Kyoto University

Graduate School of Pharmaceutical Sciences

Faculty of Pharmaceutical Sciences

2019

Contents

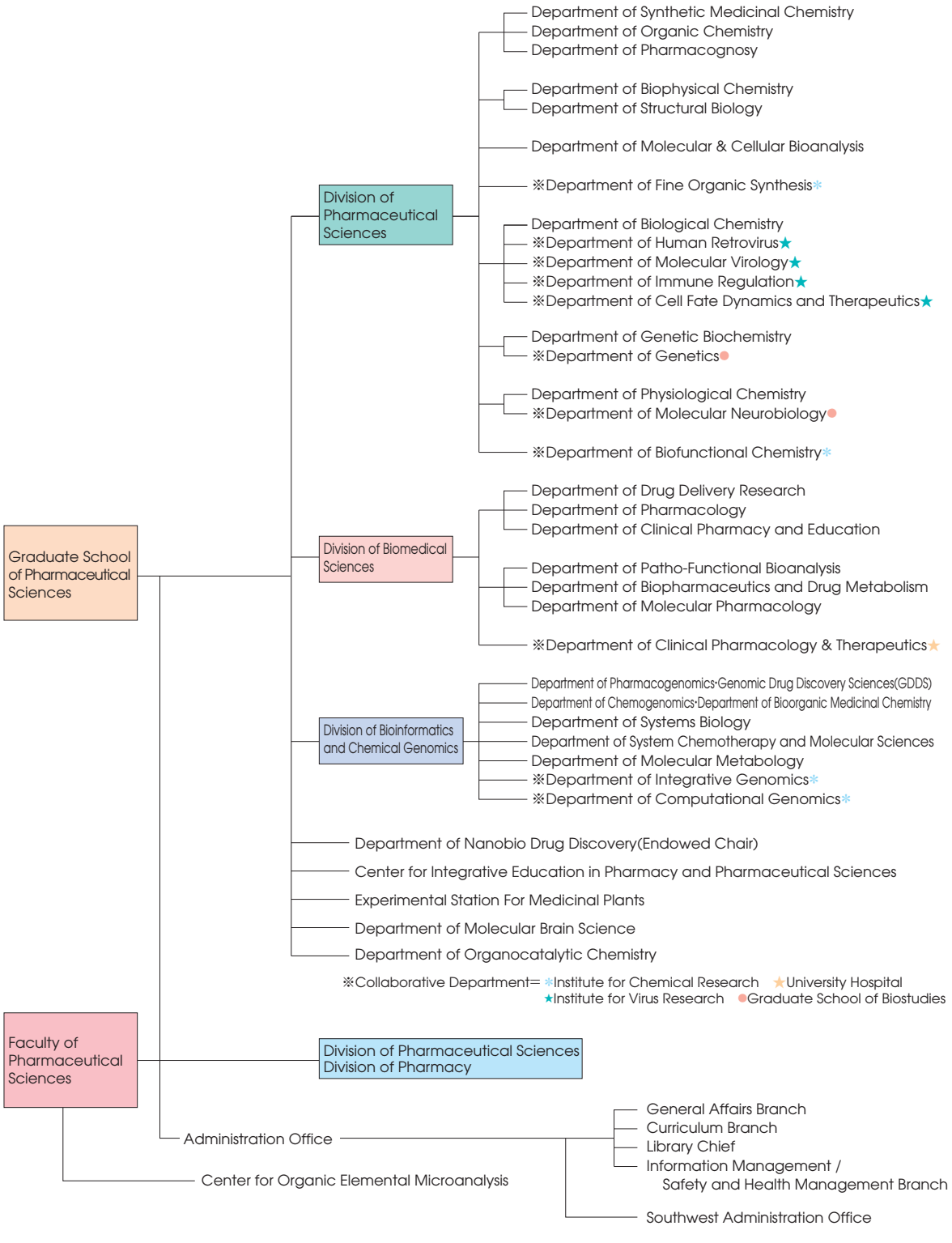
1. History

1939 March: School of Pharmacy established in the Faculty of Medicine, Kyoto University Department of Analytical Chemistry and Department of Synthetic Medical Chemistry established in the School of Pharmacy.
1940 June: Department of Organic Chemistry established.
1940 December: Department of Inorganic Chemistry established.
1941 April: Department of Pharmacognosy established.
1941 December: Doctor of Pharmaceutical Sciences newly added to academic degrees. First graduation ceremony for the School of Pharmacy in the Faculty of Medicine.
1949 May: Kyoto University reorganized under the new educational system introduced by the National School Establishment Law.
1951 April: Department of Pharmaceutics established.
1952 April: Department of Biological Chemistry established.
1953 April: Division of Pharmacy, Kyoto University Graduate School of Pharmaceutical Sciences established.
1954 April: Center for Organic Elemental Microanalysis established in the Faculty of Medicine.
1960 April: Faculty of Pharmaceutical Sciences (Division of Pharmacy) established and the following departments instituted: Analytical Chemistry, Synthetic Medical Chemistry, Organic Chemistry, Inorganic Chemistry, Pharmacognosy, Pharmaceutics, Biological Chemistry. (In accordance with establishing Faculty of Pharmaceutical Sciences, the same seven departments in the School of Pharmacy in the Faculty of Medicine were disestablished.) Center for Organic Elemental Microanalysis attached to the Faculty of Pharmaceutical Sciences.
1961 April: Division of Pharmaceutical Chemistry established. Department of Medicinal Plant Chemistry established.
1962 April: Department of Chemical Pharmacology and Department of Pharmaceutical Engineering established.
1963 April: Department of Physical Chemistry and Department of Hygienic Chemistry established.
1964 April: Department of Radiopharmaceutical Chemistry established.
1965 April: Division of Pharmaceutical Chemistry in the Graduate School of Pharmaceutical Sciences established.
1966 April: Department of Chemical Pharmacology was renamed the Department of Pharmacology, Department of Biological Chemistry renamed Department of Biochemistry.
1973 April: Experimental Station For Medicinal Plants affiliated with the Faculty of Pharmaceutical Sciences established.
1987 May: Department of Pharmaceutical Engineering renamed Department of Microbiology.
1993 April: Master's program in Pharmaceutical Control Systems (independent division) established in the Graduate School of Pharmaceutical Sciences; Pharmaceutical Informatics (transferred from the Department of Inorganic Chemistry in the Division of Pharmacy), Molecular Pharmacology (new) and Genetic Biochemistry (new) established as core departments; and Patho-Functional Bioanalysis, Drug Delivery System, Bioorganic Chemistry (Institute for Chemical Research), Biofunctional Chemistry (Institute for Chemical Research), Clinical Pharmacy (Kyoto University Hospital) established as affiliate departments.
1995 April: Doctoral program in Pharmaceutical Control Systems (independent division) established in the Graduate School of Pharmaceutical Sciences.
1997 April: Focused on the Graduate School, Divisions of Pharmaceutical Sciences, Pharmaceutical Chemistry and Pharmaceutical Control Systems reorganized into 8 major departments within 3 divisions: Drug Discovery Sciences, Pharmaceutical Life Sciences, Pharmacy and Biomedicinal Sciences.Divison of Pharmacy and Division of Pharmaceutical Chemistry in the Faculty of Pharmaceutical Sciences reorganized into one Division of Integrative Pharmaceutical Sciences.
1998 April: Experimental Station For Medicinal Plants affiliated with the Faculty of Pharmaceutical Sciences transferred to the Graduate School of Pharmaceutical Sciences.
1999 April: In accordance with establishment of the Graduate School of Biostudies, Department of Cell Biology and Department of Molecular Neurobiology established.
2002 April: Department of Pharmaceutical Informatics renamed Department of Genomic Drug Discovery Science. Department of Structural Biology established.
2002 October: Construction of new research building for the Graduate School of Pharmaceutical Sciences completed.
2003 April: Endowed chair "Neuroscience for Drug Discovery Research" established. Core Department of Drug Discovery-Medicine Collaborative Pharmaceutical Sciences affiliated with the Graduate School of Pharmaceutical Sciences established.
2003 August: Endowed chair "Theoretical Drug Design" established.
2003 September: In accordance with adoption of the 21st Century COE Program, the Department of Life Knowledge Systems established (Period: duration of implementation period for the 21st Century COE Program).
2004 April: Kyoto University becomes a national university corporation under the National University Corporation Act.
2006 April: Division of Integrative Pharmaceutical Sciences in the Faculty of Pharmaceutical Sciences reorganized into Division of Pharmaceutical Sciences and Division of Pharmacy.Center for Integrative Education of Pharmacy Frontier, affiliated with the Graduate School of Pharmaceutical Sciences, established. (Core Department of Drug Discovery-Medicine Collaborative Pharmaceutical Sciences abolished.) Department of Clinical Pharmacy and Education established.
2007 March: Renovation work on the main building for the Graduate School of Pharmaceutical Sciences completed.
2007 April: Division of Bioinformatics and Chemical Genomics established in the Graduate School of Pharmaceutical Sciences.
2007 May: Endowed chair "Nanobio Drug Discovery" established.
2008 October: Endowed chair "Systems Bioscience for Drug Discovery" established.
2009 April: Institute for Innovative NanoBio Drug Discovery and Development established.
2010 April: World-leading Drug Discovery Research Center established. Center for Development of Integrative Education in Pharmacy and Pharmaceutical Sciences established.
2012 April: Endowed chair "Pharmceutical Policy and Health Economics" established.
2014 May: Moving Experimental Staion For Medicinal Plants.
2017 March: Construction of Med-Pharm Collaboration Building Completed.

2. Chronological Lists of Deans and Directors

Toshihisa, YAMAMOTO	(1960. 4, Acting Director)	Fumiro, YONEDA	(1988. 5 ～ 1990. 4)
Masao, TOMITA	(1960. 5 ～ 1964. 4)	Akira, YOKOYAMA	(1990. 5 ～ 1994. 4)
Shojiro, UEO	(1964. 5 ～ 1968. 4)	Atsushi, ICHIKAWA	(1994. 5 ～ 1996. 4)
Kiichiro, KAKEMI	(1968. 5 ～ 1970. 4)	Masamichi, SATO	(1996. 5 ～ 1998. 4)
Shojiro, UEO	(1970. 5 ～ 1972. 4)	Toshisuke, KAWASAKI	(1998. 5 ～ 2000. 4)
Toyozo, UNO	(1972. 5 ～ 1974. 4)	Terumichi, NAKAGAWA	(2000. 5 ～ 2002. 4)
Yasuo, INUBUSHI	(1974. 5 ～ 1976. 4)	Mitsuru, HASHIDA	(2002. 5 ～ 2006. 3)
Hirouki, INOUE	(1976. 5 ～ 1978. 4)	Kiyoshi, TOMIOKA	(2006. 4 ～ 2007. 12)
Masayuki, NAKAGAKI	(1978. 5 ～ 1980. 4)	Nobutaka, FUJII	(2008. 1 ～ 2008. 9)
Hirohi, TAKAGI	(1980. 5 ～ 1982. 4)	Nobuyuki, ITOH	(2008. 10 ～ 2010. 3)
Haruaki, YAZIMA	(1982. 5 ～ 1984. 4)	Hideo, SAJI	(2010. 4 ～ 2014. 3)
Hisashi, TANAKA	(1984. 5 ～ 1986. 4)	Yoshinobu, TAKAKURA	(2014. 4 ～ 2016. 3)
Hitoshi, SEZAKI	(1986. 5 ～ 1988. 4)	Kazuhisa, NAKAYAMA	(2016. 4 ～)

3. Organization



4. Staff (As of June 1, 2019)

① Administration Officers

·Dean	Kazuhisa NAKAYAMA	·Member of University Council	Hiroaki KATO
·Vice-Dean	Yoshiji TAKEMOTO	·Member of University Council	Hiroshi TAKESHIMA
·Vice-Dean	Hideaki KAKEYA	·Head of Administration Office	Toshio USHIDA

② Present Number of Staffs

Faculty members					Administrative staffs	Technical staffs	Grand Total
Professor	Associate Professor	Lecturer	Assistant Professor	Subtotal			
14	14	7	8	43	7	4	54

③ Academic Staffs and Departments

Division	Department	Professor	Associate Professor	Lecturer	Assistant Professor
Pharmaceutical Sciences	Synthetic Medicinal Chemistry	Kiyosei TAKASU		Hiroshi TAKIKAWA	Yousuke YAMAOKA
	Organic Chemistry	Yoshiji TAKEMOTO		Yusuke KOBAYASHI	Takeshi NANJO
	Pharmacognosy		Michiho ITO		
	Biophysical Chemistry	Katsumi MATSUZAKI	Masaru HOSHINO	Yoshiaki YANO	
	Structural Biology	Hiroaki KATO	Toru NAKATSU		
	Molecular & Cellular Bioanalysis	Yasushi ISHIHAMA	Naoyuki SUGIYAMA		Akiyasu YOSHIZAWA Kosuke OGATA
	Fine Organic Synthesis ❄	Takeo KAWABATA			Yoshihiro UEDA Kazuhiro MORISAKI
	Biological Chemistry	Hiroshi TAKESHIMA	Sho KAKIZAWA		Atsuhiko ICHIMURA
	Human Retrovirus ★			Jun-ichirou YASUNAGA	Kazuya SHIMURA
	Molecular Virology ★	Yoshio KOYANAGI			Yusuke NAKANO Yuki FURUSE
	Immune Regulation ★	Koichi IKUTA			Takahiro HARA Guangwei CUI Keiko TAKEMOTO
	Cell Fate Dynamics and Therapeutics ★	Takahiro ITO			Kenkyo MATSUURA
	Genetic Biochemistry			Ayumi MIYAKE	
	Genetics ●	Tatsushi IGAKI			Masato ENOMOTO Kiichiro TANIGUCHI
	Physiological Chemistry	Kazuhisa NAKAYAMA	Hye-won SHIN		Yohei KATOH
	Molecular Neurobiology ●		Hironori KATOH		
	Biofunctional Chemistry ❄	Shiroh FUTAKI		Miki IMANISHI	Ken-ichi KAWANO
Biomedical Sciences	Drug Delivery Research	(Fumiyoshi YAMASHITA)	Yuriko HIGUCHI		
	Pharmacology	Toshiaki KUME (V)			
	Clinical Pharmacy and Education		Atsushi YONEZAWA		
	Patho-Functional Bioanalysis	Masahiro ONO		Hiroyuki WATANABE	Shimpei IIKUNI
	Biopharmaceutics and Drug Metabolism	Yoshinobu TAKAKURA	Yuki TAKAHASHI		
	Molecular Pharmacology	Shuji KANEKO	Hisashi SHIRAKAWA		Kazuki NAGAYASU
	Clinical Pharmacology & Therapeutics ★	Kazuo MATSUBARA	Takayuki NAKAGAWA	Satoshi IMAI	Shunsaku NAKAGAWA Yuki SATO
Bioinformatics and Chemical Genomics	Pharmacogenomics / Genomic Drug Discovery Sciences(GDDS)		Akira HIRASAWA		
	Chemogenomics / Bioorganic Medicinal Chemistry	Hiroaki OHNO	Shinya OISHI		Shinsuke INUKI
	Systems Biology	Masao DOI		Yoshiaki YAMAGUCHI	Takahito MIYAKE
	System Chemotherapy and Molecular Sciences	Hideaki KAKEYA	Akira HATTORI		Takefumi KURANAGA
	Molecular Metabolology		Jean-Michel Fustin		
	Integrative Genomics ❄	Hiroyuki OGATA			Romain Blanc-Mathieu Hisashi ENDO
	Computational Genomics ❄	Hiroshi MAMITSUKA			Canh Hao Nguyen
Nanobio Drug Discovery (Endowed Chair)		Yutaka SHIMADA (V) Kazuharu SHIMIZU (V) Tetsuo SUDO (V)		Yoshinori TAKEI	
		Shin YONEHARA (V)			
Center for Integrative Education of Pharmacy and Pharmaceutical Sciences	Applied Pharmaceutics and Pharmacokinetics	Fumiyoshi YAMASHITA		Masahiro TSUDA	Kanako SOH Masaya DENDA
Experimental Station For Medicinal Plants		Kazuhisa NAKAYAMA			
Molecular Brain Science		Hitoshi OKAMURA (S)			
Organocatalytic Chemistry		Keiji MARUOKA (S)			

V: Visiting Professor
S: Specially Appointed Professor

5. Students (As of May 1, 2019)

Undergraduate

Year / Division	Capacity	1st			2nd			3rd			4th			5th			6th			Total		
		Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
Not Assigned		54	29	83	44	34	78													98	63	161
Pharmaceutical Sciences	65	(1) 1	(1) 2	(2) 3	(2) 3	4	(2) 7	41	14	55	(1) 49	(1) 15	(2) 64							(4) 94	(2) 35	(6) 129
Pharmacy	15				1	1	2	16	14	30	14	17	31	14	14	28	18	20	38	63	66	129
Total (Foreign Students)		(1) 55	(1) 31	(2) 86	(2) 48	39	(2) 87	57	28	85	(1) 63	(1) 32	(2) 95	14	14	28	18	20	38	(4) 255	(2) 164	(6) 419

	Male	Female	Total
Research Students	3	1	4

	Male	Female	Total
Non-Degree Students	3	0	3

Graduate School

Master's Course

Year / Division	Capacity	1st			2nd			Total		
		Male	Female	Total	Male	Female	Total	Male	Female	Total
Pharmaceutical Sciences	50	(3) 40	(5) 13	(8) 53	(5) 44	(3) 16	(8) 60	(8) 84	(8) 29	(16) 113
Bioinformatics and Chemical Genomics	14	11	3	14	(1) 15	5	(1) 20	(1) 26	8	(1) 34
Total (Foreign Students)		(3) 51	(5) 16	(8) 67	(6) 59	(3) 21	(9) 80	(9) 110	(8) 37	(17) 147

Doctoral Course

Year / Division	Capacity	1st			2nd			3rd			Total		
		Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
Pharmaceutical Sciences	22	(3) 14	(1) 3	(4) 17	(5) 11	(1) 1	(6) 12	(2) 9	(1) 7	(3) 16	(10) 34	(3) 11	(13) 45
Bioinformatics and Chemical Genomics	7	(1) 3	(1) 1	(2) 4	(2) 3		(2) 3	(1) 2	(1) 1	(2) 3	(4) 8	(2) 2	(6) 10
Total (Foreign Students)		(4) 17	(2) 4	(6) 21	(7) 14	(1) 1	(8) 15	(3) 11	(2) 8	(5) 19	(14) 42	(5) 13	(19) 55

	Male	Female	Total
Research Students	0	0	0

	Male	Female	Total
Non-Degree Students	1	0	1

Doctoral Course (4 years)

Year / Division	Capacity	1st			2nd			3rd			4th			Total		
		Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
Pharmacy	15	7	1	8	8	4	12	6	6	12	4	1	5	25	12	37
Total (Foreign Students)		7	1	8	8	4	12	6	6	12	4	1	5	25	12	37

6. Number of Graduates

① Faculty of Pharmaceutical Sciences

Classification		Number
Old System	1941.12 ～ 1953. 3	402
New System		
Faculty of Medicine, Division of Pharmacy	1953. 3 ～ 1960. 3	300
Faculty of Pharmaceutical Sciences	1961. 3 ～ 2019. 3	4,489
Total		5,191

② Master's Degrees Conferred

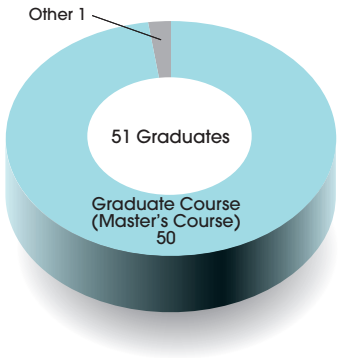
	Number
1955. 3 ～ 2019. 3	2,806

7. Doctorates Conferred

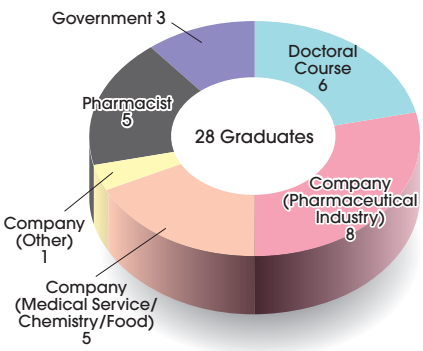
Classification		Number
Old System (including Doctor of Medical Sciences)	1943.10 ～ 1962. 2	308
New System		
Through Graduation from Doctoral Course	1958. 9 ～ 2019. 3	948
Through Submission of Research Papers	1961. 9 ～ 2019. 3	773
Total		2,029

8. Status Post-Graduation (Graduates of 2018 academic year)

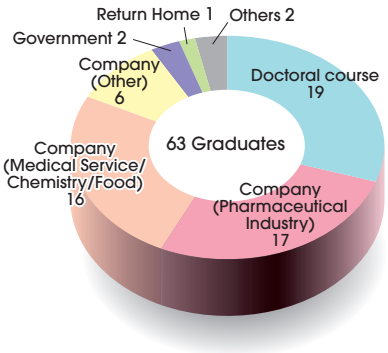
Division of Pharmaceutical Sciences



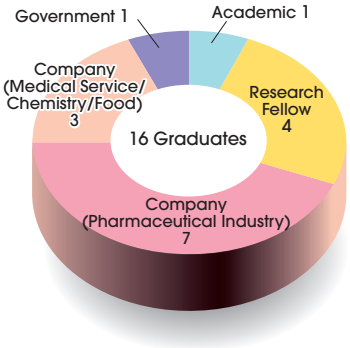
Division of Pharmacy



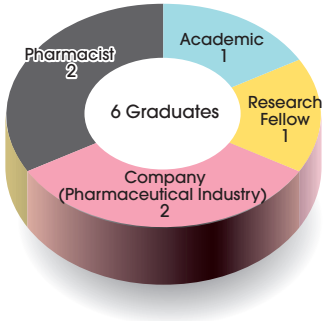
Master's course



Doctoral course (3 years)



Doctoral course (4 years)



9. Books and Journals in the Library (As of April 1, 2019)

Classification	Japanese	Foreign	Total
Books	12,290	22,265	34,555
Periodicals	176	604	780
Electronic journals	about 90,000	(Available In all University staff and students)	

10. Finances*

	Accounts Closing (Fiscal 2018)	Budget (Fiscal 2019) (As of May 1, 2019)
Operating Cost Subsidies		
Personnel Expenses	547,872	
Cost of Supplies	185,855	193,870
Contract Research and Research Cooperation with Industry	306,829	218,587
Donation for Research	127,455	65,000
Grants-in-Aid for Scientific Research	307,568	297,003
Other Grants	23,718	9,840
Total	1,499,297	784,300

(* : Unit: thousand yen)

11. Campus and Buildings (As of May 1, 2019)

	land area	building
Pharmaceutical Sciences Campus	19,339 ㎡	
Main Pharmaceutical Building		9,329 ㎡
Lecture Building		1,056 ㎡
Annex		884 ㎡
New Research Building		5,615 ㎡
Med-Pharm Collaboration Building		11,922 ㎡
Conservatory House		215 ㎡
Experimented Water Drainage Facility		144 ㎡
Flammable Storage Warehouse		40 ㎡
Warehouse		27 ㎡
Total	19,339 ㎡	29,232 ㎡

Research Fields

Division of Pharmaceutical Sciences

Departments Leaders	Research Programs
Synthetic Medicinal Chemistry Professor Kiyosei TAKASU	1. Synthesis of Biologically Active Molecules 2. Development of New Synthetic Methodology for Complex Molecules 3. Molecular Architecture of Unique Organic Materials 4. Chemistry of Strained Molecules 5. Control of Molecular Dynamism
Organic Chemistry Professor Yoshiji TAKEMOTO	1. Design of environmentally friendly organocatalysts for process chemistry 2. Development of new reagents and synthetic methods using unique elements. 3. Total synthesis of biologically important synthetic and natural products 4. Molecular modification using multi-functional heterocycles 5. Innovative catalysts for large- and medium-sized glycopeptides
Pharmacognosy Associate Professor Michiho ITO	1. Molecular cloning of enzymes responsible for biosynthesis of secondary metabolites 2. Pharmacological studies on therapeutic use of incense and balm. 3. Phytochemical analyses of bio-active substances found in medicinal plants 4. Field surveys on medicinal plants for their diversity and sustainable use
Biophysical Chemistry Professor Katsumi MATSUZAKI	1. Elucidation of the action mechanisms of antimicrobial peptides 2. Initiation mechanism of Alzheimer's disease 3. Elucidation of membrane protein folding 4. Regulation of function of G-protein coupled receptors 5. Protein structure determination by NMR
Structural Biology Professor Hiroaki KATO	1. Structural-basis for pharmacologic behaviour of ATP binding Cassette transporters 2. Development of a new technique of X-ray crystallography by X-ray free electron laser
Molecular & Cellular Bioanalysis Professor Yasushi ISHIHAMA	1. Development of novel analytical technologies for proteomics 2. Functional analysis of human proteomes by high resolution LC/MS/MS 3. Elucidation of intracellular phosphorylation network analysis 4. Intelligent proteome analysis by biomolecular mass spectrometry integrated with statistical signal processing 5. Metaproteome analysis of microbiomes in human diseases.
Fine Organic Synthesis Professor Takeo KAWABATA	1. Asymmetric synthesis based on the concept of memory of chirality 2. Organocatalytic site-selective functionalization of carbohydrates 3. Total synthesis of natural glycosides via site-selective functionalization 4. Remote asymmetric induction by organocatalysis 5. Asymmetric synthesis of supramolecules 6. Site-selective C-H functionalization 7. Remote asymmetric induction by C-H functionalization
Biological Chemistry Professor Hiroshi TAKESHIMA	1. Ca^{2+} signaling from intracellular stores 2. Novel signaling in central nervous system 3. Structure and function of muscle membrane systems
Human Retrovirus Lecturer Jun-ichirou YASUNAGA	1. Molecular pathogenesis of human retroviruses (human T-cell leukemia virus type 1 and human immunodeficiency virus) 2. Replication of human retroviruses 3. Development of anti-HIV, and anti-HTLV-1 drugs 4. Development of animal model for human retroviral infections
Molecular Virology Professor Yoshio KOYANAGI	1. Analysis of mechanism of virus infection 2. Analysis of host factors in retroviral replication 3. Analysis of HIV-induced immunodeficiency 4. Development of novel anti-viral therapy
Immune Regulation Professor Koichi IKUTA	1. IL-7 receptor signals for differentiation and maturation of immune cells 2. Control of IL-7 receptor expression during differentiation and response of immune cells 3. Circadian control of dynamics and functions of immune cells by glucocorticoids 4. Visualization and local functions of cytokine-producing cells
Cell Fate Dynamics and Therapeutics Professor Takahiro ITO	1. Regulatory mechanisms of cell fates in stem cells and cancer 2. Metabolic regulation of cancer cell fates in leukemia 3. Cell fate regulation by RNA binding proteins in myogenesis and muscle functions 4. Drug discovery and modulation of cell fates as therapeutic strategies in human malignancies
Genetic Biochemistry Lecturer Ayumi MIYAKE	1. Identification of genes for novel intercellular signaling molecules (growth factors, differentiation factors and hormones) 2. Structure and function of signaling molecules, and regulation of their gene expression 3. Roles of signaling molecules in metabolic regulation 4. Roles of signaling molecules in vertebrate development

Departments Leaders	Research Programs
Genetics Professor Tatsushi IGAKI	1. Mechanism of cell competition 2. Genetic basis of tissue growth regulation 3. Molecular basis of tumor progression and metastasis
Physiological Chemistry Professor Kazuhisa NAKAYAMA	1. Molecular mechanisms of ciliogenesis and protein trafficking in cilia 2. Mechanism of transbilayer lipid asymmetry in biological membranes and cellular functions 3. Regulation mechanism of intracellular protein transport
Molecular Neurobiology Associate Professor Hironori KATOH	1. Signal transduction pathways involved in cancer progression. 2. Regulation of amino acid metabolism in cancer cells.
Biofunctional Chemistry Professor Shiroh FUTAKI	1. Creation of bioactive proteins that control cell function and genes 2. Development of peptide-based intracellular delivery systems for biomacromolecules 3. Design of peptides and proteins that induce membrane curvature 4. Design of artificial transcription factors that regulate gene expression 5. Assembly control of membrane proteins and the regulation of biological signals

Division of Biomedical Sciences

Departments Leaders	Research Programs
Drug Delivery Research Associate Professor Yuriko HIGUCHI	1. Development of novel nano-DDSs for better therapeutic outcomes 2. Association analysis of pharmacokinetics and toxicokinetics of nano-DDSs with their physicochemical properties 3. Research on DDS technology for cell-based medicine
Pharmacology Visiting Professor Toshiaki KUME	1. Elucidation of pathogenesis and exploratory study of preventive and therapeutic agents of neurodegenerative diseases 2. Development of animal models of brain diseases using zebrafish 3. Study on function of nicotinic acetylcholine system in CNS 4. Study on neuroprotective compounds derived from food 5. Study on survival and regeneration of dopaminergic neurons
Clinical Pharmacy and Education Associate Professor Atsushi YONEZAWA	1. Clinical pharmacological research for a personalized treatment with therapeutic antibody drugs. 2. Involvement of renal organic cation transporters involved in the side effects of drugs 3. Identification of novel riboflavin transporter RFVT and pathophysiology of rare diseases BVVLS
Patho-Functional Bioanalysis Professor Masahiro ONO	1. Development of molecular probes for the in vivo analysis of biological function, etiological mechanisms, and action mechanisms of drugs 2. Development of radiopharmaceuticals for functional diagnosis and radionuclide therapy 3. Clarification of the biological actions of trace metals and development of physiologically active metals complexes
Biopharmaceutics and Drug Metabolism Professor Yoshinobu TAKAKURA	1. Development of drug delivery system using exosomes 2. Establishment of immunotherapy based on gene delivery technology 3. Development of delivery systems of proteins and nucleic acid drugs utilizing nucleic acid-based nanostructures 4. Development of multifunctional cell therapeutics for in vivo cell therapy
Molecular Pharmacology Professor Shuji KANEKO	1. Physiology, pathology, molecular mechanisms, pharmacology, ligand screening and genome science with respect to the membrane transport proteins, especially toward TRP channels 2. The roles of neuron-glia-immune cell interaction in CNS pathology and drug action 3. Substantial background of pain and action mechanism of analgesics 4. Molecular and cellular mechanisms of drug actions and aversive effects
Clinical Pharmacology & Therapeutics (University Hospital) Professor Kazuo MATSUBARA	1. Reverse translational research for adverse effects of anti-cancer drugs: elucidation of the mechanisms and development of novel preventive and treatment strategies 2. Clinical and basic studies on Pharmacokinetics and Pharmacodynamics 3. Molecular and neural mechanisms underlying pathological pain and dysesthesia 4. Study of the pathogenic mechanism of Parkinson's disease in order to identify a potential novel cure 5. Application of biomarkers to individualized pharmacotherapy

Division of Bioinformatics and Chemical Genomics

Departments Leaders	Research Programs
Pharmacogenomics ·Genomic Drug Discovery Sciences (GDDS) Associate Professor Akira HIRASAWA	1. Discovery of novel drug target and its validation by integrative genome science 2. In Silico drug discover and design by bioinformatics 3. Ligand fishing of "orphan G-protein-coupled receptors" and structure-function analysis 4. Functional genomic study using transgenic/knockout animals
Chemogenomics and Bioorganic Medicinal Chemistry Professor Hiroaki OHNO	1. Synthesis of structurally complex bioactive compounds 2. Novel methods for the synthesis of complex structures and their applications 3. Identification of functional molecules based on designs, synthetic studies and chemical modifications of biomolecules 4. Development of a novel screening platform using synthetic peptides and proteins 5. Drug screening programs using in-house chemical libraries
Systems Biology Professor Masao DOI	1. Molecular mechanisms of circadian time systems in mammals 2. Circadian brain G-protein coupled receptor signaling for sleep and metabolism 3. Molecular mechanisms of ageing-associated circadian rhythm disorders 4. Translational research on circadian time-associated lifestyle diseases 5. Development of new drugs for tuning circadian time systems
System Chemotherapy and Molecular Sciences Professor Hideaki KAKEYA	1. Advanced chemical biology research for establishing system chemotherapy in order to cure multi-factorial diseases; e.g. cancer, infectious diseases, heart failure, immunodeficiency, diabetes, and neuronal diseases 2. HCS (high-contents screening) and HTS (high throughput screening) for identifying useful small molecules (bioprobes) 3. Natural product chemistry and medicinal chemistry for mining novel bioactive small molecules 4. Biosynthetic studies of natural products and their application to combinatorial biosynthesis
Molecular Metabology Associate Professor Jean-Michel FUSTIN	1. Molecular basis of cross-talk between methyl metabolism and biological rhythms 2. Physiological functions of RNA methylation 3. Roles and regulation of Casein Kinase 1 Delta isoforms
Integrative Genomics Professor Hiroyuki OGATA	1. Genomics of viruses 2. Interactions between microbial communities and their environments 3. Integration of chemical, genomics, and biomedical knowledge for drug discovery and environmental preservation
Computational Genomics Professor Hiroshi MAMITSUKA	1. Bioinformatics by integrative data mining on structured/semi-structured data in life science 2. Developing cutting-edge computer science technology, particularly machine learning and data mining, for drug discovery and molecular-level biological information analysis 3. Machine learning-based systems biology for understanding life phenomena

Center for Integrative Education in Pharmacy and Pharmaceutical Sciences

Departments Leaders	Research Programs
Department of Applied Pharmaceutics and Pharmacokinetics Professor Fumiyoshi YAMASHITA	1. Development of tissue/intracellular targeted drug delivery systems using biomolecular recognition mechanisms 2. Development of pharmacokinetics and toxicity evaluation systems using the microfluidic devices 3. Information analysis of adverse event databases and its application to risk assessment 4. Molecular dynamics and pharmacological analysis of adverse reaction and research on development for prevention and treatment

Endowed Chair

Departments Leaders	Research Programs
Department of Nanobio Drug Discovery Professor Hiroshi Shimada Kazuharu SHIMIZU Tetsuo SUDO	1. Drug Discovery by using miRNA microarray. 2. Research esophageal squamous cell carcinoma, (ESCC) for the molecular target. 3. Development of antibody drugs using tissues and cell lines of ESCC 4. Molecular mechanisms and physiological roles of apoptotic and nonapoptotic cell death 5. Physiological and pathological functions of cell death-related molecules 6. Analyses of embryogenesis, tumorigenesis and immune system from the view point of cell death
Shin Yonehara	7. Molecular mechanisms and physiological roles of apoptotic and nonapoptotic cell death 8. Physiological and pathological functions of cell death-related molecules 9. Analyses of embryogenesis, tumorigenesis and immune system from the view point of cell death

Department of Molecular Brain Science

Departments Leaders	Research Programs
Department of Molecular Brain Science Professor Hitoshi OKAMURA	1. Molecular mechanisms of sleep-wake rhythm indiurnal primates 2. Social entrainment of the primate circadianrhythms 3. Chronometabolism: Molecular analysis of biological timing

Department of Organocatalytic Chemistry

Departments Leaders	Research Programs
Department of Organocatalytic Chemistry Professor Keiji MARUOKA	1. Design of High-Performance Organocatalysts 2. Development of New Organocatalyzed Reactions 3. Design of New Organoradical Catalysts for Organoradical Chemistry 4. New and Efficient Methodologies for Peptide Drug Synthesis

Department of Synthetic Medicinal Chemistry

Professor: Kiyosei Takasu, Lecturer: Hiroshi Takikawa,
Assistant Professor: Yousuke Yamaoka

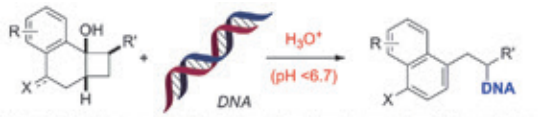
Research Projects:

Generation of new organic molecules is essential to develop new medicines and medical substances. Organic chemists can create novel organic molecules (drug candidates and nano-machines) with chemical reactions. We must think over "What molecules do we design?", "How do we synthesize them?" and "How do we analyze their actions?" Our groups aim to contribute for the life sciences through discovery of new reactions and molecular structures.

1) Development of new synthetic methodology towards rapid molecular construction: A variety of natural and non-natural substances that contain polycyclic rings and an assortment of stereogenic centers have been found to exhibit attractive and specific biological activities. Owing to this, synthetic organic chemists are constantly confronted with the task of developing new reactions that can be used to prepare these complex targets in concise fashions starting from simple and readily available materials. An innovative strategy developed for this purpose relies on the use of highly convergent domino reactions. Major advantages of these, in which multiple covalent bonds are formed in single steps, include operational simplicity, time- and cost-saving, atom economy, environmental benignancy, and applicability to diversity-oriented synthesis and combinatorial chemistry.

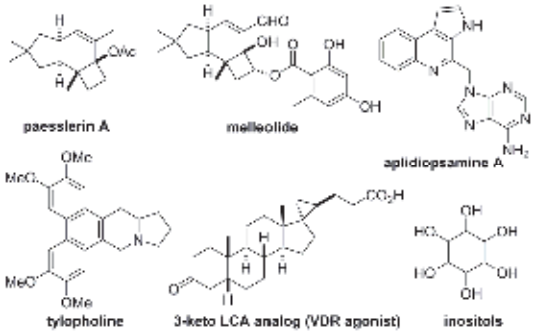
We have explored several classes of domino reactions using anionic, cationic, radical and pericylic chemistry. We recently focus on "tandem catalysis" in domino reactions, in which catalyst(s) promote more than two fundamentally different reactions in a single reactor. We have achieved rapid syntheses of structurally complex molecules including antitumor active natural products and anti-trypanosomal compounds.

2) Design and Synthesis of Artificially Useful Molecules and Materials: When we wish to design artificial biologically active molecules, it is necessary to grasp their dynamic behavior and to imagine their specific interaction with biomolecules. We are now challenging to develop original biofunctional molecules based on fine organic chemistry. Recently, we developed low-pH sensitive DNA cleaving agents based on originally developed organic reactions.

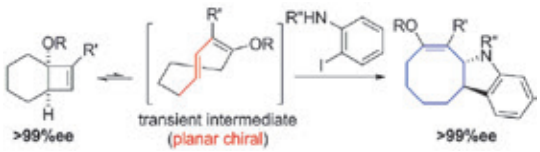


Plasmid DNAs are nicked by the molecule under weak acidic condition

3) Total Synthesis of Biologically Active Compounds: Synthesis of natural products needs comprehensive power of organic chemistry, including knowledge of a variety of organic reactions, reaction mechanism as well as structural organic chemistry. We continuously concentrate on the synthesis of natural products possessing novel chemical structure as well as potent and/or unique biological activities.



4) New Frontier of Strained Molecules: Small and medium-sized cyclic molecules show unique three-dimensional structure, unique chemical reactivity and biological activity owing to their ring strain. However, the chemistry has not been sufficiently explored. We are investigating the methodology for the synthesis of the strained molecules and molecular transformation into organic materials showing unique characters.



Recent publications

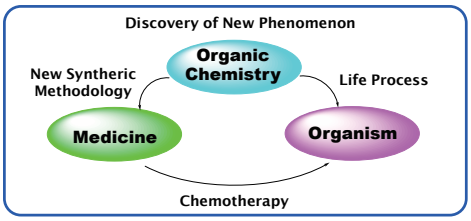
- Mogi, Y.; Inanaga, K.; Tokuyama, H.; Ihara, M.; Yamaoka, Y.; Yamada, K.; Takasu, K Rapid Assembly of Protoilludane Skeleton through Tandem Catalysis; Total Synthesis of Paesslerin A and Its Structural Revision. *Org. Lett.* **2019**, *21*, 3954–3958.
- Ogawa, N.; Yamaoka, Y.; Takikawa, H.; Tsubaki, K.; Takasu, K. Synthesis and Properties of Tribenzocarbazoles via an Acid-Promoted Retro (2+2)-Cycloaddition of Azapropellanes. *J. Org. Chem.* **2018**, *83*, 7994–8002.
- Ogawa, N.; Yamaoka, Y.; Yamada, K.; Takasu, K. Synthesis of π-Extended Fluoranthenes via a KHMDS-Promoted Anion and Radical Reaction Cascade. *Org. Lett.* **2017**, *19*, 3327–3330.
- Kuroda, Y.; Harada, S.; Oonishi, A.; Kiyama, H.; Yamaoka, Y.; Yamada, K.; Takasu, K. Use of a Catalytic Chiral Leaving Group for Asymmetric Substitutions at sp3-Hybridized Carbon Atoms: Kinetic Resolution of β-Amino Alcohols by *p*-Methoxybenzylation. *Angew. Chem. Int. Ed.* **2016**, *55*, 13137–13141.

Department of Organic Chemistry

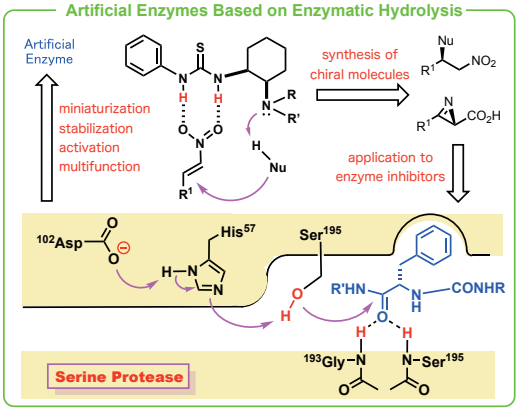
Professor: Yoshiji Takemoto, Lecturer: Yusuke Kobayashi,
Assistant Professor: Takeshi Nanjo

Research Projects:

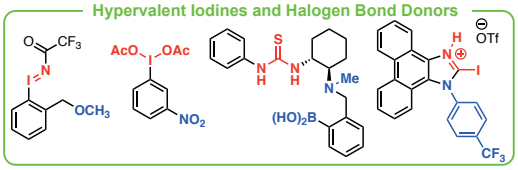
The aims of organic chemistry are structure analysis, reactivity studies, and the synthesis of organic molecules. The importance of organic chemistry in pharmaceutical sciences is clearly illustrated by the following facts: medicines are organic molecules that regulate life processes to cure diseases, and the targeted life processes are composed of organic reactions. With these properties in mind, our research programs are directed to the efficient construction of bioactive molecules and the development of new methodologies for the investigation of biological processes by utilizing these organic molecules.



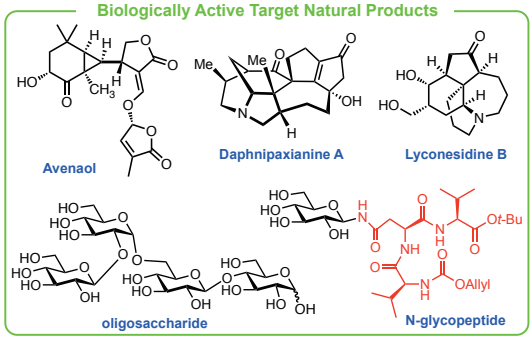
1) Development of Artificial Enzymes and Their Applications: Is it possible to create organic molecules capable of catalyzing reactions in place of enzymes? This was the starting point of our journey to the development of artificial enzymes, so-called organocatalysts. A close investigation of enzymes, such as serine protease, gave us an idea to design a small molecule, which possesses a hydrogen bonding site together with a basic amino group. After screening, a series of bifunctional thioureas were found to catalyze a wide range of stereoselective transformations. Asymmetric total syntheses using the thioureas have been achieved. We now have a broad catalyst library for a wide range of asymmetric reactions.



2) New Reagents and Catalysts for Glycopeptides: Chemical reactions without catalysts or promotors have great potential for the late-stage functionalization of bioactive compounds and prodrug synthesis. We designed *N*-acyliminoiodinanes activated by photoirradiation at 370 nm to allow a series of amination reactions with various electron-rich alkenes and alkynes. For example, the reaction with silyl enol ethers gave α-aminoketone derivatives in good yields. Furthermore, we found that α-ketoacids were converted to reactive acylating agents with hypervalent iodine(III) species, and established a novel decarboxylative acylation of amino acids and alcohols into peptides and esters without producing toxic wastes.



3) Total Synthesis of Natural Products: Natural products found in nature are expected to be the seeds of medicine in the future. We are engaged in the total synthesis of highly functionalized complex natural products in order to find the best way to rapidly synthesize an unprecedented structure. We thought that transition metals would have a key to finding an efficient solution. We have accomplished the total synthesis of avenaol using Rh and Ir catalysts. To challenge complex glycoproteins such as branched oligosaccharides and N-glycosyl peptides, new boron-hybrid catalysts and halogen bond donor catalysts have been developed. We aim to discover medicinal seeds by achieving rapid and gram-scale synthesis of valuable molecules.



Recent publications

- Hayama, N.; Kuramoto, R.; Földes, T.; Nishibayashi, K.; Kobayashi, Y.; Pápai, I.; Takemoto, Y. *J. Am. Chem. Soc.*, **2018**, 140 (38), 12216-12225.
- Nanjo, T.; Kato, N.; Takemoto, Y. *Org. Lett.*, **2018**, 20 (18), 5766-5769.
- Kobayashi, Y.; Nakatsuji, Y.; Li, S.; Tsuzuki, S.; Takemoto, Y. *Angew. Chem. Int. Ed.* **2018**, *57(14)*, 3646-3650.
- Kobayashi, Y.; Masakado, S.; Takemoto, Y. *Angew. Chem. Int. Ed.* **2018**, *57(3)*, 693-697.

Department of Pharmacognosy

Associate Professor: Michiho Ito

Research Projects:

We human beings have a long history of using various natural resources as plants and animals for curing disease and wounds. Natural medicines selected among those trials have been handed down to this century and still being used in our daily life. Also numerous pharmaceutics have been developed from compounds of natural products which were isolated from plants and microbes. However, natural medicines still include mysteries to be uncovered and potentials for creation of another pharmaceutics. Our studies on these mysteries and potentials are performed based on fieldworks and the following combination of lab-works.

1) Therapeutic use of incense and balm: "Kho-Doh", an incense ceremony, is one of the most elegant and traditional culture of Japanese; a small piece of agarwood of the highest quality is heated on a thin mica plate above charcoal, and a subtle fragrant arise from the piece is breathed in for tasting. Recent pharmacological studies indicate that the fragrant of agarwood might be a potent sedative, and which was experimentally exhibited using our new assay system. Further analyses on the active compounds and their mode of actions are under performance in the lab. Fragrant natural medicines other than agarwood, such as patchouli and spikenard that are often found in ingredients of Japanese sachet, are also analyzed for their potentials for therapeutic use.

2) Biosynthetic enzymes of secondary metabolism in medicinal plants: A large number of medicinal natural products are categorized as secondary metabolites, which differ from primary metabolites and are unique to plants. Among these we are focusing on fragrant volatiles which are mainly found in essential oil and resin of plants. Biosynthetic pathways and enzymes committed to them are studied through a combination of molecular biological techniques and conventional genetics. Agarwood, which was already mentioned in 1), and perilla, a common Labiatae kitchen herb, are materials of recent topics.

3) Fieldwork: In order to understand mechanisms and functions of secondary metabolism in plants, it is essential for researchers to know and experience the target with their own five senses, we suppose. Therefore, we perform field surveys (= fieldworks) and cultivate plant materials (= works) in our experimental station (= field); collecting experimental materials is certainly an object of the fieldworks, however, new ideas might be generated as results of watching and touching the target in the fieldworks. An interview to old healer in village is a common means for collecting information of folk medicines, which sometimes seems unlikely to the pharmaceutical sciences; how one could make mutual understanding with interviewee would be a key for these interviews. Our recent field is Indochina (Viet Nam, Lao PDR, Thailand, etc.) for pursuing agarwood, perilla, and unknown folk medicines.

4) Regulatory sciences on natural medicines (crude drugs): Medicinal plants and other natural materials are used as Kampo medicines and ingredients of different natural medicines. Many of these are used not only as pharmaceuticals but also as spices and materials for health foods; they have both natures of medicines and foods. A natural medicine that has different names in different countries may make troubles when it is traded internationally; it may be used in a wrong way to evoke unexpected side effects. In order not to happen the negative events in use of natural medicines, and to secure the safe use of natural medicines and their products, proper identification methods and other techniques and knowledge that will be useful for regulation on natural medicines are required. These knowledge and techniques are another tagets for our studies.

Recent publications

- Miho Hirai, Michiho Ito, Sedative effects of the essential oil and headspace air of Ocimum basilicum by inhalation in mice. J. Natural Medicines, **73**:283-288 (2019).
- Sakura Takamatsu, Michiho Ito, Agarotetrol: a source compound for low molecular weight aromatic compounds from agarwood heating. J. Natural Medicines, **72**, 537-541 (2018).
- Yumi Fujiwara, Michiho Ito, Molecular cloning and characterization of a Perilla frutescens cytochrome P450 enzyme that catalyzes the later steps of perillaldehyde biosynthesis. Phytochemistry, **134**, 26-37 (2017).

Department of Biophysical Chemistry

Professor: Katsumi Matsuzaki, Associate Professor: Masaru Hoshino,
Lecturer: Yoshiaki Yano

Research Projects:

Biomembranes, which play important roles in cell functions, can be considered as supramolecular complexes composed of proteins (such as receptors and ion channels), diverse lipids, and oligosaccharides attached to proteins and lipids. Therefore, to elucidate the structures and functions of biomembranes, understanding of protein-lipid interactions is essential. Our current research projects are listed below.

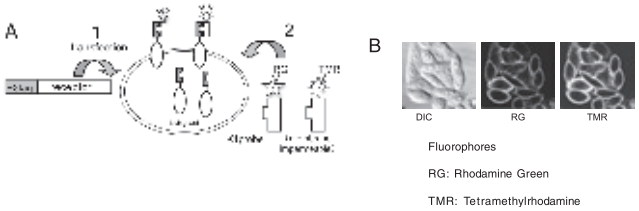
1) Elucidation of the action mechanisms of antimicrobal peptides: Antimicrobial peptides, which play an important role in innate immunity, have been isolated from many living species including human for 20 years. Shortly after the discovery of magainin 2, the first antimicrobial peptide from vertebrates, our laboratory started studying the action mechanism of antimicrobial peptides, such as magainin 2 and tachyplesin 1. We revealed for the first time that these peptides bound to bacterial membranes selectively, followed by forming dynamic peptide · lipid supramolecular-complex pores that allow mutually coupled transmembrane transport of ions, lipids, and peptides themselves. We are currently designing hybrid peptides and macromolecule-attached peptides to develop novel therapeutic agents.

2) Alzheimer's disease: The conversion of soluble, nontoxic amyloid beta peptide (Aβ) to aggregated, toxic Aβ is considered to be the key step in the pathogenesis of Alzheimer's disease. However, the mechanism of the aggregation remains unknown. It has been shown that, in Alzheimer's disease brain, Aβ is bound to the glycosphingolipid GM1-ganglioside (GM1). We have focused on microdomains in plasma membranes, called 'lipid rafts', which are mainly composed of cholesterol and sphingolipids including GM1, and revealed that Aβ specifically recognizes a ganglioside cluster, the formation of which is facilitated by cholesterol in raft-like liposomes, then undergoes a conformational transition to a β-sheet-rich structure, and the conformationally altered form of Aβ serves as a seed for the aggregation of the protein. Based on these findings, we have proposed "GM1-mediated Aβ accumulation model". In recent studies, we fluorescently visualized time- and concentration-dependent accumulation of Aβ on living cell membranes for the first time.

3) Elucidation of membrane protein folding: The folding principles of membrane proteins should be quite different from those of water-soluble proteins. However, experimental examination of the folding of membrane proteins is rather challenging due to their poor solubility and the difficulty in their isolation and purification. Our strategy is to elucidate thermodynamic parameters for forces that generally drive the folding of membrane proteins (e.g., van der Waals, H-bond, and ionic interactions) by using model transmembrane helices (folding units of membrane proteins) in the context of helix-lipid and helix-helix interactions.

4) Regulation of function of G-protein coupled receptors: We are developing new methods to control functions of GPCRs, which are important drug targets. We recently developed a labeling method named 'coiled-coil tag-probe labeling system' to quickly label cell-surface receptors in living cells with synthetic fluorescent probes, enabling easy and sensitive detection of receptor internalization after agonist stimulation. We are currently using this technology to elucidate and control complex behaviors of GPCRs in living cell membranes.

5) Protein structure determination by NMR: High-resolution NMR spectroscopy is established as a fundamental tool for the determination of detailed three dimensional structures of biomolecules such as proteins and nucleic acids in solution. This technique provides us detailed information about not only static but also dynamic nature of proteins, including protein folding, conformational change upon ligand binding at amino acid residue resolution. We are investigating the folding process of proteins and model peptides by using high-resolution NMR. We are also developing a novel method to analyze highly aggregative proteins to which current NMR is not applicable.



Coiled-coil labeling method.
(A) Labeling principle. (B) Confocal images for RG-K4 and TMR-K4 aquired 5 min after incubation with CHO cells expressing E3-β2 adrenoceptors.

Recent publications

- Okada et al. Toxic amyloid tape: A novel mixed antiparallel/parallel β-sheet structure formed by Aβ on GM1 clusters. ACS Chem. *Neurosci.*, **10**, 563 (2019)
- Nakamura et al. High performance plasma amyloid-β biomarkers for Alzheimer's disease. *Nature* **554,** 249 (2018)
- Yano et al. GXXXG-mediated parallel and antiparallel dimerization of transmembrane helices and its inhibition by cholesterol: Single-pair FRET and 2D IR studies. Angew. Chem. *Int. Ed. Engl.* **56**, 1756 (2017)

Department of Structural Biology

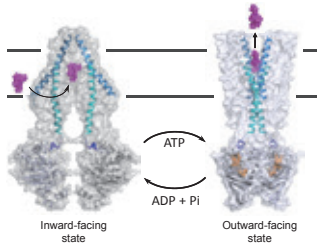
Professor: Hiroaki Kato, Associate Professor: Toru Nakatsu

Research Projects:

Three-dimensional structure determination of biological macromolecules is the most powerful way to elucidate the molecular mechanisms underlying biological phenomena. However, owing to the distinct structural changes of the molecules during their action, static views of those structures are not enough to explain their mechanisms. General objectives of this department are to understand the mechanisms of protein molecules based on dynamic motion involved in their functions. We aim to capture the structural changes of molecular machinery at atomic resolution by X-ray crystallography and to develop new crystallographic techniques that acquire time-resolved dynamic structural changes of proteins in action. Our current research subjects are listed below.

1) Structural-basis for pharmacologic behavior of ATP binding Cassette transporters: ATP Binding Cassette (ABC) transporters comprise a family of structurally related membrane proteins sharing well-conserved nucleotide binding domains. They commonly use ATP hydrolysis as an energy source for transport of the substrate across the lipid bilayer membrane. Membrane protein transporters generally function by the alternating access model of transport in which the substrate binding site alternately faces either side of the membrane through conformational changes between an inward- and outward-facing state. One of the most famous and pharmacologically important ABC transporter is P-glycoprotein, also called ABCB1 or MDR1. It is a multi-drug transporter that not only plays essential roles in normal physiology by protecting tissues from various toxic xenobiotics and endogenous metabolites but also contributes to multidrug resistance (MDR) in tumors, a major obstacle to effective chemotherapeutic treatment. Understanding the mechanism of the multidrug transport is crucial for designing drugs of good bioavailability and efficient cancer chemotherapy. Because of low thermal stability and low crystallizability of human P-glycoprotein (hP-gp), we searched for ABC transporters closely resembling hP-gp in the genome of *Cyanidioschyzon merolae*, a thermophilic unicellular eukaryote and found CmABCB1 whose amino acid sequence, multidrug specificity, and kinetics of ATP hydrolysis are the most similar to those of hP-gp. We have determined a high-resolution crystal structure of CmABCB1 at an inward-facing conformation and elucidated its gating mechanism during the substrate transport. In addition, we have discovered a novel inhibitor, which disables the diverging outward motions of the trans-membrane helices by clamping them from the outside of the transporter, and the mode of action of the inhibitor supports our proposed gating mechanism. Recently, we have succeeded in determining both inward- and outward-facing structures of the same molecule of P-gp molecule for the first time. These structures will allow us to explain the pharmacologic behavior of ABC transporters, especially to solve the ambivalent issue in controlling multi-drug transporters. We aim to determine the dynamic structures between the inward- and outward-facing conformations by fluorescence and NMR spectroscopies and kinetic (time-resolved) X-ray crystallographic techniques.

2) Development of new techniques for Structural analysis using X-ray free electron laser: The biggest issues in X-ray crystallography are necessity of large and good quality crystals, and radiation damage that hampers accurate structure determination. Thus, their overcome is the most important research subject for the current crystallographers. To overcome these issues, a new light source has emerged in the form of the X-ray free electron laser (XFEL), with improvements upon many of the properties of synchrotron radiation sources. The SPring-8 Angstrom Compact free electron LAser (SACLA) in Japan generates X-rays a billion times brighter than a third generation synchrotron, SPring-8. The extremely bright XFEL pulses enable data collection using micrometer-sized microcrystals without radiation damage at room temperature through the diffraction-before-destruction principle. We have been involved in the development of serial femtosecond crystallography (SFX) measurement systems using XFEL at the SACLA. We have succeeded in experimental phasing that enables *de novo* structure determination by SFX. We are planning to advance SFX development that can revolutionize the way in which we study matter at the atomic and molecular level. We are expecting to capture atomic resolution snapshots on the ultrafast timescale associated with the intrinsic atomic motions of proteins in action.



Inward-facing (left) and outward-facing (right) structures of CmABCB1.
The outward-facing structure is complexed with ATP analog, AMP-PNP (orange). The trans-membrane helix, TM1s (cyan) and TM3s (blue) are drawn with ribbons. The ATP binding is proposed to connect two cytosolic nucleotide binding domains (NBDs) and its structural change of NBDs drives conformational changes of the transmembrane helices. These conformational changes reduce the volume of an inner-chamber where the substrate binds at the inward-facing state, thus squeezes out the substrate from the inner chamber into the extracellular space at the outward-facing state.

Recent publications

- Kodan *et al.* Inward- and outward-facing X-ray crystal structures of homodimeric P-glycoprotein CmABCB1. *Nat Commun*, **10**(1), 88, 2019.
- Yamashita *et al.* Experimental phase determination with selenomethionine or mercury- derivatization in serial femtosecond crystallography. *IUCrJ*, **4**, 639, 2017.
- Yamashita *et al.* An isomorphous replacement method for efficient de novo phasing for serial femtosecond crystallography. *Sci Rep*, **5**, 14017, 2015.
- Kodan *et al.* Structural basis for gating mechanisms of a eukaryotic P-glycoprotein homolog. *Proc Natl Acad Sci USA*, **111**, 4049, 2014.

Department of Molecular & Cellular Bioanalysis

Professor: Yasushi Ishihama,
Associate Professor: Naoyuki Sugiyama,
Assistant Professor: Akiyasu Yoshizawa, Kosuke Ogata

Research Projects:

We have advocated elucidating the cellular functions through the measurement of biomolecules based on analytical chemistry. In particular, we have focused on proteome science consisting of mass spectrometry, nano-separation science, computational science and cell biology to develop the methodologies for the functional analysis of cells. More specifically, we are conducting research on the following five topics;

1) Development of novel analytical technologies for proteomics
2) Functional analysis of human proteomes by high resolution LC/MS/MS
3) Elucidation of intracellular phosphorylation network analysis
4) Intelligent proteome analysis by biomolecular mass spectrometry integrated with statistical signal processing
5) Metaproteome analysis of microbiomes in human diseases

Unlike genomic and transcriptomic researches, proteomics is still immature in terms of the measurement technologies and the complete analysis of proteome has not been established yet. The final goals of proteomics are to uncover the cellular protein events such as (1) protein expression/degradation, (2) protein localization, (3) protein interaction, (4) protein post-translational modifications (PTM) and (5) protein processing/splicing in proteome-wide.

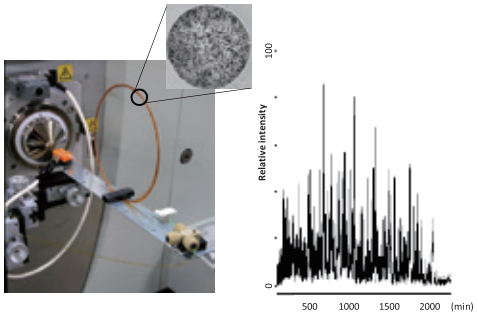


Figure 1 Complete proteome analysis by nanLC-MS.
(Left) nanoLC-MS system with 3.5 meter monolithic silica column. (Right) Total ion current chromatogram of E. coli proteome. Analysis on a microarray scale was achieved

We are aiming to develop novel approaches to tackle the technical barriers and to explore proteomic researches for clarifying the biological problems. In order to analyze the entire proteome expressed in cells, we are focusing on developing efficient separation systems based on nanoLC-MS using meter-long monolithic silica capillary columns with the world's highest performance beyond theoretical plate number 1,000,000. So far, our systems allowed to expand the measurable dynamic range of highly complex proteomics samples, achieving the analysis of Escherichia coli expressed proteome on a microarray scale (see Figure1). This system is currently applied to more complex proteome such as human.

We are also developing new technologies for quantitative proteomics by integrating intelligent statistical and bioinformatics approaches. In parallel, we built a public repository and database for proteomics datasets named jPOST and are collecting a variety of datasets acquired in world-wide, which are re-analyzed by the standardized approach and are shared with researchers according to open science policy.

In cellular signal transduction network, reversible phosphorylation is one of the key events to transduce the signal into nucleus to control the gene expression. Approximately 90% of human proteins were estimated to be phosphorylated. We have developed a highly selective enrichment method for phosphopeptides and applied to proteome-wide acquisition of cellular phosphorylation status. Currently we are working to intertwine the kinases with their substrates for revealing the whole picture of signaling network by using experimental and computational approaches.

Our proteomics system has been also employed to carry out metaproteome analysis of microbiomes in human gut, faces and oral environments to study several human diseases through the interaction between the bacteria and the host.

Recent publications

- Moriya et al., The jPOST environment: an integrated proteomics data repository and database. *Nucleic Acids Res.* **47** (D1), D1218-24, 2019.
- Tsai et al., Large-scale determination of absolute phosphorylation stoichiometries in human cells by motiftargeting quantitative proteomics. *Nat. Commun.*, **6**, 6622, 2015.
- Yamana et al., Rapid and deep profiling of human induced pluripotent stem cell proteome by one-shot nanoLC-MS/MS analysis with meter-scale monolithic silica columns. *J. Proteome Res.* **12**, 214-21, 2013.
- Imami et al., Temporal profiling of lapatinib-suppressed phosphorylation signals in EGFR/HER2 pathways. *Mol. Cell. Proteomics* **11**, 1741-57, 2012.

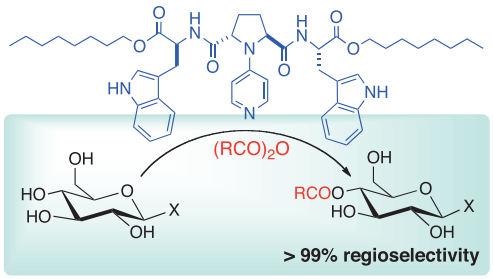
Department of Fine Organic Synthesis

Professor: Takeo Kawabata,
Assistant Professor: Yoshihiro Ueda, Kazuhiro Morisaki

Research Projects:

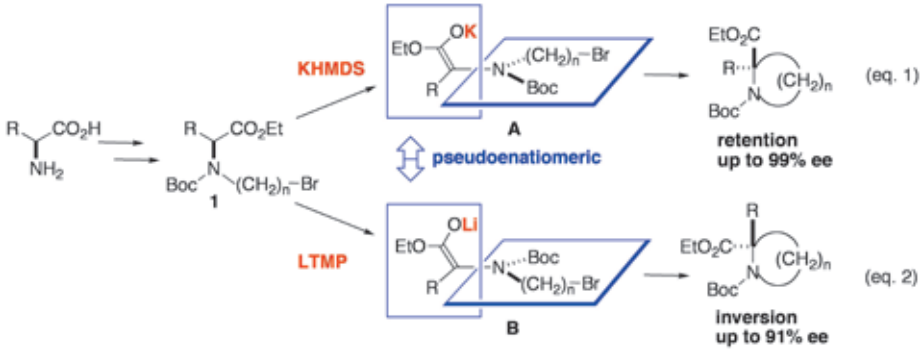
The research interests of the laboratory include the development of advanced molecular transformation, total synthesis of biologically active products, and molecular recognition. Programs are active in the areas of asymmetric alkylation of carbonyl compounds based on "memory of chirality", nucleophilic catalysis for fine organic syntheses, synthesis of unusual amino acids and nitrogen heterocycles, creation of axially chiral compounds with an inner hydrogen bond, synthesis and properties of homochiral oligonaphthalenes, and the structural and functional investigation of heterochiral oligomers. Current research topics are shown below.

1) Regioselective Acylation of Carbohydrates by Nucleophilic Catalysis: Direct regioselective functionalization of multi-functionalized substrates is one of the goals of current research toward the development of advanced molecular transformation for the next generation. We have developed a highly regioselective acylation of carbohydrates with chiral nucleophilic catalysts. Treatment of a glucose derivative with 1 mol% of a catalyst and 1.1 mol eq. of isobutyric anhydride in chloroform at -20 °C gave the 4-acylated glucose derivative and the 3-acylated surrogate in a 99:1 ratio in 98% yield. The corresponding 6-isomer, 2-isomer, and the di-acylated isomers were not detected at all. The corresponding reaction with 10 mol% of 4-dimethylaminopyridine proceeded in a random way, giving 6-, 4-, 3-, and 2-isomers in a ratio of 38:23:38:1 in a combined yield of 69% together with 19% of the di-acylated isomers and 10% recovery. Thus, discrimination of four hydroxyl groups of the glucose derivative has been achieved by the catalyst via dynamic molecular recognition.



2) Stereochemical Diversity in Asymmetric Cyclization via Memory of Chirality

N-(ω-bromoalkyl)-α-amino acid derivatives **1**, readily prepared from L-α-amino acids, gave cyclic amino acids with a tetrasubstituted carbon center by the treatment with KHMDS in DMF. Chirality of the parent amino acids was almost completely preserved during an enolate-formation and cyclization process, giving aza-cyclic amino acids in up to 99% ee with retention of configuration (eq. 1). Mechanistic investigation indicated that the asymmetric cyclization proceeds via an axially chiral enolate intermediate **A**. On the other hand, generation of the pseudoenantiomeric enolate **B** was accomplished simply by changing the conditions for enolate-formation. As the consequence, treatment of **1** with lithium 2,2,6,6-tetramethylpiperidide (LTMP) in THF gave cyclic amino acids with inversion of configuration in up to 91% ee. Thus, both enantiomers of cyclic amino acids with a tetrasubstituted carbon center were prepared in high enantiomeric purity from readily available L-α-amino acids.



Recent publications

- Kawabata, T. *et. al.*, Total Synthesis of Ellagitannins via Regioselective Sequential Functionalization of Unprotected Glucose. *Angew. Chem. Int. Ed.* **2015**, *54*, 6177-6180.
- Kawabata, T. *et. al.*, Asymmetric Induction via Short-Lived Chiral Enolates with a Chiral C-O Axis. *J. Am. Chem. Soc.* **2013**, *135*, 7102-7105.
- Kawabata, T. *et. al.*, Chemoselective Oxidation by Electronically Tuned Nitroxyl Radical Catalysts. *Angew. Chem. Int. Ed.* **2013**, *52*, 8093-8097.
- Kawabata, T. *et. al.*, Asymmetric α-Arylation of Amino Acid Derivatives by Clayden Rearrangement of Ester Enolates via Memory of Chirality. *J. Am. Chem. Soc.* **2013**, *135*, 13294-13297.

Department of Biological Chemistry

Professor: Hiroshi Takeshima, Associate Professor: Sho Kakizawa,
Assistant Professor: Atsuhiko Ichimura

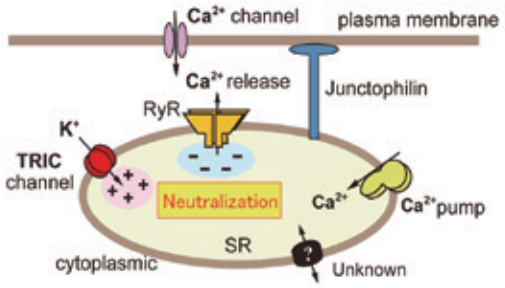
Research Projects:

In living organism, biomacromolecules physically and functionally interact with each other and induce chemical reactions to establish flexible life processes. We are dealing with biochemical and gene-handling studies on membrane proteins toward unlocking the molecular basis of life. Our efforts contribute to not only the global progress of basic biology, but also the healthcare field, for example, identifying reliable target proteins for pharmaceutical development and locating mutations in human genetic diseases. Our current research projects are listed below.

1) Ca^{2+} signaling from intracellular stores: Intracellular Ca^{2+} signaling is triggered by Ca^{2+} influx and Ca^{2+} release for the physiological regulation of a wide variety of cellular functions. In excitable cells, machinery for Ca^{2+} release from the endo/sarcoplasmic reticulum (ER/SR) is well organized and is essential for regulating muscle contraction and neural excitability. We are focusing on the Ca^{2+} release mechanism and molecular architecture of the ER/SR as intracellular stores. Our current aims are to clarify physiological roles of Ca^{2+} release mediated by ryanodine receptors, sets of ryanodine-sensitive intracellular Ca^{2+} release channels, to define functions of junctophilin contributing to junctional membrane complexes between the plasma membrane and the ER/SR, and to identify novel protein ER/SR components essential for Ca^{2+} store functions. The figure below shows major components in the juctional membrane complex for cardiac excitation-contraction coupling. Our previous studies demonstrated that cardiac Ca^{2+} signaling absolutely requires Ca^{2+} channel, TRIC channel, RyR and JP. Knockout mice lacking the components exhibit heart failure at early embryonic stages. Genetic mutations in Ca^{2+} channel and RyR cause familial cardiac myopathy and arrhythmia, respectively. Moreover, our biochemical analysis identified several membrane proteins with unknown functions in the ER/SR.

2) Novel signaling in central nervous system: Information processing and cellular organization in the central nervous system (CNS) is in mystery. Uncharacterized protein components from the brain indicates the existence of unknown intercellular and intracellular signaling in CNS. Our group identified several receptor-like membrane proteins specifically expressing in the brain, including DNER (delta-notch-type EGF repeat containing protein) and BSRPs (brain-specific receptor-like proteins), and started to survey their roles in brain development and function. Interestingly, both knockout mice lacking DNER and BSPRs show motorcoordination defects and probably share cerebellar dysfunction.

3) Structure and function of muscle membrane systems: There are many strange membrane structures in striated muscle cells, for example the transverse tubule, Z-tubule, triad and diad, junctional SR and longitudinal SR (see textbook for histology). Because molecular mechanisms for such membrane structures are almost unknown, we would like to identify proteins contributing to these membrane structures. Our previous screening identified a series of muscle membrane proteins with unknown functions, namely the "mitsugumin" family. Recent studies found that mitsugumin 29 partially restricts the ultrastructure of the transverse tubule and is involved in physiological and histological defects during muscle aging. Moreover, mitsugumin 53 is involved in repair of membrane damage in striated muscle. Therefore, it is thought that mitsugumin 53 is a responsible gene of muscular dystrophy.



Components for Ca^{2+}-induced Ca^{2+} release (CICR) in cardiac muscle cells. Ca^{2+} influx mediated by Ca^{2+} channels induces channel opening of ryanodine receptors (RyR) and triggers Ca^{2+} release from the sarcoplasmic reticulum (SR). This CICR requires the colocalization of Ca^{2+} channel and junctophilin within junctional membrane complex supported by junctophilin because the loss of the close association between Ca^{2+} channels and RyR disconnects Ca^{2+} effects. TRIC channels are likely to act as counter-ion channels that function in synchronization with Ca^{2+} release from intracellular stores and maintain an efficient Ca^{2+} release. Moreover, unidentified SR protein components might have important roles as channels and Ca^{2+} binding proteins. Our findings are expected as not only development of biochemistry but also the clinical application.

Recent publications

- Qian N et al. TRPM7 channels mediate spontaneous Ca^{2+} fluctuations in growth plate chondrocytes that promote bone development. ***Sci Signal***. Apr 9;12(576). pii: eaaw4847. 2019.
- Zhao C et al. Mice lacking the intracellular cation channel TRIC-B have compromised collagen production and impaired bone mineralization. ***Sci Signal***. May 17;9(428):ra49. 2016.
- Kakizawa S. et al. Nitric oxide-induced calcium release via ryanodine receptors regulates neuronal function. ***EMBO J***. 31, 417-428, 2012.

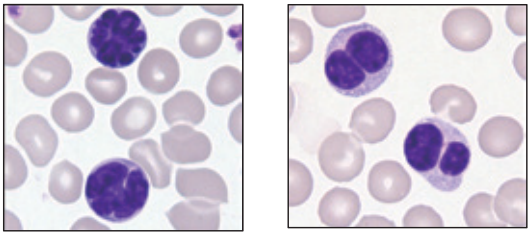
Department of Human Retrovirus

Lecturer: Jun-ichirou Yasunaga,
Assistant Professor: Kazuya Shimura

Research Projects:

Both human T-cell leukemia virus type 1 (HTLV-1) and human immunodeficiency virus (HIV) are pathogenic human retroviruses. HTLV-1 promotes proliferation of $CD4^+$ T cells, which leads to leukemia while HIV destroys $CD4^+$ T cells resulting in onset of acquired immunodeficiency syndrome (AIDS).

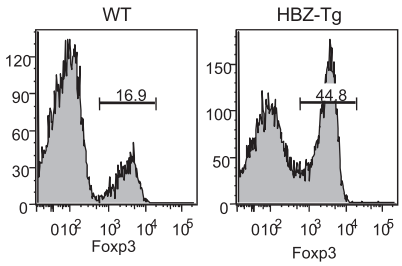
HTLV-1 causes a neoplastic disease, adult T-cell leukemia (ATL), and the inflammatory diseases, such as HTLV-1 associated myelopathy in a small part of the HTLV-1-infected individuals. We found that HTLV-1 bZIP factor (HBZ) gene is expressed in all ATL cells and supports growth of T-cells. In addition, we have established HBZ transgenic mice (HBZ-Tg), and observed that HBZ-Tg developed T-cell lymphomas and systemic inflammatory diseases, suggesting that HBZ is critical in pathogenesis of HTLV-1. Immunological analyses revealed that T-lymphoma tissues in HBZ-Tg frequently expressed Foxp3, a master molecule of regulatory T cell (Treg). Interestingly, the suppressive function of Tregs from HBZ-Tg was impaired compared with non-Tg littermates, suggesting that HBZ expression increases dysfunctional Tregs resulting in malignant transformation and inflammatory disorders *in vivo*. Those phenotypes of HBZ-Tg are very similar to those of HTLV-1 carriers. HBZ is considered to play the important roles in oncogenesis, although the precise mechanism has not been clarified. HBZ modulates various signaling pathways, such as NF-κB, TGF-β, and NFAT signaling pathways. We are analyzing their significances in leukemogenesis of HTLV-1-infected cells.



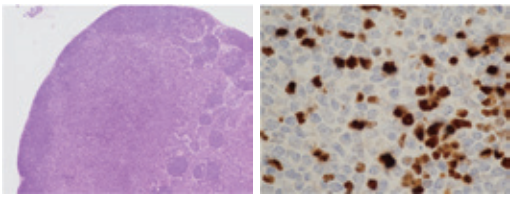
ATL cells have a hyper-segmented nuclei.

AIDS is caused by HIV infection, through the depletion of $CD4^+$ T lymphocytes. In early times, AIDS was feared as one of the most fatal diseases, with a very poor prognosis. However, due to the development of potent anti-HIV drugs, efficient anti-HIV/AIDS therapies were established, and HIV infection has become a relatively controllable chronic viral disease. Nevertheless, complete eradication of the virus from infected patients has not been achieved yet. In order to suppress viral replication and prevent the development of AIDS, lifelong medication with anti-HIV drugs is required. Drug-resistance mutations are often identified even in treatment-naïve HIV-infected patients. To overcome these obstacles, we are developing novel anti-HIV drugs, and studying anti-HIV drug resistance mechanism.

We previously developed fusion inhibitors that block the fusion step between the virus and the host cell, and an integrase inhibitor that interferes with the integration of the viral DNA into the host chromosome. Recently, we focused on quite novel anti-HIV compounds targeting viral replication step(s) other than those targeted by preexisting drugs. Interestingly, these inhibitors show anti-viral activity not only against HIV but also, against other viruses such as hepatitis C virus and human herpes simplex virus. We are now evaluating anti-viral activity *in vivo*.



Regulatory T-cells are increased in HBZ transgenic mouse (HBZ-Tg).



HBZ-Tg develop T-cell lymphomas, which express Foxp3.

Recent publications

- Mahgoub M, Yasunaga JI, Iwami S, Nakaoka S, Koizumi Y, Shimura K, and Matsuoka M. Sporadic on/off switching of HTLV-1 Tax expression is crucial to maintain the whole population of virus-induced leukemic cells. Proc Natl Acad Sci USA, 2018. doi: 10.1073/pnas.1715724115.
- Furuta R, Yasunaga JI, Miura M, Sugata K, Saito A, Akari H, Ueno T, Takenouchi N, Fujisawa JI, Koh KR, Higuchi Y, Mahgoub M, Shimizu M, Matsuda F, Melamed A, Bangham CR, Matsuoka M. Human T-cell leukemia virus type 1 infects multiple lineage hematopoietic cells in vivo. PLoS Pathog, 13(11):e1006722, 2017.
- Kinosada H, Yasunaga JI, Shimura K, Miyazato P, Onishi C, Iyoda T, Inaba K, Matsuoka M. HTLV-1 bZIP Factor Enhances T-Cell Proliferation by Impeding the Suppressive Signaling of Co-inhibitory Receptors. PLoS Pathog, 13(1):e1006120, 2017.
- Sugata K, Yasunaga JI, Kinosada H, Mitobe Y, Furuta R, Mahgoub M, Onishi C, Nakashima K, Ohshima K, Matsuoka M. HTLV-1 Viral Factor HBZ Induces CCR4 to Promote T-cell Migration and Proliferation. Cancer Res, 76:5068-5079, 2016.

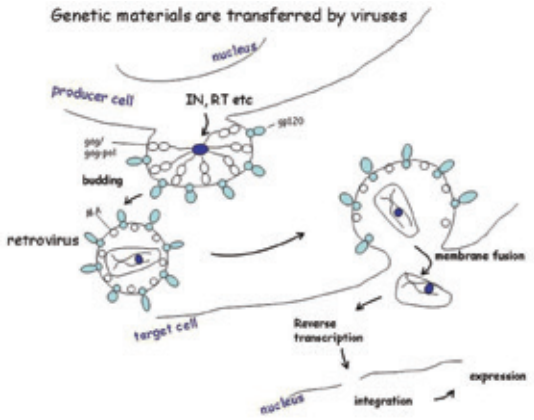
Department of Molecular Virology

Professor: Yoshio Koyanagi,
Assistant Professor: Yusuke Nakano, Yuki Furuse

Research Projects:

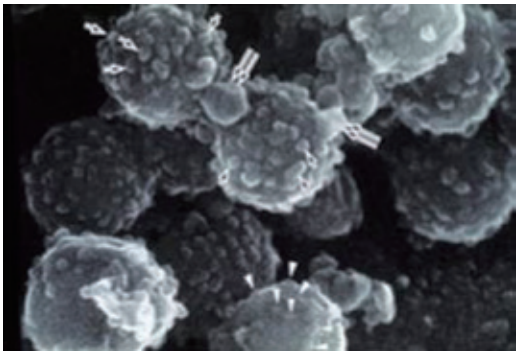
It is clear that virus researches have provided strong advances to Cell Biology. Therefore, we believe that our efforts will contribute to Medical and Pharmaceutical Sciences. Our research themes have been arranged below.

1) How virus infects cell and replicates? Viral genome moves from virion-produced cell to adjacent naive cells (See figure below). This is a most significant characteristic of virus. Elucidation of the mechanism of this infection event is a primary theme.



2) How cellular factors influence viral replication? Virus cannot replicate without cells. Since it has been found that many cellular factors promote or suppress human immunodeficiency virus (HIV) replication, we wish to learn the mechanisms from aspect of Immunology and Virology (See right figure).

3) Why HIV causes immunodeficiency in human? The mechanism of the immunodeficiency remains unclear. We have been analyzing how the immunodeficiency occurs using *in vitro*-cell culture models and *in vivo*-animal models. We developed a mouse system that human immune system is transplanted in SCID mouse and in this human-chimera mouse, abundant CD4 cell killing can be reproduced with HIV infection.



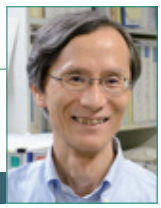
4) Why do we need novel anti-viral therapy? Although development of anti-HIV therapies has been accelerating, treatment for HIV cure has not yet been established. Therefore, we have tried to develop novel strategy for HIV proviral DNA from genome editing technology.

Recent publications

- Sato K, Takeuchi SJ, Misawa N, Izumi T, Kobayashi T, Kimura Y, Iwami S, Takaori-Kondo A, Hu WS, Aihara K, Ito M, An DS, Pathak VK, and Koyanagi Y. APOBECD and APOBECF potently promote HIV-1 diversification and evolution in humanized mice. PLoS Pathog, 10:e1004453, 2014.
- Ebina H, Misawa, Kanemura Y, and Koyanagi Y. Harnessing the CRISPR/Cas9 system to disrupt latent HIV-1 provirus. Sci. Rep. 3 : 2510, 2013.
- Sato, K, Misawa N, Iwami S, Satou Y, Matsuoka M, Ishizaka Y, Ito M, Aihara K, An DS, and Koyanagi Y. HIV-1 Vpr accelerates viral replication during acute infection by exploitation of proliferating $CD4^+$ T cells *in vivo*. PLoS Pathog, 9:e1003812. 2013.

Lab URL : http://www.virus.kyoto-u.ac.jp/Lab/KoyanagiHP/saito/TOP.html

Department of Immune Regulation



Professor: Koichi Ikuta,

Assistant Professor: Takahiro Hara, Guangwei Cui, Keiko Takemoto

Research Projects:

The immune system has acquired sophisticated control mechanisms as a result of evolution through the wars between host and microorganism. Cytokines are the molecules important for regulation of the immune system. Our laboratory aims to elucidate control mechanisms on development and response of the immune system by cytokines. Interleukin-7 (IL-7) is a cytokine essential for development and homeostasis of lymphocytes and lymphoid organs. Focusing on IL-7 and IL-7 receptor (IL-7R), our laboratory is now pursuing the following research projects.

1) Function of IL-7R in development and response of T lymphocytes

The transcription factor STAT5, which is activated by the IL-7R, controls chromatin accessibility and rearrangements of the T cell receptor (TCR) γ locus by histone acetylation (Figure 1). Although STAT binding motifs are conserved in Jγ promoters and Eγ enhancers, little is known about their precise roles in rearrangements of the TCRγ locus in vivo. To address this question, we established mouse lines with mutations in the STAT binding motifs in the Jγ promoters and Eγ enhancers. These mutant mice exhibit severe reduction in V-J rearrangements and chromatin structural changes in the TCRγ locus, suggesting an essential role of STAT5 binding to the promoters and enhancers.

T lymphocytes lacking the IL-7R exhibit impaired activation after recognizing antigens, suggesting that IL-7 signal is involved in T cell fitness for proper immune response. We are analyzing the mechanism in relation with regulatory T cells and immunometabolism.

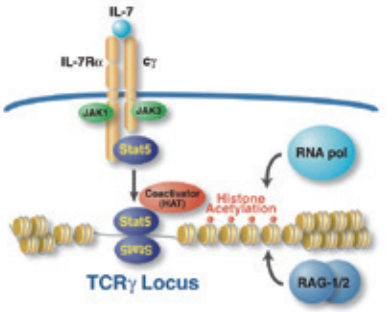


Figure 1. Control of DNA recombination of the TCRγ locus by IL-7 receptor and STAT5.
The transcription factor STAT5, which is activated by the IL-7R, controls chromatin accessibility and rearrangements of the TCRγ locus by histone acetylation.

2) Regulation of IL-7R expression

Expression of the IL-7R is strictly regulated during development of lymphocytes. We previously reported that glucocorticoid receptor (GR) binds to a proximal enhancer and transactivates the IL-7Rα promoter. However, it remained unclear whether glucocorticoids control T cell homeostasis and response at physiological concentrations. We found that GR induces IL-7R expression in mouse T cells in vivo, with a peak at midnight and a trough at midday. This diurnal induction of IL-7R supports the survival of T cells, and their redistribution between lymph nodes, spleen, and blood, by controlling expression of the chemokine receptor CXCR4. In mice, T cell accumulation in the spleen at night enhances immune responses against soluble antigens and systemic bacterial infection (Figure 2). Thus, we identified a physiological role of glucocorticoids as a bioprotective hormone. We are currently investigating the circadian rhythm and sex difference in the immune system.

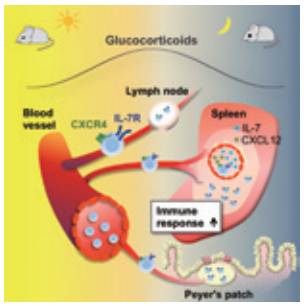


Figure 2. Immunoenhancing effects of glucocorticoids.
Glucocorticoids drive diurnal oscillations in T cell distribution and responses by inducing IL-7R and CXCR4.

(3) Visualization and function of cytokine-producing stromal cells

In addition to lymphocytes, lymphoid tissues contain stromal cells that form the microenvironment supporting development and response of lymphocytes. We established IL-7 and IL-15 reporter mice to analyze cytokine-producing stromal cells in various lymphoid organs (Figure 3). We found that IL-7 is specifically produced by lymphatic endothelial cells, whereas IL-15 is produced by blood vascular endothelial cells, in addition to previously reported IL-7– and IL-15–producing stromal cells in lymphoid organs. Furthermore, we established IL-7–floxed and IL-15–floxed mice to analyze local functions of the cytokines produced by each type of stromal cells by conditional knockout mice. We are currently characterizing the stromal cells which support development of innate lymphoid cells and NK cells. We are also analyzing a novel subset of NKT cells which depends on thymic epithelial cell-derived IL-15 and plays an important role in anti-tumor immunity in the lung.

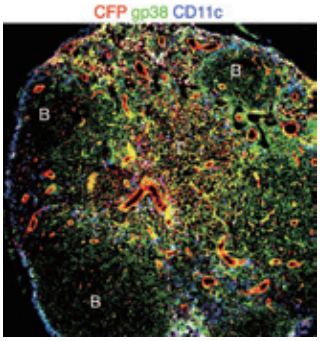


Figure 3. Detection of IL-15 (CFP)-expressing cells in lymph nodes of IL-15-CFP knock-in mouse.
IL-15-expressing cells are mainly detected in T cell zone, medulla, and blood vessels.

Recent publications

- Shimba A, et al. Glucocorticoids drive diurnal oscillations in T cell distribution and responses by inducing interleukin-7 receptor and CXCR4. ***Immunity***, **48**, 286-298, 2018.
- Gomes AC, Hara T, et al. Hematopoietic stem cell niches produce lineage-instructive signals to control multipotent progenitor differentiation. ***Immunity***, **45**, 1219-1231, 2016.
- Abe A, et al. An enhancer of the IL-7 receptor α-chain locus controls IL-7 receptor expression and maintenance of peripheral T cells. ***J Immunol***, **195**, 3129-3138, 2015.
- Wagatsuma K, et al. STAT5 orchestrates local epigenetic changes for chromatin accessibility and rearrangements by direct binding to the TCRγ locus. ***J Immunol***, **195**, 1804-1814, 2015.

Department of Cell Fate Dynamics and Therapeutics

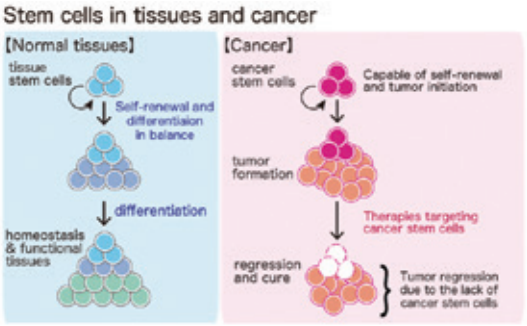
Professor: Takahiro Ito,
Assistant Professor: Kenkyo Matsuura

Research Projects:

What determines how stem cells and cancer behave?

My laboratory studies the molecular basis of cell fate regulation in normal and malignant stem cells. We are currently investigating several pathways of hematopoiesis and skeletal muscle systems in mice and human. Stem cells have a remarkable ability to propagate themselves, which is called "self-renewal". It allows tissue regeneration and repair after injury. But this ability is a double-edged sword; the same mechanism of self-renewal can be a target of malignant transformation and lead to cancer development. In the past decades, we have learned a great deal about the mechanisms of cancer-causing transformation, and yet finding effective ways to eradicate cancer cells has remained an elusive goal in many types of cancers. This is partly because tumors are often complex and heterogeneous mixtures of neoplastic cells with different self-renewal and differentiation abilities. Unlike many differentiated cells within a tumor, some cancer cells are capable of self-renewal. These self-renewing cancer cells, or cancer stem cells, are therapy-resistant and can drive tumor relapse and metastasis following treatment cessation. Recent studies, including our own work, suggest that the normal and malignant stem cells operate on cell fate regulatory signals that are common or specific to each population.

The primary goal of our research is to understand the molecular basis of self-renewal and differentiation in stem cells and cancer, i.e. cell fates. Specifically, we study hematopoiesis and myogenesis to identify cellular machinery that regulates tissue homeostasis and regeneration as well as molecular drivers of transformation that lead to human malignancies such as leukemia. We are particularly interested in the stem cell regulatory circuits governed by RNA binding proteins and cellular metabolism. Recent studies from our lab and others have demonstrated that understanding molecular machineries operating in stem cells and cancer could help us to develop new effective approaches to treat human diseases including cancers. Our research program seeks to improve our understanding of stem cell and cancer biology, and to apply this knowledge to the development of novel and effective approaches to treat human disease and cancer.



Recent publications

- Hattori A *et al.*, Cancer progression by reprogrammed BCAA metabolism in myeloid leukemia. ***Nature*** 545:500-504 (2017).
- Hattori A *et al.*, RNA binding protein MSI2 positively regulates FLT3 expression in myeloid leukemia. ***Leuk Res*** 54:47-54 (2017).
- Hattori A *et al.*, Regulation of stem cell self-renewal and oncogenesis by RNA-binding proteins. ***Adv Exp Med Biol*** 907:153-88 (2016).
- Fox RG *et al.*, Image-based detection and targeting of therapy resistance in pancreatic adenocarcinoma. ***Nature*** 534:407-11 (2016).
- Zimdahl B, Ito T, *et al.* Lis1 regulates asymmetric division in hematopoietic stem cells and in leukemia. ***Nat Genet*** 46:245-52 (2014).

Department of Genetic Biochemistry

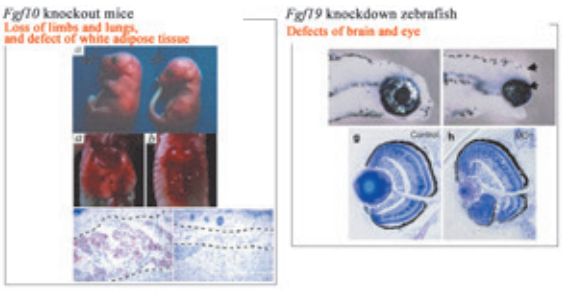
Lecturer: Ayumi Miyake

Research Projects:

In vertebrates, multiple cells interact each other to form various tissues. Secreted proteins are implicated in the intercellular interaction. Therefore, secreted proteins have come to be recognized as key mediators of many fundamental processes in embryonic development. We have focused on elucidating the roles of secreted proteins in the embryonic development and investigated the mechanisms controlling the form of a developing tissue by using the reverse genetics approach. In reverse genetics, a novel gene of unknown function is first identified, and the function of the novel gene is examined by using the loss-of- and gain-of-function approaches. Our efforts contribute to not only the global progress of life science, but also the development of the regenerative medicine. More detailed explanation about each project is described below.

1) Identification of novel Fgfs and their roles in the embryonic development: The prototypic fibroblast growth factors (Fgfs) were originally isolated from bovine brain as mitogen for fibroblasts. Afterwards, several proteins, which were identified by the process of various experiments, were named as Fgf by structural homology. The Fgf family consisted of nine members (Fgf1~9). Most Fgfs are secreted from cells and have multiple biological activities including roles in mitogenesis and cellular differentiation. Fgfs have been shown to play important roles in development of multiple tissues in addition to angiogenesis and repair of tissue injury. These important roles of Fgfs in the development prompted us to identify a novel Fgf based on structural homology. We identified nine *FGFs* including *Fgf10, 16, 17, 18, 19, 20, 21, 22,* and *23*. We examined their roles in the development of tissues. We generated *Fgf10* and *Fgf18* knockout mice. Fgf10 plays crucial roles in multi-organ development including the limbs, lungs, and adipose tissue. Fgf18 plays crucial roles in normal development of the bones and lungs. We examined the activity of Fgf20 using culture cells. We have shown that Fgf20 enhances the survival of dopaminergic neurons and promotes their differentiation from ES cells. Degeneration of dopaminergic neurons causes Parkinson's disease. Therefore, Fgf20 is expected to be useful in prevention and treatment of Parkinson's disease. Furthermore, we have shown that Fgf19 is required for normal development of forebrain and eye, and that Fgf21 is required for the formation of erythrocytes by an analysis of *Fgf19*- and *Fgf21*-knockdown zebrafish embryos, respectively. In hematopoiesis, the molecular mechanism behind the activity of Fgf21 is different from that of erythropoietin, which is used as a therapeutic agent for anemia. Therefore, Fgf21 is expected to be applied to the development of medicine. We are now investigating the roles of Fgfs and the molecular mechanisms behind the action of Fgfs in the development by using the loss-of-function approaches in mice and zebrafish,

2) Identification of novel secreted proteins other than Fgf and their roles in the embryonic development: Recently, a lot of genes of unknown function are shown by DNA database. It is expected that a lot of genes encoding the secreted proteins are included in that. We identified many cDNAs encoding the novel putative secreted proteins by searching DNA databases. Furthermore, we investigated the spatiotemporal expression patterns of the genes and selected several novel secreted proteins that might contribute to the organ's. Among them, *ectodin*, a secreted bone morphogenetic protein (BMP) inhibitor, is expressed as a "negative" image of mouse enamel knots. We propose that *ectodin* is critical for robust spatial delineation of enamel knots and cusps by an analysis of *ectodin*-deficient mice. We have shown that *fibin* expressed in the lateral plate mesoderm is a secreted signal essential for pectoral fin bud initiation in that it potentially acts downstream of retinoic acid and wnt signaling by an analysis of *fibin*-knockdown zebrafish embryos. In addition, we have identified several novel secreted proteins that might contribute to the brain's formation and investigated the roles in brain development.



***Fgf10* knockout mice and**
***Fgf19* knockdown zebrafish embryos**

In *Fgf10* knockout mice (right panels), loss of limbs and lungs (upper and middle panels, respectively) and defects of white adipose tissue (lower panels) were observed. In *Fgf19* knockdown zebrafish embryos (right panels), defects of brain and small eyes were observed (upper panels) cmpared with wild-type embryos (left panels). In eyes of *Fgf19* knockdown zebrafish embryos (left panels), lens defects and abnormal patterning of retina were observed (lower panels).

Recent publications

- Miyake et al., Brorin is required for neurogenesis, gliogenesis, and commissural axon guidance in the zebrafish forebrain . *PLoS One* **12**, e0176036, 2017.
- Miyake *et al.*, Fgf16 is required for specification of GABAergic neurons and oligodendrocytes in the zebrafish forebrain. *PLoS One* **9**, e110836, 2014.
- Miyake *et al.*, Fgf22 regulated by Fgf3/Fgf8 signaling is required for zebrafish midbrain development. *Biol. Open* **2**, 515, 2013.

Department of Genetics

Professor: Tatsushi Igaki,
Assistant Professor: Masato Enomoto, Kiichiro Taniguchi

Research Projects:

Cell-cell interactions in multicellular organisms play crucial roles in coordination of cell proliferation, differentiation, and cell death during development and homeostasis. However, little is known how cells communicate each other within animals to establish a multicelular system. We are exploring the molecular basis of cell-cell communication utilizing a powerful genetics of *Drosophila*. Especially, our research focuses on the mechanisms of cellular 'competition' and 'cooperation' within epithelium.

1) Mechanism of cell competition

'Cell competition' is a form of cell-cell interaction in which cells with higher fitness ('winners') survive and proliferate at the expense of neighboring cells with lower fitness ('losers'). Loser cells, but otherwise viable cells, are eliminated by cell death when confronted with winner cells. It has been suggested that cell competition is involved in a variety of biological processes such as organ size control, tissue homeostasis, cancer progression, and the maintenance of stem cell population. In developing *Drosophilai* maginal epithelia, clones of cells mutant for apico-basal polarity genes such as *scribble* (*scrib*) or discs large(*dlg*) lose their epithelial integrity and are eliminated by cell competition when confronted with wild-type cells. We have discovered that the *Drosophila* tumor necrosis factor (TNF) Eiger and its downstream JNK signaling play a central role in this process. Interestingly, Eiger-JNK signaling is required for both losers and winners to drive cell competition. Elevated Eiger signaling in mutant 'loser' cells promotes JNK-dependent cell death of these cells (Igaki *et al.*, *Dev Cell*, 2009), while elevated Eiger signaling in surrounding wild-type 'winner' cells facilitates elimination of mutant neighbors through JNK-dependent engulfment machinery (Ohsawa *et al.*, *Dev Cell*, 2011) (Fig. 1). Our study reveals that cell competition could be an evolutionarily conserved fail-safe mechanism by which animals protect against neoplastic development. To dissect the upstream mechanisms of cell competition, we have established and performed a genetic screen for genes that regulate this cell elimination. We have also established new models of cell competition using different types of mutations to understand the molecular mechanism and the physiological roles of cell competition.

2) Mechanism of tissue growth and tumor progression through cell-cell communication

Cell-cell interactions between oncogenic cells and surrounding normal cells in the tumor microenvironment play crucial roles in cancer progression. However, the mechanisms by which each oncogenic alteration cooperates with others to drive tissue growth and tumor progression through cell-cell communication remain elusive. We have been studying the mechanism of tumor growth and metastasis using the *Drosophila* model of tumor progression (Igaki *et al.*, *Curr Biol*, 2006). Furthermore, we have performed a genetic screen in *Drosophila* imaginal epithelium to identify mutations that cause 'non-autonomous' tumor progression through cell-cell communication. The results from our screen revealed that defects in mitochondrial respiratory function in conjunction with Ras activation potently induce tumor progression of surrounding tissue. Mechanistically, Ras activation and mitochondrial dysfunction cooperatively stimulate production of ROS, which causes activation of JNK signaling. JNK cooperates with oncogenic Ras to inactivate the Hippo pathway, leading to upregulation of the inflammatory cytokine Unpaired (Upd, an IL-6 homolog). The secreted Upd further cooperates with Ras signaling in neighboring cells with normal mitochondrial function, causing benign tumors to exhibit metastatic behavior (Ohsawa *et al.*, *Nature*, 2012) (Fig. 2). These findings provide a novel mechanistic basis for interclonal tumor progression driven by 'oncogenic inflammation' through Ras activation and mitochondrial dysfunction, the frequent alterations in human malignancies. We have also discovered that oncogenic cells with elevated Src activity promote growth of surrounding tissue via JNK-dependent regulation of the Hippo pathway (Enomoto and Igaki, *EMBO Rep*, 2012). are also establishing new models of cellular 'cooperation' that regulate tissue growth and/or tumor progression through cell-cell communications.

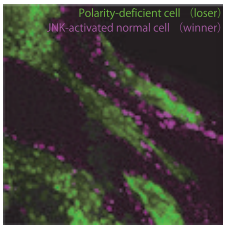


Fig. 1 Cell competition in *Drosophila* epithelium

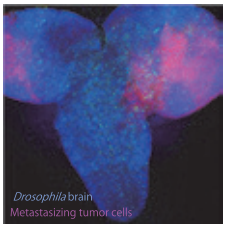


Fig. 2 Tumor metastasis in the *Drosophila* brain

Recent publications

- Yamamoto *et al.*,
  The ligand Sas and its receptor PTP10D drive tumour-suppressive cell competition"
  Nature, 542, 246-250 (2017)
- Vaughen and Igaki
  Slit-Robo repulsive signaling extrudes tumorigenic cells from epithelia"
  Developmental Cell, 39, 683-695 (2016)
- Nakamura *et al.*,
  Mitochondrial defects trigger proliferation of neighbouring cells via a senescence-associated secretory phenotype in *Drosophila*
  Nature Communications 5, 5264 (2014)
- Ohsawa *et al.*,
  Mitochondrial defect drives non-autonomous tumour progression through Hippo signalling in *Drosophila*.
  Nature, 490, 547-551 (2012)
- Ohsawa *et al.*,
  Elimination of oncogenic neighbors by JNK-mediated engulfment in *Drosophila*.
  Developmental Cell 20, 315-328 (2011)

Department of Physiological Chemistry

Professor: Kazuhisa Nakayama, Associate Professor: Hye-won Shin,
Assistant Professor: Yohei Katoh

Research Projects:

1) Studies of intracellular membrane traffic and intraflagellar transport:

Normal functions of a human body, which consists of ~3.7 × 10^{13} cells, rely strictly on the normal function of every cell. There are a variety of intracellular organelles, in which specific proteins are localized (Figure 2). Furthermore, it is essential for each cell to function properly that each protein is transported from an organelle where it is synthesized to another organelle or the plasma membrane where it fulfills its function. We are studying protein and lipid transport systems called membrane traffic mediated by membrane-enclosed structures.

We are working to elucidate the mechanism of protein transport in an organelle called primary cilium. Primary cilia serve as "cellular antennae", because they contain many receptors for extracellular signals. Defects in trafficking of ciliary proteins prevent the cilia from functioning as antennae, leading to a variety of hereditary disorders, generally called the "ciliopathies." The primary cilium has an axoneme composed of microtubules. The intraflagellar transport (IFT) machinery powered by motor proteins, kinesin and dynein, regulates anterograde and retrograde protein trafficking along the axoneme (Figure 1). The IFT machinery is a highly complicated molecular machine containing 5 multiprotein complexes composed of ~40 subunits in total. We are revealing the molecular basis of regulation of ciliary protein trafficking by elucidating the architectures of the multiprotein complexes in the IFT machinery, the roles of each subunit, and the recognition mechanisms of cargo proteins. Our research will also lead to the elucidation of the molecular basis of the ciliopathies.

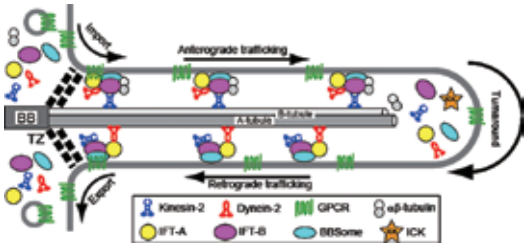


Figure 1. Bidirectional ciliary protein trafficking mediated by the IFT machinery

2) Roles of transbilayer lipid dynamics in cellular functions:

Lipids are asymmetrically distributed between lipid bilayers of biological membranes. In the plasma membrane, PS, PE, and PI are primarily confined to the cytoplasmic leaflet, and PC and SM are enriched on the exoplasmic leaflet. The transbilayer lipid asymmetry is regulated by ATP-dependent flippases (translocate phospholipids from the exoplasmic to the cytoplasmic leaflet; red) and floppases (translocate phospholipids in the opposite direction; blue) (Figure 2), and scramblases (ATP-independent bidirectional transporters). Spatiotemporal transbilayer lipid asymmetry plays a crucial role in many cellular processes (*e.g.*, thrombogenesis, immune response, clearance of apoptotic cells and erythrocytes, fusion of myocytes, cell division, cell migration, sperm capacitation, and membrane traffic), however, the regulatory mechanisms underlying the asymmetry remain unknown. We aim at elucidating roles of P4-ATPases (flippases) in cellular functions, such as membrane traffic, cell migration, and cell polarity, *etc.* Since some mutations of P4-ATPases are responsible for genetic diseases, we also aim at understanding how lipid dynamics are committed to pathophysiological conditions.

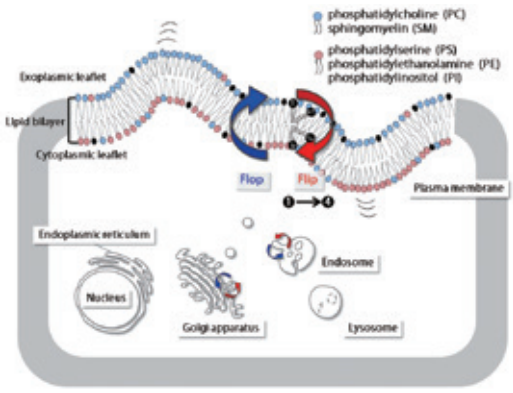


Figure 2. Regulation of transbilayer lipid asymmetry in biological membranes

Recent publications

- Tsurumi, Y. *et al.* (2019) Interactions of the dynein-2 intermediate chain WDR34 with the light chains are required for ciliary retrograde protein trafficking. ***Mol. Biol. Cell*, 30,** 658-670.
- Funabashi, T. *et al.* (2018) Interaction of heterotrimeric kinesin-II with IFT-B-connecting tetramer is crucial for ciliogenesis. ***J. Cell Biol.*, 217,** 2867-2876.
- Hamada, Y. *et al.* (2018) Interaction of WDR60 intermediate chain with TCTEX1D2 light chain of the dynein-2 complex is crucial for ciliary protein trafficking. ***Mol. Biol. Cell*, 29,** 1628-1639.
- Takahara, M. *et al.* (2018) Ciliopathy-associated mutations of IFT122 impair ciliary protein trafficking but not ciliogenesis. ***Hum. Mol. Genet.*, 27,** 516-528.
- Takada, N. *et al.* (2018) Phospholipid-flipping activity of P4-ATPase drives membrane curvature. ***EMBO J.*, 37,** e97705.
- Takatsu, H. *et al.* (2017) Phospholipid flippase ATP11C is endocytosed and downregulated by Ca^{2+}-mediated protein kinase C (PKC) activation. ***Nat. Commun.*, 8,** 1423.

Department of Molecular Neurobiology

Associate Professor: Hironori Katoh

Research Projects:

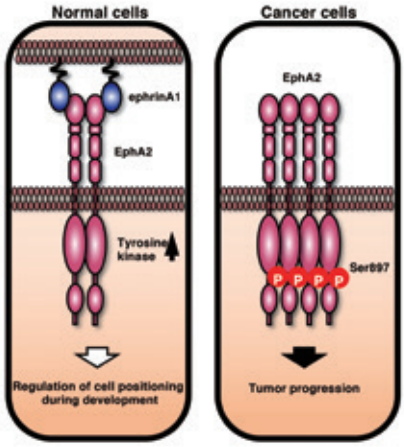
Our laboratory is seeking to understand the mechanisms underlying cancer development and progression. In particular, we study the relationship between cellular metabolism and signal transduction in cancer cells. Our current research focuses on the following subjects:

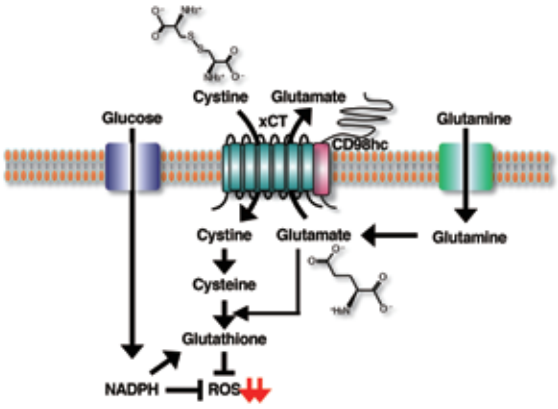
1) Signal transduction pathways involved in cancer progression.

It is well known that cancer development and progression occur through complicated multistep processes. In particular, signal transduction pathways involved in cell proliferation and migration are often aberrantly activated in cancer cells. Eph receptors are receptor tyrosine kinases, and in normal tissues, binding to their ligands ephrins causes activation of their tyrosine kinase activity and regulates many cellular processes during development and tissue homeostasis. On the other hand, when the balance of Eph-ephrin signaling is disrupted, aberrant Eph signaling occurs, leading to cancer malignancy. Among them, EphA2 is frequently overexpressed in a variety of cancer cells, including glioblastoma, lung, kidney, pancreas, and breast cancer cells, and its expression correlates with poor patient survival. EphA2 in cancer cells promotes cell proliferation and migration without ligand stimulation. In our research group, we aim to elucidate the molecular mechanisms of ligand-independent signaling by EphA2 in glioblastoma cells. We also try to reveal the relationship between Eph receptor signaling and metabolism in glioblastoma cells.

2) Regulation of amino acid metabolism in cancer cells.

It is known that amino acid metabolism is altered in many cancer cells compared to normal cells. The activities and expression of enzymes and transporters involved in amino acid metabolism are frequently elevated in cancer cells. The amino acid transporter xCT (SLC7A11) forms a complex with CD98 (4F2hc, SLC3A2) and exchanges extracellular cystine for intracellular glutamate at the plasma membrane. The imported cystine is reduced to cysteine and used to produce glutathione, which protects cells from excessive oxidative stress. Therefore, expression of xCT is up-regulated in various cancer cells including glioblastoma. We study the relationship between xCT activity and signaling pathways involved in glioblastoma progression. We also try to investigate the regulation of other amino acid transporters in glioblastoma cells.





Recent publications

- Toyama et al. EphA3 is up-regulated by epidermal growth factor and promotes formation of glioblastoma cell aggregates. Biochem. Biophys. Res. Commun. **508**, 715-721, 2019.
- Hamaoka et al. Tyrosine kinase activity of EphA2 promotes its S897 phosphorylation and glioblastoma cell proliferation. Biochem. Biophys. Res. Commun. **499**, 920-926, 2018.
- Goji et al. Cystine uptake through the cystine/glutamate antiporter xCT triggers glioblastoma cell death under glucose deprivation. J. Biol. Chem. **292**, 19721-19732, 2017.
- Hamaoka et al. EphA2 is a key effector of the MEK/ERK/RSK pathway regulating glioblastoma cell proliferation. Cell. Signal. **28**, 937-945, 2016.
- Harada et al. HGF-induced serine 897 phosphorylation of EphA2 regulates epithelial morphogenesis of MDCK cells in 3D culture. J. Cell Sci. **128**, 1912-1921, 2015.

Department of Biofunctional Chemistry

Professor: Shiroh Futaki, Lecturer: Miki Imanishi,
Assistant Professors: Ken-ichi Kawano

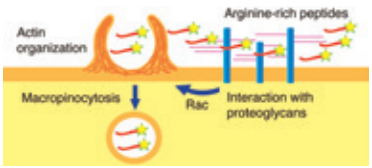
Research Projects:

The ultimate goal of our research is the regulation of cellular functions by designed peptides and proteins, aiming at elucidation of biological functions as well as development of novel functional bio-devices having therapeutic potentials.

1) Development of membrane-permeable peptide vectors: Arginine-rich peptides, including octaarginine (R8), HIV-1 Tat, and branched-chain arginine-rich peptides, belong to one of the major classes of cell-permeable peptides which deliver various proteins and macromolecules to cells. The importance of the endocytic pathways has recently been demonstrated in the cellular uptake of these peptides. We have previously shown that macropinocytosis is one of the major pathways for cellular uptake and that organization of the F-actin accompanies this process. In this study, using proteoglycan-deficient CHO cells, we have demonstrated that the membrane-associated proteoglycans are indispensable for the induction of the actin organization and the macropinocytic uptake of the arginine-rich peptides. We have also demonstrated that the cellular uptake of the Tat peptide is highly dependent on heparan sulfate proteoglycan (HSPG), whereas the R8 peptide uptake is less dependent on HSPG. This suggests that the structure of the peptides may determine the specificity for HSPG, and that HSPG is not the sole receptor for macropinocytosis. Comparison of the HSPG specificity of the branched-chain arginine-rich peptides in cellular uptake has suggested that the charge density of the peptides may determine the specificity. The activation of the Rac protein and the actin organization was observed within a few minutes after the peptide treatment. These results strongly suggest the possibility that the interaction of the arginine-rich peptides with the membrane-associated proteoglycans quickly activates the intracellular signals and induces actin organization and macropinocytotis.

2) Creation of artificial transcription factors manipulating circadian rhythm: Regulation of a target gene at will is one of the most prospective themes in the post-genomic era. An artificial transcription factor with desired DNA binding specificity could work as a powerful tool to control target genes. The C_2H_2-type zinc finger motif is one of the most typical DNA binding motifs. We have developed artificial zinc finger transcription factors with novel DNA binding specificity and succeeded in inducing resetting of circadian clock or regulating gene expression patterns to be circadian rhythmic. In addition, we are developing TALE proteins that have new DNA binding specificities. Our approach using artificial DNA binding proteins would be useful for elucidating the mechanisms of biological phenomena such as circadian rhythm.

3) Design of artificial receptor channel proteins: Ion channels and receptors are among the most biologically important classes of membrane proteins that transmit outside stimuli into cells. The creation of artificial proteins with these functions is a challenge in peptide/protein engineering in view of the creation of novel functional nano-devices as well as understanding the biological machinery. We have developed a novel Fe(III)-gated ion channel system that is comprised of assemblies of a channel forming peptide alamethicin bearing an extramembrane segment. The extramembrane segment contains a pair of diiminoacetic acid derivatives of lysine (Ida) residues. Addition of Fe(III) lead to the conformational switch in the extramembrane and the eventual increase in the channel current. This strongly suggests the possibility of establishing novel channel and sensor systems by transmitting an extramembrane conformational switch to the channel current levels. In addition, there are many reports on the creation of artificial ion channels that have a sensing function of the external ligands. However, most of them have been designed so that the interaction with ligands leads a decreased channel current, and very few of them have a function that can detect the ligand with the increased membrane current as are usually seen in natural ligand-gated ion channels. The system established by us is rather simple and may need further sophistication. However, we believe that this concept can extensively be applicable for the creation of various ligand-gated ion channels with novel receptor functions.



Interaction of arginine-rich peptides with membrane-associated proteoglycans leads activation of Rac protein followed by actin organization and induction of macropinocytosis. Thus, arginine-rich peptides are efficiently taken up by the cells with cargo molecules.

Recent publications

- Nakase et al. Molecular interplays involved in the cellular uptake of octaarginine on cell surfaces and the importance of syndecan-4 cytoplasmic V domain for the activation of protein kinase Cα. *Biochem Biophys Res Commun* **446**, 857, 2014.
- Tsuji et al. Creating a TALE protein with unbiased 5'-T binding. *Biochem Biophys Res Commun* **441**, 262, 2013.
- Noshiro et al. Construction of a Ca^{2+}-gated artificial channel by fusing alamethicin with a calmodulin-derived extramembrane segment. *Bioconjug Chem* **24**, 188, 2013.

Department of Drug Delivery Research

Associate Professor: Yuriko Higuchi

Research Projects:

A drug administered systemically is distributed nonspecifically all over the body. It is necessary to optimize the drug's pharmacokinetics in order to enhance the therapeutic effect and reduce side effects. The concept of controlling drug disposition to optimize therapeutic effect is called drug delivery system (DDS). Recently, in addition to low molecular weight compounds, proteins, nucleic acids, and cells have been a new modality for treatment. DDS should be rationally designed based on the physicochemical and biological properties of the drug, as well as based on the structure and function of the target organ. Furthermore, new analytical techniques must be established to evaluate the pharmacokinetics and therapeutic effects of drugs and DDSs according to their features. We are working on the following research topics.

1) Development of novel nano-DDSs for better therapeutic outcomes

We are developing nanoparticles-based DDSs (nano-DDSs) comprising of lipids, synthetic polymers, or biopolymers for targeted drug delivery. We optimize the formulation and manufacturing of nano-DDSs according to the structural and physicochemical properties of the encapsulated drug, in order to improve the encapsulation efficiency and control the retention and release. We also introduce targeting ligands to the nanocarriers to deliver the drug to the target tissue or cells specifically. The specialized systems of interest include glycan-lectin recognition and antigen-antibody interaction. We are also challenging targeted subcellular delivery of mid- and large-size drugs such as peptide and proteins.

2) Association analysis of pharmacokinetics and toxicokinetics of nano-DDSs with their physicochemical properties

Rational design of DDS is inevitable to reduce the side effect of drugs and carriers. We have been evaluating the toxicity of nano-DDSs, but only focusing on the change in well-established specific toxicity markers. In recent years, LS/MS/MS has been enabling for highly sensitive and comprehensive detection of protein expression variation in tissue and cells. Therefore, we are searching for new toxicity markers for nano-DDSs using this method. Particularly, we are interested in the hepatotoxicity of nano-DDSs because the liver is a major organ for the nano-DDSs to accumulate. We evaluate the variation of protein expression and phosphorylation in cultured hepatocytes or mouse liver following nano-DDS treatment, and identify hepatotoxicity-associated maker molecules through pathway analysis. Furthermore, we compare fluctuations in protein expression level in single and multiple doses and analyze the mechanism of long-term chronic toxicity that occurs in multiple doses.

3) Research on DDS technology for cell-based medicine

Mesenchymal stem cell-based medicine is now available for clinical therapy. Similar with conventional drugs such as low molecular weight compounds, high molecular weight compounds and nucleic acids, the concept of DDS that addresses the delivery of drugs to the target tissue should be propagated to cell-based therapy to improve the treatment outcomes. We apply the knowledge and technology that we have cultivated up to now, and are developing a method of controlling pharmacokinetics of mesenchymal stem cells. First, for the purpose of enhancing cell-cell adhesion to a therapeutic target cell, we are developing a method of modifying a ligand molecule on the cell membrane. In addition, we are also creating cells that exhibit therapeutic effects selectively at the treatment target site by changing their function in response to the micro environment in tissue.

Recent publications

- Rahimova N, et al., Development of mKO2 fusion proteins for real-time imaging and mechanistic investigation of the degradation kinetics of human IκBα in living cells. Biochim Biophys Acta Mol Cell Res. (2019) 1866(2),190-198.
- Babazada H, et al., Binding and structure-kinetic relationship analysis of selective TLR4-targeted immunosuppressive self-assembling heparin nanoparticles. Int J Pharm. (2018) 552(1-2), 76-83.
- Chantarasrivong C, et al., Synthesis and Functional Characterization of Novel Sialyl LewisX Mimic-Decorated Liposomes for E-selectin-Mediated Targeting to Inflamed Endothelial Cells. Mol Pharm. (2017) 14(5), 1528-1537.

Department of Pharmacology

Visiting Professor: Toshiaki Kume

Research Projects:

The trouble with a higher brain dysfunction due to neurodegenerative disease such as the Alzheimer's diseases and Parkinson s diseases and cerebral ischemia has features in the neuronal death of the neuron group of a specific area of brain by the process of apoptosis and necrosis. We investigate the mechanisms of the neuronal death and the exploratory research of low-molecular compounds that control the neuronal death accompanied by the neurodegenerative disease and cerebral ischemia and using the techniques of in vivo experiment system that used the brain disease model animal and in vitro system including the primary neuronal cultures. Our current research projects are listed below.

1) Elucidation of pathogenesis and exploratory study of preventive and therapeutic agents of neurodegenerative diseases

"Amyloid hypothesis," which amyloid β protein (Aβ) that plays an important role in the development of Alzheimer's disease, has been recognized, but the toxic mechanisms of Aβ have still unsolved. We previously identified the toxic conformer of Aβ42 with a turn at positions 22 and 23 ("toxic turn"). Our recent study suggested that oxidative stress is a key factor of the oligomerization and cognitive impairment induced by Aβ overproduction in vivo. However, the involvement of the toxic conformer in Aβ42-induced oxidative damage remains unclear. To investigate this mechanism, we examined the levels of intracellular reactive oxygen species (ROS) and neurotoxicity in rat primary neurons using E22P-Aβ42, a mutant that induces a turn at positions 22 and 23. E22P-Aβ42 induced greater ROS production than Wt-Aβ42 in addition to potent neurotoxicity. Trolox (a radical scavenger) and Congo red (an aggregation inhibitor) significantly prevented the neurotoxicity and intracellular ROS induced by E22P- Aβ42 and Wt- Aβ42, respectively. These results suggest that A≤42-mediated toxicity is caused by the turn that favors toxic oligomers, which increase generation of ROS. We currently investigate the in vivo effect of toxic conformer of Aβ42.

2) Study on function of nicotinic acetylcholine system in CNS

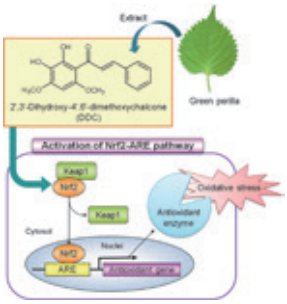
We previously reported that long-term exposure to nicotine of cerebral cortical neurons prevented neuronal death induced by glutamate and amyloid β protein. Furthermore, we also reported that central-type acetylcholinesterase inhibitors including donepezil protected cortical neurons against glutamate neurotoxicity via the stimulation of nicotinic acetylcholine receptors. Then, we are currently examining detailed mechanisms of the neuroprotective effect of acetylcholinesterase by the nicotinic receptor stimulation.

3) Study on neuroprotective compounds derived from food

For overcoming these neurodegenerative diseases, it is necessary to manage them from the point of view of preventive medicine because neuronal death has already occurred at the onset. In addition, it is important to slow the progress not only by the drug treatment but also by the auxiliary use of food with the neuroprotective effect because the symptoms gradually progress for a long period of several years or more. Our aim is to explore and analyze the neuroprotective or neuroregenerative compounds derived from food for the management of aging risk, such as dementia. We previously identified DDC from green perilla as a novel functional component and clarified that DDC induced upregulation of intracellular antioxidant enzymes. We are currently investigating the neuroprotective actions of several components derived from foods including green perilla

4) Study on survival and regeneration of dopaminergic neurons

With respect to Parkinson disease characterized by selective loss of dopaminergic neurons in the substantia nigra, we reported that dopaminergic neurons were particularly vulnerable to cellular stress because they are rich in dopamine, which can easily undergo autoxidation, as a neurotransmitter. Therefore, we are exploring the compounds which regulate the abnormal autoxidation of dopamine as candidates for dopaminergic neuroprotective drugs. In addition, dysfunction of protein quality control is implicated in Parkinson disease. We are examining the novel neuroprotective mechanisms by clarifying the role of proteasome and autophagy in dopaminergic neuronal death. Furthermore, the study aimed at regeneration of the nigrostriatal dopaminergic projection is in progress. By the use of original methods, the mechanism by which dopaminergic axons innervate striatal neurons is investigated. Findings which will be obtained from this study might be applicable to stem cell-derived cell transplantation therapy.



Schematic representation of cytoprotective mechanism of DDC.
DDC was extracted and isolated from the leaves of green perilla. DDC activated Nrf2-ARE pathway, a cellular defense system against oxidative stress. Nrf2, a transcriptional factor, is translocated to the nucleus and bound to antioxidant response element (ARE), resulting in the transcriptional activation of a number of antioxidant enzymes. Cells treated with DDC acquired resistance to oxidative damage.

Recent publications

- Izumi *et al.* Endogenous dopamine is involved in the herbicide praquat-induced dopaminergic cell death. *Toxicol Sci.* **139**, 466, 2014
- Wakita *et al.* Staurosporine induces dopaminergic neurite outgrowth through AMP-activated protein kinase/mammalian target of rapamycin signaling pathway. *Neuropharmacology.* **77**, 39, 2014
- Izuo *et al.* Toxicity in rat primary neurons through the cellular oxidative stress induced by the turn formation at positions 22 and 23 of Aβ42. *ACS Chem Neurosci.* **3**, 674, 2012
- Izumi *et al.* Isolation, identification, and biological evaluation of Nrf2-ARE activator from the leaves of green perilla (Perilla frutescens var. crispa f. viridis). *Free Radic Biol Med.* **53**, 669, 2012

Department of Clinical Pharmacy and Education

Associate Professor: Atsushi Yonezawa

Research Projects:

1) Clinical pharmacological research for a personalized treatment with therapeutic antibody drugs.
2) Involvement of renal organic cation transporters involved in the side effects of drugs
3) Identification of novel riboflavin transporter RFVT and pathophysiology of rare diseases BVVLS

Many medical drugs have been developed so far, and they have greatly contributed to the development of drug treatment. On the other hand, Japan's medical care has various problems such as aging, rising cost of medical care, complication of diseases and medicines, increase of intractable diseases and rare diseases. In recent years, "Precision Medicine" which conducts the treatment and prevention of diseases based on genetic information, individual differences in living environments and lifestyles, has attracted attention. In the department of clinical pharmacy education, we are promoting reverse translational research, which forms a scientific basis for solving problems found in clinical drug treatment, and translational research, which develop new drug treatments based on basic research results, and are aiming to develop personalized treatments that achieve optimal care for each patient (Figure 1). The following is an overview of research themes developed in this field.

1) Clinical pharmacological research for a personalized treatment with therapeutic antibody drugs.

The importance of antibody drugs in drug treatment has increased in recent years. On the other hand, despite the fact that only about 50 antibody drugs have been approved and marketed, the medical economic point is a social issue, such as accounting for half of the TOP10 drug sales in 2014. In other words, it is urgent to develop biomarkers to predict clinical effects and to realize personalized medicine.

We have established a method for structural analysis of antibody drugs using TOF-MS, and also used pharmacokinetic evaluation techniques for immune cell activation markers using Flow Cytometry and CyTOF, the next-generation Flow Cytometry. We are developing personalized therapies by (PK) and pharmacodynamics (PD) analysis (Figure 2). In addition to cell and animal experiments, we are also promoting clinical research through joint research with the clinical department at Kyoto University Hospital. Furthermore, we are focusing on drug repositioning to search for drugs that enhance the effects of antibody drugs. The research results will lead to the appropriate use of antibody drugs, and will also contribute to the reduction of medical expenses and the development of antibody drugs such as biosimilars.

2) Pharmacokinetics and pharmacological research for transporters

2-1) Involvement of renal organic cation transporters involved in the side effects of drugs

The side effects of drugs are not only caused by pharmacodynamics but also by pharmacokinetics. We have focused on the organic cation transporters OCT2 (uptake type) and MATE (efflux type) in the kidney. We succeeded in identifying the human kidney-specific transporter MATE2-K that belongs to the MATE family. In addition, we have clarified that OCT expression distribution, substrate recognition characteristics, and functional change of MATE are important factors for renal specific toxicity of cisplatin and induction of lactate acidosis by metformin. The elucidation of the mechanism of side effects caused by these transporters is considered to be useful information for drug selection in patients with various pathological conditions, and also leads to the design of a side effect avoidance method in drug discovery.

2-2) dentification of novel riboflavin transporter RFVT and pathophysiology of rare diseases BVVLS

We have successfully identified the first riboflavin transporters RFVT1 (formerly known as RFT1) and RFVT2 (formerly named RFT3) in mammals. Collaborating with foreign laboratories, we also found that this gene defect causes the rare disease Brown-Vialetto-Van Laere syndrome (BVVLS). BVVLS is a disease that causes hypotonia and respiratory failure, but the details of its mechanism were unknown. By performing animal experiments, it was revealed that blood plasma riboflavin concentration is unchanged in RFVT2 deficiency and riboflavin concentration is lowered in RFVT3 deficiency, and BVVLS pathologies with different degrees of severity are exhibited. Based on this research result, each genetic disease was registered in the human genetic disease database OMIM as BVVLS2 (OMIM # 614707) and BVVLS1 (OMIM # 211530). We are currently working on knock-out mice to elucidate the pathophysiology of BVVLS and to develop treatments.

図1. 医薬品の体内動態と薬効・毒性に関する基礎と臨床

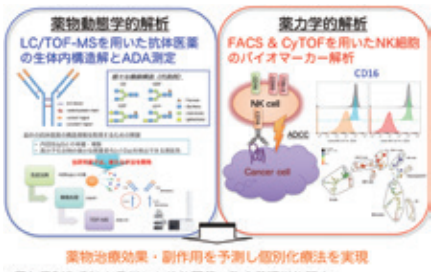


図2. 個別化療法を目指した抗体医薬の臨床薬理学的研究

Recent publications

- Yonezawa A, Otani Y, Kitano T, Mori M, Masui S, Isomoto Y, Tsuda M, Imai S, Ikemi Y, Denda M, Sato Y, Nakagawa S, Omura T, Nakagawa T, Yano I, Hayakari M, Takaori-Kondo A, Matsubara K. Concentration and Glycoform of Rituximab in Plasma of Patients with B Cell Non-Hodgkin's Lymphoma. *Pharm Res.* 36(6):82, 2019
- Otani Y, Yonezawa A, Tsuda M, Imai S, Ikemi Y, Nakagawa S, Omura T, Nakagawa T, Yano I, Matsubara K. Time-Dependent Structural Alteration of Rituximab Analyzed by LC/TOF-MS after a Systemic Administration to Rats. *PLoS One.* 12: e0169588, 2017
- Yoshimatsu H, Yonezawa A, Yamanishi K, Yao Y, Sugano K, Nakagawa S, Imai S, Omura T, Nakagawa T, Yano I, Masuda S, Inui K, Matsubara K. Disruption of Slc52a3 gene causes neonatal lethality with riboflavin deficiency in mice. *Sci Rep* 6:27557, 2016

Department of Patho-Functional Bioanalysis

Professor: Masahiro Ono, Lecturer: Hiroyuki Watanabe,
Assistant Professor: Shimpei Iikuni

Research Projects:

A wide range of biological functions are established via the interactions of many biomolecules; therefore, the clarification of such molecular interactions is necessary for the elucidation of biological functions. Our department is developing analytical methods that visualize the interactions among molecules occurring in living and functioning bodies (*in vivo*) as real-time spatial and temporal images using photon technology (molecular imaging), studying biological functions and etiology using this method, and developing clinical diagnostic methods and therapeutic agents based on the characterization of pathological conditions. Our current research projects are outlined below.

1) Development of molecular probes for the *in vivo* analysis of biological function, etiological mechanisms, and action mechanisms of drugs

We are currently conducting research on development of radiolabeled and optical molecular probes, which are reagents for molecular imaging, on the basis of analysis of the relationships among the structure, activity, and distribution. For example, we have succeeded in development of radiolabeled molecular imaging probes, imaging and the quantitative evaluation of β-amyloid plaques and neurofibrillary tangles in the brain in Alzheimer's disease patients (Figure 1). Furthermore, we also develop radiolabeled molecular probes effective for molecular imaging of receptors of endocrine peptides and transporters of pharmaceuticals. Moreover, we have developed a self-quenching activatable fluorescence probe for *in vivo* near-infrared optical imaging, which is activated by the interaction with specific molecule or under cellular microenvironment *in vivo* and emits fluorescence. In addition, with the molecular design concept of bifunctional compounds having both a moiety related to physiologic activities and a moiety that emits detection signals of radiation and fluorescence, within the same molecule, we are conducting research for the development of molecular probes derived from physiologically active peptides or proteins.

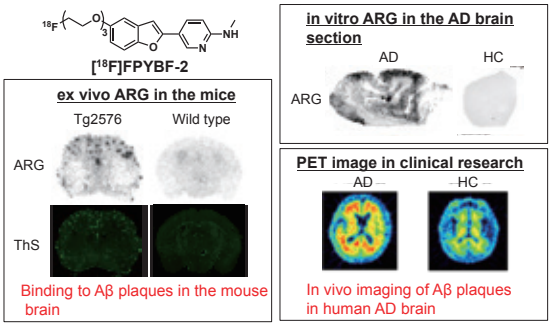


Figure 1. In vivo imaging of Aβ plaques in the brain from Alzheimer's disease (AD) patients

2) Development of radiopharmaceuticals for functional diagnosis and internal radionuclide therapy

The nuclear medicine techniques, in which a radioactive compound (radiopharmaceutical) is administered to patients, and radioactivity from the radioactive compound is detected and processed into images, are used as a clinical imaging method excellent for functional diagnosis. We are conducting research into the development and clinical use of radiopharmaceuticals based on the characterization of physiological conditions and diseases. We are simultaneously conducting research for the development of ^{99m}Tc-labeled radiopharmaceuticals for nuclear medicine diagnosis; that is, we are systematically investigating the formation of complexes of Tc, a transition metal, and developing functional radiopharmaceuticals labeled with ^{99m}Tc. We are also developing cancer radiotheranostics that includes nuclear medical imaging and internal radionuclide therapy targeting carbonic anhydride IX (CA-IX) (Figure 2).

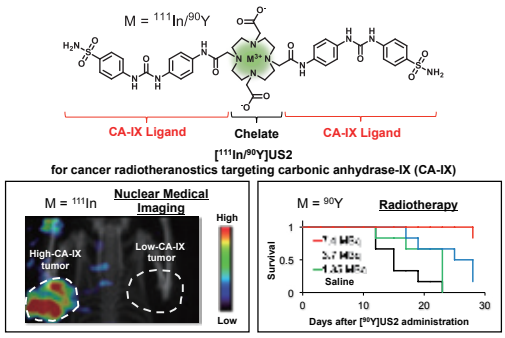


Figure 2. Development of cancer radiotheranostics agents targeting CA-IX

Recent publications

- Iikuni S, et al., Cancer radiotheranostics targeting carbonic anhydrase-IX with ^{111}In- and ^{90}Y-labeled ureidosulfonamide scaffold for SPECT imaging and radionuclide-based therapy. *Theranostics*, **8** (11), 2992-3006 (2018).
- Watanabe H, et al., Novel benzothiazole derivatives as fluorescent probes for detection of β-amyloid and α-synuclein aggregates. *ACS Chem. Neurosci.*, **8** (8), 1656-1662 (2017).
- Ono M, et al., Radioiodination of BODIPY and Its Application to a Nuclear and Optical Dual Functional Labeling Agent for Proteins and Peptides. *Sci. Rep.*, **7**, 3337 (2017).

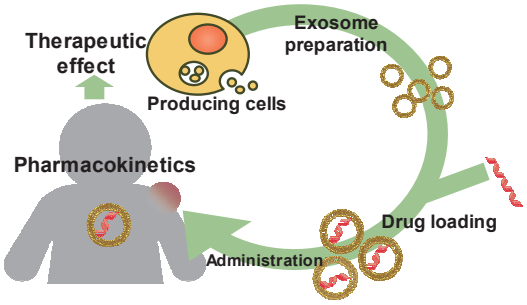
Department of Biopharmaceutics and Drug Metabolism

Professor: Yoshinobu Takakura, Associate Professor: Yuki Takahashi

Research Projects:

To realize ideal drug therapy by optimizing drug design and delivery, we are focusing on the studies on the drug-body interaction based on the scientific background of biopharmaceutics, pharmacokinetics and drug delivery system. Our current research projects are listed below.

1) Development of drug delivery system using exosomes: Exosomes are small membrane vesicles secreted from various cells. Exosomes work as endogenous delivery carriers for protein, RNA and DNA, so that they are expected to be delivery system for these molecules. To develop exosome-based delivery systems, we have been trying to establish a method to control the tissue distribution of exogenously administered exosomes. We have succeeded in pharmacokinetic analysis of exosomes by developing methods that can specifically label exosomes. A series of studies also have been conducted to overcome pharmaceutical challenges of exosomes; i.e., selection of exosome-producing cells, preparation methods of exosomes, methods to load drugs onto exosomes, and storage of exosomes. We have also demonstrated that genetically engineered exosome can be useful delivery carriers for tumor antigens targeting antigen presenting cells. Moreover, we have succeeded in the evaluation of not only exosomes collected from *in vitro* cultured cells but also exosomes collected from the blood of mice. Our currently ongoing studies include the regulation of in vivo behavior of exosomes that endogenously exist in the body. Moreover, now we are focusing on various novel types of cell-derived extracellular particles other than exosomes.



Development of exosome-based drug delivery system

2) Establishment of immunotherapy based on gene delivery technology: Recently, immunotherapy using intrinsic immune systems such as immune checkpoint therapy and CAR-T therapy have been attracting a huge amount of attention. Membrane proteins such as MHC and soluble proteins such as cytokines plays major roles in immune system. A method to regulate behavior and activity of proteins of interest is desirable for the development of therapy utilizing immune system and gene delivery of the proteins of interest can be the method. We have succeeded in developing plasmid vectors that express interferon, a cytokine, for a long period of time and proved their efficacy on the treatment for cancer, atopic dermatitis, and multiple sclerosis. We have also succeeded in designing a variety of fusion proteins to control their tissue distribution after in vivo gene transfer.

3) Development of delivery systems of proteins and nucleic acid drugs utilizing nucleic acid-based nanostructures: DNA containing CpG motifs (CpG DNA) induce cytokine production through Toll-like receptor-9 (TLR-9), so CpG DNA is expected to be applied to the treatment of cancer, autoimmune diseases and allergic diseases. We have successfully developed unique DNA assemblies with branches; multiple pods extend from the center of the assembly. We have demonstrated that CpG DNA-induced immune activation is significantly increased by building it up into such branched structures. Dendritic DNA and DNA hydrogels were also prepared by connecting the assemblies. We have also succeeded in the preparation of DNA nanostructures prepared by using long single stranded DNA. Studies are ongoing to develop novel delivery systems using these nucleic acid nanostructures.

4) Development of multifunctional cell therapeutics for in vivo cell therapy: Recent progress in the technology for culture and differentiation of a variety of cells, including induced pluripotent stem cells, has increased the possibility of cell-based therapy. We have been studying on the development of multi-functional cell therapeutics that can be applicable for the next generation therapy. We have established a technology to construct multicellular spheroids, and demonstrated that the spheroid formation is useful to increase the survival of cells transplanted and effective for the treatment of diabetic model mice and the treatment of tumor-bearing model mice.

Recent publications

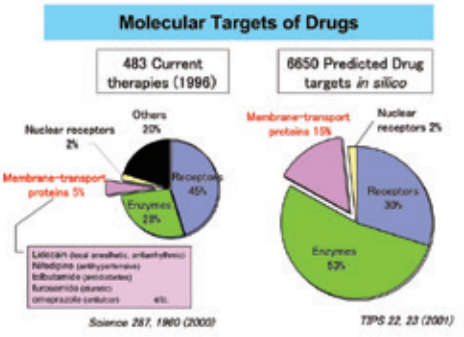
- Morishita et al. Exosome-based tumor antigens-adjuvant co-delivery utilizing genetically engineered tumor cell-derived exosomes with immunostimulatory CpG DNA. *Biomaterials* **111**, 55-65, 2016.
- Charoenviriyakul C et al. Role of Extracellular Vesicle Surface Proteins in the Pharmacokinetics of Extracellular Vesicles. *Mol Pharm.* **15**, 1073-1080, 2018.
- Umeki et al. Combined encapsulation of a tumor antigen and immune cells using a self-assembling immunostimulatory DNA hydrogel to enhance antigen-specific tumor immunity. *J Control Release* **288**, 189-198, 2018.
- Tanaka et al. Control of polarization and tumoricidal activity of macrophages by multicellular spheroid formation. *J Control Release* **270**, 177-183, 2018.

Department of Molecular Pharmacology

Professor: Shuji Kaneko, Associate Professor: Hisashi Shirakawa,
Assistant Professor: Kazuki Nagayasu

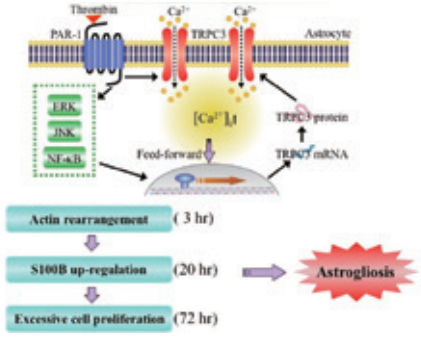
Research Projects:

A comprehensive analysis of molecular targets of drug therapy revealed that the largest subgroup is cell membrane receptors (45%), and the next is enzymes (28%), while membrane-transport proteins account for only 5% of all current drug targets; however, the drugs targeting membrane-transport proteins are strong, effective and frequently used in the therapeutic treatment. On the other hand, 6650 predicted proteins of potential drug targets are composed of 30% cell membrane receptors, 53% enzymes, and 15% membrane-transport proteins (see left panel). Consequently, it is considered that membrane-transport proteins will be the promising molecular targets of drug development. In our Department of Molecular Pharmacology, membrane-transport proteins, especially ion channels and transporters in the central nervous system, are focused on, and a variety of studies are in progress as follows:



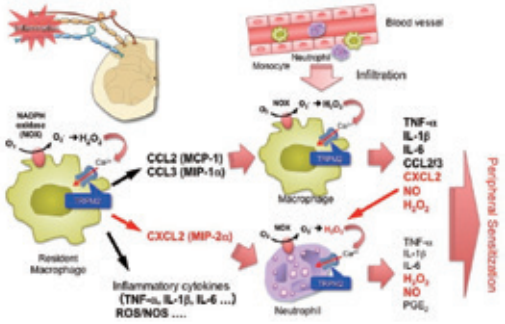
1) Study on the role of TRP channels involved in the pathophysiology of cerebrovascular diseases

Cerebrovascular diseases including cerebral infarction and intracerebral hemorrhage are severe neurological deficits in which generation of reactive radical moieties and inflammatory responses cause neuronal death and abnormal activation of glial cells after excessive overflow of neurotransmitters. On the other hand, TRP (transient receptor potential) channel is a family of nonselective cation channels, which may have important roles in nonexcitable cells, such as glial cells and immune cells. Therefore we focused on the mechanisms of abnormal glial activation that are involved in the chronic pathogenesis of cerebral stroke. So far, we have identified the pivotal role of TRPC3 in the thrombin-induced activation of astrocytes (see right panel). We now address the physiological and pathophysiological roles of other TRP channels in glial cells including astrocytes, microglia and oligodendrocyte precursor cells using genetically modified animals.



2) Study on the roles of TRP channels and transporters involved in the chronic pain

Injury of sensory neurons and surrounding inflammatory lesions cause chronic pain that is not always responsive to conventional analgesics. Since the mechanism underlying chronic pain is now well understood, we focused on the roles of glial cells and immune cells in the interaction with sensory neurons that aggravate pain sensation. We have clarified the role of astroglial glutamate transporter GLT-1 in the generation of neuropathic pain, and are investigating the algesic roles of TRPM2 expressed in monocytes/macrophages and microglia (see right panel). In addition, we are analyzing the involvement of TRP channels in the grave peripheral neuropathy induced by several kinds of antineoplastic agents such as oxaliplatin.



3) Study on the action mechanisms of antidepressants and addictive drugs

We have established an in-vitro chronic experimental system in which midbrain and limbic slices are cocultured for the study of addictive mechanisms of psychostimulants, narcotic analgesics, other addictive drugs such as MDMA on dopaminergic neuronal networks. We also developed an in-vitro raphe slice culture for the study of chronic effects of antidepressants such as SSRI, SNRI and tricyclic antidepressants on serotonergic neuronal networks.

Recent publications

- Munakata et al., Transient receptor potential canonical 3 inhibitor Pyr3 improves outcomes and attenuates astrogliosis after intracerebral hemorrhage in mice. **Stroke** 44, 1981-1987 (2013)
- Nagayasu et al., Chronic effects of antidepressants on serotonin release in rat raphe slice cultures: high potency of milnacipran in the augmentation of serotonin release. **Int J Neuropsychopharmacol**. 16, 2295-306 (2013)
- Zhao et al., Acute cold hypersensitivity characteristically induced by oxaliplatin is caused by the enhanced responsiveness of TRPA1 in mice. **Mol Pain**. 8, 55 (2012)
- Haraguchi et al., TRPM2 contributes to inflammatory and neuropathic pain through the aggravation of pronociceptive inflammatory responses in mice. **J Neurosci**. 32, 3931-3941 (2012)

Department of Clinical Pharmacology & Therapeutics

Professor: Kazuo Matsubara, Associate Professor: Takayuki Nakagawa,
Lecturer: Satoshi Imai, Assistant Professor: Shunsaku Nakagawa, Yuki Sato

Research Projects:

The aim of our laboratory is to establish the scientific bases of appropriate drug usage and pharmaceutical practice. The efficacy and safety of drugs are closely related to their pharmacokinetics and pharmacodynamics. We have systematically developed the research from drug transport analyses based on the molecular levels to the clinical pharmacokinetics. We are also trying to elucidate the mechanisms underlying adverse effects of anti-cancer reagents, and are studying the mechanisms underlying neurodegenerative disease. To settle the problem found in the pharmacotherapy, we attempt to feedback the achievements of basic research to clinical practice. Topics currently undergoing are outlined below:

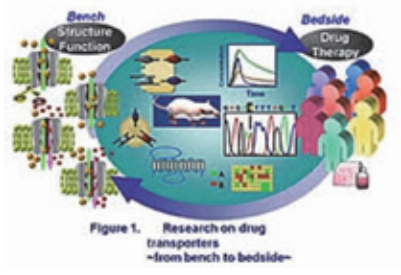
1) Reverse translational research for adverse effects of anti-cancer drugs: elucidation of the mechanisms and development of novel preventive and treatment strategies: Anti-cancer drugs used in chemotherapy frequently exhibit a variety of adverse effects. Some of them are dose-limiting adverse effects in anti-cancer chemotherapy, but effective clinical preventive and treatment strategies have not been established. We are trying to elucidate the molecular mechanism underlying the uncontrolled adverse effects, in which the findings are originally obtained from the bedside, by in vitro and in vivo experiments (reverse-translational research), and to propose effective preventive and treatment strategies. We are now investigating the mechanism of nephrotoxicity induced by cisplatin, interstitial lung disease induced by EGFR inhibitors (gefitinib and erlotinib), peripheral neuropathy induced various types of anti cancer drugs in cell cultures and animal models.

2) Clinical and basic studies on Pharmacokinetics and Pharmacodynamics: Pharmacokinetics consists of four processes, which are regulated by several pharmacokinetic factors, such as drug transporters and drug-metabolizing enzymes. We carry out clinical and basic studies on Pharmacokinetics and Pharmacodynamics (PK/PD). For example, it has been clarified that the efficacy and adverse effects of platinum anticancer drug cisplatin and anti-diabetic drug metformin depended on the characteristics of organic cation transporters. Also, novel riboflavin transporter RFVT has been identified. It has been indicated that RFVT mutation caused a rare disease. Then, we now try to clarify the mechanism and discover new therapeutic drugs of this rare disease.

3) Molecular and neural mechanisms underlying pathological pain and dysesthesia: The physiological (acute) pain is transient and necessary for the alarm system that warns us and helps to protect from tissue damage, while pathological (chronic) pain is usually persistent and unnecessary for survival and protective role. Pathological pain is mediated through plastically altered pain pathways induced by a variety of causes, while it is often resistant to current therapeutic approaches. To elucidate the molecular mechanisms underlying pathological pain/dysesthesia, we are investigating (1) the roles of nociceptors (mainly TRP channels) expressed in sensory neurons in the generation of pathological pain/dysesthesia, and (2) the possible involvement of neuroimmune response mediated by the reciprocal interaction between peripheral/central nervous and immune systems.

4) Study of the pathogenic mechanism of Parkinson's disease in order to identify a potential novel cure: Parkinson's disease (PD) is the most common movement disorder caused by dopaminergic neuronal degeneration. It is characterized by the symptoms of resting tremor, rigidity, and akinesia. Many medical treatments have been developed; however, there is no fundamental cure. The goal of our research is to reveal the pathogenic mechanism of PD and to identify a novel cure. We recently reported that zonisamide (antiepileptic drug) and oxicam (non-steroidal anti-inflammatory) prevent cell death in a PD model, and we seek to advance our research to improve the clinical outcome for PD patients.

5) Application of biomarkers to individualized pharmacotherapy: Design of a dosing plan for immunosuppressiveagents, tacrolimus and cyclosporine, is difficult because of large intra- and interindividual variability in the pharmacokinetics. To overcome these clinical problems, the development of individual immunosuppressive therapies based on the genomic, biochemical and population pharmacokinetic analyses have been attempted. We also focus on biomarkers that predict drug-mediated kidney injury.



Figures 1. Research on drug transporters –from bench to bedside–

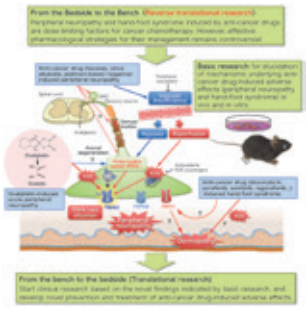


Figure 2. Reverse translational research for adverse effects of anti-cancer drugs

Recent publications

- Omura T, Sasaoka M, Hashimoto G, Imai S, Yamamoto J, Sato Y, Nakagawa S, Yonezawa A, Nakagawa T, Yano I, Tasaki Y, Matsubara K. Oxicam-derived non-steroidal anti-inflammatory drugs suppress 1-methyl-4-phenyl pyridinium-induced cell death via repression of endoplasmic reticulum stress response and mitochondrial dysfunction in SH-SY5Y cells. *Biochem Biophys Res Commun* **503**: 2963-2969 (2018)
- Nakagawa S, Nakaish M, HashimotoM, Ito H, Yamamoto W, Nakashima R, Tanaka M, Fujii T, Omura T, Imai S, Nakagawa T, Yonezawa A, Imai H, Mimori T, Matsubara K: Effect of medication adherence on disease activity among Japanese patients with rheumatoid arthritis. *PLOS ONE* **13**: e0206943 (2018)
- Omura T, Matsuda, H, Nomura L, Imai S, Denda M, Nakagawa S, Yonezawa A, Nakagawa T, Yano I, Matsubara K. Ubiquitin ligase HMG-CoA reductase degradation 1 (HRD1) prevents cell death in a cellular model of Parkinson's disease. *Biochem Biophys Res Commun* **506**: 516-521 (2018)
- Imai S, Koyanagi M, Azimi Z, Nakazato Y, Matsumoto M, Ogihara T, Yonezawa A, Omura T, Nakagawa S, Wakatsuki S, Araki T, Kaneko S, Nakagawa T, Matsubara K: Taxanes and platinum derivatives impair Schwann cells via distinct mechanisms. *Sci Rep* **7**: 5947 (2017)

Department of Pharmacogenomics · Genomic Drug Discovery Sciences (GDDS)

Associate Professor: Akira Hirasawa

Research Projects:

What's GDDS

The genomic drug discovery science is the science field of discovery of the new drug, the medicine of the effect to be higher and the medicine with few side effects, using the genome information. Our research projects are performed on major 3 themes; 1. Function of G protein-coupled receptors (GPCR), which are in cell membrane and play important roles on bio-reactions. 2. Development of microarray techniques, which are took notice as the techniques of comprehensive gene analysis. 3. Bioinformatics sciences, which is necessary to analyze a lot of information including genomic information.

GPCR

The Human Genome Project is now completed, and that enables access to every human G-protein coupled receptor (GPCR), which represents the single most important drug targets for medical therapy. Many of novel GPCR discoveries were based solely upon their shared sequence identities and characteristic seven transmembrane-spanning structure encoded therein. This sequence conservation allowed for powerful cloning techniques through DNA technology (in particular PCR technology) and in silico screening of GPCRs using genome or cDNA sequence data. Information from genome sequencing estimated the existence of 700-800 GPCRs in the human genome: about 250 of GPCRs are identified as receptors for known ligands, and the rest are still orphan receptors (oGPCRs). Recognized for the potential of oGPCRs as targets of novel drug discovery, oGPCRs have attracted a tremendous level of attention in terms of continued identification of their endogenous ligands and elucidation of their physiological functions.

Microarray

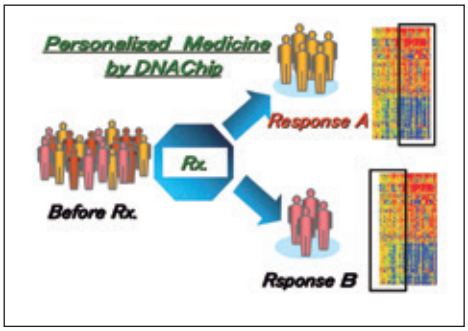
A microarray is one of the most important basic technology for drug discovery from the aspect of genomics. The focus of genome research will be shifted to functional analysis of genes including the determination of precise transcript unit as transcriptome.

The target validation is one of the critical point of drug discovery. The gene expression pattern (i.e. profile) of disease specific status could be obtained by DNA chip technology that makes it accelerate to find candidate molecules of drug target. Microarrays enable the comprehensive analysis of gene expression of various disease status including model animals and cellular activity. The process of target validation would be aided by the databases which conjugate the data of gene expression and that of pharmacology, physiology, biochemistry, molecular biology and so on. We are interested in the construction of databases of gene expression data, gene expression analysis of disease model animals and human disease status, and finally discovering the candidate molecule of the effective novel drug target and revealing the mechanisms of human disorders.

Pharmacogenomics

Pharmacogenomics is the study of how an individual's genetic inheritance affects the body's response to drugs. The term comes from the words pharmacology and genomics and is thus the intersection of pharmaceuticals and genetics. Pharmacogenomics holds the promise that drugs might one day be tailor-made for individuals and adapted to each person's own genetic makeup. Environment, diet, age, lifestyle, and state of health all can influence a person's response to medicines, but understanding an individual's genetic makeup is thought to be the key to creating personalized drugs with greater efficacy and safety. Pharmacogenomics combines traditional pharmaceutical sciences such as biochemistry with annotated knowledge of genes, proteins, and transcriptome scanning in particular.

Expression monitoring by DNA microarray is the most biologically informative application of this technology at present. Microarray technology has important applications in pharmacogenomics: drug discovery and development, drug safety and molecular diagnostics. DNA chips will facilitate the integration of diagnosis and therapeutics, as well as the introduction of personalized medicines.



Recent publications

- Takeuchi M, Hirasawa A, Hara T, Kimura I, Hirano T, Suzuki T, Miyata N, Awaji T, Ishiguro M, Tsujimoto G. FFA1-selective agonistic activity based on docking simulation using FFA1 and GPR120 homology models. Br J Pharmacol. **168**(7): 1570-1583, 2013.
- Ichimura A et al. Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. *Nature*. **483**(7389): 350-354, 2012.
- Hirasawa A et al. Free fatty acids regulate gut incretin glucagons-like peptide–1 secretion through GPR120. *Nat. Med.* **11**: 90-94, 2005.

Department of Chemogenomics and Bioorganic Medicinal Chemistry

Professor: Hiroaki Ohno, Associate Professor: Shinya Oishi,
Assistant Professor: Shinsuke Inuki

Research Projects:

The phenomena of life are driven by well-regulated chemical reactions and equilibrium of many organic compounds. Abnormalities in this regulation can cause disease, and organic compounds are often used as the medicine to treat disease. It is thus essential to understand the underlying organic chemistry of drug discovery and treatment of disease. Since highly effective medicines have already been developed for many druggable therapeutic targets, we have to consider how to discover drugs for difficult-to-treat diseases. This challenging situation can be solved by making available compounds that are hard to synthesize with existing methods in drug discovery, or by controlling the interaction of biomolecules that have hitherto been difficult to control. In our laboratory, the following research projects are underway that focus on organic chemistry-driven drug discovery.

1) Synthesis of structurally complex bioactive compounds: Small molecule drug discovery has become increasingly challenging. Despite advances in combinatorial synthesis and high-throughput screening, such technologies still have limitations. Another problem is the lack of therapeutic targets since drugs have already been developed for many diseases. Our approach is to use complex molecules to target biomolecular interactions that have not yet been probed in drug discovery. We are interested in the synthesis of biologically-active compounds, such as alkaloids containing highly complex ring systems, and macrocyclic peptides.

2) Novel methods for the synthesis of complex structures and their applications: Structure-activity relationship (SAR) studies and structural optimization are needed to improve the biological activity and bioavailability of potential drug candidates. This becomes very costly in terms of time and money when using very complex molecules. We are developing new synthetic methodologies that can be used to construct complex core structures commonly found in biologically active molecules. We are particularly interested in atom-economic transition metal catalysis using elements such as gold and palladium. Such methodologies are applied to drug discovery and structural studies to evaluate the utility of the developed reactions.

3) Identification of functional molecules based on designs, synthetic studies and chemical modifications of biomolecules: Biomolecules such as glycans, lipids, and peptides possess a wide range of biological activities, and are involved in various physiological functions and pathological conditions. By implementing rational designs, synthetic studies and chemical modifications on the basis of organic chemistry and bioorganic chemistry, we aim to develop functional molecules that can modulate and help us understand physiological functions and pathologies. Furthermore, by using these functional molecules as biological probes, we investigate the localization and intracellular behavior of target molecules for the elucidation of biological mechanisms and the discovery of new drug leads.

4) Development of a novel screening platform using synthetic peptides and proteins: Recombinant DNA technology facilitates the preparation of peptides and proteins. In contrast, chemical synthesis of peptides and proteins *via* the stepwise assembly of amino acids and/or chemical ligation can provide an alternative approach for the preparation of bioactive peptides with unique structures (secondary metabolites) and peptides/proteins containing post-translational modifications. Using these synthetic peptides and proteins, we are developing a unique screening platform to identify unprecedented drug leads from unexplored classes of substances.

5) Drug screening programs using in-house chemical libraries: Identification of novel bioactive compounds as drug candidates is an important subject in drug discovery. We have synthesized natural products with unique bioactivity (e.g. alkaloids) and biomolecules with important physiological functions (e.g. peptide hormones) and constructed a chemical library containing these compounds. Synthetic intermediates of these functional molecules are also included in our library. These compounds cannot be obtained commercially and we are engaged in a number of ongoing collaborative screening projects.

Recent publications

- Inuki *et al.* Construction of Quaternary Carbon Stereocenter of α-Tertiary Amine through Remote C–H Functionalization of Tris Derivatives: Enantioselective Total Synthesis of Myriocin. *Org. Lett.*, **21**, 5485 (2019).
- Ohno *et al.* Gold(I)-Catalyzed Cascade Cyclization Reactions of Allenynes for the Synthesis of Fused Cyclopropanes and Acenaphthenes. *Angew. Chem. Int. Ed.*, **58**, 7792 (2019).
- Oishi *et al.* Development of Mirror-Image Screening Systems for XIAP BIR3 Domain Inhibitors. *Bioconjug. Chem.*, **30**, 1395 (2019).
- Inuki *et al.* Potent Th2 Cytokine-Bias of Natural Killer T Cell by CD1d Glycolipid Ligands Based on "Anchoring Effect" of Polar Groups in Their Lipid Component. *Angew. Chem. Int. Ed.*, **57**, 9655 (2018).
- Ohno *et al.* Direct Synthesis of Aryl-Annulated (c)Carbazoles by Gold(I)-Catalysed Cascade Reaction of Azide-Diynes and Arenes. *Chem. Sci.*, **9**, 8416 (2018).
- Oishi *et al.* Structure–Activity Relationship Study of Cyclic Pentapeptide Ligands for Atypical Chemokine Receptor 3 (ACKR3). *J. Med. Chem.*, **61**, 3745 (2018).

Department of Systems Biology

Professor: Masao Doi, Lecturer: Yoshiaki Yamaguchi,
Assistant Professor: Takahito Miyake

Research Projects:

The major interest of our lab is and has been centered on Research and Development of Innovative Chronomedicine and Therapies Based on Circadian Clock. Research topics undergoing in our lab are characterized as follows:

Fig. Drug discovery based on TIME:
Let's tune the world for better health!

1) Clock Gears: State-of-the-art research on circadian rhythms began with a seminal finding of "clock genes" that are common to human beings and other mammals. The field since then has expanded into a rich and diverse interdisciplinary academic field that deals with both basic sciences and clinical interrogations of daily rhythms in physiology. There are still a number of mysteries in the molecular clock gears, which would reveal as yet undeciphered molecular links between daily physiologies and circadian clock.

2) Central Clock: A section of the hypothalamus called the suprachiasmatic nucleus (SCN) lies at the center of the body's master clock and gets input directly from light sensors in the eyes, keeping the rest of the body on schedule. My lab studies the molecules, cells, and circuits underlying these circadian rhythms in the SCN using techniques that include real-time cellular imaging and multiple genetic manipulations, i.e. mutants, knockouts, transgenics, optogenetics, etc. This approach is producing insight into the roles of specific neuropeptides and G-protein-coupled receptor-mediated signaling circuits in the rich repertoire of daily behaviors and physiologies.

3) Time Medicine: What will be a representative medical application of time control medicine? Sleep-wake cycles are profoundly influenced by the abnormalities of the circadian clock. We have recently demonstrated that Gpr176 is an orphan G-protein-coupled receptor that sets the pace of the central clock in the brain. Gpr176 is thus a potential drug target to treat sleep-wake cycle dysfunction. Development of a chemical modulator of Gpr176 function is our next on-going challenge.

4) Lark vs. Owl: Do you usually wake up early or sleep in the morning? Genome-wide association studies featured RGS16 as a "morning person"-associated gene. These human studies corroborate our original research finding using knockout mice. RGS16 shows strong circadian expression in the brain's pacemaker neurons and its deletion leads to elongation of period of circadian locomotor activity rhythm. We are currently interested in defining brain G-protein signaling that sets the time to go to sleep and wake up every day.

5) Clock Disorders: One of the most significant conceptual changes brought about by the analysis of circadian clock-deficient mice is that abnormalities in the circadian clock are linked not only to sleep arousal disorder but also to a wide variety of common diseases, including hypertension, diabetes, obesity, and cancer. We previously revealed a molecular mechanism linking circadian malfunction to salt-sensitive hypertension. Elucidation of clock dysfunction behind diseases will continue to be a main project of our lab.

6) Circadian Therapies: The development of anti-cancer drugs and therapies for lifestyle-related illnesses, such as cardiovascular disorder, obesity, and diabetes mellitus, would not solely involve manipulation of the core clock proteins *per se*, but will also be equally facilitated by manipulating the function of the group of "output" genes, which are regulated by the circadian clock. Manipulating the "input" signals that adjust the phase and amplitude of the circadian clock will also expand the path to drug discovery for circadian disorders. Symptoms of illness and efficacies of drugs are known to change according to time of our body and therefore can be predicted. It is hoped that the body time information will be used as clinical evidence to maximize the effectiveness of existing drugs and also to reduce their potential adverse side-effects.

Recent publications

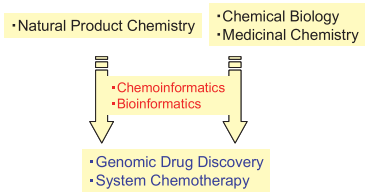
- Doi et al. Salt-sensitive hypertension in circadian clock-deficient *Cry*-null mice involves dysregulated adrenal Hsd3b6. **Nature Medicine** 16, 67 (2010)
- Doi et al. Circadian regulation of intracellular G-protein signaling mediates intercellular synchrony and rhythmicity in the suprachiasmatic nucleus. **Nature Commun**. 2, 327 (2011)
- Yamaguchi et al. Mice genetically deficient in vasopressin V1a and V1b receptors are resistant to jet lag. **Science** 342, 85 (2013)
- Doi et al. Gpr176 is a Gz-linked orphan G-protein-coupled receptor that sets the pace of circadian behaviour. **Nature Commun**. 7, 10583 (2016)
- Chao et al. Circadian clock regulates hepatic polyploidy by modulating Mkp1-Erk1/2 signaling pathway. **Nature Commun**. 8, 2238 (2017)
- Goda, Doi et al. Calcitonin receptors are ancient modulators for rhythms of preferential temperature in insects and body temperature in mammals. **Genes Dev**. 32, 140 (2018)
- Doi, Shimatani et al. Non-coding *cis*-element of *Period2* is essential for maintaining organismal circadian behaviour and body temperature rhythmicity. **Nature Commun**. 10, 2563 (2019)
- Miyake and Doi, Reconstitution of organismal liver clock function requires light. **Trends Endocrinol Metab**. in press

Department of System Chemotherapy and Molecular Sciences

Professor: Hideaki Kakeya, Associate Professor: Akira Hattori,
Assistant Professor: Takefumi Kuranaga

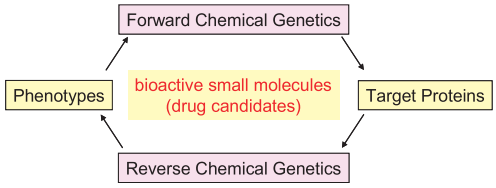
Research Projects:

Chemical biology based on forward/reverse chemical genetics is a new research paradigm that accelerates drug development and the functional analysis of genes and proteins. Diversity of small molecules is one of the most important points to facilitate the success of chemical biology. As such, we have been screening two types of chemical libraries: a natural products library and a synthetic chemical library. After identifying bioactive small molecules, their modes of actions and targets are investigated using a chemical biology-based approach.



Recent major projects are as follows:

1. **Advanced chemical biology research for establishing system chemotherapy in order to cure multi-factorial diseases; e.g. cancer, heart failure, immunodeficiency, infectious diseases, diabetes, and neuronal diseases.**
2. **HCS (high-contents screening) and HTS (high-throughputs screening) for identifying useful small molecules (bioprobes).**
3. **Natural product chemistry and medicinal chemistry for mining novel bioactive small molecules.**
4. **Biosynthetic studies of natural products and their application to combinatorial biosynthesis.**

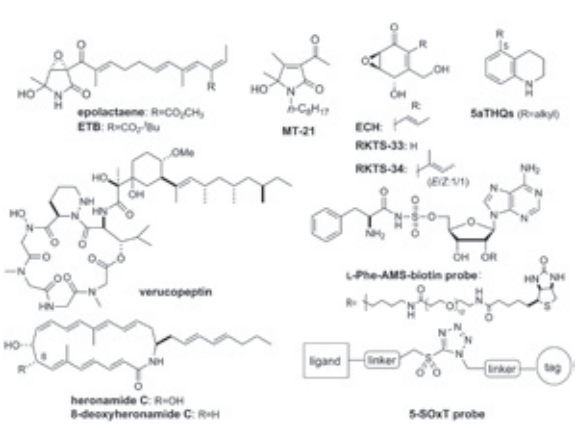


We have discovered epolactaene from *Penicillium* sp. to be a neuronal differentiation inducer, and have identified MT-21 and ETB (epolactene *tert*-butyl ester) as potent apoptosis inducers based on Structure-Activity Relationships (SAR) studies. Using a biotin-labeled probe of epolactaene/ ETB, human Hsp 60 (heat-shock protein 60) was identified as a binding protein of epolactaene/ ETB in vitro as well as in situ. Moreover, it was suggested that Cys442 of Hsp60 is responsible for the covalent binding with epolactaene/ETB as well as the inhibition of chaperone activity by epolactaene/ ETB. Epolactaene/ETB would be highly useful tools to understand the function of human Hsp60 and the mechanisms of molecular chaperones.

We have also found a small molecule, ECH, produced by a fungal strain that selectively inhibits apoptosis induced by the death-receptor system. Using chemical biology-based approaches, we revealed that ECH inhibits Fas-mediated apoptosis by blocking activation of procaspase-8 in the DISC (death-inducing signaling complex). In addition, ECH also inhibits Fas ligand-dependent apoptosis in CTL-mediated cytotoxicity. Based on the detailed SAR studies of ECH, RKTS-33&34 were developed as novel nonpeptide inhibitors targeting death receptor-mediated apoptosis.

Hypoxia-inducible factor (HIF) is deeply involved in cancer progression. During the course of our screening for HIF-signaling modulators, we re-discovered verucopeptin, produced by *Streptomyces* sp., as a new HIF-signaling inhibitor. We determined the absolute stereochemistry of verucopeptin by the spectroscopic analysis and synthetic approaches. Verucopeptin decreased the amount of HIF-1α protein, whereas it did not affect the level of HIF-1β protein. Further analysis of the inhibitory mechanism by verucopeptin is on going.

Irreversible modification is one of the most promising strategies to identify cellular receptors of bioactive small molecules. Recently we developed a 5-sulfonyl tetrazole probe, which enabled chemical tagging of binding proteins against a ligand. The studies on modes of action for antifungal molecules heronamides and 5aTHQs (5-alkyl-1,2,3,4-tetrahydroquinolines), as well as the development of an affinity probe to identify adenylation domain-containing modules in nonribosomal peptide synthetase (NRPS)-polyketide synthase (PKS) hybrids and NRPSs are also undertaken.



Recent publications

- Ozaki, T. *et al.* Identification of the common biosynthetic gene cluster for both antimicrobial streptoaminals and antifungal 5-alkyl-1,2,3,4-tetrahydroquinolines. *Org. Biomol. Chem.* **17**, 2370, 2019.
- Lu, S. *et al.* Discovery of Presaccharothriolide X, a Retro-Michael Reaction Product of Saccharothriolide B, from the Rare Actinomycete *Saccharothrix* sp. *Org. Lett.* **20**, 4406, 2018.
- Ozawa, H. *et al.* Curcumin beta-D-glucuronide plays an important role to keep high levels of free-form curcumin in the blood. *Biol. Pharm. Bull.* **40**, 1515, 2017.
- Sugiyama, R. *et al.* Disocovery and total synthesis of streptoaminals, antimicrobial (5,5)-spirohemiaminals from the combined-culture of *Streptomyces nigrescens* and *Tsukamurella pulmonis*. *Angew. Chem. Int. Ed.* **55**, 10278, 2016.
- Kakeya, H. Natural products-prompted chemical biology: Phenotypic screening and a new platform for target identification. *Nat. Prod. Rep.* **33**, 648, 2016.
- Goto, Y. *et al.* UCHL1 provides diagnostic and antimetastatic strategies due to its deubiquitinating effect on HIF-1α. *Nat Commun.* **6**, 6153, 2015.
- Moriyama, A. *et al.* In vivo linking of membrane lipids and the anion transporter band 3 with thiourea-modified amphiphilic lipid probe. *Sci. Rep.* **5**, 17427, 2015.
- Sugiyama, R. *et al.* Structure and biological activity of 8-deoxyheronamide C from a marine-derived *Streptomyces* sp.: heronamides target saturated hydrocarbon chains in lipid membranes. *J. Am. Chem. Soc.* **136**, 5209, 2014.

Department of Molecular Metabology

Associate Professor: Jean-Michel Fustin

Research Projects:

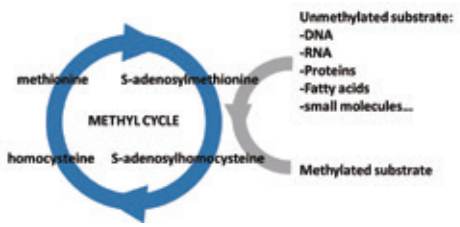
The circadian clock is a molecular mechanism that orchestrates physiology and behaviour of the whole organism, segregating at different times of the day incompatible metabolic processes. While the circadian clock is a self-sustained mechanism that can keep ticking in constant conditions, it can be synchronized with the light-dark cycles, enabling the organism to anticipate rhythmic changes in the environment. The circadian clock involves a negative transcription-translation feedback loop in which so-called "clock genes" regulate their own expression and that of output genes of metabolic significance. In virtually all tissues in the body, about 10-15% of genes are rhythmically controlled by the circadian clock. These widespread oscillations in gene expression, accompanied by oscillations in proteins function and metabolites abundance and ultimately behaviour and physiology, depend on the many regulatory steps in transcriptional control, splicing and RNA processing, translation and RNA degradation, and protein modifications (phosphorylation, ubiquitination, SUMOylation…). Research in the past decades have contributed to the establishment of a relatively well-understood model on how the clock functions at the molecular level and how it regulates physiology, and animal models enabling the molecular clock to be "visualized" in real-time, using luciferase or fluorescent proteins have been established.

Cells must constantly react to their environment and integrate the signals received in order to produce an appropriate response. Immediate responses will involve rapid changes in the level of metabolites such as cAMP and NAD^+, followed by the regulation of immediate early genes. More sustained responses, triggered by chronic stimulations or long-term fluctuations in the environment, will involve stable changes in metabolism and gene expression regulated at the epigenetic and epitranscriptomic levels, notably via phosphorylation, acetylation and methylation of histones, and methylation of RNA.

The research at the Laboratory of Molecular Metabology focusses on the crosstalk between metabolism and gene expression: How metabolism regulates gene expression, and how gene expression in turns regulate metabolism. We use the circadian clock as a tool to measure and understand how gene expression, and ultimately physiology and behaviour, is regulated by physiological and pathological metabolic changes.

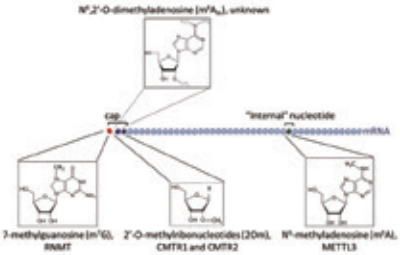
1. Molecular basis of cross-talk between intracellular methylation rhythm and clock gene regulation

All transmethylations are influenced by the metabolic state of the cell due to their sensitivity to the availability of S-adenosylmethionine, the universal methyl donor cosubstrate synthesized from methionine in a ubiquitous metabolic pathway called the methyl cycle. We are investigating how the circadian clock and the methyl cycle are linked.



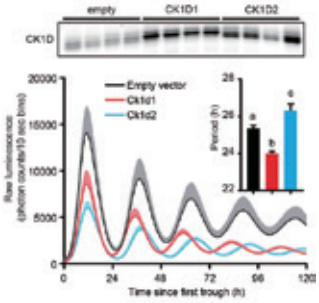
2. Physiological functions of mRNA methylation

It is now known that many nucleotides in mRNA are methylated and is an essential step in the processing and degradation of the transcript. Most of these methylations are essential for cell differentiation and development, and their disruption is embryonic lethal. However, their presence in differentiated somatic tissues suggest they may have a physiological function in adults. We the role of mRNA methylation in the function of the circadian clock.



3. Roles and regulation of Casein Kinase 1 Delta isoforms

Casein Kinase 1 Delta (CK1D) is a key regulator of circadian rhythms and cell division. We have identified that the expression of *Ck1d* regulated by mRNA methylation (internal N^6-methyladenosine of the 3'-UTR) and reported the existence of two alternatively spliced and functionally different isoforms of CK1D, CK1D1 and CK1D2. We are investigating how the expression of these two isoforms is controlled.



Recent publications

- Masao Doi, Hiroyuki Shimatani, Yuta Atobe, Iori Murai, Hida Hayashi, Yukari Takahashi, Jean-Michel Fustin, Yoshiaki Yamaguchi, Hiroshi Kiyonari, Nobuya Koike, Kazuhiro Yagita, Choogon Lee, Manabu Abe, Kenji Sakimura, Okamura Hitoshi. Non-coding cis-regulatory element E'-box of Period2 is essential for daily maintenance of organismal behavior and physiology. Nature Communications, May 2019.
- Jean-michel Fustin and Kashiwazaki Yasuo
  RNA のメチル化による概日リズム制御
  実験医学 , 36(19), Nov 2018. Translated title: "*Control of circadian rhythms by RNA methylation.*"
- Jean-michel Fustin and Okamura Hitoshi
  RNA メチル化 生体の科学 , 69(5) 2-3, Nov 2018. Translated title: "*RNA methylation*"
- Jean-Michel Fustin, Rika Kojima , Kakeru Itoh , Hsin-Yi Chang , Ye Shiqi , Bowen Zhuang , Asami Oji , Shingo Gibo , Rajesh Narasimamurthy , David M. Virshup , Gen Kurosawa , Masao Doi , Ichiro Manabe , Yasushi Ishihama , Masahito Ikawa , Hitoshi Okamura.
  Two Ck1δ transcripts regulated by m6A methylation code for two antagonistic kinases in the control of the circadian clock.
  Proc Natl Acad Sci U S A. Jun 5;115(23):5986-5991, 2018
- Rajesh Narasimamurthy, Sabrina R. Hunt, Yining Lu, Jean-Michel Fustin, Hitoshi Okamura, Carrie L. Partch, Daniel B. Forger, Jae Kyoung Kim, David M. Virshup
  CK1δ/ε protein kinases prime the PER2 circadian phosphoswitch.
  Proc Natl Acad Sci U S A. Jun 5;115(23):5980-5985, 2018

Department of Integrative Genomics

Professor: Hiroyuki Ogata,

Assistant Professor: Romain Blanc-Mathieu, Hisashi Endo

Research Projects:

Our laboratory aims to understand the diversity and functioning of complex living systems based on large scale life science data towards application in biomedical sciences and environmental conservation. We develop new bioinformatics methods allowing integrated analyses of molecular data such as drug structure, metabolites, and genomic information and higher level knowledge about cells, organisms, populations and environments. Current research projects involve viral and microbial genomics, prediction of drug-microbiome interactions, and investigation of the functional link between microorganisms and the environmental changes.

1. Genomics of viruses

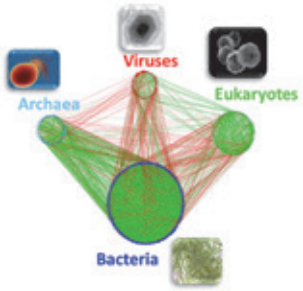
Viruses are generally considered as small biological objects with only a handful of genes sufficient for their rapid replication. However, relatively large viruses such as herpesviruses and poxviruses possess a few hundreds genes. Furthermore, recent studies have revealed the existence of much larger viruses encoding more than 300 up to 2,500 genes. Such giant viruses, comparable to cells in their dimension, show a huge genomic diversity. Including these giant viruses, viruses possess various mechanisms to evade host defense systems and to reprogram intracellular machinery of their hosts for their replication. Viruses are also considered to contribute to the evolution of their hosts through horizontal gene transfer and the host-parasite arms race. However, our knowledge on viruses is limited compared to the knowledge on cellular organisms. We are interested in revealing functions of their genomes and the roles of viruses in various ecosystems through comparative genomics. We also develop new bioinformatics methods helping such comparative genomics.

2. Interactions between microbial communities and their environments

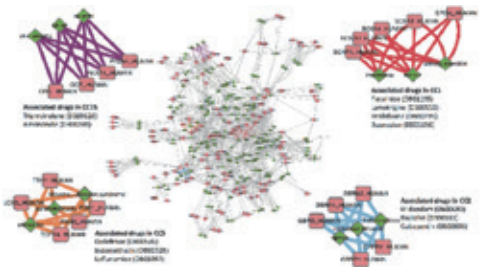
Bacteria and unicellular eukaryotes play important roles in various environments. We study microorganisms (from viruses, bacteria, unicellular eukaryotes to zooplankton) in animal gut and marine ecosystems in terms of their community structure and functioning. Our focus is on the characterization of their diversity and the interactions among them as well as the relationships between the dynamics of microbial communities under varying environmental conditions. Our research interests include the identification of enzymes and secondary metabolites with new pharmacological activity from large scale genetic data.

3. Integration of chemical, genomics, and biomedical knowledge for biomedical sciences and environmental preservation

To help the research communities in genomics and biomedical sciences, we develop a suite of bioinformatics tools and various databases through an integrated web environment named GenomeNet (http://www.genome.jp/). GenomeNet integrates major molecular biology databases such as the KEGG database (http://www.kegg.jp/) developed in Kyoto University, as well as other databases of genes, proteins, enzyme reactions, metabolic compounds, drugs, and drug side effects. Currently, we put much effort in integrating metagenomic data generated at a population level (e.g. Human microbiomes) or at a global scale (e.g. *Tara* Oceans expedition data). We also started an integrated database project for proteomics data from various species including human. Certain pathogens evade the host immune system by altering the surface proteins ("antigenic variation"), but its mechanism is still unclear. In this regard, we collect and organize the information of antigenic variations and relevant gene families to uncover the mechanism and to utilize it in clinical practice (varDB, http://www.vardb.org/). These resources are freely available through GenomeNet to the communities. We also develop various bioinformatics and statistical methods for medical and pharmacological sciences such as prediction methods for the side effects of drugs.



Prediction of species interaction networks



Prediction of drug-drug interactions

Recent publications

- Yoshikawa G., Blanc-Mathieu R., Song C., Kayama Y., Mochizuki T., Murata K., Ogata H., Takemura M.; Medusavirus, a novel large DNA virus discovered from hot spring water. *J. Virol.*, **93**, e02130-18 (2019).
- Li Y., Hingamp P., Watai H., Endo H., Yoshida T., Ogata H.; Degenerate PCR primers to reveal the diversity of giant viruses in coastal waters. *Viruses*, **10**, 496 (2018).
- Endo H., Ogata H., Suzuki K.; Contrasting biogeography and diversity patterns between diatoms and haptophytes in the central Pacific Ocean. *Sci. Rep.*, **8**, 10916 (2018).
- Yoshida, T., Nishimura, Y., Watai, H., Haruki, N., Morimoto, D., Kaneko, H., Honda, T., Yamamoto, K., Hingamp, P., Sako, Y., Goto, S., and Ogata H.; Locality and diel cycling of viral production revealed by a 24 h time course cross-omics analysis in a coastal region of Japan. *ISME J.*, **12**, 1287-1295 (2018).

Department of Computational Genomics

Professor: Hiroshi Mamitsuka, Assistant Professor: Canh Hao Nguyen

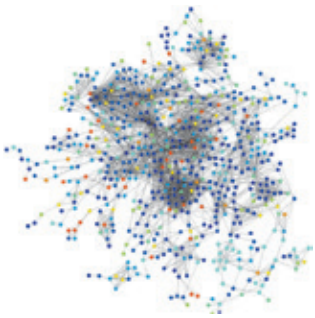
Research Projects:

Recent development in experimental biotechnology and nation-wide or international projects in life sciences have generated a variety of different types of biological data. They are currently stored in a lot of publicly available databases, which can be accessed through the internet. These databases, however, do not seem to have been used thoroughly in terms of understanding the mechanisms of life sciences. So it must be useful to systematically analyze the data by using the techniques in information sciences. This approach is generally called "bioinformatics", and in particular, so-called machine learning, data mining and statistics would be key techniques for this purpose. Machine learning (and data mining as well) is a research field in computer science to develop the methods which efficiently capture the property, such as patterns, rules and hypothesis etc., of given data. The data format which has been used for a long time in this field is a simple table (each example is a row, and each attribute of an example is a column). To deal with this type of structured data, a lot of techniques have been already proposed in machine learning and statistics. On the other hand, there exist a lot of different types of datasets in life sciences, such as genome sequences, chemical structures and signal transduction pathways, which are unstructured (Note that a table is called structured data). Each of them cannot be a table easily, and even if it can, some important information might be dropped off when we transform unstructured data into structured data. Thus it would be valuable to develop a new approach of machine learning for unstructured data. We note that this approach for unstructured data must be worth contributing not only to promoting the findings in life sciences but also to the development of computer science itself. Currently our laboratory has developed a variety of new techniques in the above direction, which will be kept in this fiscal year as well. Below we will briefly show three topics which we have conducted in our laboratory, including on-going projects.

1) Integrative mining from unstructured and structured data: Recent biological data are, in many cases, represented by graphs, such as gene regulatory networks, metabolic pathways and protein-protein interactions, etc. We are developing a technique for combining this type of unstructured information with structured data. An example is clustering genes using both gene networks (unstructured data) and cDNA microarray expressions (structured data). This approach is for predicting the function of an arbitrary gene. We currently focus on the network with high modularity, and in the future, our attention will be extended to variety types of networks, including that with the scale-freeness

2) Data mining from tree structured glycans: We have developed a probabilistic model-based approach for mining patterns from labeled ordered trees, or two-dimensional chemical structures of glycans (carbohydrate sugar chains). Our approach realizes multiple tree alignment and the findings of some patterns peculiar to each class of glycans. We, in the future, will develop a method for automatically classifying a variety of glycans based on our current approach.

3) Text mining from biomedical documents: A typical example of unstructured data which are accumulated in a rapid speed is biomedical documents. We are now developing roughly three approaches for text mining, i.e. acquiring new information from biomedical documents. The first is a method, which can be categorized into the field of information retrieval, to find the most relevant document to a given query phrase like "what is the function of a gene of Mad Cow Disease?" The second is a probabilistic model-based approach for finding a new co-occurrence of biological entities, like a pair of some small molecule and a disease, from a currently available set of co-occurrences. The third is a probabilistic model-based approach for clustering documents with multiple fields.



Left: Clustered genes by structured data only
Right : Clustered genes by both structured and unstructured data

Each color is a gene function. The right figure is more organized than the left, meaning that unstructured data works well to labeling gene functions.

Recent publications

- Takigawa *et al.* Mining Significant Substructure Pairs for Interpreting Polypharmacology in Drug-Target Network. *PLoS One*, **6**(2), e16999, 2011.
- Takigawa and Mamitsuka. Graph Mining: Procedure, Application to Drug Discovery and Recent Advance. *Drug Discovery Today*, **18**(1-2), 50-57, 2013.
- Ding *et al.* Similarity-based Machine Learning Methods for Predicting Drug-target Interactions: A Brief Review. *Briefings in Bioinformatics*, **15**(5), 737-747, 2014.

Department of Nanobio Drug Discovery

Visiting Professor: Yutaka Shimada, M.D., Kazuharu Shimizu, Ph.D., Tetsuo Sudo,
Lecturer: Yoshinori Takei, Ph.D.

Research Projects:

1. Background and aims

Recent advances in the field of engineering, including nano, material, and analytical technology, contribute to produce huge amount of bio-information, which helps progress of genomic, genetic, epigenetic, and proteomic studies. Systems biology, a new approach on the basis of those accumulated bio-information, allows novel methods for both discovery of novel drugs and biomakers, and creation of innovative diagnostic tools and therapeutic methods."**Nanobio**" research, an integrated research between "nano" -material technology and "bio"-logy, will not only provide cutting-edge bio-information to the drug discovery science field, but also expand the range of research options in the medical and pharmacological field.

In our laboratory, we will use Nanobio technology not only for elucidation of changes in genome function under physiological and pathological conditions, but also for establishment of novel diagnostic and therapeutic tools of cancers.

2. Research directions

Using nanobio analytical devices, such as DNA microarray, we will collect novel genome-wide bio-information that cannot be obtained by conventional analytical devices. In combination of these new information and high quality clinical specimens, we seek to develop (1) new methods for diagnosis, (2) tailor-made therapy and (3) targeted therapy of cancers.

1: From mRNA expression profiling to "Tailor-made" therapy.

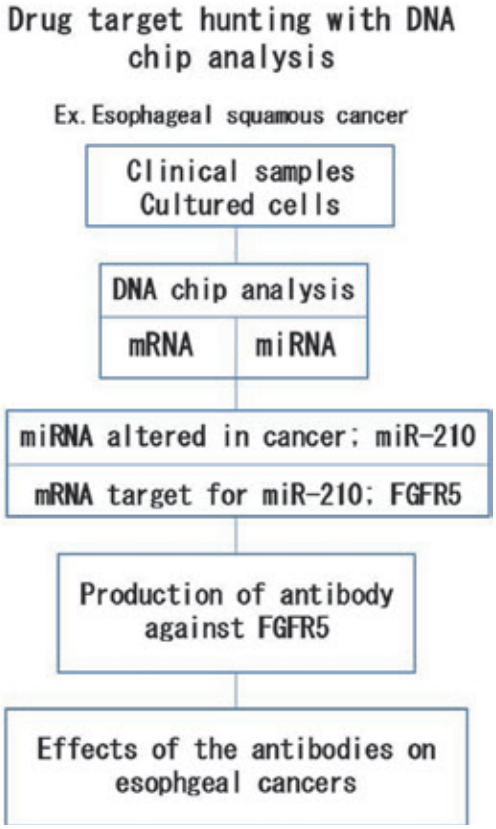
Most of current gene counseling are to diagnose diseases or to stratify patients by a single gene marker. In contrast, using a comprehensive gene expression data set, we are trying to establish mathematical models to predict a survival rate, sensitivity to chemo-radiation therapy, and distant metastasis for patients with various malignancies, especially esophageal cancer. To establish a reliable stratification strategy using these prediction models will enable us to perform "Tailor-made" therapy.

2: Functional analysis of microRNA (miRNA)

MicroRNAs are short RNA molecules that do not code proteins. With microarray technique we are investigating microRNA functions in normal cell differentiation and in malignant characteristics of tumor cells. As an outcome from this project, we indicated that low expression level of miR-210 is correlated to good prognosis of patients with esophageal cancers.

3: Development of antibody drugs

We found that FGF5 is a target for miR-210 and that high expression level of FGFR5 is correlated to good prognosis of patients with esophageal cancers. An antibody against FGFR5 inhibits cell proliferation of primary cultured cells derived from esophageal cancers. We are studying mechanism of the inhibition of cell proliferation, in order to develop new antibody drugs against esophageal cancers.



Recent publications

- S.Tsuchiya et al. MicroRNA-210 regulates cancer cell proliferation through targeting fibroblast growth factor receptor-like 1 (FGFRL1). J Biol Chem. 286,420-428,2011
- Y.Shimada et al. Expression analysis of fibroblast growth factor receptor-like 1 (FGFRL1) in esophageal squamous cell carcinoma. Esophagus 11 (1), 48-53, 2014

Department of Nanobio Drug Discovery

Visiting Professor: Shin Yonehara, Ph.D.

Research Projects:

Apoptosis, or programmed cell death, plays an important role in many biological processes, including embryogenesis, development of immune system, maintenance of tissue homeostasis, and elimination of virus-infected and tumor cells. We found cell surface Fas antigen (Fas), which can directly mediate apoptosis-inducing signals into cells by stimulation with agonistic anti-Fas monoclonal antibodies or Fas ligand. Our main research project is to understand the intracellular signal transduction mechanism of cell death including apoptosis and caspase-independent novel types of cell death, and the biological significance/physiological role of cell death and cell death-regulating molecules. In conjunction with these studies, we have been trying to identify other cell death-related molecules that play a key role in embryogenesis, tumorigenesis or immune system. Investigations of molecular mechanisms and physiological roles of cell death and cell death-related molecules are important for a better understanding of mammalian embryogenesis, tumorigenesis and immune system.

1. Interferon-γ (IFN-γ)-induced programmed necrosis/ necroptosis

We found that not only tumor necrosis factor (TNF) but also IFN-γ could induce necroptosis when caspase-8-dependent apoptotic pathway was inhibited. Although necroptosis has been considered to be induced by the function of RIPK1 and RIPK3 to activate MLKL which executes necrosis by inducing plasma membrane rupture (Figure 1), we found that RIPK1 is not necessary, but RIPK3 and MLKL are necessary, to induce necroptosis in the absence of caspase-8 protein. Protease activity of caspase-8 suppresses RIPK1 and RIPK3 to inhibit necroptosis, but adaptor activity of caspase-8 is necessary for RIPK1 to activate RIPK3 and MLKL.

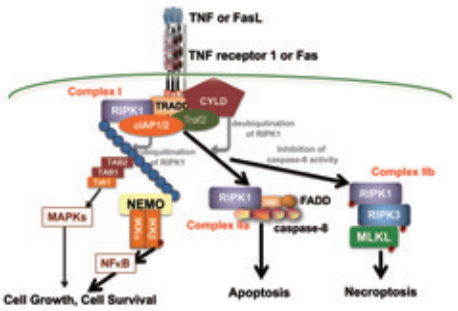


Figure 1. Cell death and survival signals mediated by Death Receptors

Phosphatidylserine (PS), an anionic phospholipid enriched in the inner leaflet of the plasma membrane, is exposed to the outer leaflet during apoptosis and the cell surface PS plays an essential role in apoptotic cells to be engulfed by macrophages as "eat me" signal. PS exposure was recently shown to be induced during TNF-induced necroptosis. We found that PS exposure mediated by RIPK3-activated MLKL was induced by a treatment with IFN-γ for more than 10 hours before the induction of necroptosis by membrane rupture. We suppose that the main activity of IFN-γ may be the induction of PS exposure, not necroptosis.

2. Retinoic acid (RA) signaling is regulated by the signaling molecules of necroptosis.

We found that knockdown of *Caspase-8* expression in embryoid bodies derived from ES cells markedly enhances RA-induced cell differentiation and necroptosis, both of which are dependent on *Ripk1* and *Ripk3*; however, the enhancement of RA-induced cell differentiation is independent of *Mlkl*. RA treatment obviously enhanced the expression of RA-specific target genes having the RA response element in their promoters to induce cell differentiation, and induced or enhanced the expression of RIPK1, RIPK3 and MLKL to stimulate necroptosis. *Caspase-8* knockdown induced RIPK1 and RIPK3 to translocate into the nucleus and to form a complex with RA receptor (RAR), and RAR interacting with RIPK1 and RIPK3 showed much stronger binding activity to RA response element than RA receptor without RIPK1 or RIPK3. In *Caspase-8*-deficient as well as *Caspase-8*- and *Mlkl*-deficient mouse embryos, the expression of RA-specific target genes and the expression of RIPK1, RIPK3 and MLKL were obviously enhanced. Thus, Caspase-8, RIPK1, and RIPK3 coordinately regulate RA-induced cell differentiation and necroptosis both *in vitro* and *in vivo* (Figure 2).

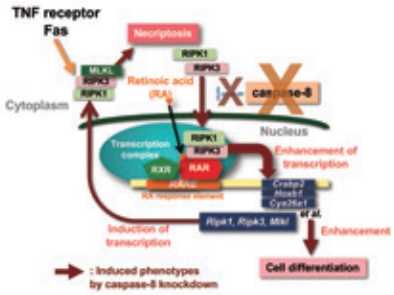


Figure 2. A model of the retinoic acid signaling in the presence and absence of Caspase-8.

3. A novel type of cell death specifically induced in carcinoma-derived cells.

By utilizing a tetracycline/doxycycline (Dox)-inducible short hairpin RNA (shRNA) expression (Tet-On) system, we established various cell lines from untransformed and transformed cells, in which knockdown of specific target genes could be induced by the treatment with Dox. Among these cells, induced knockdown of the expression of SMC2 or CAPRIN1 was shown to induce cell death specifically in transformed cells derived from human carcinoma. In case of knockdown of SMC2, a component of the condensin complex, a couple types of cell death including apoptosis and nonapoptotic cell death were observed in carcinoma-derived tetraploid cells, while a senescence-like phenotype associated with cell growth retardation was induced in untransformed diploid cells. In case of CAPRIN1 knockdown, cell growth retardation was observed in all the cells; however, nonapoptotic cell death was observed specifically in carcinoma-derived cells. The cell death with comparatively normal nucleus is not known types of cell death dependent on caspase or RIPKs, and is associated with vigorous membrane raffling and enforced detachment (abruptio) from the substrate and neighboring cells (Figure 3), both of which are dependent on a small G protein, Rac1. We named the cell death to be "Abruptosis". We are now analyzing the molecular mechanism and physiological roles of these novel types of cell death.

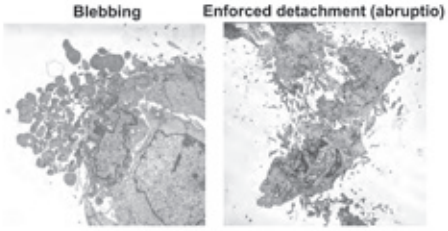


Figure 3. Analysis of Abruptosis under transmission electron microscope

Recent publications

- Shibata *et al.* 2017. Protein-driven RNA nanostructured devices that function in vitro and control mammalian cell fate. *Nat Commun*, **8**, 540, 2017.
- Chalabi-Dchar *et al.* Loss of somatostatin receptor subtype 2 promotes growth of KRAS-induced pancreatic tumors in mice by activating PI3K signaling and overexpression of CXCL16. *Gastroenterology*, **148**, 1452, 2015.
- Nakanishi *et al.* Dclk1 distinguishes between tumor and normal stem cells in the intestine. *Nat Genet*, **45**, 98, 2013.
- Fukuoka *et al.* 2013. Identification of a novel type 2 innate immunocyte with ability to enhance IgE production. *Int Immunol*, **25**, 373, 2013.

Department of Applied Pharmaceutics and Pharmacokinetics

Professor: Fumiyoshi Yamashita, Lecturer: Masahiro Tsuda,
Assistant Professor: Kanako Soh

Research Projects:

With the aging of the population, the disease pattern is changing, and medical care is becoming increasingly complex. Although pharmaceuticals have been contributing significantly to the health care, medications for patients in clinical practice are variable among patients, depending on their genetic background, living environment, and health conditions. Therefore, even if it is a drug whose efficacy and safety are guaranteed through clinical trials, unexpected problems often occur such as its limited efficacy, side effects due to drug-drug or drug-food interactions, and idiosyncratic side effects. In order to reduce the risk of such problems arising, it is of extreme importance to establish the risk assessment method in drug development, to develop pharmaceutical technology for risk mitigation and avoidance, and to establish a clinical medication design and monitoring method. From these points of view, our laboratory is currently working on the following research.

1) Development of tissue/intracellular targeted drug delivery systems using biomolecular recognition mechanisms

When administered to the body a drug distributes nonspecifically throughout vascular and interstitial tissues, nonspecific tissue distribution. However, if specific molecular recognition mechanisms of endogenous substances are available, it is possible to selectively deliver the drug to the site of action. We are developing a targeted drug delivery system in which vesicles composed of lipids and synthetic polymers are used as drug carriers and the vesicle surface is modified with receptor recognition molecules. Specifically, the drug is efficiently taken up into the target cells using a sugar chain that recognizes E-selectin expressed in inflammatory blood vessels, and a peptide that binds to transferrin receptor highly expressed in cancer cells. We are trying to control intracellular dynamics and enhance pharmacological effects by using molecular recognition related to endoplasmic reticulum transport.

2) Development of pharmacokinetics and toxicity evaluation systems using the microfluidic devices

In order to understand the pharmacokinetics of candidate substances in drug discovery research, in vitro pharmacokinetic tests using cultured cells are usually conducted. At this time, with the ideal of reflecting the function of the cells used to human beings, expectations for human iPS cells have recently increased. Currently, we are developing a system that can evaluate the pharmacokinetics and toxicity of human tissues on a chip by seeding cells on a microfluidic device and performing perfusion culture in a 2D or 3D environment.

3) Information analysis of adverse event databases and its application to risk assessment

The side effects of medicines impose not only a clinical burden on patients in which they occur but a social and economic burden on medical practice and pharmaceutical companies. By epidemiologically analyzing large-scale data on post-marketing safety information, it is possible to find feature quantities in the occurrence of side effects and to formulate an action plan for risk reduction and avoidance. Currently, we are conducting researches on comprehensive searches for drugs with hepatotoxicity, their biomarkers, and applications to risk assessment.

4) Molecular dynamics and pharmacological analysis of adverse reaction and research on development for prevention and treatment

The side effects of medicines are a frequent problem in the clinical setting, which reduces the quality of life of patients and interferes with treatment. By clarifying the mechanism of its expression from the pharmacokinetics and pharmacological aspects, it is possible to cope with the side effects, which can lead to the prevention of the occurrence and the alleviation of the symptoms. At present, we are conducting researches on pharmacokinetic control factors in anti-HIV drugs and selection of appropriate anti-HIV drugs for controlling the onset of HIV-related neurocognitive disorders, and on mechanism analysis of taste disorders, which are side effects generated during cancer chemotherapy.

Recent publications

- Babazada H, Yanamoto S, Hashida M, Yamashita F. Binding and structure-kinetic relationship analysis of selective TLR4-targeted immunosuppressive self-assembling heparin nanoparticles. Int J Pharm. 552(1-2):76-83, 2018.
- Tsuda M, Otani Y, Yonezawa A, Masui S, Ikemi Y, Denda M, Sato Y, Nakagawa S, Omura T, Imai S, Nakagawa T, Hayakari M, Matsubara K. Analysis of glycoforms and amino acids in infliximab and a biosimilar product using new method with LC/TOF-MS. Biol Pharm Bull. 41(11):1716-1721, 2018
- Yamashita F, Fujita A, Sasa Y, Higuchi Y, Tsuda M, Hashida M. An evolutionary search algorithm for covariate models in population pharmacokinetic analysis. J Pharm Sci. 106(9):2407-2411, 2017.

Center for Integrative Education in Pharmacy and Pharmaceutical Sciences

The center is an adjunct facility of the Faculty and Graduate School of Pharmaceutical Sciences, and was established in April, 2010 as a base unit for the development of integrative education/research in pharmacy and pharmaceutical sciences. The center consists of three departments: Department of Education for Drug Developmental Sciences, Department of Education for Drug Discovery Sciences, and Department of Education of Clinical Pharmacy. The specialists in the Division of Bioinformatics and Chemical Genomics are in charge of education/research into information sciences. Through close collaboration, the center offers the human resource development program described below.

The mission of the Faculty and Graduate School of Pharmaceutical Sciences is to establish a global center for innovative drug discovery and development, and optimization of pharmacotherapy. Through its curricula at both the undergraduate and graduate levels, the school aims to train world leaders in the pharmaceutical sciences. In accordance with this mission, the education programs cover not only the basic sciences, but also specialized sciences associated with industrial drug discovery and development, the theorization of pharmacotherapy theories at medical institutions, and the sciences behind governmental pharmaceutical regulations.

The mission of the center is to create an integrated education/research system to meet the recent advances in drug discovery and development, and in pharmacotherapy. The human resource development program includes:

1. Pharmaceutical R&D exercise I

The students take on roles as members of the research section of a pharmaceutical company, and discuss the strategy to discover a novel drug candidate. Through small group discussion, the team, consisting of 5-6 students, makes a presentation to supposed representatives of the company on the concept of development, social and therapeutic contributions, revenue and post-marketing management of the drug.

2. Pharmaceutical R&D exercise II

The students take on roles as members of the developmental section of a pharmaceutical company. They make a presentation to supposed doctors and/or other medical staff about clinical research on a novel drug candidate. To do this, the students prepare presentation documents/slides using the investigator's brochure, protocol, clinical report form and informed consent. "Exercise I" is assumed to be on early stage of R&D, and "Exercise II" is a later stage.

3. Integrated pharmaceutical exercise

This exercise consists of two exposure programs. Early exposure to pharmacy and pharmaceutical sciences is scheduled immediately after admission, whereas exposure to industrial drug discovery and development is in the third year.

4. Laboratory for medical ethics

This exercise deals with medical safety issues, such as adverse events and medical errors/malpractice. Through experiences as medical staff and small group discussion with medical staff, the students learn about safety management strategies and prerequisites for provision of the best medical care.

Experimental Station for Medicinal Plants

Medicinal plants have served human beings as traditional medicines and been used for making pharmaceutical products. Recent revival of interest in Kampo medicine made it to be established in Japanese community, while a variety of compounds produced by plants were shown to be a good stock for new drug development. Many people in the world have realized that plants harbored a huge pool of a variety of compounds for drug development. As this awareness becomes common, wild plants are now generally recognized as potential resources for useful compounds, and which leads countries to make strict rules on import and export of plant materials.

The station covers an area of 3,042 square meters consisting of herbarium gardens, nurseries, experimental fields and greenhouses. Various species of important medicinal plants appeared in Japanese Pharmacopoeia and rare plants collected in fieldworks abroad are cultivated in these places and used for training of undergraduate students as well as for research works of pharmaceutical sciences.

1) Herbarium Gardens: This area shows medicinal plants of Japanese Pharmacopoeia, Japanese and European folk medicines, Labiatae and other herbs and some medicinal trees of temperate zone. These plants are used for student training and also for exercises of the training course for "Pharmacist accredited with knowledge of Kampo and natural medicines".

2) Greenhouses: Medicinal plants of the tropical zone, such as cinnamon, frankincense, turmeric, long pepper, and more, are grown in the greenhouses.

3) Nurseries and Experimental Fields: Since 1980's, genetic and phylogenic studies and breeding of perilla have been performed in the fields. Pure strains of perilla kept here counted more than 5700 now.

4) Herbarium and Natural Medicine Specimens: Specimens collected in fieldworks in countries of the Middle and Near East, Central and South-East Asia are kept in the herbarium and utilized for teaching and research works.

Organic Elemental Microanalysis Center

Organic Elemental Microanalysis Center provides the necessary data in structure determination of novel compounds for the Faculty of Pharmaceutical Sciences, other universities, and other research organization.
We analyzing C, H, N, O, S, F, Cl, Br, I and P included in the organic compound mainly.

Department of Molecular Brain Science

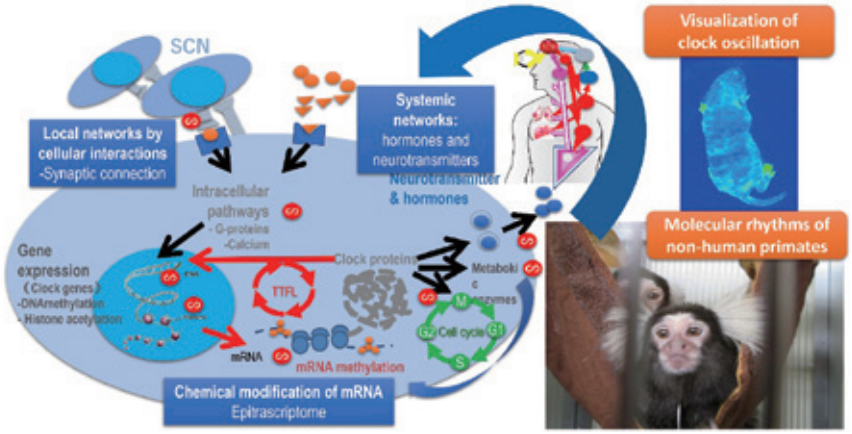
Specially Appointed Professor: Hitoshi Okamura

Research Projects:

Organisms live in a spatiotemporal world. Even for humans, regular diet and sleep are the basis of a healthy life. Epidemiological studies have demonstrated that the incidence of life-style related disorders such as diabetes, hypertension and obesity has increased in parallel with the development of our 24-hours society.

We are researchers of the suprachiasmatic nucleus (SCN) where the master clock of the mammalian circadian rhythms localizes. In the past decades, we have been pioneers in the exploration of molecular mechanism underlying the biological clock since the isolation of the mammalian *Period* genes (Nature 1997; Cell 1997; Science 1999; Science 2001, Science 2003a). In recent years, we have shown that the methylation of mRNA determines the period length of the clock (Cell 2013; PNAS 2018), that novel orphan G protein-coupled receptors and their regulation are critical for the function of the SCN and the synchronization to the light/dark cycles (Nature Comm 2011, 2016). We have also examined the impact of a disrupted clock on the development of diseases including hypertension (Nature Med 2010), jet lag (Science 2013; iScience 2018), and on the timing control of cell division, especially during the development and regeneration of hepatocytes (Science 2003b; Nature Com 2017).

The disruption of circadian rhythms cannot be explained simply based on the transcription-translation feedback loop (TTFL), since metabolism also greatly influences the formation and adjustment of rhythms, and so is the ability of cells to synchronize their intracellular rhythms. In nocturnal animals, synchronization to the environmental cycles mostly occurs via light exposure, while non-photic information (darkness, melatonin, etc.) is critical in diurnal animals such as humans. Therefore, to understand sleep rhythm disorders in humans, it is important to develop experimental paradigms in non-human primates. In 2018 we set up the first Japanese biorhythms measurement room for marmosets, and observed their strong circadian rhythms and social synchronization, as well as how their rhythms can be reset by dark-pulses. At the molecular level, we now try to visualize gene expression oscillations in marmoset brain, and compare this to that of mice brain.



Recent publications

- Doi M et al. Non-coding cis-element of Period2 is essential for maintaining organismal circadian behaviour and body temperature rhythmicity, ***Nature Commun*** 10: ***, 2019.
- Yamaguchi Y & Okamura H Vasopressin signal inhibition in aged mice decreases mortality under chronic jet lag, ***iScience*** 5: 118–122, 2018.
- Fustin, JM et al. Two Ck1δ transcripts regulated by m6A methylation code for two antagonistic kinases in the control of the circadian clock. ***Proc Natl Acad Sci USA*** 115:5980-5985, 2018.
- Chao H-W et al. Circadian clock regulates hepatic polyploidy by modulating Mkp1-Erk1/2 signaling pathway. ***Nature Commun*** 8: 2238, 2017.
- Yamaguchi Y et al. Mice genetically deficient in vasopressin V1a and V1b receptors are resistant to jet lag. ***Science*** 342: 85-90, 2013.
- Fustin JM et al. RNA-methylation-dependent RNA processing controls the speed of the circadian clock. ***Cell*** 155: 793-806, 2013.
- Doi M et al. Salt-sensitive hypertension in circadian clock-deficient mice involves dysregulated adrenal Hsd3b6. ***Nature Med*** 16: 67-74, 2010.
- Yamaguchi S et al. Synchronization of cellular clocks in the suprachiasmatic nucleus. ***Science*** 302: 1408-1412, 2003.
- Matsuo T et al. Control mechanism of the circadian clock for timing of cell division in vivo. ***Science*** 302: 255-259, 2003.
- Shigeyoshi Y et al. Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript. ***Cell*** 91: 1043-1053, 1997.

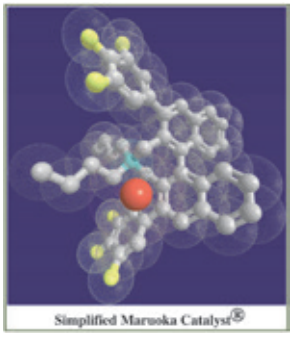
Department of Organocatalytic Chemistry

Specially Appointed Professor: Keiji Maruoka

Research Projects:

"Organocatalyst" has recently attracted considerable attention as the third catalyst in organic synthesis in addition to the conventional "biocatalyst" and "metal catalyst". In such an organocatalytic field, the design of "high-performance organocatalysts", if possible, enables the achievement of new reactivity and selectivity, hitherto not obtainable in the conventional "biocatalysts" and "metal catalysts". The aim of our research is to realize the rational design of high-performance organocatalysts, which is divided into four main categories consisting of "organobase catalysts", "organoacid catalysts", "organoacid/base bifunctional catalysts" and "organoradical catalysts". Throughout both the basic and applied researches in this project, we have developed the design and synthesis of a series of truly high-performance organocatalysts for practical organic transformations.

1) Rational design of chiral phase-transfer catalysts: A most important and significant aspect of our work is the impact it has had on the field of phase-transfer chemistry. We rationally designed and synthesized a series of chiral binaphthyl-modified spiro-type phase-transfer catalysts as "Maruoka Catalyst®" for asymmetric synthesis of various natural- and unnatural-type amino acid derivatives starting from simple glycine derivative. We further challenged the simplification of these phase-transfer catalysts with high catalytic activity, and finally succeeded to design super-active "Simplified Maruoka Catalyst®" (*i.e.*, S/C = 5,000~10,000) with virtually complete enantioselectivity. This catalyst is produced by Nagase & Co., Ltd. in a Kg-scale, and now commercially available from Sigma-Aldrich Co., Strem Co. and Kanto Chemical Co. In addition, Nagase & Co., Ltd. and Kishida Chemical Co., Ltd. have started the large-scale production of unnatural α-alkyl and α,α-dialkyl amino acids (100 g~300 kg scale) as new pharmaceutical intermediates by using "Simplified Maruoka Catalyst®". Such large-scale production of unnatural amino acids is truly important because about 20% (about 100 kinds) of the top-500 best selling medicines in the world markets utilize α-amino acids as starting materials and pharmaceutical intermediates.



Our asymmetric phase-transfer chemistry has been successfully applied to asymmetric aldol reaction, Mannich reaction, conjugate addition, epoxidation, Strecker reaction, alkylation of β-keto esters, and diastereoselective terminal alkylation of peptides, etc.

2) Design of chiral bifunctional organocatalysts: We designed an axially chiral hexamethoxybiphenyl amino acid catalyst to achieve the very high catalytic efficiency in the asymmetric direct aldol reaction. Based on the information, we succeeded to design an axially chiral binaphthyl-modified amino Tf-amide catalyst for hitherto unknown *anti*-selective asymmetric Mannich reaction of aldehydes with activated imines and *syn*-selective direct aldol reaction of two different aldehydes.

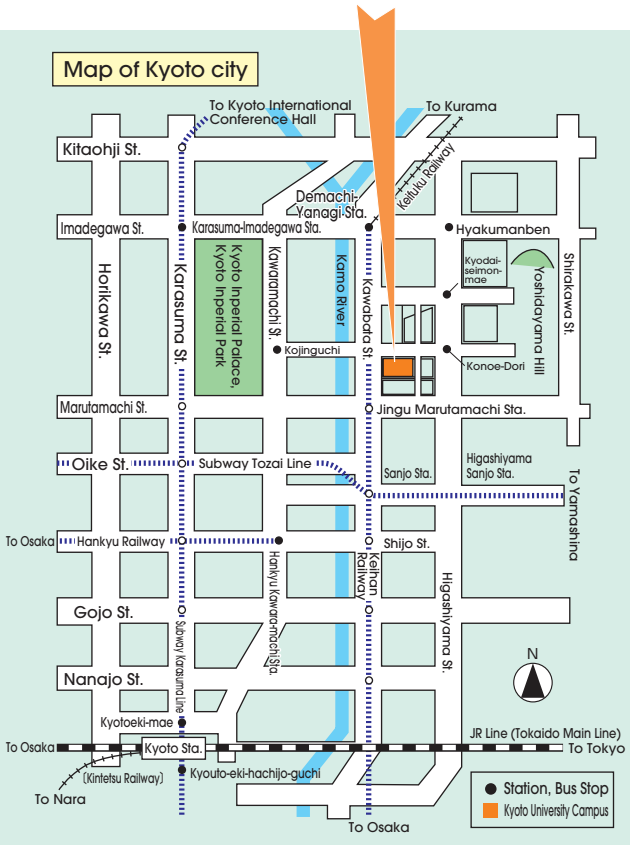
3) Design of chiral organo diacid catalysts: Although the asymmetric hydrogen-bonding catalysis mainly relies on the use of thiourea, diol, and phosphoric acid as hydrogen-bonding donors, we newly designed axially chiral binaphthyl-modified dicarboxylic acids for asymmetric Mannich reaction of arylaldehyde *N*-Boc imines with diazoacetates; imino aza-enamine reaction of arylaldehyde *N,N*-dialkylhydrazones; and trans-selective aziridination of diazoacetamides with *N*-Boc imines.

4) Design of chiral organoradical catalysts: We began our own organoradical chemistry by the generation of several iodanyl radical species from hypervalent iodine compounds under visible photocatalysis for site-selective functionalization of saturated hydrocarbons and selective generation of acyl radicals for selective C-C bond formation. We also achieved the design of chiral organothiol catalysts to generate chiral thiyl radicals for asymmetric (3+2) cyclization.

Recent publications

- Design of N-Spiro C_2-Symmetric Chiral Quaternary Ammonium Bromides as Novel Chiral Phase–Transfer Catalysts: Synthesis and Application to Practical Asymmetric Synthesis of α-Amino Acids, Ooi, T.; Kameda, M.; Maruoka, K. *J. Am. Chem. Soc.* **2003**, 125, 5139-5151.
- An Organic Thiyl Radical Catalysed Enantioselective Cyclization, Hashimoto, T.; Kawamata, Y.; Maruoka, K. *Nature Chem.*, **2014**, 6, 702-705.
- A Chiral Electrophilic Selenium Catalyst for Highly Enantioselective Oxidative Cyclization, Kawamata, Y.; Hashimoto, T.; Maruoka, K. *J. Am. Chem. Soc.*, **2016**, 138, 5206-5209.

Graduate School of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences



by Train and Bus

Railway Station		get a bus at	Bus #	Route	Bus stop
JR Kyoto Station North Exit		Bus terminal D2	City Bus #206	for Kita-ohji bus terminal via Higashiyama street	Konoe-dori
		Bus terminal A2	City Bus #17	for Kinrin bus termninal via Kawaramachi Street	Koujin-guchi
		Bus terminal A2	City Bus #205	for Kita-ohji bus terminal via Kawaramachi Street	
		Bus terminal C3 Kyoto Bus stop	Kyoto Bus #17	for Ohara bus via kawabata Street	Koujin-bashi
JR Nara Line / Keihan Main Line	Kyoto Subway Line	Karasuma-marutamachi (east bound)	City Bus #202	for toufukuji/kujo-shako via kawaramachi-marutamachi	Marutamachi Keihan-mae
	Maru-ta-machi		City Bus #204	for Ginkakuji via kawaramachi-marutamachi	
Keihan Jingu-Marutamachi Station		8 min Walk from `Jingu-Marutamachi' on the Keihan Main Line			
Hankyu Kawaramachi Station		Shijo-Kawaramachi F bus stop on Shijo Street (East bound)	City Bus #201	for Hyakumanben via Higashi-oji Street	Konoe-dori
			City Bus #31	for kumano/iwakura via Higashi-oji Street	
		Shijo-Kawaramachi H bus stop on Kawaramachi Street (North bound)	City Bus #3	for kitashirakawa-sibuse-cho via Imadegawa Street	Koujin-guchi
			City #17	for Kinrin bus termninal via Kawaramachi Street	
			Kyoto Bus #17	for Ohara via Kawabata Street	Koujin-bashi

Kyoto University
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Faculty of Pharmaceutical Sciences

August, 2019

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