine (II)	Dept. of Internal Medicine University of Alberta	1986.1.24 -1986.4.6
Mamoru Aoki Professor Dept. of Physiology (II)	Dept. of Physiology University of Alberta University of Calgary	1986.10.20 -1986.11.3
Shoichi Tanaka Associate Professor Dept. of Obstetrics & Gynecology	Dept. of Obstetrics & Gynecology University of Calgary	1987.1.13 -1987.4.5
Morio Akiyama Associate Professor Physics	Dept. of Biochemistry University of Alberta	1987.9.2 -1987.10.22
Koichi Itaya Professor Hospital Pharmacy	Dept. of Pharmacology University of Alberta University of Calgary	1987.11.15 -1987.11.30
Takashi Horikoshi Assistant Professor Dept. of Dermatology	Dept. of Dermatology University of Alberta	1988.7.4 -1988.11.2
Takashi Nakagawa Professor Dept. of Ophthalmology	Dept. of Ophthalmology University of Alberta University of Calgary	1988.9.25 -1988.10.8
Tomio Abe Associate Professor Dept. of Surgery (II)	Dept. of Surgery University of Calgary	1989.8.21 -1989.10.20
Kazuo Hashi Professor Dept. of Neurological Surgery	Division of Neurosurgery University of Alberta	1990.3.17 -1990.3.29
Hideshi Tomita Instructor Dept. of Pediatrics	Dept. of Pediatrics University of Alberta	1990.10.1 -1990.12.31
Yoshiaki Kumamoto Professor Dept. of Urology	Dept. of Urology University of Alberta University of Calgary	1991.3.24 -1991.4.3
Yasufumi Asai Associate Professor Division of Emergency & Critical Care Medicine	Division of Emergency & Critical Care University of Alberta	1991.7.1 -1991.10.2
Haruyuki Tatsumi Associate Professor Dept. of Anatomy (I)	Dept. of Anatomy University of Alberta University of Calgary	1991.9.10 -1991.9.27
Susumu Chiba Assistant Professor Dept. of Neurology	Dept. of Neuropsychiatry University of Alberta University of Calgary	1992.1. 31 -1992. 8. 13
Akira Mizuguchi Instructor Dept. of Physiology (II)	Dept. of Physiology University of Alberta	1992.8.3 -1992. 10. 31
Shunzo Chiba Professor Dept. of Pediatrics	Dept. of Pediatrics University of Alberta	1993. 6.5 -1993. 6.22
Hidenori Yoshino Associate Professor Chemistry	Dept. of Medical Biochemistry University of Calgary	1993. 9.15 -1993. 11. 15
Atsushi Miyamoto Associate Professor Dept. of Pharmacology	Dept. of Pharmacology University of Alberta	1994. 6. 4 -1994. 7. 19
Yukiharu Sawada Associate Professor Dept. of Molecular Biology Cancer Research Institute	Dept. of Medical Biochemistry University of Calgary	1994. 6. 30 -1994. 8. 26
Toshihiko Ogino Professor Dept. of Physical Therapy	Dept. of Plastic Surgery University of Alberta	1995. 8. 11 -1995. 8. 28
Kei Fujinaga Professor Dept. of Molecular Biology Cancer Research Institute	Dept. of Medical Biochemistry University of Calgary	1995. 8.28 -1995. 9.8
Terukatsu Sasaki Professor Dept. of Biochemistry Cancer Research Institute	Dept. of Medical Biochemistry & Oncology University of Calgary	1996. 8. 14 -1996. 8. 24
Hideyo Ohshika Professor Dept. of Pharmacology	Dept. of Pharmacology University of Alberta	1996. 10.17 -1996. 11.3
Nobuyuki Tanaka Assistant Professor Dept. of Oral Surgery	Dept. of Surgery University of Calgary	1997. 10. 30 -1997. 12. 10
Shigeto Fuse Instructor Dept. of Pediatrics	Dept. of Pediatrics University of Alberta	1998. 1. 17 -1998. 3. 6

NAME & TITLE	HOST DEPARTMENT	PERIOD
Toshiaki Yamaki Instructor Dept. of Neurosurgery	Dept. of Clinical Neurosciences University of Calgary	1998. 3. 1 -1998. 3. 16
Toshiaki Tanaka Assistant Professor Dept. of Surgery (II)	Dept. of Surgery University of Alberta	1998. 3. 16 -1998. 3. 28
Masato Nagashima Assistant Professor Dept. of Physiology (I)	Dept. of Physiology & Biophysics University of Calgary	1998. 10. 29 -1998. 11. 11
Hiroyuki Koba Associate Professor Dept. of Internal Medicine (III)	Division of Pulmonary Medicine University of Alberta	1999. 3. 1 1999. 3. 13
Toshihiko Yamashita Assistant Professor Dept. of Orthopaedic Surgery	Dept. of Surgery University of Calgary	1999. 10. 24 -1999. 11. 7
Masaki Katayose Instructor Dept. of Physical Therapy	Faculty of Rehabilitation Medicine University of Alberta	2000. 1. 13 -2000. 1. 23
Kazumitsu Koito Assistant Professor Dept. of Radiology	Dept. of Radiology University of Calgary	2000. 3. 16 -2000. 4. 16
Hisako Izumi Instructor Dept. of Nursing	Faculty of Nursing University of Alberta	2000. 7. 28 -2000. 8. 11
Takuro Wada Assistant Professor Dept. of Orthopaedic Surgery	Dept. of Orthopaedic Surgery University of Calgary	2000. 10. 8 -2000. 10. 22
Kanshi Komatsu Assistant Professor Dept. of Surgery (II)	Dept. of Surgery University of Alberta	2001. 2. 5 -2001. 2. 20
Hiroshi Tanaka Assistant Professor Dept. of Internal Medicine (III)	Dept. of Medicine University of Calgary	2001. 3. 12 -2001. 4. 1

UNIVERSITY OF MASSACHUSETTS

→ SAPPOR MEDICAL UNIVERSITY

NAME & TITLE	HOST DEPARTMENT	PERIOD
Richard C. Marks Professor Dept. of Surgery & Neurology	Dept. of Neurological Surgery	1996. 3. 2 -1996. 3. 31
Richard V. Aghababian Professor Dept. of Emergency Medicine	Division of Traumatology & Critical Care Medicine	1996. 3. 23 -1996. 3. 31
Francis P. Renzi Associate Professor Dept. of Emergency Medicine	Division of Traumatology & Critical Care Medicine	1996. 11. 3 -1996. 11. 14

SAPPORO MEDICAL UNIVERSITY

→ UNIVERSITY OF MASSACHUSETTS

NAME & TITLE	HOST DEPARTMENT	PERIOD
Fumio Ito Instructor Dept. of Internal Medicine (I)	Dept. of Medicine	1995. 2. 20 -1995. 3. 24
Masamitsu Kaneko Professor Division of Traumatology & Critical Care Medicine	Dept. of Emergency Medicine	1995. 3. 18 -1995. 3. 31
Satoru Sagae Assistant Professor Dept. of Obstetrics & Gynecology	Dept. of Obstetrics & Gynecology	1996. 2. 10 -1996. 3. 16
Teruhisa Kazui Assistant Professor Dept. of Surgery (II)	Dept. of Thoracic & Cardiac Surgery	1996. 3. 17 -1996. 3. 31
Satoru Sagae Assistant Professor Dept. of Obstetrics & Gynecology	Dept. of Obstetrics & Gynecology	1996. 12. 11 -1997. 1. 17
Noritsugu Tohse Associate Professor Dept. of Physiology (I)	Dept. of Physiology	1997. 2. 5 -1997. 2. 20
Tomio Abe Professor Dept. of Surgery (II)	Division of Cardiothoracic Surgery	1997. 10. 20 -1997. 11. 2
Osamu Honmo Instructor Dept. of Neurosurgery	The Cancer Center	1997. 12. 1 -1997. 12. 20
Gen Murakami Professor Dept. of Anatomy (II)	Division of Cell Biology & Radiology	1998. 11. 9 -1998. 12. 23
Yasushi Itoh Instructor Division of Traumatology & Clitical Care Medicine	Dept. of Emergency Medicine	1999. 3. 15 -1999. 3. 28
Kowichi Jimbow Professor Dept. of Dermatology	Dept. of Medicine	1999. 11. 28 -1999. 12. 4
Ken-ichiro Hirata Assistant Professor Division of Diagnostic Ultrasound & Medical Electronics	Dept. of Surgery	1999. 11. 11 -1999. 12. 29
Masayuki Morikawa Assistant Professor Dept. of Surgery (II)	Dept. of Surgery	2000. 11. 1 -2000. 12. 15
Tomihiro Imai Assistant Professor Division of Neurology	Dept. of Neurology	2001. 3. 18 -2001. 4. 1

VISITING RESEARCH FELLOWS

For the purposes of widening the exchange of scientific research and contributing to the development of scientific techniques, Sapporo Medical University, upon due consideration and deeming it both appropriate and non-obstructive to its professors' research, shall make the appointment of Visiting Research Fellow, if a person belonging to some other research institution should express the desire to do specialized or high level scientific research at this university for a specified length of time.

NUMBER OF FOREIGN VISITING RESEARCH FELLOWS

Fiscal year	1997	1998	1999	2000
	13	14	12	16

INTERNATIONAL CONTRIBUTION

With the hope of improving the health and welfare standards of people around the world, the university participates in various international cooperation projects to help developing countries. As part of the projects, the university has actively sent its researchers and accepted trainees from foreign countries. Many of them are long-term projects, in which many staff in the hospital are involved, such as the Kenya Infection Prevention Project and the establishment of the nursing department of Cairo University in Egypt.

INTERNATIONAL MEDICAL EXCHANGE CENTER OF SAPPORO MEDICAL UNIVERSITY

Sapporo Medical University has an "International Exchange Center" in the campus for foreign scientists, which consists of accommodation (1 twin room & 3 single rooms) a small conference room, a meeting room and an internet-equipped study room. To stay in the Center, reservation must be made through the host department in advance.

Address: South-1, West 18, Chuo-ku, Sapporo JAPAN

Sapporo Medical University

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Hokkaido, 060-8556, Japan