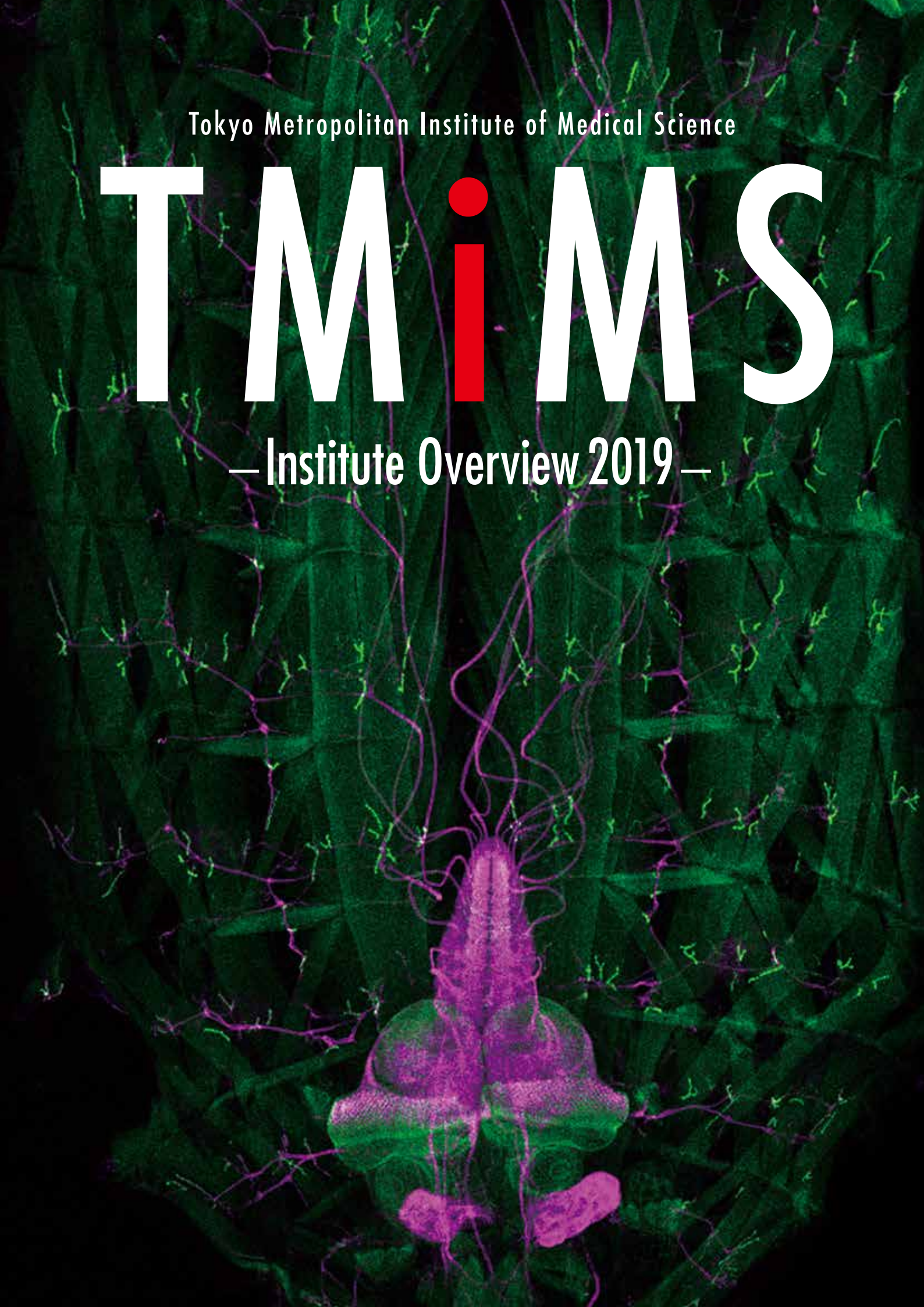
Tokyo Metropolitan Institute of Medical Science

TMiMS

—Institute Overview 2019—



Contents

Research Activities

Department of Genome Medicine

Department of Dementia and Higher Brain Function

Department of Brain Development and Neural Regeneration

Department of Psychiatry and Behavioral Sciences

Department of Sensory and Motor Systems

Department of Advanced Science for Biomolecules

Research Support

Figure. Drosophila larval neuromuscular junction. In each abdominal hemisegments of Drosophila larvae, there are 30 muscles innervated by 37 motor neurons in a highly stereotypic manner (Magenta; Presynaptic marker HRP, Green; Postsynaptic marker Discs-large).

Message from Our Chairperson

Chairperson **Keiji Tanaka**

The metropolis of Tokyo is the nerve center of Japan. Developing Tokyo into a healthy welfare state will therefore go a long way toward building a prosperous future for Japan. The mission of the Tokyo Metropolitan Institute of Medical Science (TMIMS) is to promote research in the life and medical sciences to improve the lives and health of Tokyo citizens. It is well known that Japan has the most rapidly aging society in the world. Tokyo, which reflects Japan itself, is undergoing a steady increase in cancer, infectious diseases, lifestyle-related illnesses, neural and mental disorders, and various other health problems. Naturally, curing all of these diseases is a common goal for all humankind, and considerable efforts have been made at the national level. Even so, because Tokyo is one of the largest civilized cities in the world, it is also essential for the Tokyo Metropolitan Government to take the initiative in this endeavor. Besides, Tokyo has numerous problems unique to megacities. For instance, many people suffer from rare and intractable diseases that researchers often overlook. TMIMS has been actively working on these important problems, promptly and practically addressing health-related issues with the aim of protecting the health of Tokyo citizens.

I am of the opinion that “research represents culture”. Accordingly, TMIMS aims to be acclaimed both academically and culturally for the knowledge and wisdom of its excellent researchers. Our goal is to become a symbol of the culture of Tokyo, the foremost megalopolis in the world. Academic research is often roughly divided into top-down, exit-oriented, applied research (of immediate use), and bottom-up, future-oriented fundamental research (seemingly not of immediate use). Balancing these two research strategies, TMIMS endeavors to operate in a flexible manner in order to achieve additive and synergic effects. Top-down and bottom-up research strategies are not incompatible, but can work in a cooperative and harmonious manner. Throughout the history of science, we can find numerous examples of seemingly useless research suddenly becoming useful, resulting in great service to society.

Our medical researchers are energetically pursuing their research day and night to develop preventive medicine and new therapeutic methods to protect citizens’ health. During this process, TMIMS naturally takes on the role of educating young researchers who will help develop human knowledge and wisdom, and contribute to social prosperity. All the staff members of TMIMS are working on life science research, ranging from fundamental to practical, using the most cutting-edge technologies to achieve their goals.

It is vitally important that TMIMS grow to be the world’s premiere research institute, and advancing and enriching its research power will create an institute capable of rendering wide-ranging services to society. To this end, the entire staff of TMIMS will strive to help pursue incomparable fundamental research, and pass the benefits of this research on to society. At the same time, we will continue recruiting and educating talented people to increase our momentum. We look forward to your guidance and encouragement, which are indispensable for the further development of TMIMS. Thank you for your support.

Message from Our Director

Director **Hisao Masai**

In a metropolis such as Tokyo, people suffer from various diseases and other health-related problems unique to urban life-styles. These include mental disturbances caused by stresses associated with daily life, diseases associated with longevity, diseases caused by complicated genetic traits, as well as infectious outbreaks of hepatitis, influenza, and other viral diseases. We are studying these problems using a unique combination of disciplines, technology and expertise. We conduct forefront basic research in various biological fields including genome and protein functions, stem cell development, genetic diseases, brain functions, neurobiology, neurodegeneration, viral infection, allergies, schizophrenia, and depression. Using the state-of-art technology and equipment, we are identifying molecules and mechanisms responsible for various biological phenomena as well as for disease progression.

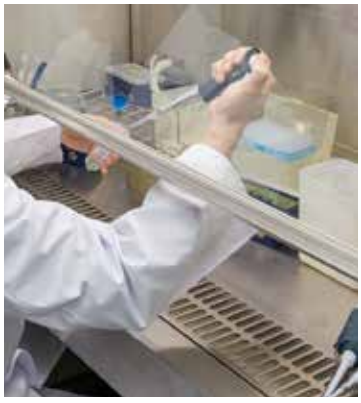
We also take sociomedical approaches to improving urban life, including using large-scale cohort studies to identify social and environmental factors affecting the mental health of youths. We are improving effective care and nursing systems for elderly people suffering from dementia, and provide patients suffering from progressive and currently incurable diseases such as ALS (amyotrophic lateral sclerosis) with innovative care systems to improve their quality of life.

We pursue research that will contribute to the prediction, prevention, diagnosis, and treatment of various diseases, and will improve patient care and longevity. We also inform the people of the Tokyo Metropolises and other areas of our progress through outreach activities including public lectures, science cafés, and lectures and classes to students. Through these efforts, we hope to serve as a leader and model institute for the life/medical science in the coming decades.

History

The Tokyo Metropolitan Institute of Medical Science (TMIMS) was established in April 2011 from the merging of three institutes; the Tokyo Metropolitan Institute for Neuroscience, the Tokyo Metropolitan Institute of Psychiatry, and the Tokyo Metropolitan Institute of Medical Science. These institutes had all been founded in the early to mid 1970s at different locations in Tokyo with support from the Tokyo Metropolitan Government, and their merger brought together scientists from three different disciplines in a quiet residential area at Kamikitazawa in Setagaya-ku, about 15 min by train from Shinjuku. The institute is under the continuous support of the Tokyo Metropolitan Government, and it's aim is to advance medical research and improve the health and welfare of people living in the metropolises through collaborative research in basic life sciences, medical sciences, social medicine, and nursing.

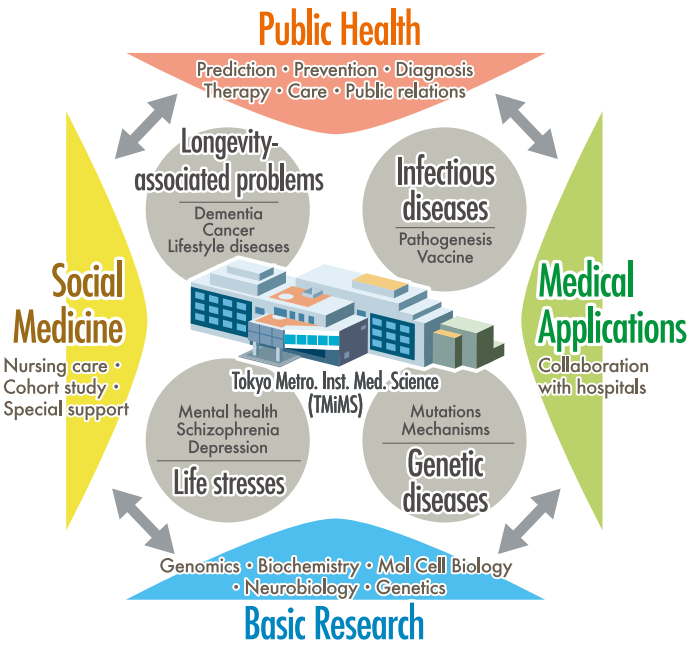
Our Mission



The mission of our institute is to pursue research that will lead to the development of solutions for health-related problems commonly observed in large urban areas and developed countries. We pursue basic research in molecular and cellular mechanisms of biological pathways and disease pathology, and collaborate with municipal hospitals and clinics to translate basic research findings into technologies that can be used to predict, prevent, and treat health problems. We are also working to identify causes of unsolved diseases in order to develop novel drugs and therapies. We pursue research that contributes to improved care with those suffering from incurable diseases such as ALS to better patients' quality of life, and also study mental diseases in order to improve care and treatment.

Our Strategies

We study various diseases and other health-related problems prominent in urban life-styles, using a unique combination of disciplines, technology and expertise. We conduct cutting-edge basic research in various biological fields including genome and protein functions, genetic diseases, brain functions, neurobiology, neurodegeneration, stem cell development, virus infection, allergies, schizophrenia, and depression. Using state-of-art technology and equipment, we are identifying molecules and mechanisms responsible for disease progression and biological phenomena. We combine sociomedical approaches with molecular and genomic approaches to discover unique and effective treatments, novel cures, and more effective nursing systems for those suffering from mental disorders or progressive diseases that are currently hard to cure. Through these efforts, we hope to develop strategies for prediction, prevention, diagnosis, and treatment of various diseases, which will ultimately lead to extended lifespans and improved quality of life for Tokyo citizens and human beings in general.

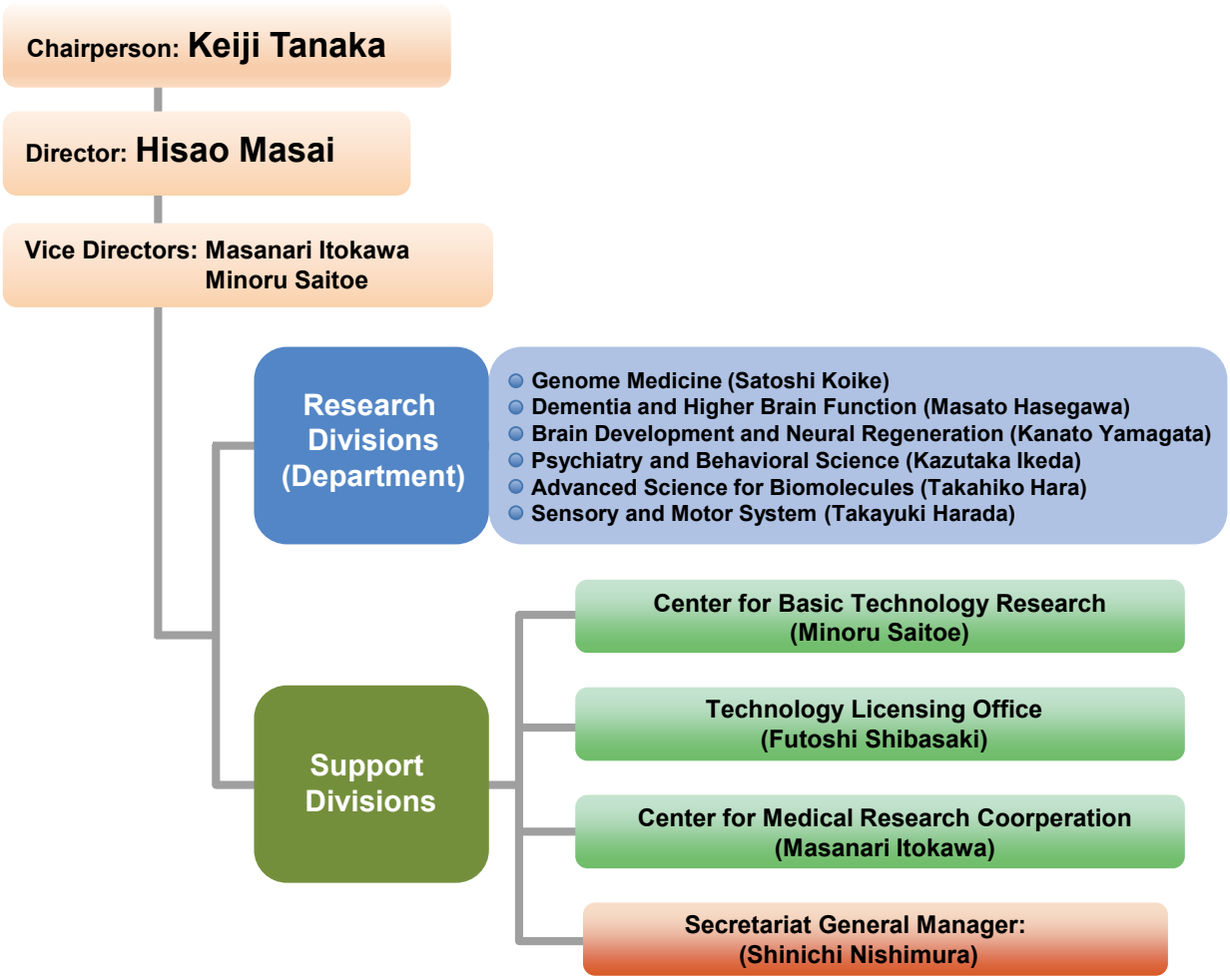


Our Goals

To pursue research that will help predict, prevent, diagnose, and treat various diseases, and improve the care of patients, leading to longer healthier lives.

To serve as a leader and model institute for life/medical science research in the coming decades.

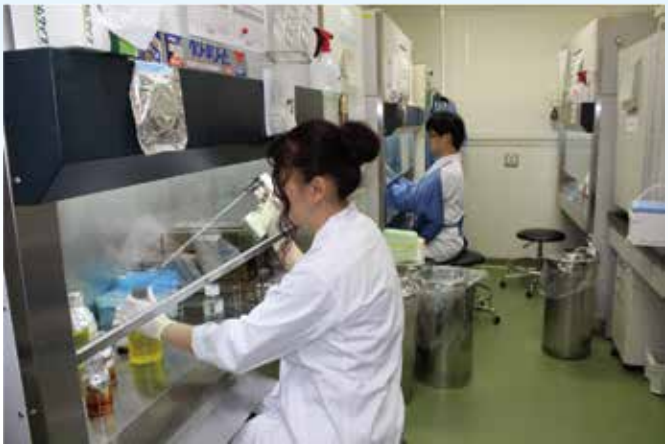
Organizational Chart

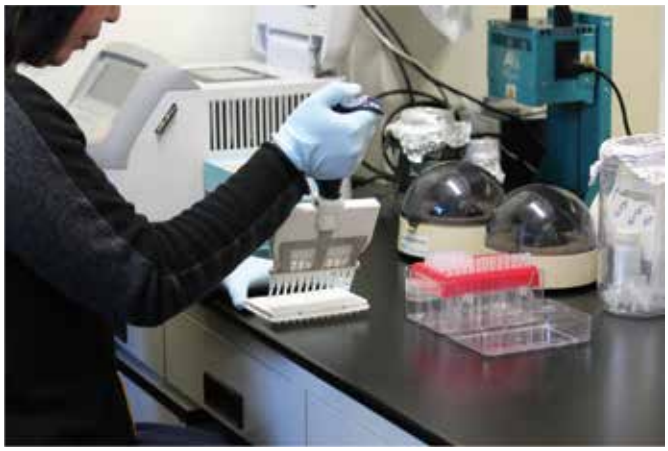


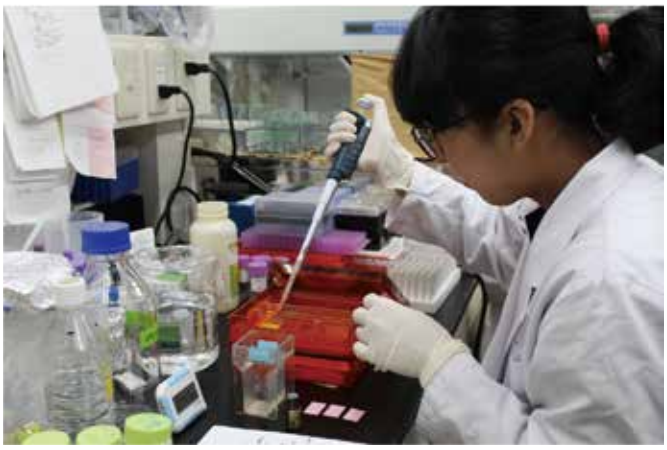
Our People at a Glance

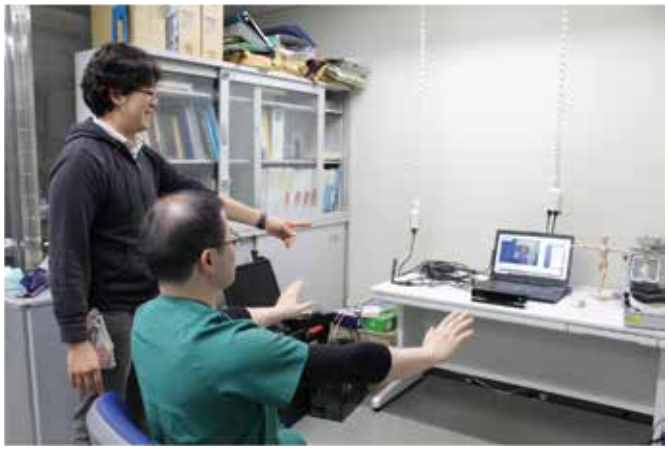
Position	Number
Researchers	156
Postdoctoral Fellows	64
Students	120
Visiting Scientists	139
Guest Scientists	122
Administrative Staff	27
Total	628

September 1, 2019







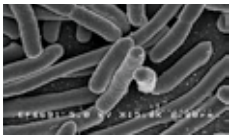


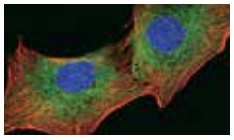
Research Activities

Project Leader **Hisao Masai** Genome Dynamics Project

Genome Replication and Maintenance:
In search of unexplored messages in the genome

Precise duplication of genetic materials is central to the stable maintenance of genomes through generations. Defects in genome copying processes would generate genomic instability which could ultimately result in various diseases including cancer. The goal of our studies is to understand the molecular basis of how huge genomes are accurately replicated and precise copies of genetic materials are inherited to the next generation. Three billion base pairs of the human genome (2 meter long) are replicated with almost no errors during a 6-8 hr time span in the cell cycle. This requires an extreme level of coordination of temporal and spatial arrangements of chromatin organization and signaling events for initiation of DNA replication.





"We are trying to decipher 'unexplored messages' of the genome that are crucial for shaping the chromosomes, copying and reading out genetic information, and even for causing detrimental diseases."

We recently discovered novel and crucial roles of non-standard DNA structures in regulating of DNA replication and transcription. Notably, we found that G-quadruplex structures, which are widely present on genomes (estimated to be present at more than 370,000 locations on the human genome), regulate organization of chromatin architecture and initiation of DNA replication. Our major goal is to establish a novel principle of genome organization by elucidating the fundamental and universal functions of G-quadruplex and other non-B type DNA structures in the regulation of various genome functions. We will also explore the possibility that mutations found in various diseases including cancer and neurodegenerative diseases are related to alteration and mal-formation of these non-B DNA structures, which are likely to be essential components of genomes.

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Genome Dynamics

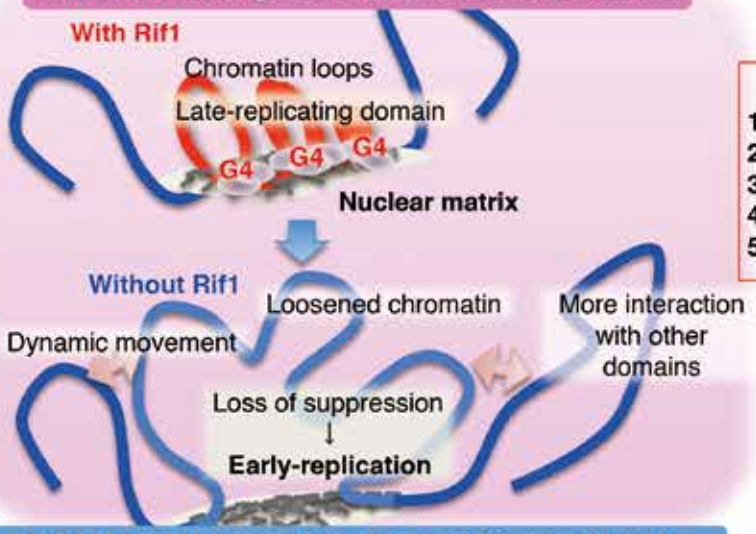
Cdc7 kinase as a modulator of chromosome transactions

Dbf4/ASK

Cdc7

- Mcm: Replication initiation
- Claspin/Mrc1: Replication Checkpoint
- Rad18: Trans-lesion DNA synthesis/ repair
- AuroraB: Trans-lesion DNA synthesis/ repair
- Mer2: Meiotic recombination
- Rec8: Lsr4: Meiotic cell division
- HP1: Heterochromatin formation
- H3 T45: Histone modification
- Caf1: Chromatin reconstruction
- Mrc1, Ams2, Eco1: Protein degradation
- Top2A: Centromere regulation
- TDP43: Protein aggregation
- Mus4-Mus81: Homologous recombination

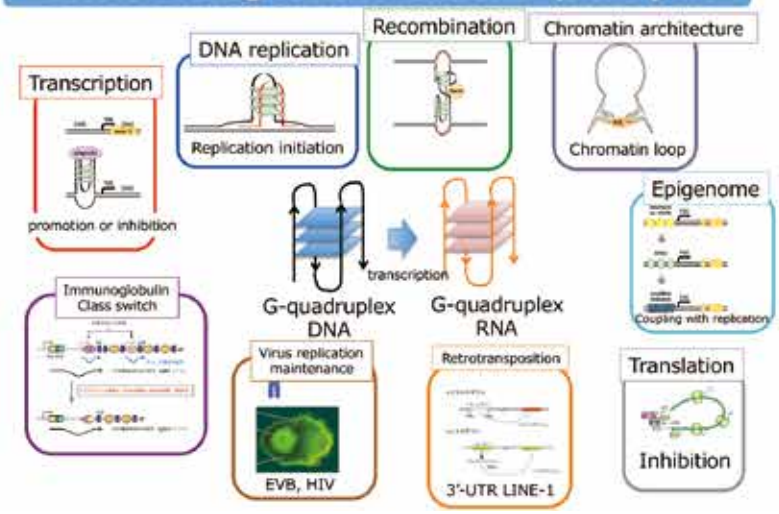
Rif1 and G4 as organizers of chromatin architecture



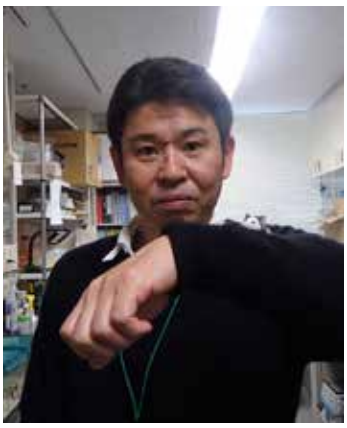
Our Major Projects

1) Universal mechanisms of DNA replication
2) Cellular responses to replication stress
3) Unusual DNA structures (G-quadruplex etc.)
4) DNA replication and development
5) Novel drugs and therapies for cancer

Diverse biological functions of G-quadruplex



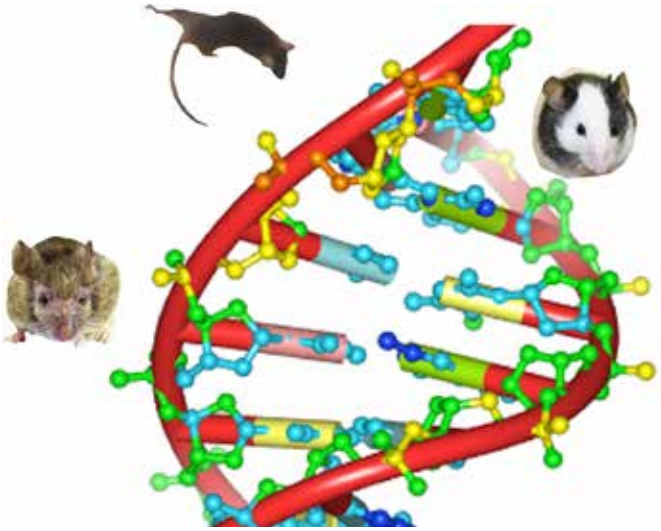
Genome Dynamics



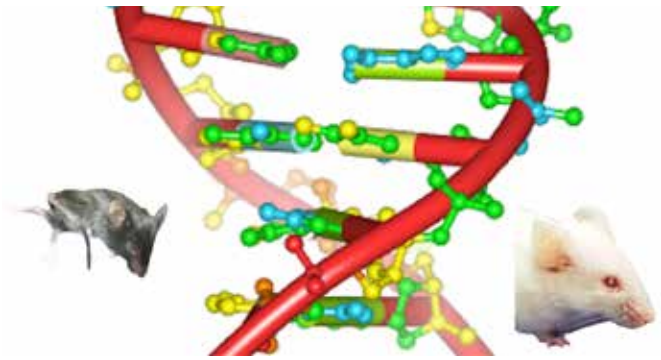
Project Leader **Yoshiaki Kikkawa** Mammalian Genetics Project

Gene discovery: Phenotype- and gene-driven approaches to identify disease-associated genes in mice

The genetic factors and molecular mechanisms behind many human genetic diseases are still unknown. Mouse disease models are important tools for identifying genes that are responsible for genetic diseases. They are also important for studying the processes that regulate the onset of genetic diseases and for evaluating the effectiveness of new drugs. We aim to develop novel mouse models of human genetic diseases via forward and reverse genetics to understand disease pathogenic mechanisms.



"We are identifying genes associated with human diseases and developing new mouse models for human diseases."



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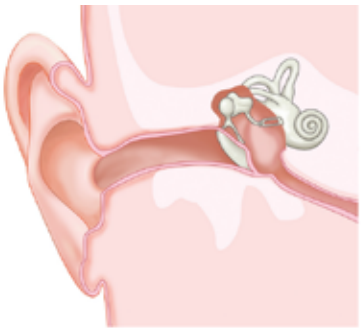
Mammalian Genetics

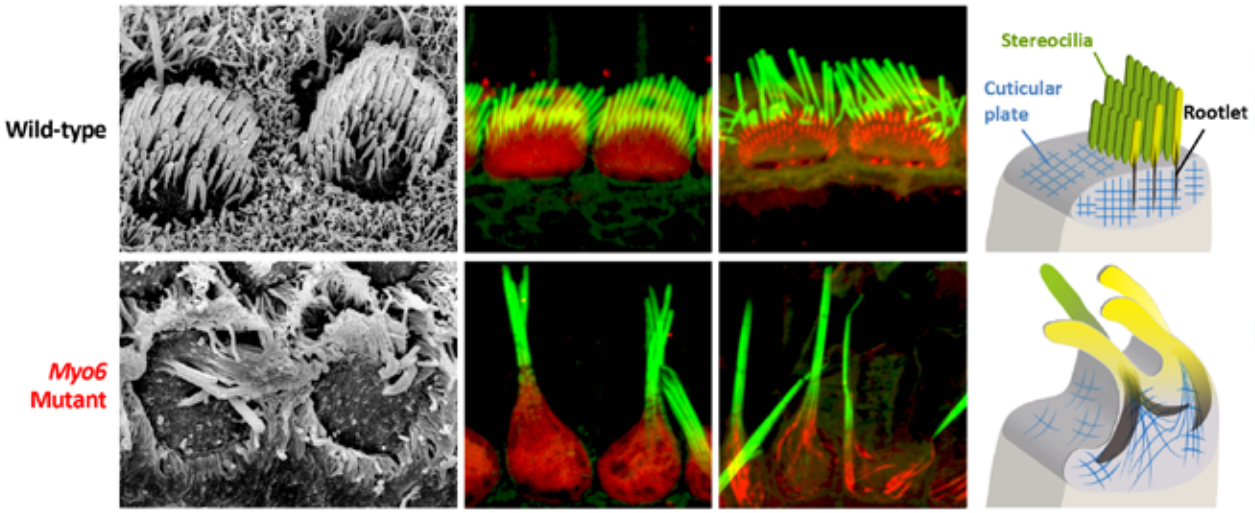
Main project: Genetics of deafness



Hearing loss is the most common sensory disease in humans, and severely affects one's quality of life. We continue to make significant advances in understanding the development, transduction, and homeostasis of the auditory system by studying corresponding mouse mutants. We exploit the similarities between the mouse and human genomes, physiology, and auditory system anatomy to identify and characterize genes related to deafness.

Current focus

Stereociliary fusion in Myo6 mutant mice caused by a disruption of actin networks in the apical region of inner ear hair cells

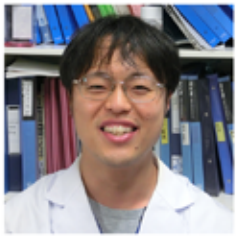


An unconventional myosin encoded by *MYO6*, a myosin VI gene, contributes to hearing loss in humans. Homozygous *Myo6* mutant mice exhibit congenital hearing defects caused by the fusion of stereocilia. We recently identified morphological changes at the base of the stereocilia in *Myo6* mouse mutants by scanning electron microscopy and analysis of the marker proteins of the cuticular plate and rootlet. In wild-type mice, stereocilia have dense rootlets that extend through the taper region of stereocilia to anchor them into the actin mesh of the cuticular plate. These structures are maintained when MYO6 is normally expressed in the stereociliary taper region, cuticular plate, and cytoplasm of the hair cells, but a reduction of MYO6 leads to stereociliary fusion accompanied by deformations of the cuticular plates and the extension of rootlets.

By Yuta Seki

Members

Kunie Matsuoka



Shumpei Yasuda

Xuehan Hou

Yuki Miyasaka

Kenta Wada

Mammalian Genetics

Project Leader **Fumihiko Yasui** Viral Infectious Diseases Project

Control of viral infectious diseases: Virology, immunology, vaccinology and therapy

Our project studies the virology, immunology, vaccinology and therapy of incurable viral diseases. We currently focus on liver diseases, influenza and dengue fever. However, the lack of suitable infection models in vitro and in vivo has hampered the clarification of viral pathogenesis. To overcome this problem, we have been developing various animal models including transgenic mice, humanized mice with human liver cells, monkeys and tree shrews. We also investigate the precise mechanisms by which host factors regulate viral growth.

"We are studying the mechanisms underlying development of severe acute inflammation and establishment of chronic infection by viruses through the development of suitable animal models that are capable of being infected by viruses."

Hepatitis

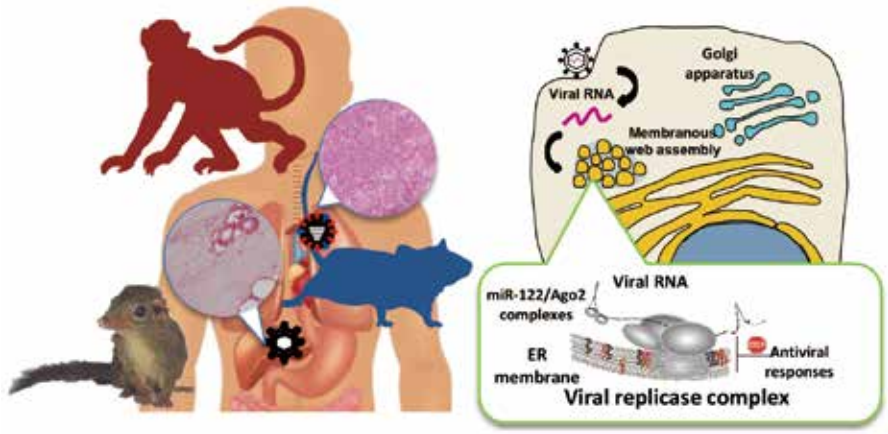
- Identification of host factors regulating virus growth.
- Elucidation of the mechanisms underlying pathogenesis caused by hepatitis virus infection.
- Development of therapeutic vaccine and drug for chronic HBV/HCV infection and other liver diseases.

Influenza

- Elucidation of the mechanisms by which highly pathogenic Flu causes severe pneumonia.
- Development of novel vaccine and therapeutic drug against highly pathogenic Flu and seasonal Flu.

Dengue fever

- Development of suitable animal models to study vaccine efficacy and pathogenesis of dengue fever.
- Development of novel vaccine for all serotypes of DENV.



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Viral Infectious Diseases

Topics of our research

Selective inhibitor of Wnt/β-catenin/CBP signaling ameliorates hepatitis C virus-induced liver fibrosis in mouse model

Chronic hepatitis C viral (HCV) infection is one of the major causes of serious liver diseases, including liver cirrhosis. We investigated the effects of a β-catenin/CBP inhibitor on liver fibrosis. PRI-724, a selective inhibitor of β-catenin/CBP, reduced liver fibrosis in HCV-Tg mice while attenuating αSMA induction. PRI-724 increased matrix metalloproteinase (MMP)-8 mRNA in the liver, and elevated levels of intrahepatic neutrophils and macrophages/monocytes. These results suggest that inhibition of hepatic stellate cell activation and induction of fibrolytic cells expressing MMP-8 contribute to the anti-fibrotic effects of PRI-724.

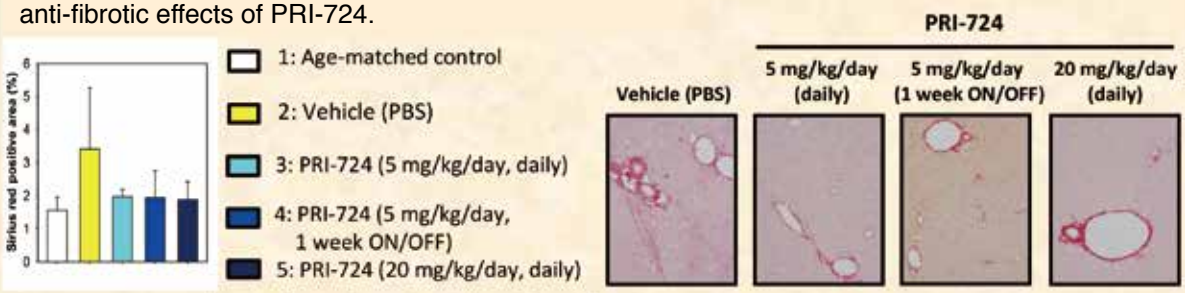
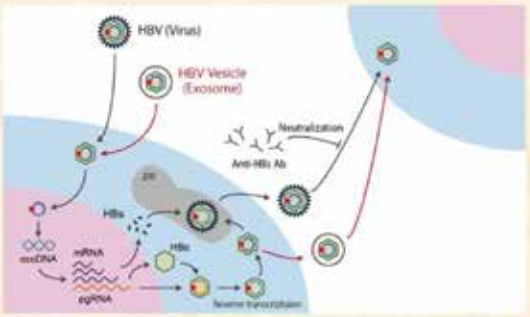


Figure. PRI-724ameliorates hepatitis C virus-induced liver fibrosis.

Transmission of HBV DNA Mediated by Ceramide-Triggered Extracellular Vesicles

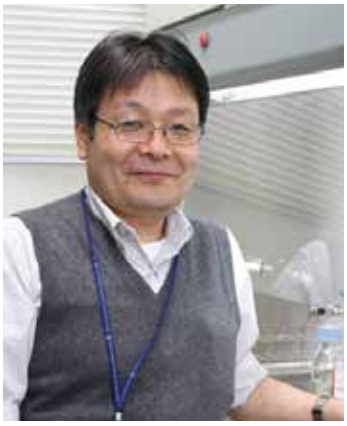
Extracellular vesicles are nanovesicles that shuttle proteins, nucleic acids, and lipids, thereby influencing cell behavior. We showed that ceramide-triggered extracellular vesicles transport hepatitis B virus-DNA and are capable of transmitting viral DNA to naive hepatocytes. Further, we demonstrated that the transmission of hepatitis B virus-DNA via these extracellular vesicles is resistant to antibody neutralization.



Members

Michinori Kohara
Tsubasa Munakata
Daisuke Yamane
Kenzaburo Yamaji
Naoki Yamamoto
Yuko Tokunaga
Takahiro Sanada
Tomoko Honda

Viral Infectious Diseases



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Project Leader **Satoshi Koike** Neurovirology Project

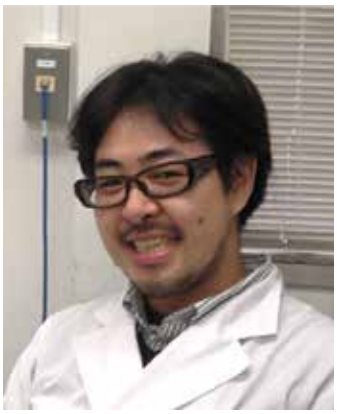
Protecting the Central Nervous System from Infectious Diseases

"The development of vaccines and anti-viral drugs and the evaluation of these agents using experimental models are important for controlling emerging and re-emerging viral infections. We study the basic principles of neurotropic enterovirus infection to further knowledge and technologies to control infectious diseases."

Enterovirus 71 (EV71), a human enterovirus species A of the genus *Enterovirus* within the *Picornaviridae* family, is known to be one of the causative agents of hand-foot-and-mouth disease (HFMD). HFMD is generally a mild and self-limiting disease. However, in some infants and young children, HFMD caused predominantly by EV71 can be complicated by neurological manifestations. Thus, EV71 infection is a serious public health concern. Unfortunately, there is still very little information concerning EV71 pathogenesis, and vaccines or anti-EV71 drugs have yet to be developed.

Members

Kyosuke Kobayashi

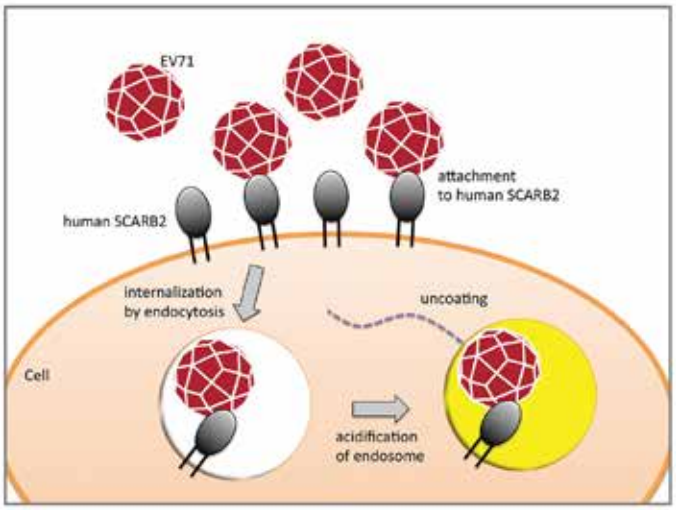


Neurovirology

Research Topics

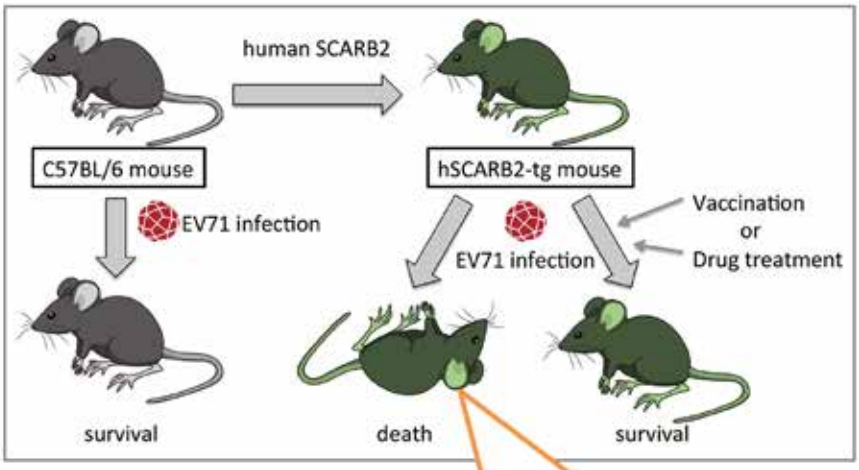
Mechanism of Enterovirus 71 infection

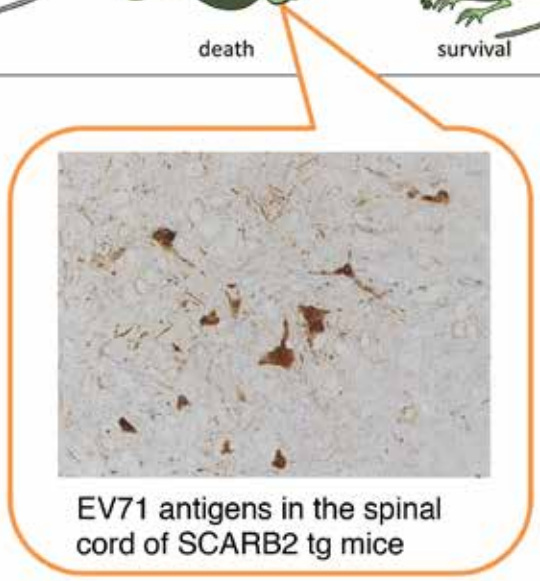
We recently found that Scavenger receptor B2 (SCARB2) is a receptor for EV71. SCARB2 plays a central role in early stages of EV71 infection. SCARB2 is able to mediate binding of the virus at the cell surface, internalization of the virus and initiation of uncoating.



Development of an animal model for Enterovirus 71 infection

Transgenic mice expressing human SCARB2 are susceptible to EV71, and are a useful model for the study of EV71 pathogenesis and vaccine efficacy.





EV71 antigens in the spinal cord of SCARB2 tg mice

Neurovirology

Project Leader **Takachika Hiroi** Allergy and Immunology Project

Allergies and Mucosal Immunology:

Investigating molecular mechanisms of sublingual immunotherapy (SLIT) and developing therapeutic biomarkers for allergic diseases

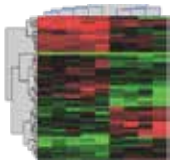
Japanese cedar pollen allergy is the major allergic disease in Japan, affecting approximately 35% of Japanese people. In recent years, sublingual immunotherapy has been recognized as an effective curative treatment for allergic diseases.

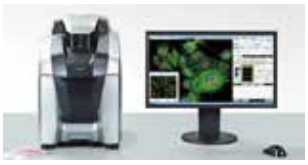
However, the molecular mechanisms of mucosal tolerance still remain unclear. In our laboratory, we focus on the following topics.

"We are developing new diagnostic and treatments for allergies."

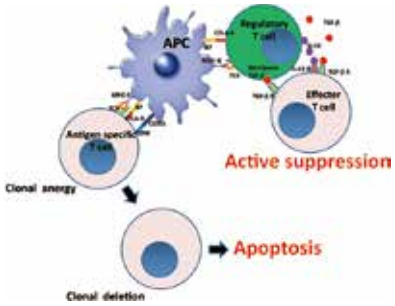
1. Search for effective biomarkers of SLIT

- CNVs
- SNPs
- Epigenome
- Proteome, etc.





2. Elucidation of molecular mechanisms by which SLIT induces immunological tolerance



- iTregs
- Apoptosis
- CTLA-4
- TGF-β
- IL-10 etc.

Gotoh M, Kaminuma O, Nakaya A, Katayama K, Motoi Y, Watanabe N, Saeki M, Nishimura T, Kitamura N, Yamaoka K,Okubo K, and Hiroi T. (2017) "Identification of biomarker sets for predicting the efficacy of sublingual immunotherapy against pollen-induced allergic rhinitis." ***International Immunology*** 29: 291-300.

Nishimura T, Kaminuma O, Saeki M, Kitamura N, Matsuoka K, Yonekawa H, Mori A, and Hiroi T. (2016) "Essential contribution of CD4+ T cells to antigen-induced nasal hyperresponsiveness in experimental allergic rhinitis." ***PLOS ONE*** 11: e0146686.

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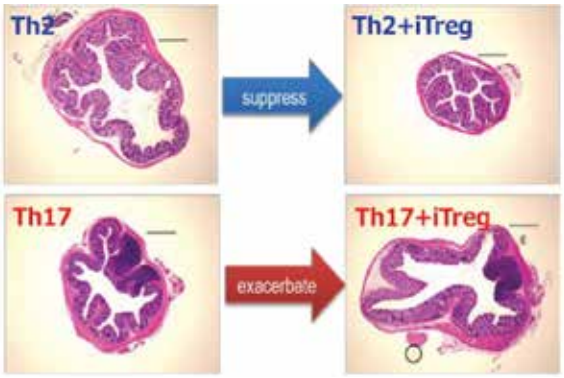
Yokoyama S, Watanabe N, Sato N, Filkoski L, Tanaka T, Miyasaka M, Waldmann TA, Hiroi T, and Perera PL. (2009) "Antibody-mediated blockade of IL-15 signaling reverses autoimmune intestinal damage in a mouse model of celiac disease." ***Proc. Natl. Acad. Sci. USA*** 106: 15849-15854.

Allergy and Immunology

Other Research

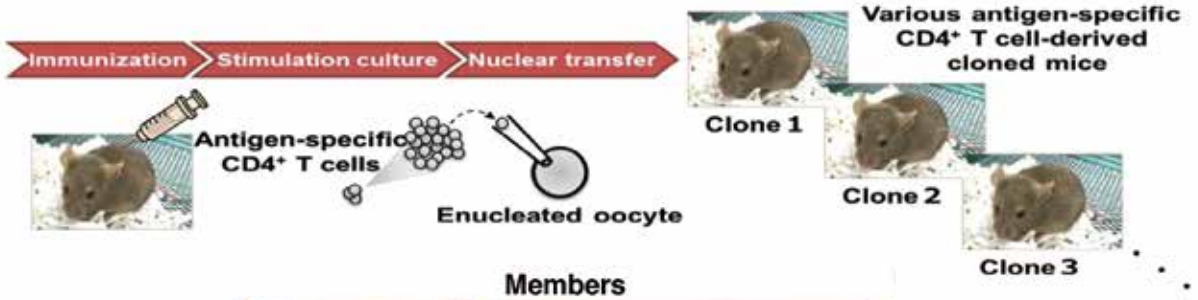
1. Antigen-specific iTreg cells stimulate Th17-mediated colon inflammation



CD4+ helper T cells play a crucial role in allergy and autoimmune diseases including inflammatory bowel diseases (IBDs). Th17 cells and Foxp3+ regulatory T cells (Tregs) are thought to promote and suppress inflammatory responses, respectively. Recently we have developed an antigen-specific and organ-targeted inflammation model by transferring antigen-specific helper T cell subsets followed by antigen administration. By adopting this strategy to colon, we have shown that antigen-specific Tregs stimulate Th17-mediated inflammation in a CTLA4-dependent manner. This finding suggests that Treg/CTLA4-based immunological treatment that are currently in use may be problematic.
(Watanabe N, et al. (2016) *PLOS ONE,* 11: e0150244.)

2. The mechanisms of allergic inflammation investigated using "cloned mice" of antigen-specific CD4+ T cells

Allergens bind to a T-cell receptor (TCR) on CD4+ T cells and induce a series of immune reaction. TCR-transgenic mice are important tools to analyze antigen-response mechanisms, but their non-endogenous TCR might induce immune responses in a manner distinct from those induced by the endogenous TCR. Cloning by the nuclear transfer method enables us to produce animals that retain the donor genotypes in all tissues including germline and immune systems. We generated cloned mice carrying TCR genes of antigen-specific CD4+ T cells that have rearranged in an endogenous manner. These cloned mice express antigen-specific TCR under the intrinsic promoter, and present a unique animal model with which one can investigate CD4+ T cell-mediated pathogenesis and cellular commitment in immune diseases.
(Kaminuma O, et al. (2017) *EMBO Rep.* 18: 885-93.)



Members

Allergy and Immunology

Li Q, et al. (2018) "Int6/eIF3e Silencing Promotes Placenta Angiogenesis in a Rat Model of Pre-eclampsia." ***Sci. Reports*** 12, 8(1):8944

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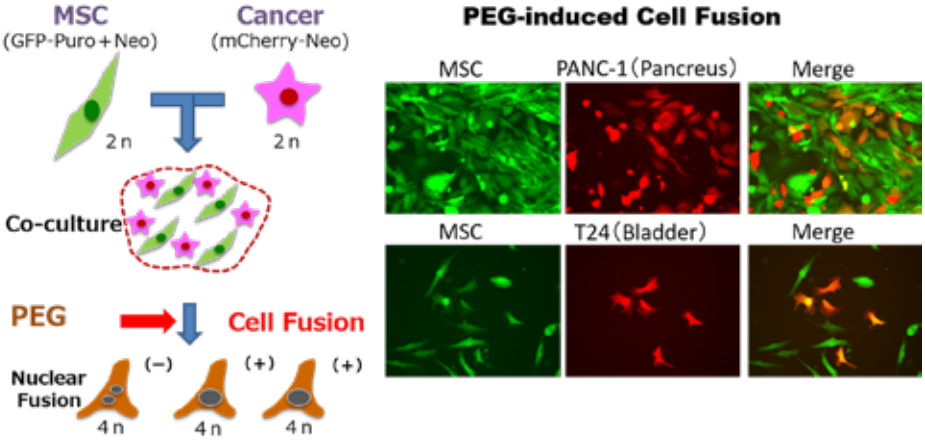
Li Chen, et al. (2007) "Mammalian Tumor Suppressor Int6 Specifically Targets HIF-2alfa To Degradation by Hypoxia- And pVHL- Independent Regulation." ***J. Biol. Chem.*** 282. 12707.

Chen L, et al. (2010) "*Int6/eIF3e* Silencing Promotes Functional Blood Vessel Outgrowth and Enhances Wound Healing by Upregulating HIF2-a Expression." ***Circulation*** 122: 910-919.

Project Leader **Futoshi Shibasaki** Molecular Medical Research Project

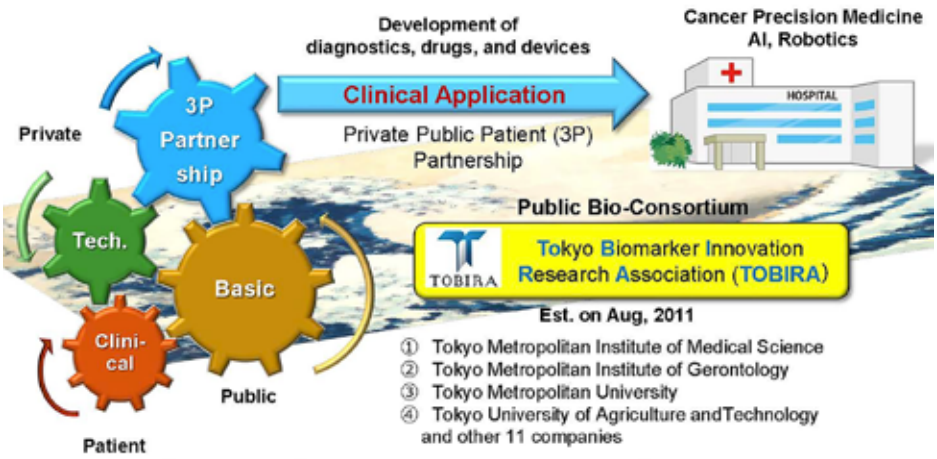
Translational Research for Cancer and Infectious Diseases: Basic to Applied Science

We uncover new mechanisms involved in the pathology of cancers and infectious diseases using novel biomarkers and technologies. This allows us to develop new drugs for the treatment of these diseases. In basic research, we focus on understanding mechanisms of cancer angiogenesis, elucidating how cell fusion induces malignant transformation and metastasis, developing drugs using siRNAs, and developing novel drug treatments for H5 influenza viral infections.



In clinical and translational research, we are establishing a platform to perform "precision medicine" by whole genome analysis using next generation sequencing, in collaboration with metropolitan hospitals. Towards this end, we have already established a bio-consortium, "Tokyo Biomarker Innovation Research Association (TOBIRA) in a private-public partnership (3P) program.

Our general goal is to perform both basic research to identify novel disease targets, and translational research to develop our findings into novel treatments for patients.

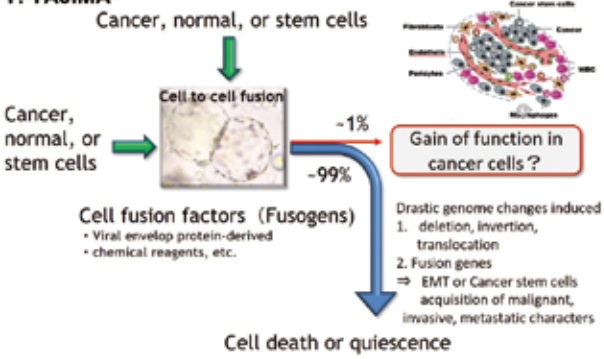


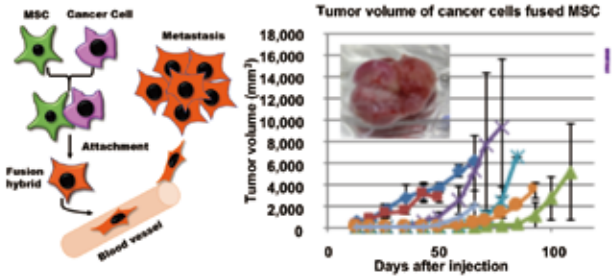
Molecular Medical Research

Malignant cancer progression after cell fusion with stem cells

Y. TAJIMA

Cancer cells fused with mesenchymal stem cell (MSC) in the micro-environment, changes the original character, and often promote dormant, malignant, or metastatic tendency.





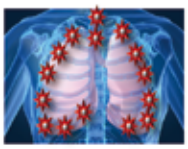
Fused cancer/MSCs promote metastasis than originals

Development of drugs for highly pathogenic H5N1 influenza viruses

N. KAJIWARA

H5N1 has multiple basic amino acids at HA cleavage site.

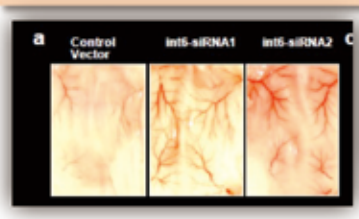
H1N1 Cleavage site
KYVRSTKLRMVTGLRNIPSIQYR----/GLF
H3N2
KYVKQNTLKLATGMRNVPEKQTR----/GLF
H5N1
HA KYVKSNRLVLATGLRNSPQRERRRKKR/GLF

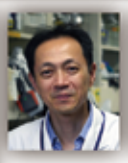


H5N1 highly pathogenic avian influenza virus causes severe pneumonia and multiple organ failure. The mortality rate is about 60%.

We focus on the mechanism of basic amino acid sequence of the split region for discovering new model of the virus entry. The goal of our research is to provide new insights into the molecular mechanism of highly pathogenic avian influenza (H5N1) infection as well as the development of novel antiviral drugs.

Drug development of Int6-siRNA



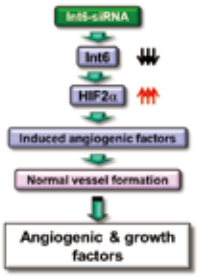


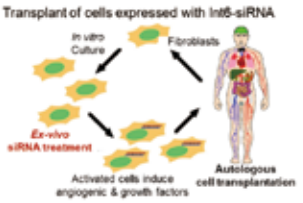
A. IRIE

S. GOTO

Int6 is a key factor to negatively regulate HIF2α-induced angiogenesis and cell protection. The specific siRNA against *int6* would be a possible candidate for cell therapy to treat emic diseases of heart, brain, lower limb, and degenerative and atrophic diseases.

Cell Therapy with *Ex vivo*-siRNA treated Cells





for Ischemia, Degeneration, Atrophy

Diagnostics and device development through Private Public Patient Partnership

Fluoro-IC Chip & Reader

IC Reader

With high sensitive fluoro-beads <15 min, >100 folds sensitive

① **Seasonal A, B Influ IC**
PMDA-approved in 2014
(100 fold higher sensitivity)

② **H5N1 Avian Influ IC**
under development

Rapid & Easy IC Chip

Rapid Colored

Semi-Quantification

Launched in 2016 from LSI Medience

① **Kits for detecting neutralizing Ab in Fabry**

② **Seasonal A, B Influ color IC**
PMDA Approved in 2014
Now on sale

③ **Kits for Cervical Cancer (Plan for sale in 2018)**

Rapid Gene Amp. Devices

Superspeed PCR & Chips

Ver.1 Ver.2 Ver.3

Rapid PCR <8 min for 40 cycles

We aim to develop a rapid and handy device to amplify the target DNAs and RNAs for diagnosis of infectious diseases and cancers.

Molecular Medical Research

Shimozawa A, Ono M, Takahara D, Tarutani A, Imura S, Masuda-Suzukake M, Higuchi M, Yanai K, Hisanaga SI, and Hasegawa M . (2017) "Propagation of pathological α-synuclein in marmoset brain." ***Acta. Neuropathol. Commun.*** 5:12.

Hasegawa M, Nonaka T, and Masuda-Suzukake M. (2016) "α-Synuclein: Experimental Pathology." ***Cold Spring Harb Perspect Med.*** 6. pii: a024273.

Tarutani A, Suzuki G, Shimozawa A, Nonaka T, Akiyama H, Hisanaga S, and Hasegawa M. (2016) "The Effect of Fragmented Pathogenic α-Synuclein Seeds on Prion-like Propagation." ***J. Biol. Chem.*** 291:18675-18688.

Tanaka Y, Nonaka T, Suzuki G, Kametani F, and Hasegawa M. (2016) "Gain-of-function profilin 1 mutations linked to familial amyotrophic lateral sclerosis cause seed-dependent intracellular TDP-43 aggregation." ***Hum. Mol. Genet.*** 25:1420-1433.

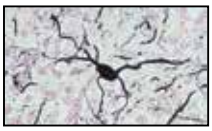
Shimonaka S, Nonaka T, Suzuki G, Hisanaga S, and Hasegawa M. (2016) "Templated Aggregation of TAR DNA-binding Protein of 43 kDa (TDP-43) by Seeding with TDP-43 Peptide Fibrils." ***J. Biol. Chem.*** 291:8896-8907.

Taniguchi-Watanabe S, Arai T, Kametani F, Nonaka T, Masuda-Suzukake M, Tarutani A, Murayama S, Saito Y, Arima K, Yoshida M, Akiyama H, Robinson A, Mann D, Iwatsubo T, and Hasegawa M. (2016) "Biochemical classification of tauopathies by immunoblot, protein sequence and mass spectrometric analyses of sarkosyl-insoluble and trypsin-resistant tau." ***Acta. Neuropathol.*** 131: 267-280.

Project Leader **Masato Hasegawa** Dementia Research Project

Prion-like propagation of tau, α-synuclein and TDP-43 in neurodegenerative diseases

Neurodegenerative diseases are characterized by progressive degeneration of subsets of neurons and gliosis. Many of these diseases are accompanied with intracellular amyloid-like protein pathologies, such as tau in Alzheimer's disease (AD), α-synuclein in dementia with Lewy bodies (DLB) and TDP-43 in amyotrophic lateral sclerosis (ALS) and frontotemporal dementias (FTLD). Importantly, the distributions and spread of these proteins are closely correlated with clinical presentation and disease progression. However, little attention had been given to the questions of why these diseases are progressive, and why the pathologies spread to different brain regions during the course of the diseases.



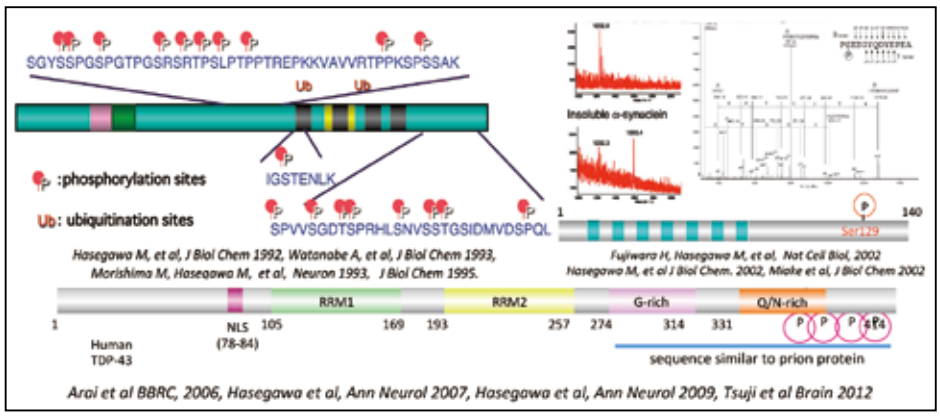
Tau in AD

TDP-43 in ALS

α-synuclein in DLB

"Emerging evidence indicates that intracellular amyloid-like proteins have prion-like properties and propagate from cell to cell by converting normal proteins into abnormal forms.
We are trying to elucidate the molecular mechanisms of this propagation"

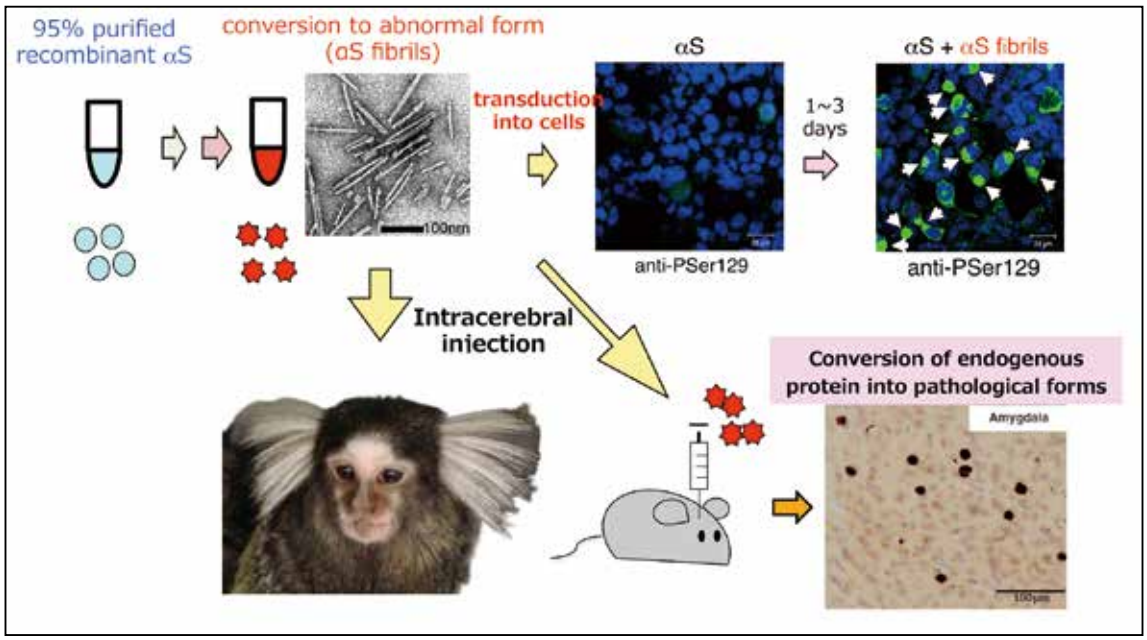
We have been investigating these intracellular abnormal proteins in brains of patients, biochemically using LC/MS/MS, immuno-histochemically with specific antibodies and ultrastructurally. And we found that all of these proteins accumulate in brains of patients as fibrous or filamentous forms in hyperphosphorylated and partially ubiquitinated states.



Schematic diagrams of human tau, α-synuclein and TDP-43.

Dementia Research

To investigate the molecular mechanisms of aggregation of these proteins, we established seed-induced aggregation models which recapitulate the pathological protein aggregation in in vitro, cultured cells and in brains of animals (mouse and marmoset), and proposed a hypothesis, “prion-like propagation of these intracellular pathological proteins in brain”. These models are highly useful not only for clarifying the molecular mechanisms involved in the pathogenesis and progression of neurodegenerative diseases but also for the development of disease modifying drugs and therapy.



Nonaka T, et al. Phosphorylation of TAR DNA-binding Protein of 43 kDa (TDP-43) by Truncated Casein Kinase 1 Triggers Mislocalization and Accumulation of TDP-43. ***J. Biol. Chem.*** 291: 5473-5483, 2016

Nonaka T, et al. Prion-like properties of pathological TDP-43 aggregates from diseased brains. ***Cell Rep.*** 4: 124-134 , 2013

Nonaka T, et al. Seeded aggregation and toxicity of alpha-synuclein and tau: cellular models of neurodegenerative diseases. ***J. Biol. Chem.*** 285: 34885-34898, 2010

Senior Research Scientist **Takashi Nonaka**

Molecular mechanisms of cell-to-cell propagation of aggregated proteins

I am studying molecular mechanisms of cell-to-cell propagation of aggregated proteins (tau, α-synuclein and TDP-43) in neurodegenerative diseases. I am also trying to make in vitro and in vivo models recapitulating abnormal features found in cells of brains of patients using cultured cells and mice. These models will contribute to a better understanding of the mechanisms involved in these diseases, and also to the development of novel therapeutic strategies.

Dementia Research

Ueno K, Suzuki E, Naganos S, Ofusa K, Horiuchi J, and Saitoe M. (2017). "Coincident postsynaptic activity gates presynaptic dopamine release to induce plasticity in Drosophila mushroom bodies." ***eLife***, 6: e21076.

Hirano Y, Ihara K, Masuda T, Yamamoto T, Iwata I, Takahashi A, Awata H, Nakamura N, Takakura M, Suzuki Y, Horiuchi J, Okuno H, and Saitoe M. (2016). "Shifting transcriptional machinery is required for long-term memory maintenance and modification in Drosophila mushroom bodies." ***Nat. Commun.*** 7: 13471.

Matsuno M, Horiuchi J, Yuasa Y, Ofusa K, Miyashita T, Masuda T, and Saitoe M. (2015). "Long-term memory formation in Drosophila requires training-dependent glial transcription." ***J. Neurosci.*** 35: 5557-5565.

Yamazaki D, Horiuchi J, Ueno K, Ueno T, Saeki S, Matsuno M, Naganos S, Miyashita T, Hirano Y, Nishikawa H, Taoka M, Yamauchi, Y, Isobe T, Honda Y, Kodama T, Masuda T, and Saitoe M. (2014). "Glial dysfunction causes age-related memory impairment in Drosophila." ***Neuron*** 84: 753-763.

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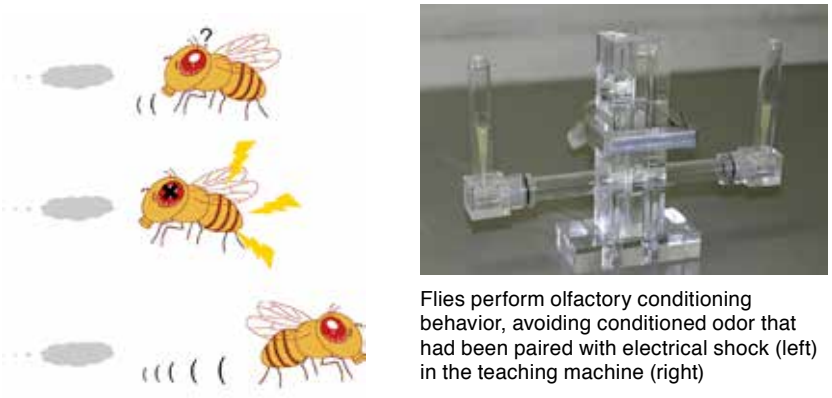
Project Leader **Minoru Saitoe** Learning and Memory Project

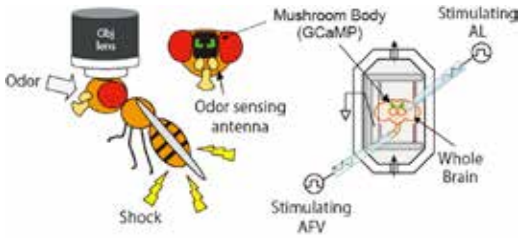
Investigating the Molecular Mechanisms that Generate Memory-encoding Neural Networks

Memories mold our personalities to make us who we are: Using powerful genetic tools, a number of genes and neural substrates underlying memory-associated behaviors have been identified in Drosophila. We have investigated when, where and how identified memory-associated gene products function to produce memory-based behavior, and how the underlying mechanism changes in response to changes in physical conditions such as aging.
In addition to behavioral genetic approaches, we employ in vivo and ex vivo imaging techniques to characterize physiological properties of memory-associated neural networks, and understand how memory-associated genes and neuromodulatory systems regulate function of these networks: how sensory information is associated, and how memory information is stored in neural substrates, and later recalled.

"Combining behavioral genetics and state-of art imaging techniques, we aim to understand how our brains form, store and retrieve memory."



Flies perform olfactory conditioning behavior, avoiding conditioned odor that had been paired with electrical shock (left) in the teaching machine (right)

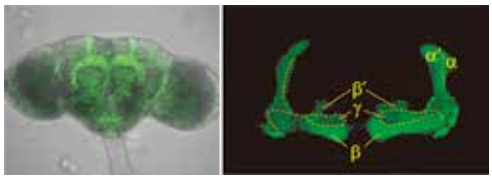


Left, schematic diagram of our in vivo imaging set-up. A living fly is fixed under a microscope and can be exposed to both odors and electrical shocks. Neuronal activity can be observed during formation, storage, and retrieval of odor-shock associative memories.Right, in our ex vivo imaging set-up, we can make artificial memories in cultured brains by stimulating odor and shock sensory pathways.

Learning and Memory

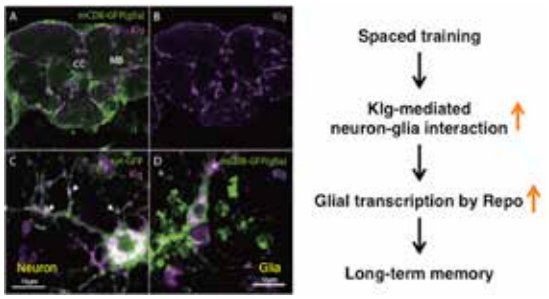
Current Research Topics

Encoding and decoding memory: In Drosophila, formation, storage, and recall of odor memories require activity in a brain region known as the mushroom bodies (MBs). There are various subsets of MB neurons including alpha/beta, alpha prime/beta prime, and gamma neurons. Similarly, there are various different phases of memory including initial learning, short-term memories, middle-term memories, anesthesia resistant memories, and long-term memories. Interestingly, different phases of memory require activity of different subsets of MB neurons. We are studying how anatomical shifting of memory phases occurs and how information is moved between different MB subtypes during different phases of memory.



Structure of MBs and their lobe
Left: MBs in the fly brain expressing GFP.
Right: Subdivision of MB lobes derived from each type of MB neurons.

Neuron-glia interactions: Communication between neurons and glia are important for memory formation. We have identified a cell adhesion molecule, Klingon (Klg) that is expressed in both neurons and glia and is required for memory-associated communication between these cell types. Currently we are studying how Klg signaling is required for memory formation, stabilization and retrieval. We are also studying how Klg signaling is altered upon aging.

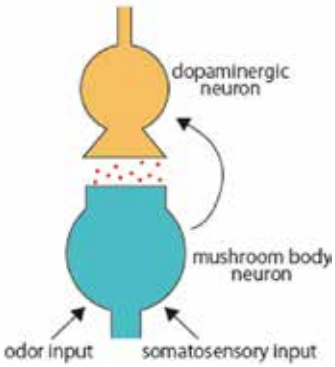


Klingon mediates neuron-glia interaction for LTM formation. A, B) Expression of Klingon (Klg) in the fly brain. C) Expression of Klg in neurons. D) Expression of Klg in glial cells.
Spaced training increases Klg-mediated neuron-glia interaction, thereby induces Repo-dependent glial transcription required for LTM.

Senior Research Scientist **Kohei Ueno**

Exploring Physiological Systems Underlying Learning and Memory

Neural plasticity in the MBs is believed to be a cellular basis of olfactory memory. To understand how odor and shock information are associated to produce plastic changes in the MB neurons, we developed an ex vivo brain imaging system. Using this system, we found that simultaneous stimulation of odor and shock input pathways to the MBs produces long-term enhancement (LTE) in MB neurons in a manner dependent on activity of D1 receptor in the MBs. We further discovered a novel mode of dopamine release locally evoked by postsynaptic MB neurons which have been coincidentally activated by odor and shock input pathways. We have investigated how coincidentally activated MB neurons direct dopamine release and whether such on-demand release mode also takes place for other neuromodulators and other animals.



If mushroom body neurons are activated by two inputs, namely odor and somatosensory inputs, the activated mushroom body requires dopamine release from dopaminergic neurons.

Learning and Memory

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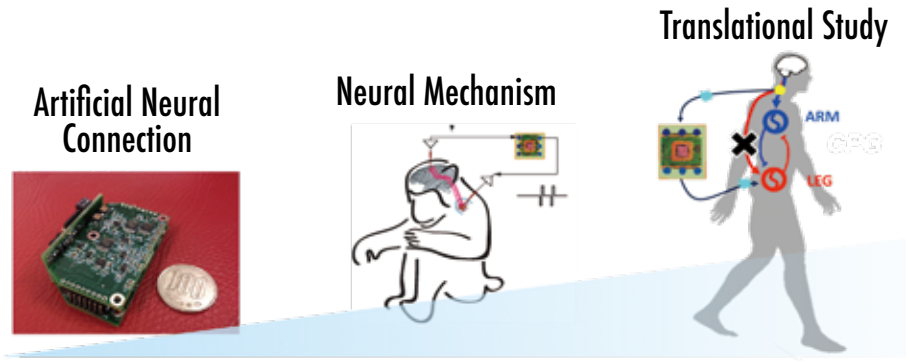
Project Leader **Yukio Nishimura** Neural Prosthesis Project

Restoring Lost Function After Neural Damage

Our research goal is to conceive innovative idea in neuro-rehabilitation to restore lost functions after impairment of the central nervous system, and to translate our findings into clinical applications capable of improving the quality of life for individuals with neural damage.

"Bridging Damaged Neural Pathways using a Neural Interface."

Regaining the function of an impaired limb is necessary for individuals experiencing paralysis. Functional loss of limb control in individuals with spinal cord injury or stroke is often caused by transection of descending and ascending pathways connecting cortical to spinal networks, with neural circuits located above and below the impaired site remaining functional.



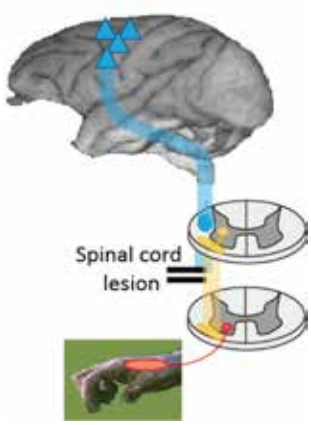
Neuron 2013 | Frontiers 2013 | J Neurosci 2014

We are developing a neural interface known as an "artificial neuronal connection (ANC)". The ANC bridges the supra-spinal system with the spinal network beyond the lesion site to restore lost function. We are conducting clinical trials to assess the effectiveness of ANCs in restoring motor function in paralyzed patients. We also investigate neural changes that occur during recovery.

Neural Prosthesis

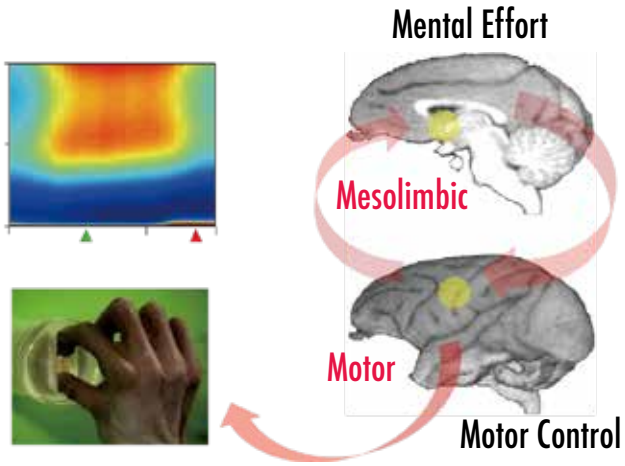
Neural Mechanisms of Functional Recovery

Using large scale multichannel recordings, pharmacological interventions, neuroanatomy, viral tools, computation, and whole brain imaging, we seek to uncover the neural mechanisms underlying voluntary limb movement in intact animals, as well as the processes in which motor functions are reestablished after neural damage such as spinal cord injury and stroke. We are also performing clinical studies to test the efficacy of ANCs in human patients.



Science. 2007, Brain 2009

Psychological Effects on Motor Control



PLoS ONE 2011, Science. 2015

Emotional states influence how we perform motor activities and how we perceive errors. Depression impedes, and motivation enhances, functional recovery after neuronal damage. However, the neuronal substrates underlying these psychological effects on functional recovery remains unclear. We investigate the neuronal substrates underlying psychological effects on motor performance in human and animal models of neural damage.

Members

Yukio Nishimura
Toshiki Tazoe
Osamu Yokoyama
Nobuya Sano
Noboru Usuda
Kei Obara
Yu Shimada
Yoshihisa Nakayama
Hiroaki Ishida
Michiaki Suzuki
Miki Kaneshige
Ryoutaro Numata
Naoya Kabe

Neural Prosthesis

Unit Leader **Makoto Hashimoto** Parkinson's disease Unit

Protection from neurodegenerative diseases

The number of patients with age-associated neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), is rapidly increasing worldwide. Consequently, huge costs for medical treatment and nursing care for these patients have become a serious socioeconomic dilemma. Nonetheless, extensive studies of amyloid immunotherapy in AD have been so far unsatisfactory. Thus, the development of an effective disease-modifying therapy is the highest priority in neurodegenerative disease research.

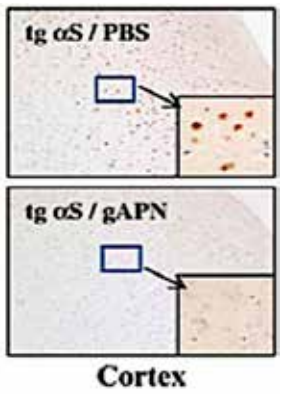


Fig. 1
Effect of adiponectin on neurodegeneration in tg mice

In our laboratory, we seek to exploit a mechanism-based disease-modifying strategy for α-synucleinopathies, such as PD and dementia with Lewy bodies. In this context, we have a particular interest in the suppressive effect of adiponectin on neurodegeneration (Fig.1). We are also identifying new molecules that could be useful for the prevention of neurodegenerative diseases. For this purpose, we currently perform Drosophila molecular genetics (Fig.2) in addition to cell biological and transgenic mice studies. Our results should be applicable to diseases, such as AD and Huntington's disease.

Members

Yoshiki Takamatsu
Masaaki Waragai
Hiromu Sugino
Ryoko Wada

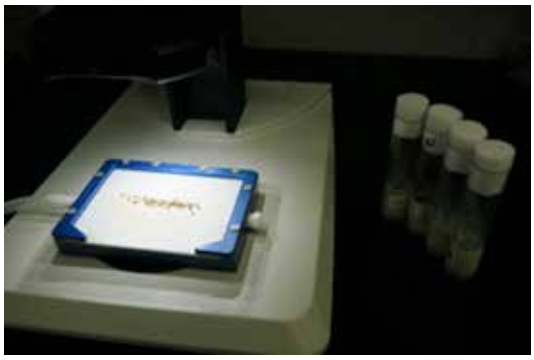


Fig. 2 Drosophila molecular genetics

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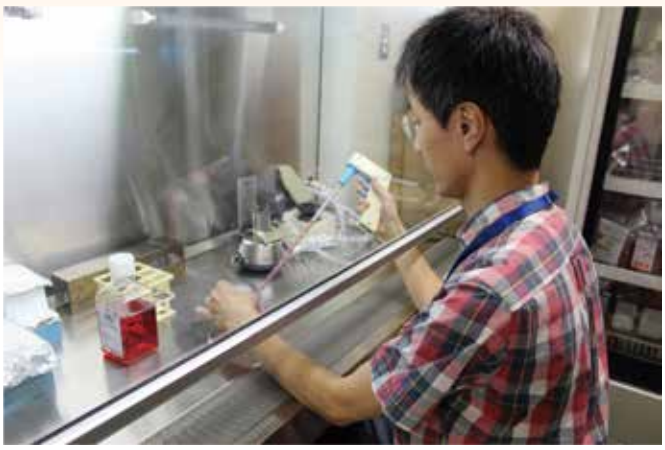

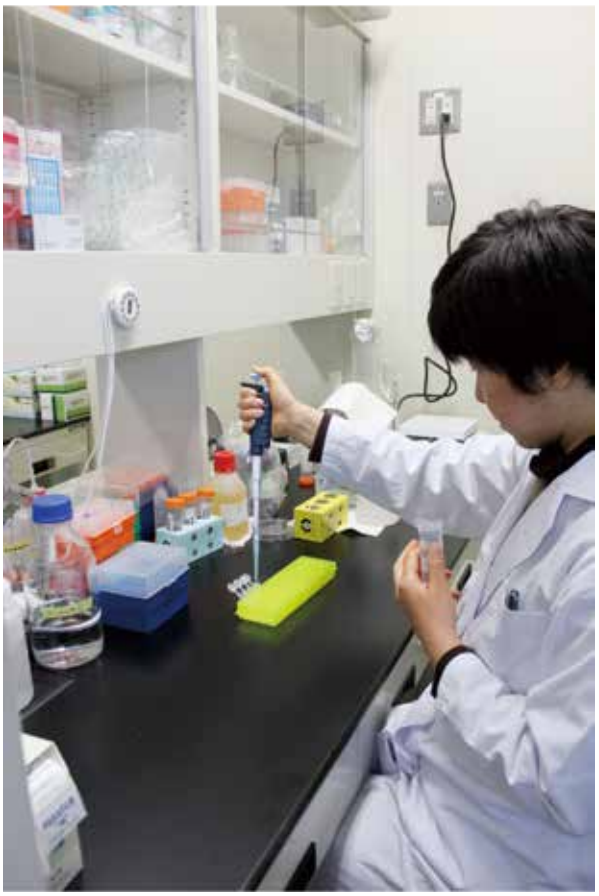

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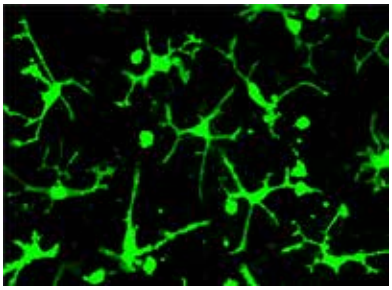
Project Leader **Hiroshi Sakuma** Developmental Neuroimmunology Project

Towards a Better Understanding of Neuro-immune Interactions in the Developing Brain

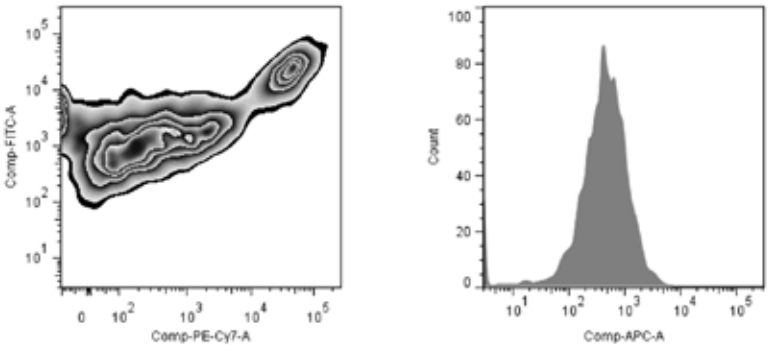


Our research focuses on the role of the immune system in the developing brain. Immune and inflammatory responses not only combat pathogens but also play a variety of physiological roles in the central nervous system. Microglia are brain-resident immune cells and play multiple roles in protection from pathogens and clearance of debris. In addition, recent studies have shed light on unexpected functions of microglia in regulating physiology. For example, microglia actively participate in the brain development by modulating synapses.

"We are investigating the mechanisms by which microglia maintain homeostasis in the developing brain."

Our main research areas include:

1) Development and differentiation of microglia
2) Neuron-microglia interaction
3) In-vitro differentiated myeloid cells for cell therapy
4) Autoantibodies associated with neurological diseases
5) New biomarkers for pediatric immune-mediated neurological diseases



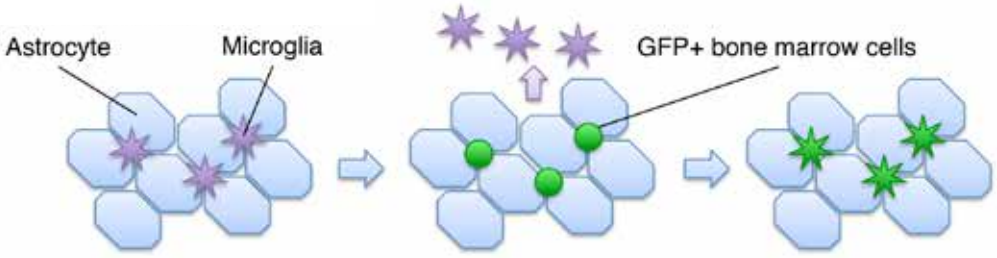
Flow cytometric analysis of microglia

Developmental Neuroimmunology

Research topics

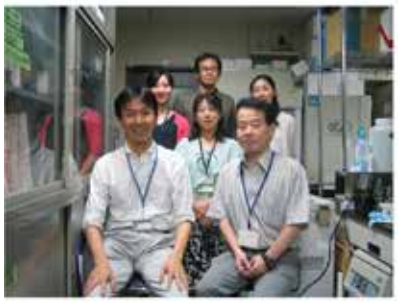
Do astrocytes nurture microglia?

Microglial progenitors originate from the yolk sac and develop into mature microglia in the fetal brain. This observation suggests that non-microglial brain cells support microglial development. We speculated that astrocyte-microglia interaction, both contact-dependent and -independent, is critical for development of microglia. Based on this hypothesis, we have tried to induce microglia from hematopoietic stem-cells by co-culture with astrocytes. When bone-marrow lineage negative cells were co-cultured on an astrocyte monolayer for one week, they developed into microglia-like cells characterized by process-bearing morphology and the expression of microglial markers including CX3CR1 and TREM-2. Differentiation of microglia-like cells was further facilitated by interleukin-34 and TGF-β. These findings provide a theoretical basis for optimizing treatment of neurological diseases by hematopoietic cell transplantation.



Members

Hiroshi Sakuma	Tomonori Suzuki	Yasuo Hachiya
Takako Matsuoka	Taiki Shima	Hiroko Tada
Kuniko Kohyama	Hiroya Nishida	Masaharu Hayashi
Setsuko Hasegawa	Ayuko Igarashi	



Developmental Neuroimmunology

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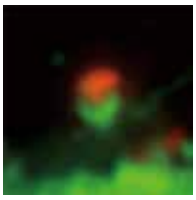
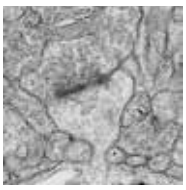
Project Leader **Kanato Yamagata** Synaptic Plasticity Project

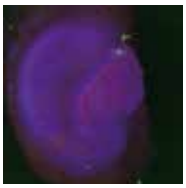
Synaptic Plasticity and Brain Diseases:

Elucidating mechanisms causing developmental epilepsy, intellectual disability, and autism

We study the molecular basis of activity-dependent synaptic plasticity. In particular, we have cloned a set of immediate early genes (IEGs) that are rapidly transcribed in neurons involved in information processing, and that are essential for long term memory. IEG proteins can directly modify synapses and provide insight into cellular mechanisms that support synaptic plasticity. Furthermore, these IEG products have been shown to be involved in developmental brain disorders, including refractory epilepsy, intellectual disability and/or autism.



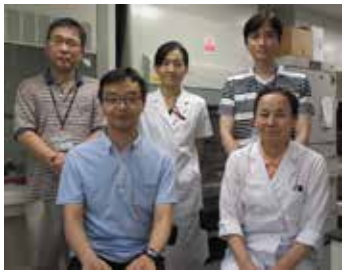




"We have clarified mechanisms of refractory epilepsy, intellectual disability and/or autism caused by impaired synaptic plasticity. Based on the novel mechanisms we found, we are trying to find new treatments for developmental brain disorders"

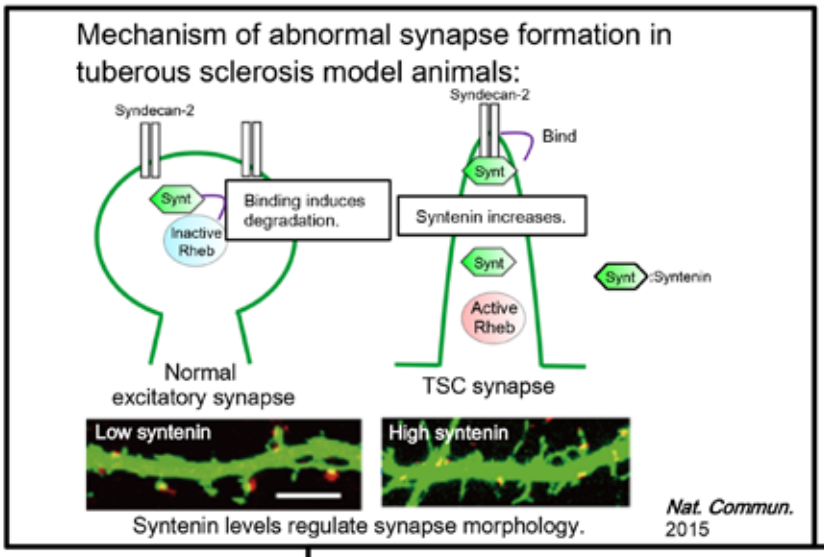
For example, COX-2 and mPGES-1 are prostaglandin synthases that exacerbate neuronal cell death after seizures, leading to intractable epilepsy. Arcadlin is a protocadherin that induces spine shrinkages after seizures, resulting in developmental delay or amnesia. Rheb regulates excitatory synapse formation via syntenin. Constitutive activation of Rheb causes TSC (tuberous sclerosis complex), which is accompanied by epilepsy, mental retardation and autism. Finally, neuritin is a secreted or membrane-anchored protein and induces neurite branching. It may be involved in temporal lobe epilepsy. Thus, analysis of rapid de *novo* transcription provides novel insights into the cellular and neural network basis of behavioral plasticity.

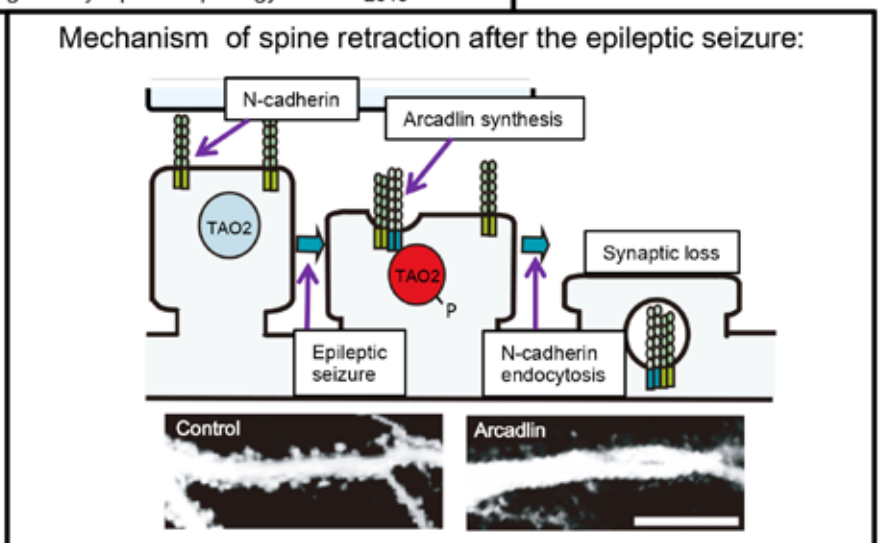
We are also exploring the possibility that these IEG products could be therapeutic targets for developmental disorders. We are making genetic mouse models of developmental disorders and are testing the effects of several drug inhibitors against IEGs.

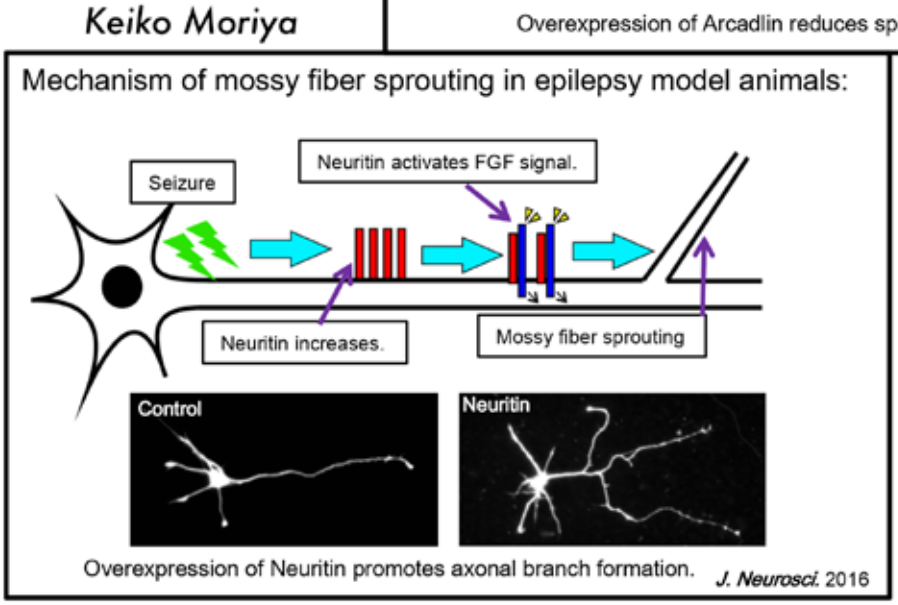


Synaptic Plasticity

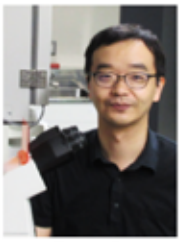
Recent Research Topics





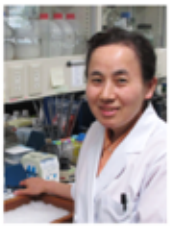


Members



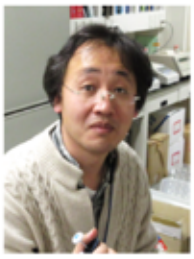
Tadayuki Shimada

Chihiro Hisatsune



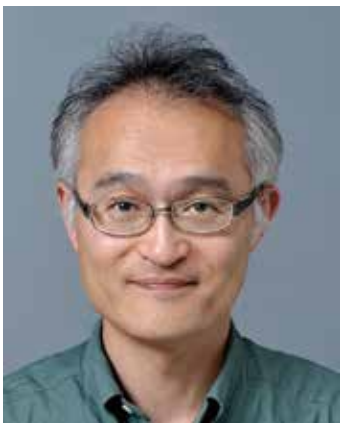
Hiroko Sugiura

Keiko Moriya



Shin Yasuda

Synaptic Plasticity



Project Leader **Haruo Okado** Neural Development Project

Brain Development and Maintenance

Various factors control differentiation of neural stem cells and survival of the resulting neurons, and aberrancy in these processes are associated with intellectual disability, age-related brain disorders, and brain tumors. We aim to elucidate the mechanisms of development and maintenance of brain functions, ultimately to develop methods for the prevention and treatment of intractable cranial nerve diseases.

Various gene-targeted mice

in utero electroporation

"We are studying the effects of various genetic and environmental factors on the molecular mechanisms of brain development and maintenance, with the ultimate goal of developing new treatments for mental diseases."

Laboratory Members

Hirai S, Hotta K, and Okado H. (2018) "Developmental Roles and Evolutionary Significance of AMPA-Type Glutamate Receptors." ***Bioessays.*** 2018 2018 Sep;40(9):e1800028.

Hirai S, Hotta K, Kubo Y, Nishino A, Okabe S, Okamura Y, and Okado H. (2017) "AMPA glutamate receptors are required for sensory-organ formation and morphogenesis in the basal chordate." ***Proc. Natl. Acad. Sci. USA.*** 114: 3939-3944.

Nakajima K, Hirai S, Morio T, and Okado H. (2015) "Benzodiazepines induce sequelae in immature mice with inflammation-induced status epilepticus." ***Epilepsy & Behavior*** 52: 180-186.

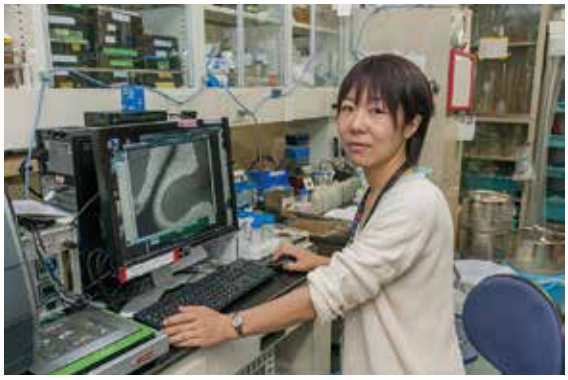
Ohtaka-Maruyama C, Hirai S, Miwa A, Heng JI, Shitara H, Ishii R, Taya C, Kawano H, Kasai M, Nakajima K, and Okado H. (2013) "RP58 regulates the multipolar-bipolar transition of newborn neurons in the developing cerebral cortex." ***Cell Rep.*** 3: 458-471.

Hirai S, Miwa A, Ohtaka-Maruyama C, Kasai M, Okabe S, Hata Y, and Okado H. (2012) "RP58 controls neuron and astrocyte differentiation by downregulating the expression of Id1-4 genes in the developing cortex." ***EMBO J.*** 31: 1190-1202.

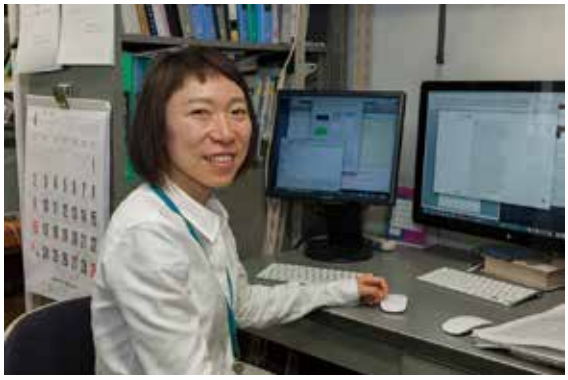
Ohtaka-Maruyama C, Hirai S, Miwa A, Takahashi A, and Okado H. (2012) "The 5'-flanking region of the RP58 coding sequence shows prominent promoter activity in multipolar cells in the sub- ventricular zone during corticogenesis." ***Neuroscience*** 201: 67-84.

Okado H, Ohtaka-Maruyama C, Sugitani Y, Fukuda Y, Ishida R, Hirai S, Miwa A, Takahashi A, Aoki K, Mochida K, Suzuki O, Honda T, Nakajima K, Ogawa M, Terashima T, Matsuda J, Kawano H, and Kasai M. (2009) "Transcriptional repressor RP58 is crucial for cell-division patterning and neuronal survival in the developing cortex." ***Dev. Biol.*** 331: 140-151.

Neural Development



Shinobu Hirai

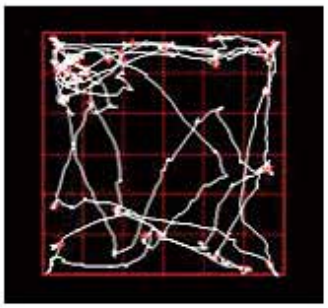


Tomoko Tanaka

Our major projects include

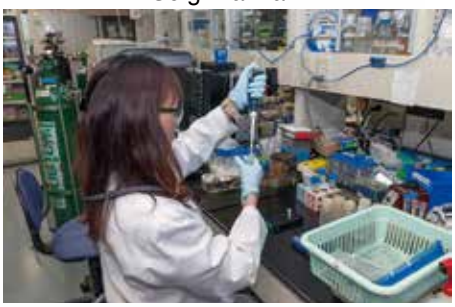
1) **Understanding how the transcriptional repressor, RP58, regulates brain development and maintenance.**
2) **Altering the nutritional environmental factors to manipulate brain development and functions.**
3) **Understanding the roles of environmental factors in development and aging of brain functions.**

Yoshie Matsumoto

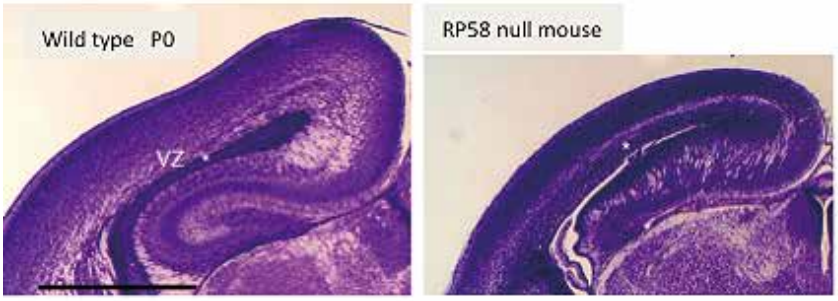


Locomotion, anxiety, memory, and sociality of mice are evaluated using a tracking system. Neuronal activity can be analyzed using an *in vivo* system.

Seigi Kanzaki



Tomoko Fukuoka



RP58 is required for development of the cerebral cortex. The cell-cycle exit of progenitor cells, neuronal radial migration and maturation of cortical neurons are impaired in RP58-deficient mice.

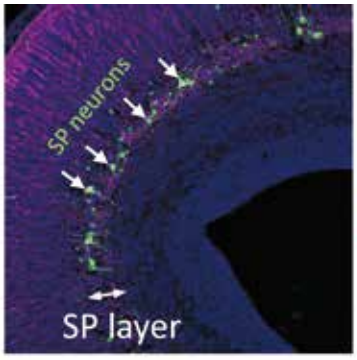
Neural Development

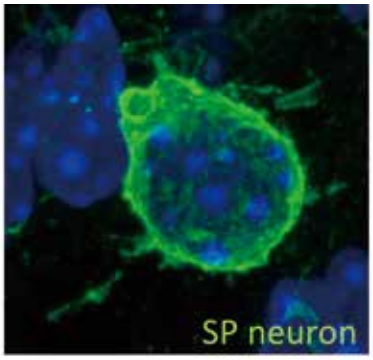
Senior Research Scientist **Chiaki Ohtaka-Maruyama**

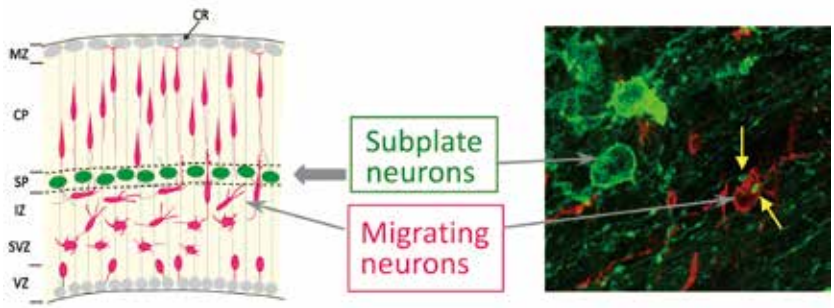
Mechanisms of Neural Network Formation: Neocortical development and synapse formation

How does the mammalian neocortex acquire the unique six-layered structure that is considered to be the structural basis for the remarkable evolution of complex neural circuits? To approach this question, we are focusing on subplate (SP) neurons which develop and mature extremely early during cortical development but disappear postnatally. Recently, we found that SP neurons play an important role in radial neuronal migration via direct interaction with young migrating neurons. Moreover, the SP layer is surrounded by a rich extracellular matrix (ECM), suggesting that it may be an important signaling center for mammalian corticogenesis. Functional elucidation of SP layer should lead to the better understanding of brain development during evolution.

"We are interested in the roles of the subplate later in the development of the cerebral cortex. It is suggested that this transient cell population plays a crucial role as a metaphorical "control tower" during neocortical formation."







Ohtaka-Maruyama C, Okamoto M, Endo K, Oshima M, Kaneko N, Yura K, Okado H, Miyata T, Maeda N ., Synaptic transmission from subplate neurons controls radial migration of neocortical neurons. ***Science 360***,313-317 (2018)

Nomura T, Ohtaka-Maruyama C, Yamashita Y, Wakamatsu Y, Murakami Y, Calegari F, Suzuki K, Gotoh H, Ono K. Evolution of basal progenitors in the developing non-mammalian brains. ***Development 143***: 66-74. (2016)

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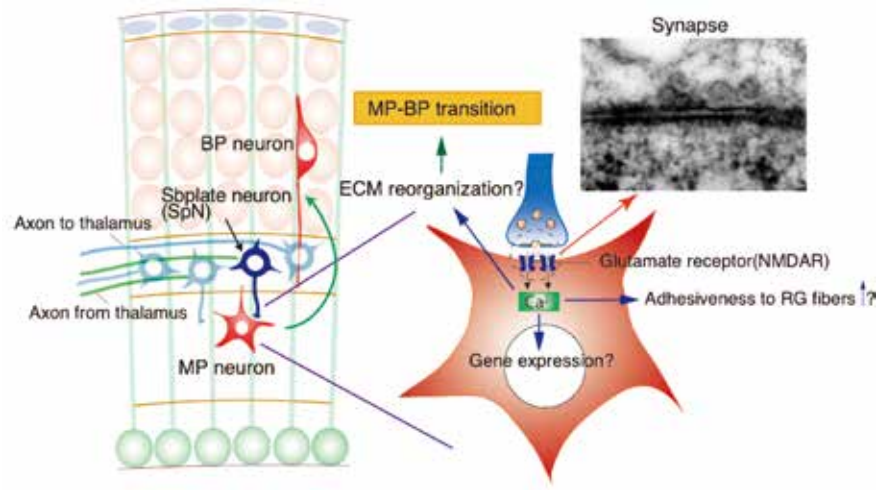
Ohtaka-Maruyama C, Hirai S, Miwa A, Heng JI, Shitara H, Ishii R, Taya C, Kawano H, Kasai M, Nakajima K, Okado H., RP58 regulates the multipolar-bipolar transition of newborn neurons in the developing cerebral cortex. ***Cell Reports.,*** 3, 458-471(2013)

Kamimura, K., Ueno, K., Nakagawa, J., Hamada, R., Saitoe, M. and Maeda, N. (2013) "Perlecan regulates bidirectional Wnt signaling at the Drosophila neuromuscular junction." ***J Cell Biol*** 200, 219-233.

Kamimura, K., Maeda, N. and Nakato H. (2011) "In vivo manipulation of heparan sulfate structure and its effect on Drosophila development." ***Glycobiology*** 21, 607-618.

Neural Network

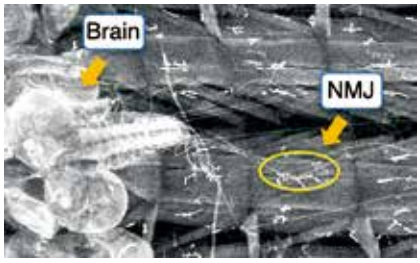


Newly born neurons initially exhibit slow multipolar migration.
Later, the migration mode switches to faster locomotion.

Our study revealed that subplate neurons send signals via synapses to multipolar migrating neurons, leading to conversion of their migration mode to faster locomotion.

Functions of proteoglycans in synapse formation

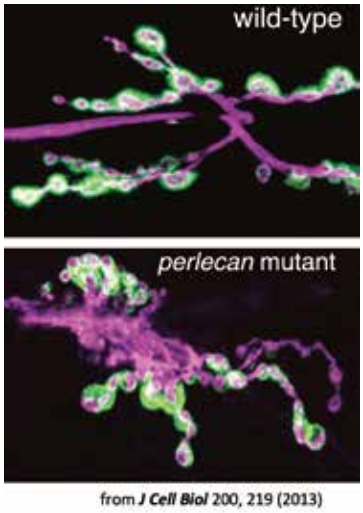
The SP layer has a rich extracellular matrix (ECM). To explore the functions of the extracellular matrix in developing neural networks, we use the Drosophila neuromuscular junction (NMJ) as a model system. The Drosophila NMJ is a readily accessible system of excitatory synapses, which resembles the glutamatergic synapses of vertebrate central nervous systems.



Brain and NMJ
of *Drosophila larva*

Perlecan is a secreted heparan sulfate proteoglycan, and its gene deletion leads to diverse defects at the Drosophila NMJ.

We demonstrated that Perlecan bidirectionally regulates pre- and post-synaptic Wnt signaling by precisely distributing Wnt at the NMJ.



from ***J Cell Biol*** 200, 219 (2013)

Members

Keisuke Kamimura
Kumiko Hirai
Aiko Odajima
Noe Kaneko
Ai Fujii
Kaori Miura

Neural Network

Nakanishi M, Niimura J, Yamasaki S, and Nishida A. (2017) "Death of dementia inpatients in Japanese psychiatric hospitals accounts for one-fifth of discharge destination in national data from 1996 to 2014" ***J. Alzheimers Dis.*** 56, 817-824.

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Nakanishi M, Nakashima T, Shindo Y, Niimura J, and Nishida A. (2016) "Japanese care location and medical procedures for people with dementia in the last month of life." ***J. Alzheimers Dis.*** 51, 747-755

Yamasaki S, Ando S, Koike S, Usami S, Endo K, French P, Sasaki T, Furukawa TA, Hasegawa-Hiraiwa M, Kasai K, and Nishida A. (2016) "Dissociation mediates the relationship between peer victimization and hallucinatory experiences among early adolescents." ***Schizophr. Res. Cogn.*** 4, 18–23

Project Leader **Atsushi Nishida** Mental Health Promotion Project

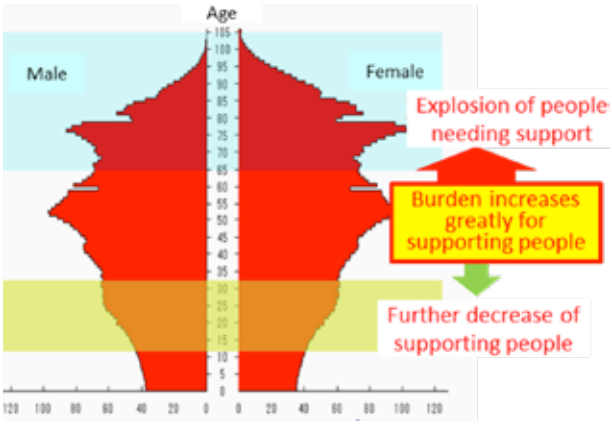
No health without Mental Health:

Mental Health promotion as the first priority in our society

While life in big cities, microcosms of today's stressful societies, is a full of risk factors for mental health, interpersonal bonds that support individuals are increasingly weakening. Hence, multifaceted research of mental health promotion via clinical medicine and sociomedical methodologies needs to be promoted.
The Mental Health Promotion Project engages in promoting mental well-being in big cities through, empirical findings from large-scale birth cohort studies conducted in partnership with municipalities in Tokyo which are experiencing increasingly aging populations and low birthrates, and developing programs in collaboration with clinical care units.

"We are trying to elucidate preventive factors for mental health problems and enhancing factors for mental well-being, and improve care for people living in communities and their families."

Our goals are as follows, 1) Elucidate preventive factors for mental health problems and enhancing factors for mental well-being in adolescence. 2) Improve care for people with dementia living in communities and their families, 3) Develop transition support programs connecting acute-phase hospital treatment and post-discharge outpatient treatment.



- **Increase in people with dementia:** Est. number in 2025 is 7 million (MHLW, 2014)
- The largest cause of health damage among **young people** is **mental illness and suicide** (Patton, Lancet, 2009)

Mental Health Promotion

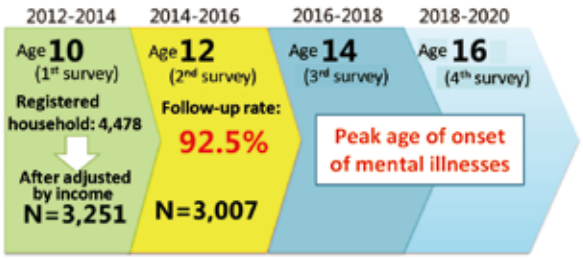
What we do

Elucidating contributing factors to adolescent mental health

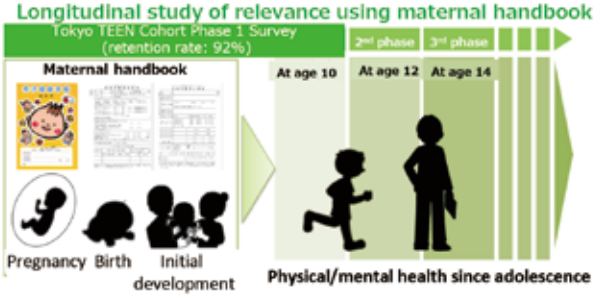
In adolescence, body and mind change significantly. Adolescents, therefore, are vulnerable to mental problems. Adolescent Health/Development Survey is a large-scale longitudinal birth cohort study included 10-year-old children and their carers living in Setagaya-ward, Chofu-city, and Mitaka-city. Currently, the study has completed the follow-up of children at their age 12; the follow-up rate is as high as 92.5%. The longitudinal relevance between the initial development at birth/childhood and the physical/mental health status since adolescence is also being studied based on information collected from maternal handbooks and various other health records.

[Adolescent Health/Development Survey]
TEEN COHORT

Progress of Health Development Survey (2017)

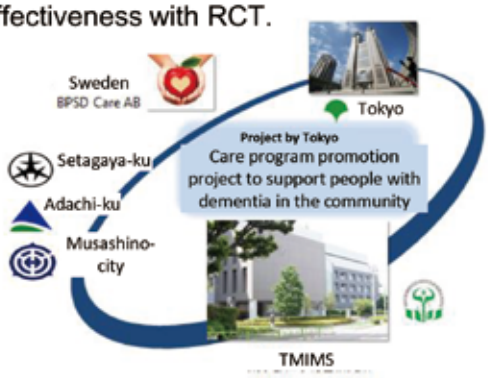


Longitudinal study of relevance using maternal handbook



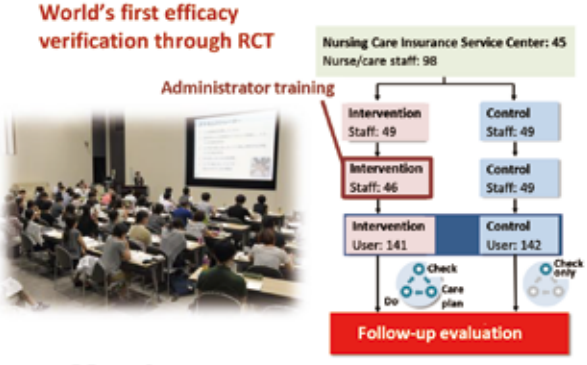
Care model development to support people with dementia at home

To support people with dementia living at home, it is indispensable to care Behavioral and Psychological Symptoms of Dementia (BPSD) as it is experienced by 90% of them. Being commissioned by Tokyo, we are working on to introduce highly-appreciated BPSD Care Program from Sweden. We aim to contribute to the dementia-related policies in Tokyo and improve the quality of dementia care through scientific verification of effectiveness with RCT.



Introduction of Sweden BPSD Care Program

World's first efficacy verification through RCT



Members

Atsushi Nishida
Syudo Yamasaki
Miharu Nakanishi
Junko Niimura
Kaori Endo
Kayo Hirooka
Yudai Iijima
Yu Yamamoto

Mental Health Promotion

Itokawa M, Miyashita M, Arai M, Dan T, Takahashi K, Tokunaga T, Ishimoto K, Toriumi K, Ichikawa T, Horiuchi Y, Kobori A, Usami S, Yoshikawa T, Amano N, Washizuka S, Okazaki Y, and Miyata T. (2018) "Pyridoxamine: A novel treatment for schizophrenia with enhanced carbonyl stress." ***Psychiatry Clin. Neurosci.*** 72: 35-44.

Miyashita M, Watanabe T, Ichikawa T, Toriumi K, Horiuchi Y, Kobori A, Kushima I, Hashimoto R, Fukumoto M, Koike S, Ujike H, Arinami T, Tatebayashi Y, Kasai K, Takeda M, Ozaki N, Okazaki Y, Yoshikawa T, Amano N, Washizuka S, Yamamoto H, Miyata T, Itokawa M, Yamamoto Y, and Arai M. (2016) "The regulation of soluble receptor for AGEs contributes to carbonyl stress in schizophrenia." ***Biochem. Biophys. Res. Commun.*** 479: 447-452.

Arai M, Miyashita M, Kobori A, Toriumi K, Horiuchi Y, Hatakeyama S, and Itokawa M. (2014) "Carbonyl stress and schizophrenia." ***Psychiatry Clin. Neurosci.*** 68: 655-665.

Miyashita M, Arai M, Kobori A, Ichikawa T, Toriumi K, Niizato K, Oshima K, Okazaki Y, Yoshikawa T, Amano N, Miyata T, and Itokawa M. (2014) "Clinical Features of Schizophrenia With Enhanced Carbonyl Stress." ***Schizophr. Bull.*** 40: 1040-1046.

Arai M, Nihonmatsu-Kikuchi N, Itokawa M, Rabbani N, and Thornalley PJ. (2014) "Measurement of glyoxalase activities." ***Biochem Soc. Trans.*** 42: 491-494.

Miyashita M, Arai M, Yuzawa H, Niizato K, Oshima K, Kushima I, Hashimoto R, Fukumoto M, Koike S, Toyota T, Ujike H, Arinami T, Kasai K, Takeda M, Ozaki N, Okazaki Y, Yoshikawa T, Amano N, Miyata T, and Itokawa M. (2014) "Replication of enhanced carbonyl stress in a subpopulation of schizophrenia." ***Psychiatry Clin. Neurosci.*** 68: 83-84.

Arai M, Koike S, Oshima N, Takizawa R, Araki T, Miyashita M, Nishida A, Miyata T, Kasai K, and Itokawa M. (2011) "Idiopathic carbonyl stress in a drug-naive case of at-risk mental state." ***Psychiatry Clin. Neurosci.*** 65: 606-607.

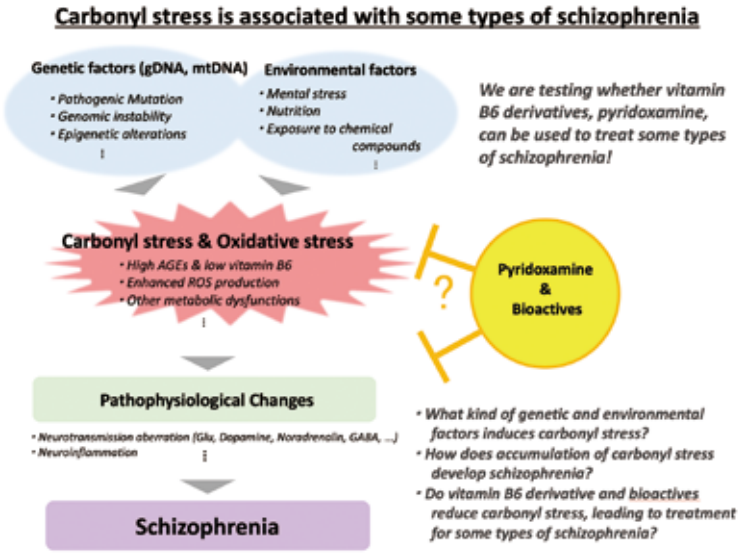
Arai M, Yuzawa H, Nohara I, Ohnishi T, Obata N, Iwayama Y, Haga S, Toyota T, Ujike H, Arai M, Ichikawa T, Nishida A, Tanaka Y, Furukawa A, Aikawa Y, Kuroda O, Niizato K, Izawa R, Nakamura K, Mori N, Matsuzawa D, Hashimoto K, Iyo M, Sora I, Matsushita M, Okazaki Y, Yoshikawa T, Miyata T, and Itokawa M. (2010) "Enhanced Carbonyl Stress in a Subpopulation of Schizophrenia." ***Arch. Gen. Psychiatry.*** 67: 589-597.

Project Leader **Makoto Arai** Schizophrenia Research Project

Identifying Biomarkers of Schizophrenia

Profiling of the peripheral metabolic system is a viable schizophrenia research strategy that can lead to earlier diagnostic methods, elucidation of molecular mechanisms, and novel strategies for the prevention and treatment of schizophrenia.
We focus on, 1) developing individualized medicine for treating schizophrenia, 2) investigating factors involved in disease onset, and 3) understanding the molecular pathology by using biomarkers to overcome the barrier of heterogeneity. Our research outcomes will be applied to drug development by establishing a new biomarker-based field of research in molecular psychiatry. Data obtained from metabolomics, genomics, induced pluripotent stem (iPS) cell models, animal models, post-mortem brain analyses, neuropsychology, and genetic counseling research will be consolidated to elucidate the genetic and environmental factors relevant to psychiatric disorders such as schizophrenia.

"Identifying biomarkers will allow us to classify schizophrenia into different types, and aid in earlier diagnoses and better treatments, leading to improvements in patients' quality of life."



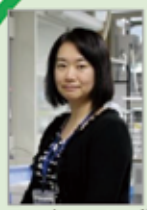
This biomarker-based approach is an innovative and creative strategy for identifying the metabolic changes associated with schizophrenia, independent of conventional pathological hypotheses. Verification in cellular and animal models can shed light on the molecular mechanisms underlying the utility of naturally-derived substances in trading schizophrenia, and is expected to lead to the future development of much safer treatments and prophylactic methods.

Schizophrenia Research

Topics of our research

- Clinical study
- Genomics
- Metabolomics
- Neuropsychology
- iPS cell models
- Mouse models
- Post-mortem brain analysis
- Genetic counseling

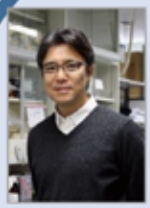
Yasue Horiuchi



Research of schizophrenia cell models and genetic counseling

Induced pluripotent stem cells (iPSCs) are believed to provide a powerful strategy to obtain and characterize central nervous system-relevant cells in vitro. We have successfully generated iPSCs, neurons and glial cells derived from patients with schizophrenia and carbonyl stress. We are confident such cellular models will supply us with a unique tool to study major mental disorders. Our other focus is making genetic counseling pervasive in the research and psychiatric field in Japan. (Please see our web site for more detail).

Mitsuhiro Miyashita



Investigating the pathophysiology and clinical relevance of schizophrenia with carbonyl stress.

We have found that carbonyl stress-related schizophrenia (SZ) presents a treatment-resistant phenotype. In our research, we try to elucidate the mechanism underlying how carbonyl stress affects onset and increases both hospitalization time and symptom severity in SZ, by investigating the elements of the AGEs-RAGE-inflammation axis. Additionally, we will examine longitudinally how carbonyl stress alters the clinical prognosis and physical complications in patients with SZ.

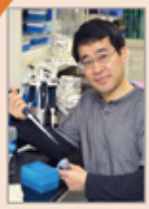
Our projects contribute to future innovation for preventive medical research in the areas of psychiatry, health, and welfare

Kazuya Toriumi

Development and analysis of mouse models based on schizophrenia pathophysiology

Based on clinical findings, we have developed genetic and/or environmental mouse models for schizophrenia, and analyzed them to uncover the molecular mechanisms underlying schizophrenia with carbonyl stress, oxidative stress and/or vitamin B6 deficiency. Moreover, using these mouse models, we have tried to explore new types of therapeutic drugs for schizophrenia that use different mechanisms of action than existing antipsychotics.

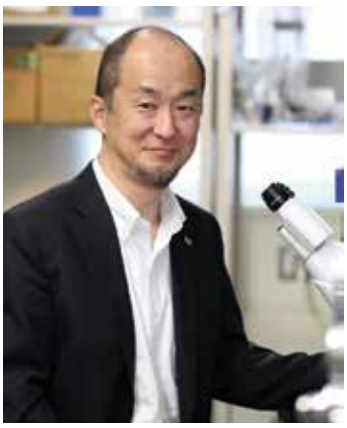
Masanari Itokawa



Clinical pharmacology of TM8001 in patients with carbonyl stress-related schizophrenia

TM8001 is a dihydrochloride of pyridoxamine, one of the vitamin B6 groups. TM8001 can act to capture reactive carbonyl compounds, and has inhibitory activity against the production of AGE by reactive carbonyl compounds. Thus, by reducing carbonyl stress, it is expected to be therapeutic in this type of schizophrenia. Removal of these substances is the key to a possible new treatment method based on the root cause of carbonyl stress-related schizophrenia.

Schizophrenia Research



Bauer M, (64 co-authors), Tatebayashi Y et al. (2014) "Relationship between sunlight and the age of onset of bipolar disorder: an international multisite study." ***J. Affect. Disord.*** 167:104-111.

Nihonmatsu-Kikuchi N, Hayashi Y, Yu XJ, and Tatebayashi Y. (2013) "Depression and Alzheimer's disease: novel postmortem brain studies reveal a possible common mechanism." ***J. Alzheimers Dis.*** 37: 11-21.

Tatebayashi Y, Nihonmatsu-Kikuchi N, Hayashi Y, Yu XJ, Soma M, and Ikeda K. (2012) "Abnormal fatty acid composition in the frontopolar cortex of patients with affective disorders." ***Transl. Psychiatry*** 2:e204.

Hayashi Y, Nihonmatsu-Kikuchi N, Hisanaga S, Yu XJ, and Tatebayashi Y. (2012) "Neuropathological similarities and differences between schizophrenia and bipolar disorder: a flow cytometric postmortem brain study." ***PLoS One.*** 7: e33019.

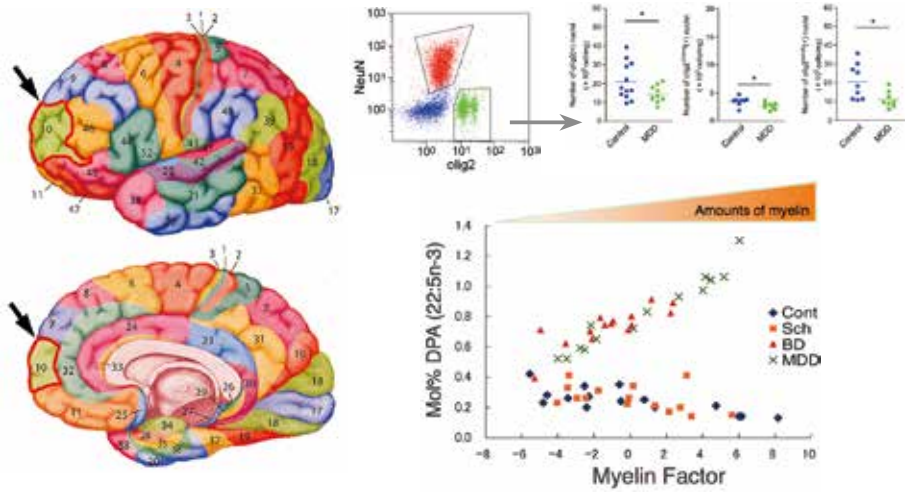
Hayashi Y, Nihonmatsu-Kikuchi N, Yu XJ, Ishimoto K, Hisanaga SI, and Tatebayashi Y. (2011) "A novel, rapid, quantitative cell-counting method reveals oligodendroglial reduction in the frontopolar cortex in major depressive disorder." ***Mol. Psychiatry.*** 16: 1155-1158.

Project Leader **Yoshitaka Tatebayashi** Affective Disorders Research Project

Our Goal is to Decipher the Neurobiological Bases of Affective Disorders.

Major depressive disorder (MDD) and bipolar disorder (BD), collectively known as affective disorders, are relapsing and remitting disorders of affect with nearly full recovery between episodes. We use human postmortem brains and animal and cell culture models to identify the processes in which stress or aging causes changes in brain to induce these disorders. A major focus of our work is stress-induced or age-related changes in cellular structure, especially that of oligodendrocyte lineage cells and lipids, within the brain's mood circuitry. We are also interested in the biological relationship between affective disorders and dementias such as Alzheimer's disease.

"Our human postmortem brain studies reveal oligodendroglial reductions and myelin-dependent fatty acid abnormalities in the frontopolar cortex in affective disorders."



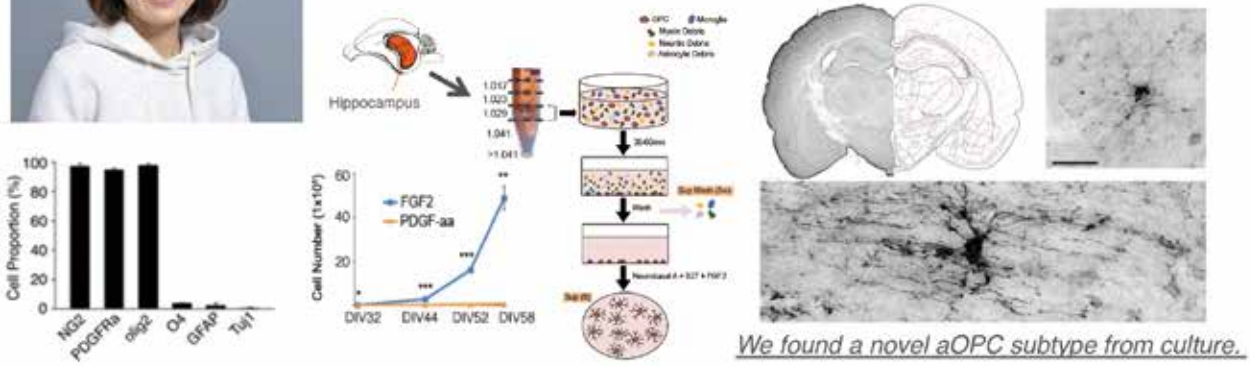
The exact functions of the human frontopolar cortex (BA10) remain enigmatic. Given that the BA10 is thought to be the most evolutionarily recent expansion of the primate prefrontal cortex, its function may uniquely reflect human adaptations in the context of selecting and updating models of reward contingency in dynamic environments. As adulthood cortical myelination is an essential process for the establishment of efficient neuronal signaling networks, any abnormalities in this process may have important roles in the pathophysiology of affective disorders.

Affective Disorders Research

"Better understanding of these phenomena will provide important insights to facilitate the more effective diagnosis, treatment and prevention of affective disorders."

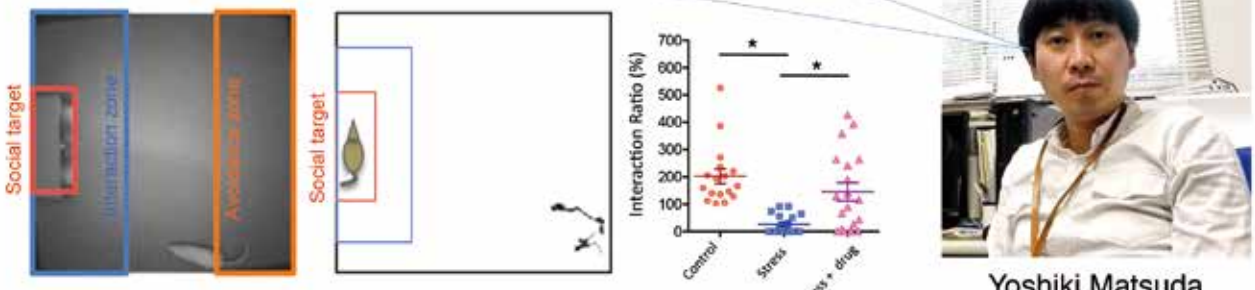
Naomi Nihonmatsu-Kikuchi

We purify and culture adult oligodendrocyte progenitor cells (aOPCs) from adult mammalian brains to understand their roles in the pathogenesis of affective disorders.



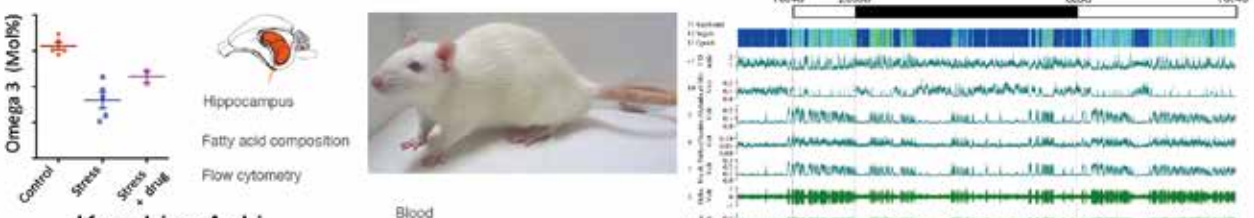
We found a novel aOPC subtype from culture.

Our animal model clearly indicates essential roles of inflammation in the pathophysiology of depression. Chronic stress induces changes not only in behavior but also in electrophysiology and cellular structure.



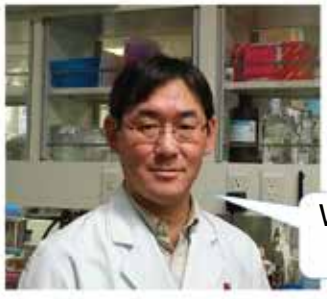
Yoshiki Matsuda

We found a therapeutic effect of anti-inflammatory drugs.



Kazuhisa Aoki

We found several candidate blood biomarkers for psychosocial stress.



We are conducting detailed "omics" analyses of our animal models to discover novel biomarkers for depression.

Affective Disorders Research

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Project Leader **Makoto Honda** Sleep Disorders Project

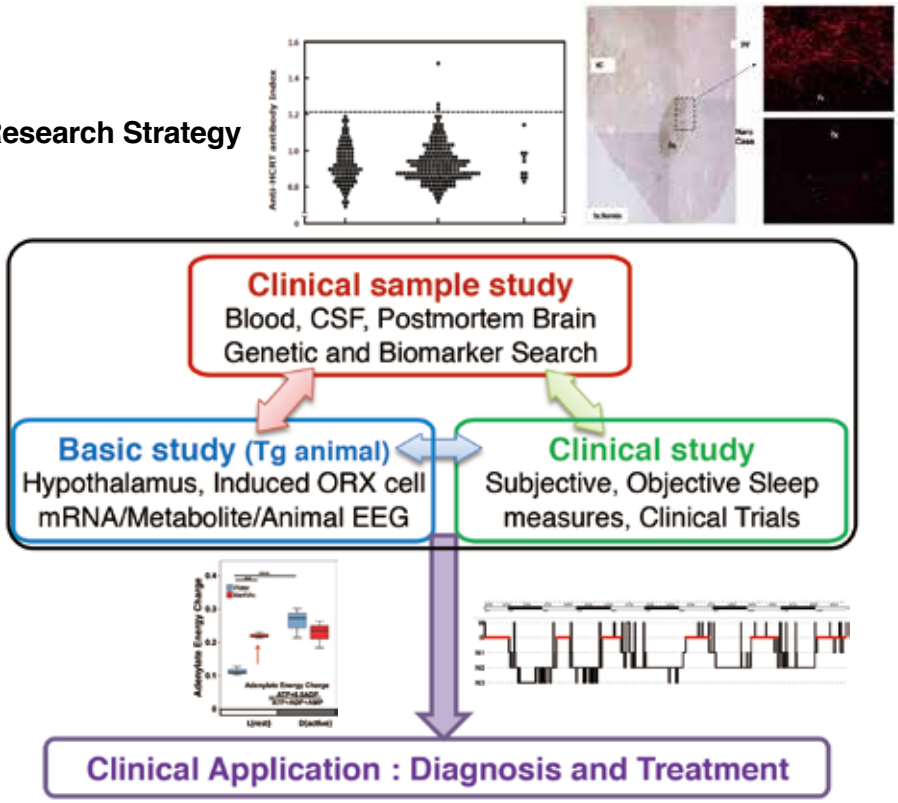
Narcolepsy and Hypersomnia:
Find the causes to develop better treatments

Narcolepsy is a sleep disorder of abnormal intrinsic sleep-wake regulation, resulting in unique symptoms including frequent lapses into sleep, nocturnal sleep instability, and REM sleep related manifestations such as cataplexy (abrupt loss of muscle tone triggered by emotion), sleep paralysis, and hypnagogic hallucination.
Narcolepsy is associated with a deficiency of wake-promoting orexin/hypocretin producing neurons localized in the hypothalamus, and virtually all the patients carry *human leukocyte antigen (HLA)-DQB1*06:02.*

"We are trying to solve the mystery of narcolepsy : Listen to the patients, get the whole picture, and improve their lives"

Narcolepsy is associated with a variety of physical and psychiatric comorbid conditions. Since appropriate wakefulness is essential for higher brain functions, abnormal sleep-wake regulation can lead to various associated features. Despite the progress in sleep research fields, we currently have inadequate symptom-based treatments for sleep disorders, including narcolepsy. We are trying to elucidate the pathophysiology of narcolepsy with multifaceted problems to improve the QOL of hypersomnia patients.

Research Strategy

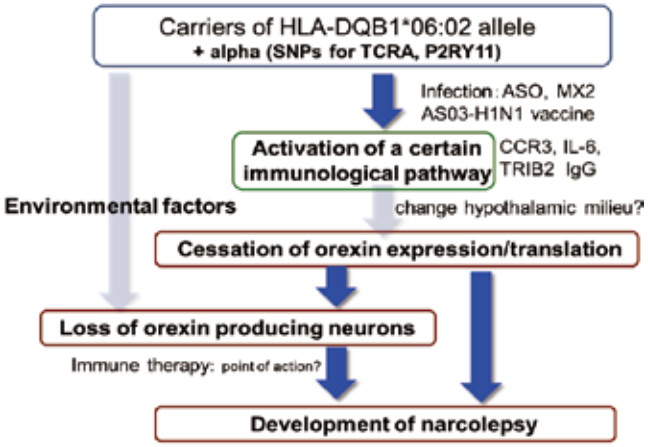


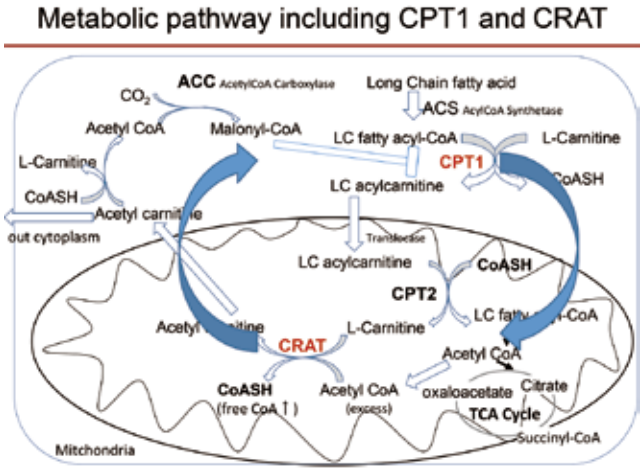
Research Interests

The hypothalamus works as a center for sleep-wake switching and integrates information from the body for this process. We are particularly interested in how the body's immune and metabolic status affect sleep. This may be a key to understanding altered sleep-wake regulation in narcolepsy.

1. HLA association and immune abnormality

In addition to a tight association with HLA, narcolepsy is also associated with the T cell receptor (TCR) alpha locus, indicating that HLA-TCR mediated immunological alterations occur in narcolepsy. Both genetic and environmental factors are implicated in narcolepsy predisposition. We have reported an altered immune status in narcolepsy, but so far could not confirm the leading hypothesis that orexin neurons are destroyed by direct autoimmune attacks. Immune mechanisms other than autoimmunity might lead to inhibition of orexin neuropeptide production.





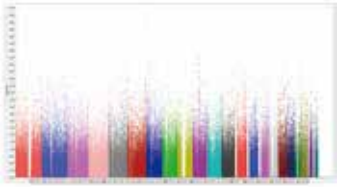
2. Metabolic aspect of narcolepsy and related hypersomnia

Through genome-wide association studies (GWAS), we have identified novel narcolepsy (and other hypersomnia) related genes. These genes encode key enzymes located in the fatty acid metabolism pathway. We have confirmed their functional relevance, performed clinical trials, and are currently analyzing the potential efficacy of a novel therapy (promoting metabolism) in hypersomnia patients.

Members of Sleep Disorders Project (2017)

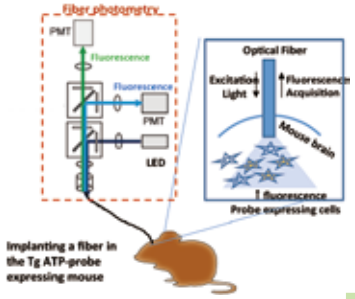
Taku Miyagawa

Understanding the genetic background and mechanism of sleep disorders.



Akiyo Natsubori

Understanding the brain metabolic dynamics of mice under sleep and wakefulness.



Sleep Disorders

Project Leader **Kazutaka Ikeda** Addictive Substance Project

Addictive Drugs are Double-edged Sword: They can be both harmful and beneficial, depending on how they are used

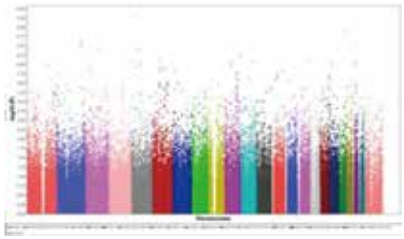
Addiction to various substances (e.g., drugs, alcohol, and tobacco) and behaviors (e.g., Internet and gambling) is a serious public health problem. The use of legal drugs has been increasing in Japan in recent years. Thus, preventing and solving problems that are related to addiction are important.

Some addictive drugs are also widely used as analgesics and for the treatment of developmental disorders. Some molecules that are involved in the actions of addictive drugs may be shared between analgesia and developmental disorders.

The goals of our project are the following: (1) developing novel treatments for addiction and prevention, (2) improving personalized pain treatment, and (3) developing novel treatments for developmental disorders.

"We are trying to improve treatment, prevention, and our understanding of addiction, pain, and developmental disorders by revealing the mechanisms that underlie addiction."

Attaining these goals will make significant contributions to society. We seek to accomplish these goals by studying the actions of addictive drugs using molecular biological, behavioral pharmacological, human genomic, and clinical approaches.



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Addictive Substance

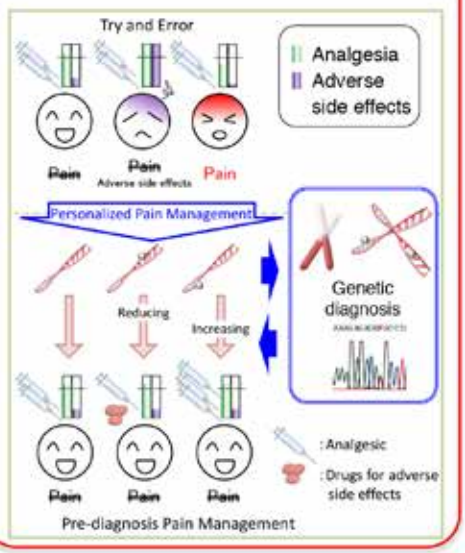
Topics of our research

Addiction research

We study the mechanisms of action of opioids, dopamine, and hallucinogens (e.g., phencyclidine) to reveal the etiology of addiction using several mouse models and behavioral pharmacological approaches. In parallel with basic research, we are also developing a clinical scale to measure addiction severity.

Pain treatment research

The sensitivity to opioid analgesics is associated with polymorphisms of several genes. Based on genomic information, we are developing personalized pain treatments.



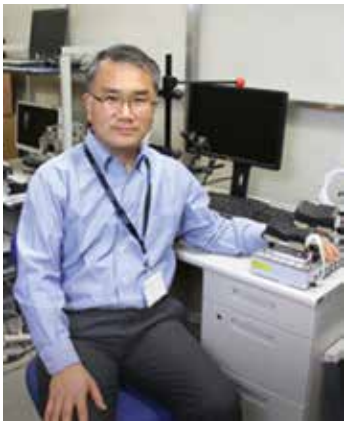
Developmental disorder research

We focus on autism and attention-deficit/ hyperactivity disorder (ADHD). Tuberous sclerosis complex 1 and 2 heterozygous knockout mice and dopamine transporter knockout mice are mainly used as models of autism and ADHD, respectively. We seek to develop novel treatments for autism.

Members

Kazutaka Ikeda
Shinya Kasai
Daisuke Nishizawa
Soichiro Ide
Seii Ohka
Masayo Fujita
Hiroko Kotajima

Addictive Substance



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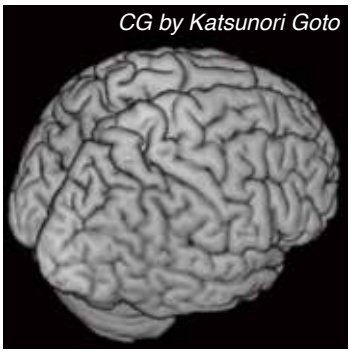
Project Leader **Shinji Kakei** Motor Disorders Project

From Neuron to Action and its Disorders

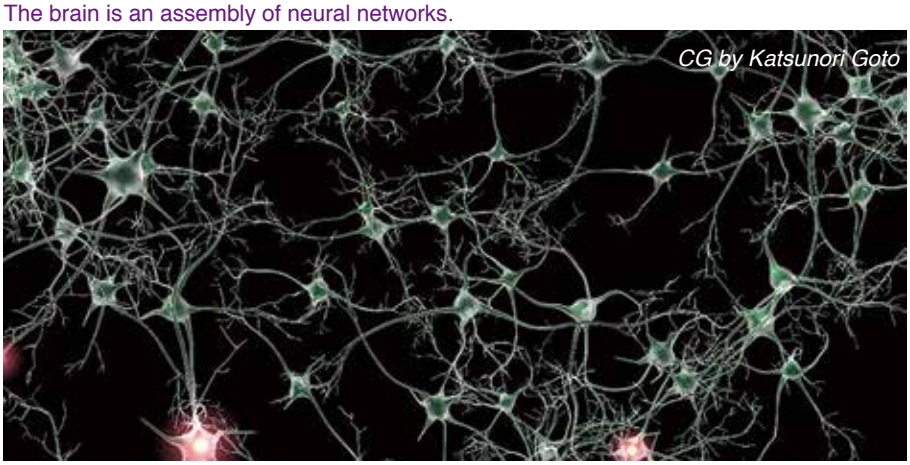
We try to understand how the brain controls our movements in the real world. We study the process of action generation at a single neuron level using animal models to understand how movements are processed in the brain. We also study actions of healthy people, as well as those with neurological disorders, such as cerebellar disorders, Parkinson's disease or strokes. We look for building-blocks of motor control with multidisciplinary approaches. Our tools include various neurophysiological recording techniques (single unit recording, electromyography (EMG) and electro-encephalography (EEG)), brain stimulation, neuroimaging, analysis of movement kinematics and a large-scale modeling. We have two long-term goals: 1) to understand the basic function of the motor structures of the brain including the cerebellum, the basal ganglia, and the motor cortex; and 2) to understand how our brain controls our movements on the basis of the findings in 1).

"Through our research, we are trying to understand the brain. The brain was first created to control movement and extended to control higher brain functions."



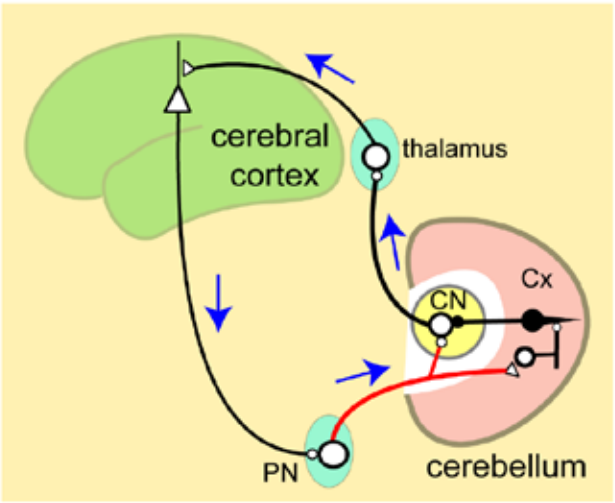
"The brain mechanism for motor control must provide a basic framework to understand higher brain functions."

The brain is an assembly of neural networks.

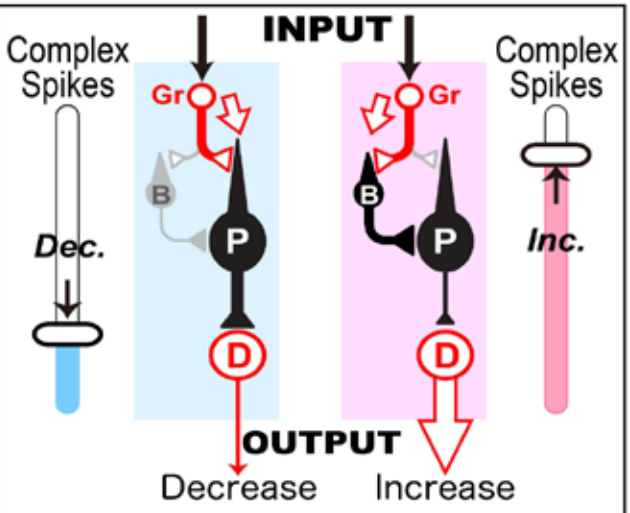


Motor Disorders

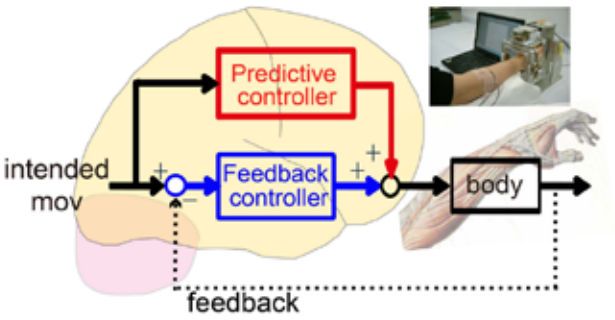
Hot Topics of Our Research



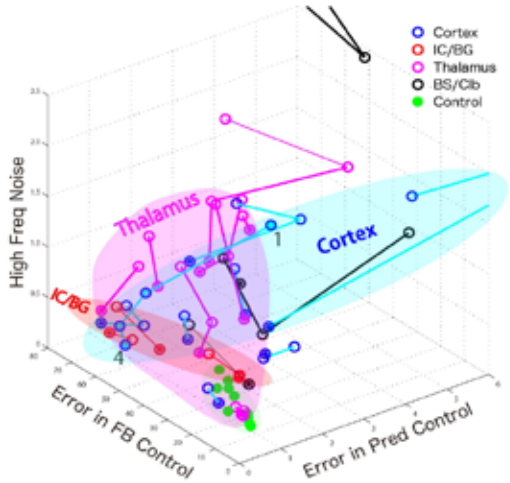
The cerebro-cerebellar communication loop plays essential roles to organize both motor control and higher brain functions such as thought and speech.



We found two modes of cerebellar input-output relationship that explain generation of precise motor commands.



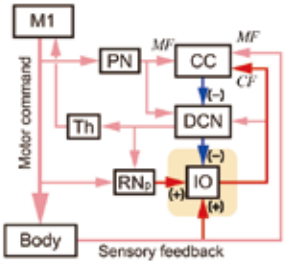
We were the first group in the world to build a system (*inset*) to dissociate predictive motor control and feedback motor control (below) in patients with neurological disorders. This system provides quantitative parameters that characterize the two controllers.



With new quantitative parameters, we were the first group to visualize different courses of recovery for stroke patients with different localization of brain lesions.

Members

Kyuengbo Min,
Jongho Lee,
Takahiro Ishikawa,
Takeru Honda



Motor Disorders

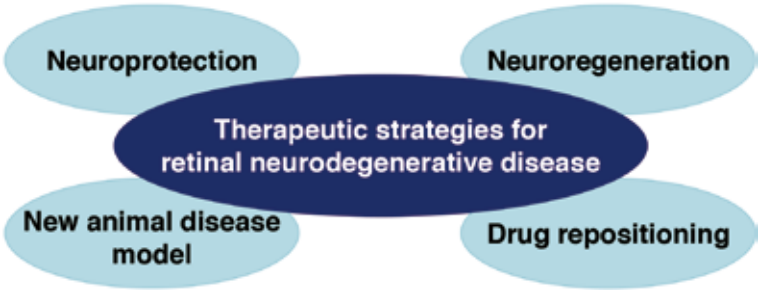
Project Leader **Takayuki Harada** Visual Research Project

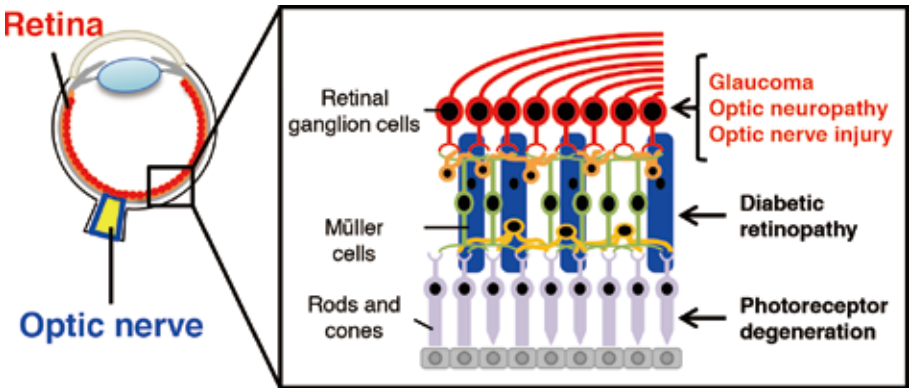
Elucidation of Pathology and Development of Therapeutic Strategies for Retinal Neurodegenerative Diseases

More than 1.6 million people in Japan are visually impaired, representing economic social losses estimated at more than 8 trillion yen. In the particular context of the increased penetration of Western lifestyles and an aging society, the increase in the number of patients with conditions such as glaucoma and diabetic retinopathy, which could be called “adult eye diseases,” has become a major social issue.
To achieve improved quality of life (QOL) for the visually impaired in an increasingly aging population, we seek to elucidate detailed pathogenic mechanisms and develop new therapies through the development of a model of intractable eye disease.

Our objectives

“We are focusing on elucidating the molecular mechanisms of neuroprotection and neuroregeneration, and our final goal is the prevention or treatment of blindness in retinal neurodegenerative disorders such as glaucoma and traumatic injury.”





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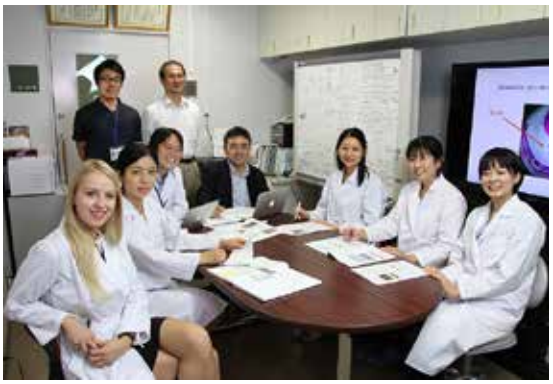
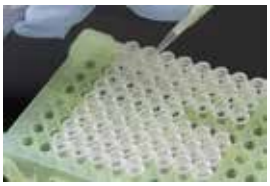
Visual Research

Our major aim

- **To develop a neuroprotective retinal therapy using animal disease models**
- **To elucidate the mechanisms involved in the onset of optic neuritis**
- **To establish a method to promote regeneration of the optic nerve**



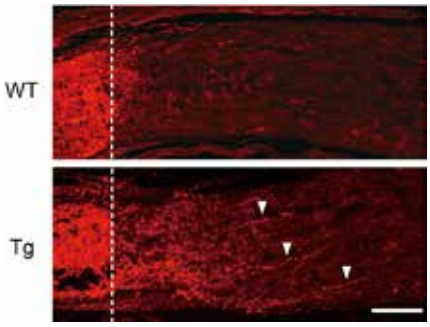




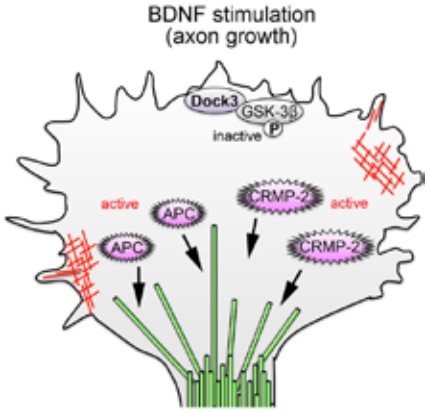
Senior Research Scientist **Kazuhiko Namekata**

Dock family proteins

The dedicator of cytokinesis (Dock) family is composed of atypical guanine exchange factors (GEFs) that induce actin polymerization. To date, 11 Dock family members have been identified. Dock3 is predominantly expressed in the central nervous system. In the growth cone, Dock3 induces actin polymerization by activating WASP family verprolin-homologous protein (WAVE) and modulates microtubule dynamics through inactivation of GSK-3β, leading to axon elongation. In addition, Dock3 plays a role in protecting retinal ganglion cells from neurotoxicity and oxidative stress. Dock3 may be a therapeutic target for optic neuropathy including glaucoma.



Axonal regeneration was enhanced in Dock3 overexpressing mouse (Tg) (Arrow heads indicate regenerating axons)



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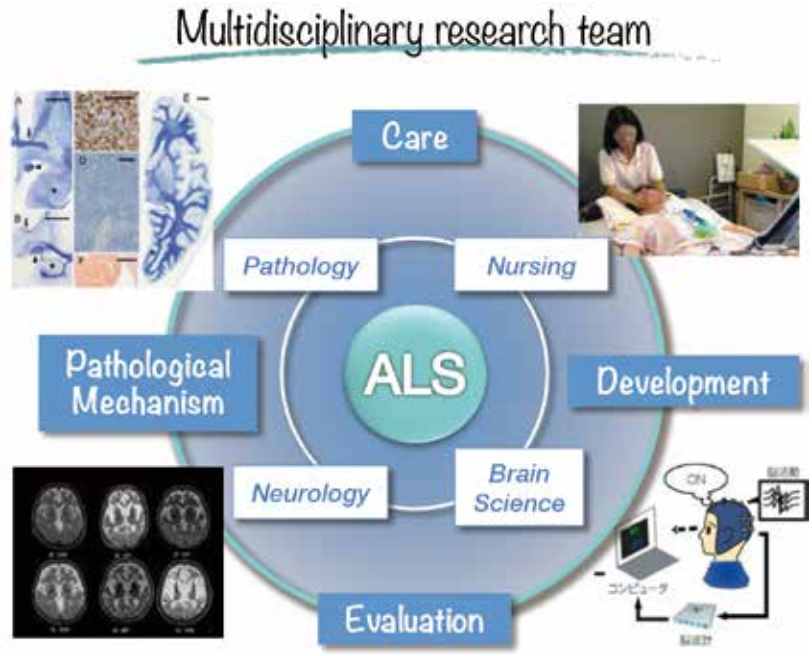
Visual Research

Project Leader **Yuki Nakayama** ALS Nursing Care Project

Improving the Quality Of Life of Patients with Amyotrophic Lateral Sclerosis

"Our mission is to establish the best practices for respiratory and communication management for ALS patients in a community-based setting . We have established a multidisciplinary research team to develop a Brain Machine Interface for ALS patients."



Nakayama Y, Shimizu T, Matsuda C, Haraguchi M, Hayashi K, Bokuda K, Nagao M, Kawata A, Ishikawa-Takata K, Isozaki E. (2019) "Body weight variation predicts disease progression after invasive ventilation in amyotrophic lateral sclerosis." ***Scientific Reports*** volume 9, Article number: 12262

Shimizu T, Nakayama Y, Matsuda C, Haraguchi M, Bokuda K, Ishikawa-Takata K, Kawata A, Isozaki E. 2019 "Prognostic significance of body weight variation after diagnosis in ALS: a single-centre prospective cohort study." ***Journal of Neurology*** .266(6), 1412–1420

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Nakayama Y, Shimizu T,Matsuda C, Mochizuki Y, Hayashi K,Nagao M, Kawata A, Isozaki E. (2018) "Non-Motor Manifestations in ALS Patients with Tracheostomy and invasive ventilation." ***Muscle and Nerve.*** 57(5):735-741.

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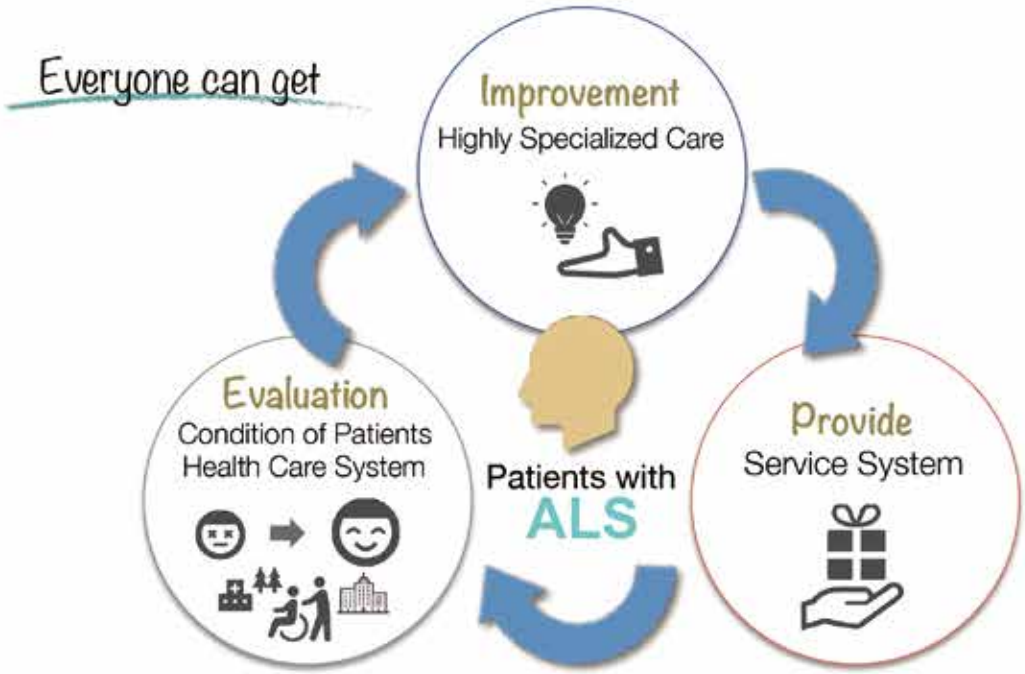
ALS Nursing Care

ALS Nursing Care Project Ground design



Administration of Community-Based Nursing

How many visiting nurse stations are there in this community?

" Do Patients live well? "

Akiko Ogura, Ph.D.

Quality Assurance of Home Care

Collaboration between visiting Nurse and care workers.
Risk management on Home Mechanical Ventilation, and Construction of information system for Medical Near-miss/adverse event.
Needs of Nursing Support in Outpatient Department on Patients with ALS.

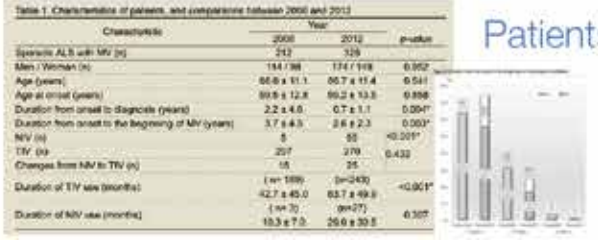
Michiko Haraguchi, Ph.D.

Establishing specialized Oral Nursing care system for advanced amyotrophic lateral sclerosis patient

Chiharu Matsuda, Ph.D.

Multivariate analysis for the occurrence of macroglossia by logistic regression

Variables	Odds ratio	95% CI	P-value
Age at beginning of TIV use, years	0.937	0.845–1.041	0.225
Duration of TIV use, months	1.022	1.000–1.044	0.050
ALSFRS-R score	0.822	0.314–2.146	0.314
Body mass index, kg/m²	1.653	1.150–2.370	0.007
Energy intake, kcal/d	1.001	0.995–1.006	0.784
Stages of communication impairments (I, II–IV, V)	3.771	1.150–12.370	0.029



Patients with Intractable Diseases Analyze their physical and psycho-social Data

Yumi Itagaki, M.S.

ALS Nursing Care

Nakamura S*, Oba M*, Suzuki M, Takahashi A, Yamamuro T, Fujiwara M, Ikenaka K, Minami S, Tabata N, Yamamoto K, Kubo S, Tokumura A, Akamatsu K, Miyazaki Y, Kawabata T, Hamasaki M, Fukui K, Sango K, Watanabe Y, Takabatake Y, Kitajima TS, Okada Y, Mochizuki H, Isaka Y, Antebi A, and Yoshimori T. (2019) "Suppression of autophagic activity by Rubicon is a signature of aging." ***Nat. Commun.*** 10:847 (*First authors)

Takaku S, Yako H, Niimi N, Akamine T, Kawanami D, Utsunomiya K, and Sango K. (2018) "Establishment of a myelinating co-culture system with a motor neuron-like cell line NSC-34 and an adult rat Schwann cell line IFRS1." ***Histochem. Cell Biol.*** 149:537-543.

Yoshida S, Hasegawa T, Suzuki M, Sugeno N, Kobayashi J, Ueyama M, Fukuda M, Ido-Fujibayashi A, Sekiguchi K, Ezura M, Kikuchi A, Baba T, Takeda A, Mochizuki H, Nagai Y, and Aoki M. (2018) "Parkinson's disease-linked DNAJC13 mutation aggravates alpha-synuclein-induced neurotoxicity through perturbation of endosomal trafficking." ***Hum. Mol. Genet.*** 27:823-836.

Niimi N, Yako H, Takaku S, Kato H, Matsumoto T, Nishito Y, Watabe K, Ogasawara S, Mizukami H, Yagihashi S, Chung SK, and Sango K. (2018) "A spontaneously immortalized Schwann cell line from aldose reductase-deficient mice as a useful tool for studying polyol pathway and aldehyde metabolism." ***J. Neurochem.*** 144:710-722.

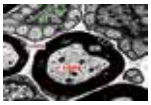
Sango K, Mizukami H, Horie H, and Yagihashi S. (2017) "Impaired axonal regeneration in diabetes. Perspective on the underlying mechanism from in vivo and in vitro experimental studies." ***Front. Endocrinol.*** 8:12.

Project Leader **Kazunori Sango** Diabetic Neuropathy Project

Pathogenesis-based Therapeutic Approaches to Diabetic Neuropathy

One of the most common complications of Diabetes Mellitus, and its symptoms such as pain and numbness can be the cause of insomnia and depression. When allowed to progress to more advanced disease stages, peripheral neuropathy can result in serious consequences such as lower limb amputation and lethal arrhythmia. In addition, recent studies have indicated that diabetes is a major risk factor for cognitive disorders such as Alzheimer's disease.

"We are trying to improve QOL for diabetics and help them to live longer lives by elucidating the pathogenesis of neurological disorders and establishing effective treatments."



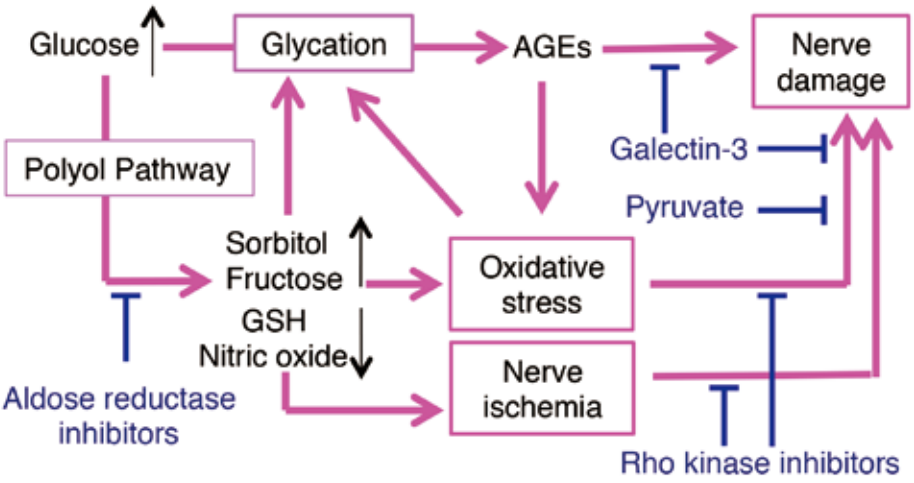
The goals of our project are as follows:

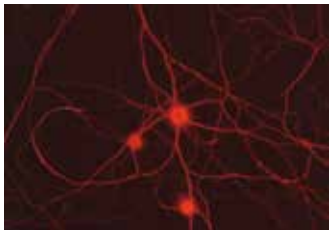
1) Establishing effective pathogenesis-based treatments for diabetic peripheral neuropathy.
2) Elucidating mechanistic links between metabolic dysfunction and neurodegenerative diseases.

Diabetic Neuropathy

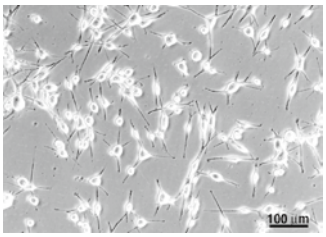
Project1: Therapeutic Approaches to Diabetic *Peripheral Neuropathy* [Sango, Yako, Niimi, Takaku, Akamine]

Metabolic disorders and vascular abnormalities caused by hyperglycemia appear to be closely related to the development and progression of diabetic peripheral neuropathy.
Using diabetic model animals and culture systems of adult rodent **dorsal root ganglion (DRG) neurons** and **immortalized Schwann cells,** we seek to establish effective pathogenesis-based treatments for peripheral neuropathy.





Adult rat DRG neurons



Immortalized mouse Schwann cells IMS32

Project2: Mechanistic link between *Metabolic dysfunction* and *Neurodegenerative* Diseases [Suzuki, Oba]

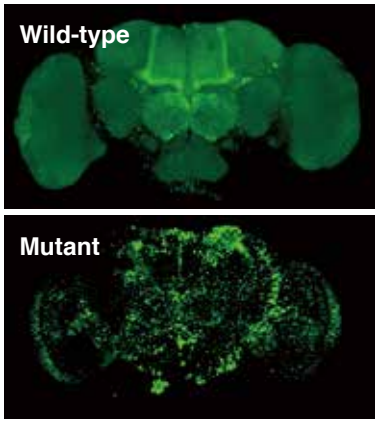
Neurodegenerative diseases are considered to share a common molecular pathogenesis involving protein misfolding and aggregation. Recently, increasing evidence suggests a relationship between metabolic syndrome and Alzheimer's disease. By using a **Drosophila model,** we aim to understand the molecular mechanism by which metabolic conditions influence misfolding protein-induced neurodegeneration.

High-nutrient diet

Nutrient-restricted diet

Drosophila models of neurodegenerative diseases

- Alzheimer's
- Parkinson's
- Polyglutamine
- ALS *etc*…



Protein aggregation (brain)

Diabetic Neuropathy

Hata S, Kitamura F, Yamaguchi M, Shitara H, Murakami M, and Sorimachi H. (2016) "A Gastrointestinal Calpain Complex, G-calpain, Is a Heterodimer of CAPN8 and CAPN9 Calpain Iso-forms, Which Play Catalytic and Reg-ulatory Roles, Respectively." ***J. Biol. Chem.,*** 291: 27313-27322.

Ono Y, Saido TC, and Sorimachi H. (2016) "Calpain research for drug discovery: challenges and potential." ***Nature Reviews: Drug Discovery,*** 15: 854-876.

Shinkai-Ouchi F, Koyama S, Ono Y, Hata S, Ojima K, Shindo M, duVerle D, Ueno M, Kitamura F, Doi N, Takigawa I, Mamitsuka H, and Sorimachi H. (2016) "Predictions of cleavability of calpain proteolysis by quantitative structure-activity relationship analysis using newly determined cleavage sites and catalytic efficiencies of an oligopeptide array." ***Mol. Cell. Proteomics,*** 15: 1262-1280.

Ojima K, Ono Y, Hata S, Noguchi S, Nishino I, and Sorimachi H. (2014) "Muscle-specific calpain-3 is phosphorylated in its unique insertion region for enrichment in a myofibril fraction." ***Genes Cells,*** 19: 830-841.

Ono Y, Shindo M, Doi N, Kitamura F, Gregorio CC, and Sorimachi H. (2014) "The N- and C-terminal autolytic fragments of CAPN3/p94/calpain-3 restore proteolytic activity by intermolecular complementation." ***Proc. Natl. Acad. Sci. USA,*** 111: E5527-5536.

Tonami K, Hata S, Ojima K, Ono Y, Kurihara Y, Amano T, Sato T, Kawamura Y, Kurihara H, and Sorimachi H. (2013) "Calpain-6 deficiency promotes skeletal muscle development and regeneration." ***PLoS Genet.,*** 9: e1003668.

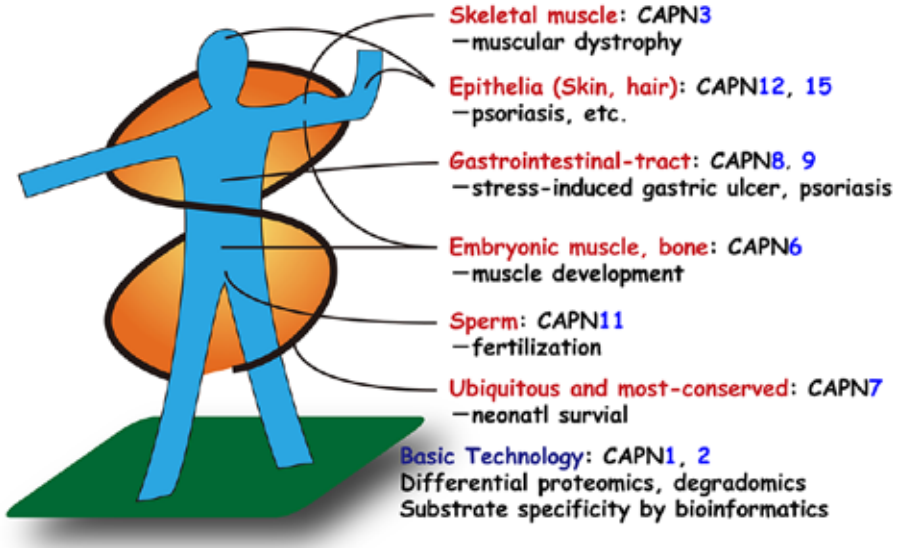
Calpain

Project Leader **Yasuko Ono** Calpain Project

Calpain: Structure-Function Relationships

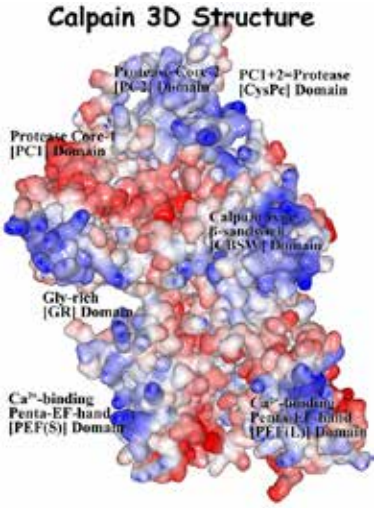
Exploring calpain-mediated biological modulation

Proteins are chains of amino acids, and their functions change when they are cut or partially cut. Calpains are enzymes that perform such "cuts" or "limited proteolytic processing" in cooperation with calcium. Humans have 15 calpain species. Defects of these species cause various deficiencies, such as muscular dystrophy, stomach ulcer, and embryonic lethality.



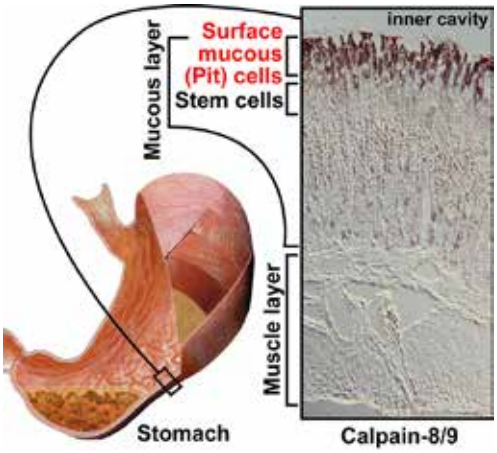
"Translational research involving calpains is still at the developmental stage. We need to learn more about the calpains themselves, as well as their impact on various physiological systems and molecular pathways." (Nat.Rev.Drug Discov.2016).

In this project, we aim to understand the biology of calpains, and translate this knowledge into improvements in health.

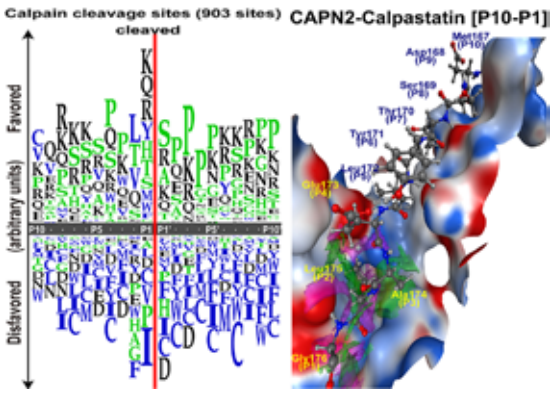


Calpains in health and disease

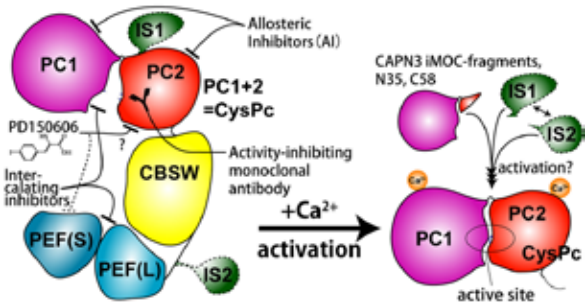
Some calpains, predominantly these expressed in specific tissue(s), are associated with genetic diseases; *e.g.*, defects in CAPN3 cause muscular dystrophy. Other calpains with more ubiquitous expression cause lethality if deficient. In addition, some calpain species express their activity through unique and unexpected mechanisms, such as intermolecular complementation (CAPN3), heterodimerization (CAPN8/9), etc.
To explore how calpains protect our health, analyses of cells/mice lacking function of specific calpain species or expected targets are being performed. We are also improving research platforms for studying calpains using proteomics, genetics, and bioinformatics.



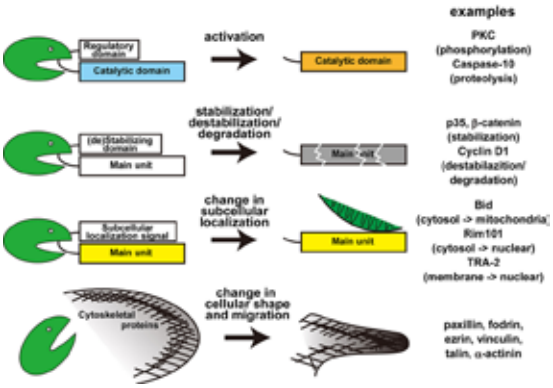
Protection of epithelial cells by heterodimeric calpain, G-calpain



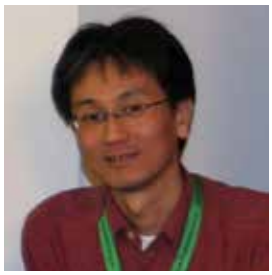
Characterization of calpain-substrate interface



Strategy for activity regulation of CAPN3



Multiplicity of calpain actions



Shoji Hata, Ph.D.
Calpains in epithelial function and tissue development

Fumiko Shinkai-Ouchi, Ph.D.
Proteomic analysis of muscular dystrophy and calpain substrate specificities

Aya Noguchi, Ph.D.
Cross talk of calpain and other proteolytic systems

Calpain

Project Leader **Noriyuki Matsuda** Ubiquitin Project

Ubiquitin-Mediated Mitochondrial Quality Control: A shield against Parkinson's disease

Ubiquitin is well-known as a signal for proteasome-dependent degradation; however, it also functions in autophagic degradation. Increasing evidence indicates that selective autophagy functions in intracellular quality control by using ubiquitin tags to delineate aggregated proteins and damaged organelles for degradation.

In 2000, Dr. Mizuno and Dr. Hattori (Juntendo Univ.), in collaboration with Dr. Suzuki and Dr. Tanaka (TMIMS) reported for the first time that Parkin, which is a causative gene product for familial Parkinson's disease (PD), is a ubiquitin-protein ligase (Nat. Genet. 2000). In addition, the identification of another gene PINK1 that is linked to familial forms of PD (Science 2004) has revealed that phosphorylation, ubiquitylation, and mitochondrial integrity are key factors in disease pathogenesis. Nevertheless, the exact mechanism underlying the functional interplay between Parkin and PINK1 remained an enigma. We are investigating how PINK1 and Parkin cooperate to keep mitochondrial integrity against mitochondrial stresses.

"We found that low-quality mitochondria are marked with ubiquitin for selective degradation, and the key factors in this process are PINK1 (a mitochondrial kinase) and Parkin (a ubiquitin ligase), two proteins implicated in Parkinson's disease."

PINK1 is a mitochondrial Ser/Thr kinase, whereas Parkin is a ubiquitin-protein ligase that catalyzes ubiquitylation of diverse mitochondrial outer membranous proteins (J. Cell Biol. 2010). We revealed that PINK1 is rapidly and constitutively degraded under steady-state conditions in a mitochondrial membrane potential-dependent manner, but that a loss in mitochondrial membrane potential stabilizes PINK1 mitochondrial accumulation (J. Cell Biol. 2010). Previously our group and others found that PINK1 acts as an upstream factor for Parkin, but how PINK1 activates latent Parkin and recruits cytoplasmic Parkin to damaged mitochondria was still obscure.

Yamano K, Wang C, Sarraf S, Münch C, Kikuchi R, Noda N, Hizukuri Y, Kanemaki M, Harper W, Tanaka K, Matsuda N, and Youle R. (2018) "Endosomal Rab cycles regulate Parkin-mediated mitophagy." ***eLife*** 7: e31326

Okatsu K, Koyano F, Kimura M, Kosako H, Saeki Y, Tanaka K, and Matsuda N. (2015) "Phosphorylated ubiquitin chain is the genuine Parkin receptor." ***J. Cell Biology*** 209:111-128

Koyano F, Okatsu K, Kosako H, Tamura Y, Go E, Kimura M, Kimura Y, Tsuchiya H, Yoshihara H, Hirokawa T, Endo T, Fon E-A, Trempe J-F, Saeki Y, Tanaka K, and Matsuda N. (2014) "Ubiquitin is phosphorylated by PINK1 to activate Parkin." ***Nature*** 510, 162-166.

Okatsu K, Oka T, Iguchi M, Imamura K, Kosako H, Tani N, Kimura M, Go E, Koyano F, Funayama M, Shiba-Fukushima K, Sato S, Shimizu H, Fukunaga Y, Taniguchi H, Komatsu M, Hattori N, Mihara K, Tanaka K, and Matsuda N. (2012) "PINK1 autophosphorylation upon membrane potential dissipation is essential for Parkin recruitment to damaged mitochondria." ***Nature Commun.*** 3 : e1016 (10 pages).

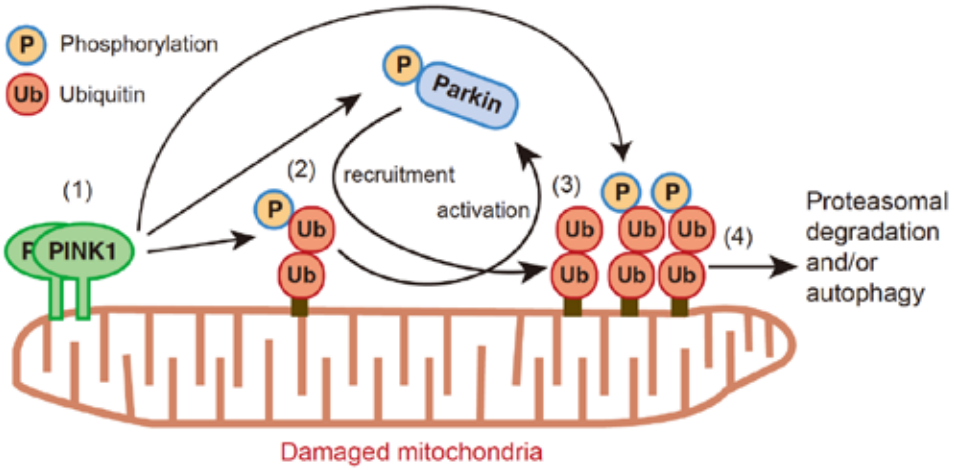
Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier C, Sou Y-S, Saiki S, Kawajiri S, Sato F, Kimura M, Komatsu M, Hattori N, and Tanaka K. (2010) "PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy." ***J. Cell Biology*** 189: 211-221.

Ubiquitin

We have found that PINK1 phosphorylates both Parkin and ubiquitin, and both types of phosphorylation contribute to activation of Parkin E3, with Ser65 phosphorylated ubiquitin acting as a Parkin activator (Nature 2014). In addition, we found that phosphorylated ubiquitin chains function as genuine Parkin receptors, recruiting it to depolarized mitochondria (J. Cell Biol. 2015). Thus ubiquitin phosphorylation allows us to comprehensively understand how PINK1 regulates Parkin to prevent Parkinson's disease.

Our study has revealed that PINK1 and Parkin cooperate in the recognition, labeling, and clearance of damaged (i.e., depolarized) mitochondria by selective mitochondrial autophagy (mitophagy). To date, ubiquitylation has been a well-known post-translational modification; however, it is becoming increasingly clear that modification of ubiquitin itself plays a critical cellular function as demonstrated by the role of S65-phosphorylated ubiquitin functions in mitochondrial quality control.

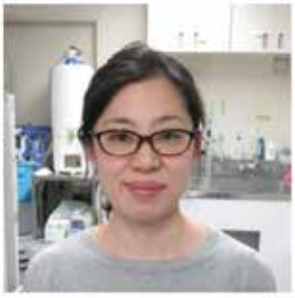
"We believe that a big mystery in mitochondrial quality control has been unraveled, and our work has established new principles of how a simple ubiquitin tag plays more varied roles than expected."



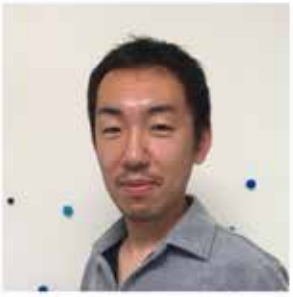
Our model for PINK1- and Parkin-catalyzed ubiquitylation for mitochondrial quality control.
We have revealed that accumulated PINK1 on damaged mitochondria (1) phosphorylates Parkin and ubiquitin, which (2) induces Parkin activation and its recruitment to the phosphorylated ubiquitin chain. Activated Parkin produces more ubiquitin chain (3), and the resultant ubiquitin is phosphorylated by PINK1 in a feed forward cycle. Parkin thus functions as an amplifier of the ubiquitin chain on depolarized mitochondria (4) for degradation.

Members

Yukiko Yoshida
Organellophagy via glycoprotein-specific ubiquitin ligase



Fumika Koyano
Molecular mechanism underlying Parkin-catalyzed ubiquitylation



Koji Yamano
Membrane dynamics upon mitochondrial quality control

Ubiquitin

Project Leader **Takahiko Hara** Stem Cell Project

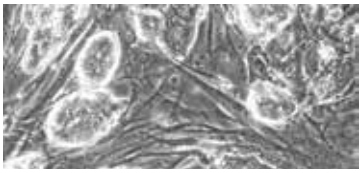
Blood regeneration from ESC/iPSC and development of novel anti-cancer drugs

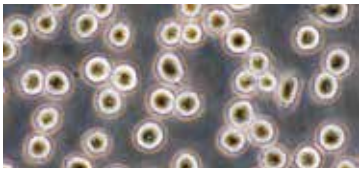
Dr. Yamanaka's inducible pluripotent stem cell (iPSC) technology has opened a new avenue to overcome incurable diseases by cell transplantation. In 2011, we discovered that overexpression of Lhx2 in hemogenic mesodermal cells resulted in *ex vivo* expansion of transplantable hematopoietic stem cells (HSCs) from mouse embryonic stem cells (ESCs) and iPSCs. Since then, we have been improving this system and applying this method to human iPSCs. We believe that comparison of the *in vitro* differentiation capacity of hematopoietic cells between mouse and human iPSCs will uncover novel and fundamental aspects of human HSC development.

"We are making efforts to derive HSCs from human iPSCs in vitro. We are also developing novel anti-leukemia drugs and chemokine-based anti-cancer drugs."

The presence of cancer stem cells has been proposed in various types of human cancer. As with tissue stem cells, cancer stem cells reside in a niche and stay dormant, thereby surviving chemotherapy and radiotherapy. Presumably, both tissue and cancer stem cells commonly express critical transcriptional regulators and signal transducers. We have already identified DDX1 and PTPN23 as essential molecules for the onset of testicular tumors.

In 2007, we discovered that CXCL14, a CXC-type chemokine, is one of the causative factors for obesity-associated diabetes. In contrast, CXCL14 is known to possess tumor-suppressive activity against lung and oral carcinomas. Recently, we discovered that CXCL14 binds to CXCR4 with high affinity, thereby inhibiting the CXCL12-mediated cell migration. This could be one of the underlying mechanisms of the CXCL14's anti-tumor function. We are vigorously investigating physiological roles of CXCL14 and its action mechanisms. CXCL14 is a promising tool for developing novel anti-cancer and anti-diabetes drugs.





Kitajima K, Kanokoda M, Nakajima M, and Hara T. (2018) "Domain-specific biological functions of the transcription factor Gata2 on hematopoietic differentiation of mouse embryonic stem cells." ***Genes Cells*** 23: 753-766.

Tanegashima K, Takahashi R, Nuriya H, Iwase R, Naruse N, Tsuji K, Shigenaga A, Otaka A, and Hara T. (2017) "CXCL14 acts as a specific carrier of CpG DNA into dendritic cells and activates Toll-like receptor 9-mediated adaptive immunity." ***EBioMed.*** 24: 247-256.

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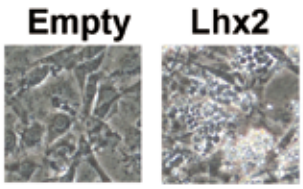
Suzuki T, Kazuki Y, Oshimura M, and Hara T. (2016) "Highly efficient transfer of chromosomes to a broad range of target cells using Chinese hamster ovary cells expressing murine leukemia virus-derived envelope proteins." ***PLoS ONE*** 11: e0157187.

Kodaka Y, Tanaka K, Kitajima K, Tanegashima K, Matsuda R, and Hara T. (2015) "LIM homeobox transcription factor Lhx2 inhibits skeletal muscle differentiation in part via transcriptional activation of Msx1 and Msx2." ***Exp. Cell Res.*** 331: 309-319.

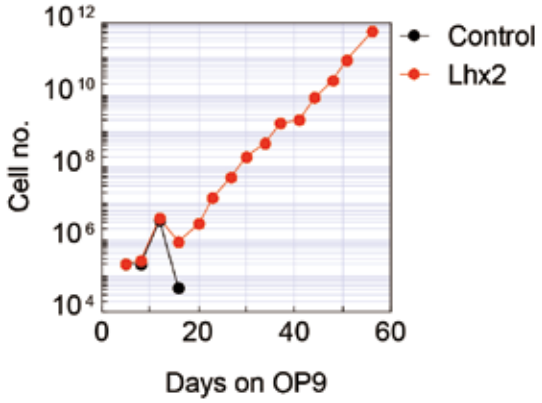
Tanaka K, Kondo K, Kitajima K, Muraoka M, Nozawa A, and Hara T. (2013) "Tumor-suppressive function of protein-tyrosine phosphatase non-receptor type 23 in testicular germ cell tumors is lost upon overexpression of miR142-3p microRNA." J. ***Biol. Chem.*** 288: 23990-23999.

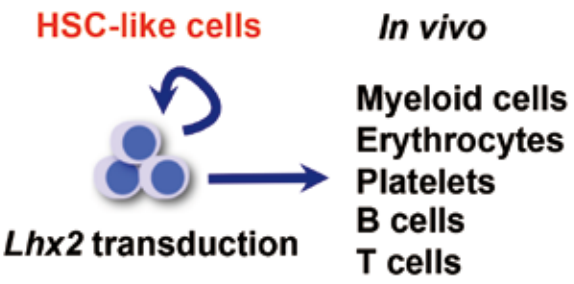
Stem Cell



Topic 1

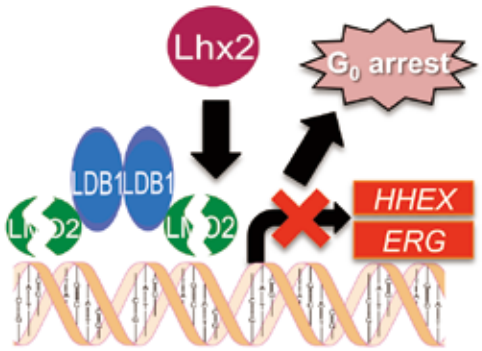
Enforced expression of *Lhx2* leads to robust *ex vivo* production of HSC-like cells from mouse ESCs/iPSCs.





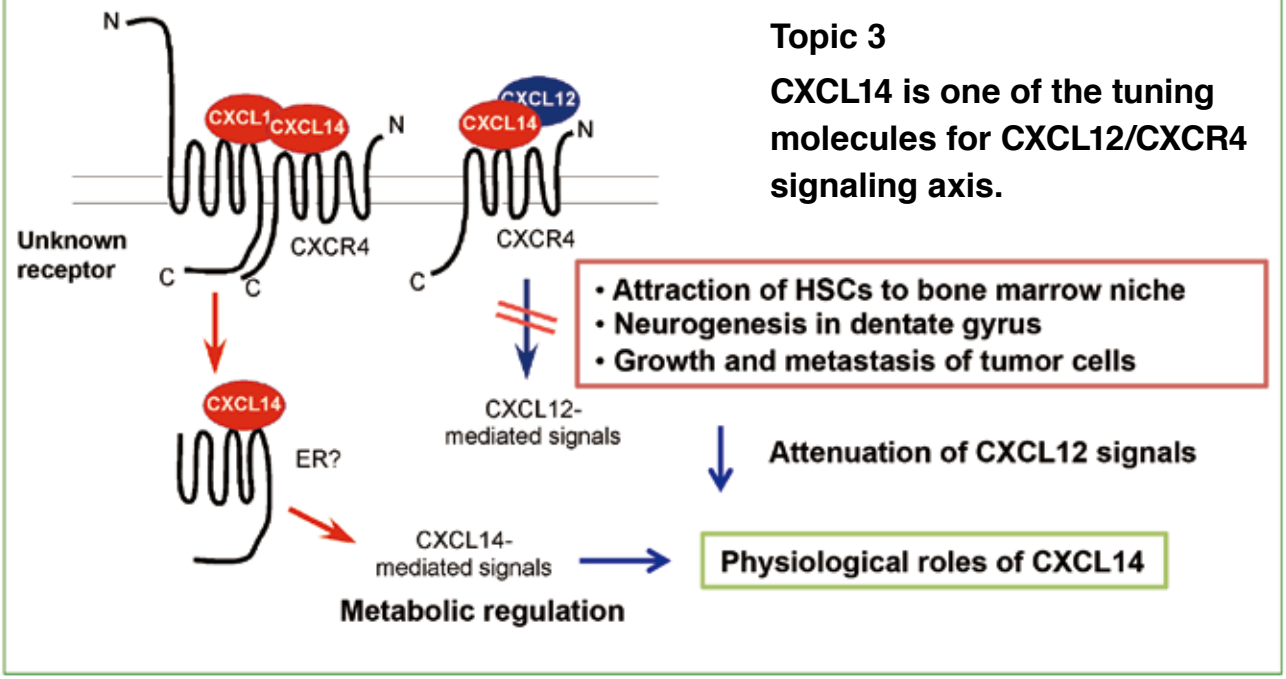
Topic 2

Lhx2 induces degradation of Lmo2 protein, thereby inhibiting proliferation of human T-cell acute lymphoblastic leukemia (T-ALL) cells.

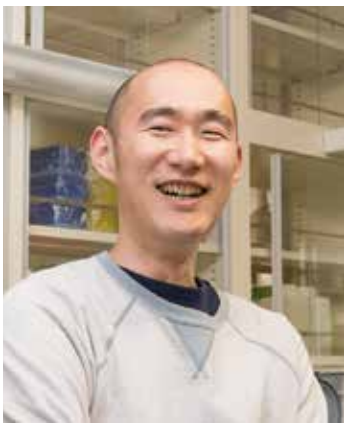


Topic 3

CXCL14 is one of the tuning molecules for CXCL12/CXCR4 signaling axis.



Stem Cell



Kato-Inui T, Takahashi G, Hsu S, and Miyaoka Y. (2018) "Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 with improved proof-reading enhances homology-directed repair." ***Nucleic Acids Res.*** 46: 4677-4688.

Miyaoka Y, Mayerl SJ, Chan AH, and Conklin BR. (2018) "Detection and Quantification of HDR and NHEJ Induced by Genome Editing at Endogenous Gene Loci Using Droplet Digital PCR." ***Methods Mol. Biol.*** 1768: 349-362.

Workman MJ, Mahe MM, Trisno S, Poling HM, Watson CL, Sundaram N, Chang CF, Schiesser J, Aubert P, Stanley EG, Elefanty AG, Miyaoka Y, Mandegar MA, Conklin BR, Neunlist M, Brugmann SA, Helmrath MA, and Wells JM. (2017) "Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system." ***Nat. Med.*** 23: 49-59.

Miyaoka Y, Chan AH, and Conklin BR. (2016) "Using Digital Polymerase Chain Reaction to Detect Single-Nucleotide Substitutions Induced by Genome Editing." ***Cold Spring Harb. Protoc.*** 2016:688-692.

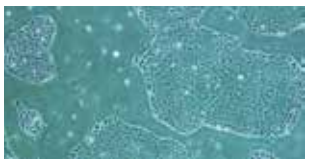
Miyaoka Y, Berman JR, Cooper SB, Mayerl SJ, Chan AH, Zhang B, Karlin-Neumann GA, and Conklin BR. (2016) "Systematic quantification of HDR and NHEJ reveals effects of locus, nuclease, and cell type on genome-editing." ***Sci. Rep.*** 6: 23549.

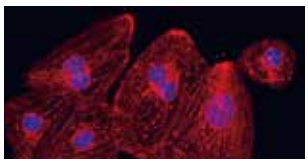
Miyaoka Y, Chan AH, Judge LM, Yoo J, Huang M, Nguyen TD, Lizarraga PP, So PL, and Conklin BR. (2014) "Isolation of single-base genome-edited human iPS cells without antibiotic selection." ***Nat. Methods*** 11: 291-293.

Project Leader **Yuichiro Miyaoka** Regenerative Medicine Project

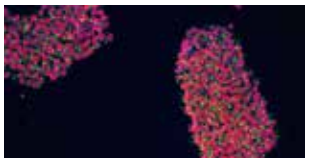
Genome Editing in Human iPS Cells: To study and cure genetic disorders

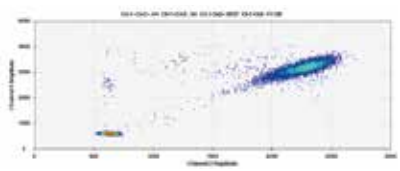
Genome editing technology allows us to rewrite the genetic information in virtually any species and any cell type including human cells. To study the pathogenesis of human diseases at the molecular level, and to develop new therapies using genome editing, we need appropriate human cellular models. Our focus is on human iPS (induced pluripotent stem) cells, a type of pluripotent stem cell that can be generated from patients' cells by introduction of specific transcription factors, and differentiated into other cell types. Our goal is to use genome editing of iPS cells to both model human diseases, and develop new therapies.





"Our goal is to develop methods to precisely and efficiently edit the genome in human iPS cells to allow us to develop both disease models using human cells, and new therapies for these diseases."



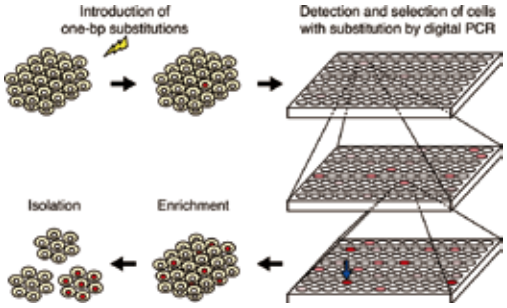


We developed an efficient method to isolate iPS cell lines containing a single nucleotide substitution. The nucleotide substitution is created by genome editing based on digital PCR, and isolation is accomplished by repeated limited dilutions in the absence of selection markers (Miyaoka, Nat. Methods 2014). Using this method, we are analyzing the pathogenesis of cardiomyopathy caused by point mutations of RBM20 (RNA-binding motif protein 20) in isogenic cardiomyocytes derived from genome-edited iPS cells. We are also improving the precision and efficiency of genome editing technology, and developing new therapies based on correcting mutations in iPS cells from patients. In addition, we are developing a strategy to directly edit the genome in cells in the human body.

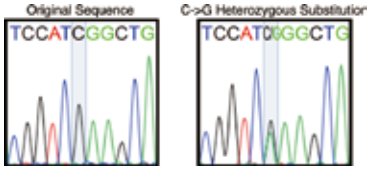
Regenerative Medicine

Changing a Single DNA Base-pair out of Thirty Billion

Single point mutations are often responsible for genetic disorders. Thus, the development of techniques to generate single point mutations is important for both modeling and curing diseases. However, thus far, it had been difficult to make specific single base-pair (bp) substitutions in the 3 billion-bp human genome. We have developed a method for isolating iPS cells with single-bp substitutions by combining genome editing, and serial limited dilutions using digital PCR.



Isolation of iPS cells with single nucleotide substitution

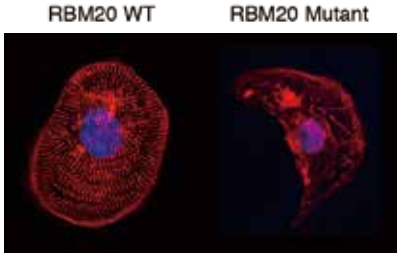


Isolated iPS cells with C→G substitution

Using this method, we can efficiently introduce single-bp substitutions at any location in the genome, allowing us to develop iPS cell-based disease models and transplantation therapies.

Heart Failure in a Dish

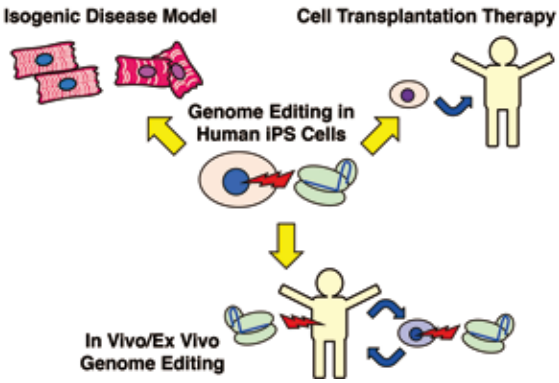
By editing the genome of iPS cells, we can study pathogenic mechanisms of genetic disorders in any cell type in a dish. For example, a point mutation in RBM20(a cardiomyopathy mutation) introduced into iPS cells caused abnormal sarcomere structures (a functional unit of muscle contraction visualized as red stripes), when these cells were differentiated into cardiomyocytes. These cells can serve as a platform for drug screening.



Sarcomere (red) and nucleus (blue) in iPS cell-derived cardiomyocytes

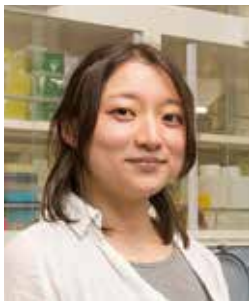
Development of Precise Ways to Edit the Genome

Current genome editing tools including CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9) have revolutionized our ability to modify the genetic information in cells. However, these tools still need to be improved for accuracy and efficiency when used in therapies. Therefore, we are developing a more precise and efficient way to edit the genome by modifying the Cas9 nuclease, and the guide RNA that directs Cas9 to the target regions. These improvements are necessary for further development of genome editing-based therapies.

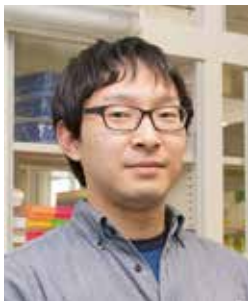


Genome editing in iPS cells to study and cure disease

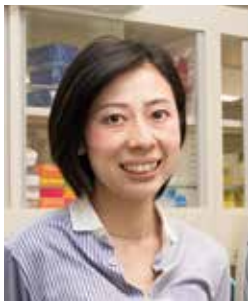
Members



Tomoko Kato-Inui



Gou Takahashi

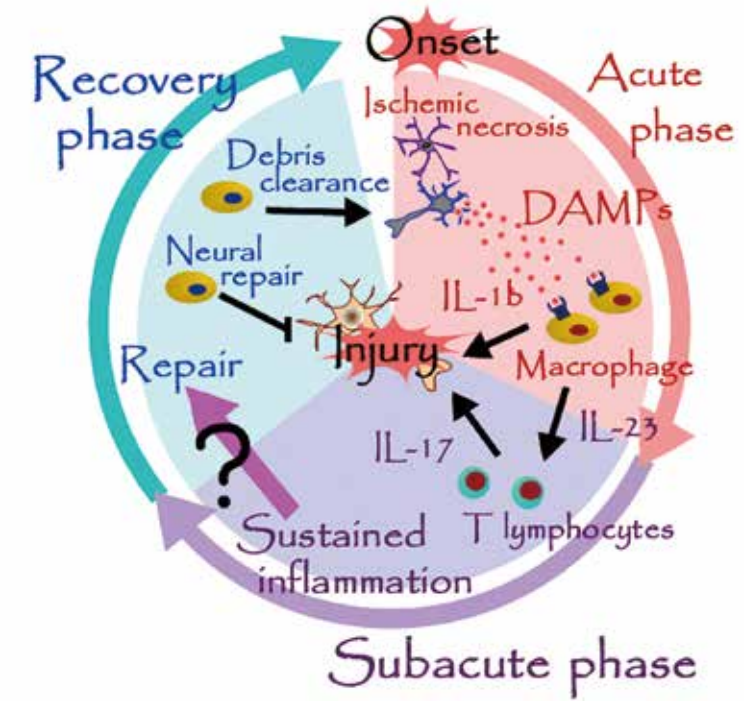


Szuyin Hsu

Regenerative Medicine

Project Leader **Takashi Shichita** Stroke Renaissance Project

Sterile Inflammation After Ischemic Stroke



"What triggers neural repair after stroke?"

We have identified peroxiredoxin family proteins as DAMPs (damage associated molecular patterns) which trigger post-ischemic inflammation (***Nat. Med.*** 2012). DAMPs induce IL-23 production from infiltrating macrophages and neutrophils, and this sustains the inflammation after ischemic stroke by promoting IL-17 production of gdT lymphocytes (***Nat. Med.***2009). Cerebral post-ischemic inflammation resolves several days after the stroke onset. The clearance of DAMPs from ischemic brain through MSR1, a scavenger receptor, plays a pivotal role in the resolution of sterile inflammation after ischemic stroke (***Nat. Med.*** 2017). Now our question is how cerebral post-ischemic inflammation switches into the process of neural repair.

Tsuyama J, Nakamura A, Ooboshi H, Yoshimura A, and Shichita T. (2018) "Pivotal role of innate myeloid cells in cerebral post-ischemic sterile inflammation." ***Semin. Immunopathol.***

Shichita T, Ito M, Morita R, Komai K, Noguchi Y, Ooboshi H, Koshida R, Takahashi S, Kodama T, and Yoshimura A. (2017) "Mafb prevents excess inflammation after ischemic stroke by accelerating clearance of danger signals through MSR1." ***Nat. Med.*** 23(6): 723-732.

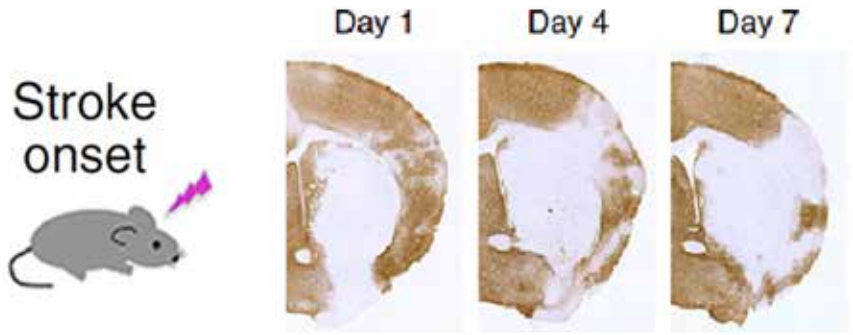
Shichita T, Hasegawa E, Kimura A, Morita R, Sakaguchi R, Takada I, Sekiya T, Ooboshi H, Kitazono T, Yanagawa T, Ishii T, Takahashi H, Mori S, Nishibori M, Kuroda K, Akira S, Miyake K, and Yoshimura A. (2012) "Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain." ***Nat. Med.*** 18(6): 911-917.

Shichita T, Sugiyama Y, Ooboshi H, Sugimori H, Nakagawa R, Takada I, Iwaki T, Okada Y, Iida M, Cua DJ, Iwakura Y, and Yoshimura A. (2009) "Pivotal role of cerebral interleukin-17-producing gammadelta T cells in the delayed phase of ischemic brain injury." ***Nat. Med.*** 15(8):946-950.

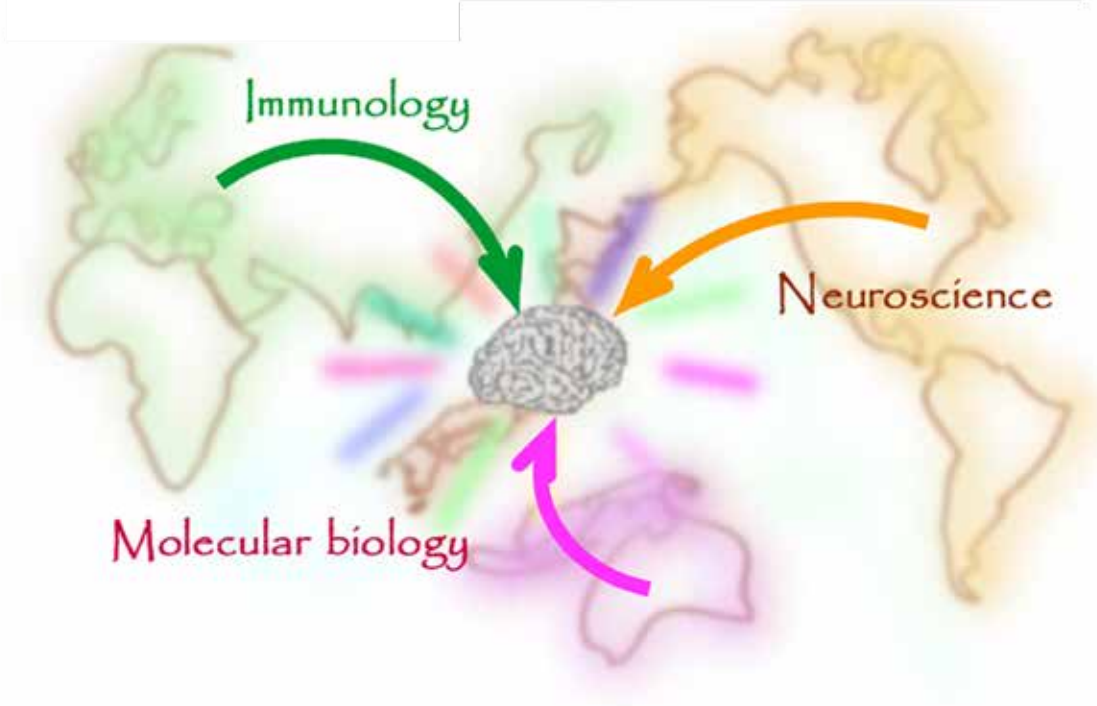
Stroke Renaissance

Stroke Renaissance Project

Stroke is a common cause of cause of severe disability and death worldwide; however, few therapeutic agents have been shown to improve the neurological deficits of stroke patients.



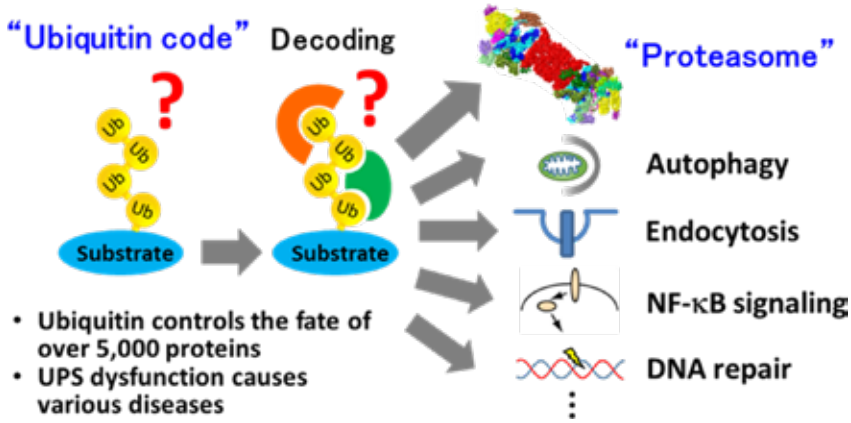
In the project, we are studying the detailed molecular mechanisms underlying the recovery of the brain after stroke. New research methods and techniques which have been recently developed in the field of immunology or neuroscience are allowing us to investigate the precise process of inflammation and regeneration in the injured brain after stroke. The purpose of our project is to develop a new therapeutic method for promoting the recovery of neurological function in patients with cerebrovascular diseases.



Stroke Renaissance

Laboratory Head **Yasushi Saeki** Protein Metabolism Laboratory

The Ubiquitin Proteasome System: Elucidation of Fundamental and Pathophysiological Mechanisms



The ubiquitin-proteasome system (UPS) plays a pivotal role in proteostasis and controls almost all cellular functions by selective protein degradation. As the maintenance of protein homeostasis is essential to human health, dysfunction of the UPS due to stresses, age-associated changes, or gene mutations causes various diseases such as cancers, inflammation, and neurodegeneration. However, we do not yet know the overall principles behind ubiquitin signaling, decoding mechanisms, and the proteasome. We aim to elucidate the fundamental mechanisms of the ubiquitin code as well as proteasome function and integrate this information into pathophysiology, to develop therapeutic strategies for UPS-related diseases.

Research Projects

1. Proteasome Dynamics and Pathophysiology

The proteasome is a highly organized proteolytic machine that degrades ubiquitylated proteins in an ATP-dependent manner. We have characterized the structure, assembly pathway, and substrate targeting mechanism of the proteasome. We found that the proteasome dynamically changes its intracellular localization and its accessory proteins under various stresses to restore proteostasis. Currently, we are generating knock-in mice to visualize proteasome localization and activity to analyze physiological changes of the proteasome during stress and aging. Furthermore, we have generated model mice with proteasomal gene mutations derived from patients with neurodevelopmental disorders. Using these mutant mice, we will elucidate the pathophysiology of proteasome mutations at the whole-body level.

Tsuchiya H, Burana D, Ohtake F, Arai N, Kaiho A, Komada M, Tanaka K, and Saeki Y. (2018) "Ub-ProT reveals global length and composition of protein ubiquitylation in cells." ***Nature Commun.*** 9, 524.

Ohtake F, Tsuchiya H, Saeki Y, and Tanaka K. (2018) "K63 ubiquitylation triggers proteasomal degradation by seeding branched chains." ***Proc. Natl. Acad. Sci. USA.*** 115, E1401-E1408.

Xie X, Matsumoto S, Endo A, Fukushima T, Kawahara H, Saeki Y, and Komada M. (2018) "Deubiquitylases USP5 and USP13 are recruited to and regulate heat-induced stress granules through deubiquitylating activities." ***J. Cell. Sci.*** 131, jcs201856

Tsuchiya H, Ohtake F, Arai N, Kaiho A, Yasuda S, Tanaka K, and Saeki Y. (2017) "In vivo ubiquitin linkage-type analysis reveals that the Cdc48-Rad23/Dsk2 axis contributes to K48-linked chain specificity of the proteasome." ***Mol. Cell*** 66, 485-502.

Ohtake F, Saeki Y, Ishido S, Kanno J, and Tanaka K. (2016) "The K48-K63 branched ubiquitin chain regulates NF-κB signaling." ***Mol. Cell*** 64, 251-266.

Yoshida Y, Saeki Y, Murakami A, Kawawaki J, Tsuchiya H, Yoshihara H, Shindo M, and Tanaka K. (2015) "A comprehensive method for detecting ubiquitinated substrates using TR-TUBE." ***Proc. Natl. Acad. Sci. USA.*** 112, 4630-4635.

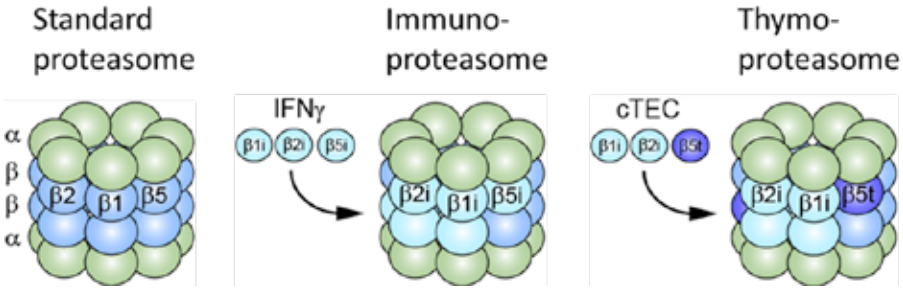
Pack Chan-Gi, Yukii H, Toh-e A, Kudo T, Tsuchiya H, Kaiho A, Sakata E, Murata S, Yokosawa H, Sako Y, Baumeister W, Tanaka K, and Saeki Y. (2014) "Quantitative live-cell imaging reveals molecular dynamics and cytoplasmic assembly of the 26S proteasome." ***Nature Commun.*** 5, 4396

Saeki Y, Toh-e A, Kudo T, Kawamura H, and Tanaka K. (2009) "Multiple proteasome-interacting proteins assist the assembly of the yeast 19S regulatory particle." ***Cell*** 137, 900-913.

Protein Metabolism

2. Roles of Specialized Proteasomes in Cell-Mediated Immunity

The proteasome has acquired diversity in the catalytic β subunits, which likely evolved during the acquisition of adaptive immunity. To date, we have discovered the vertebrate-specific alternative proteasomes, which we named the "immunoproteasome" and the “thymoproteasome”. Whereas the immunoproteasome plays a specialized role as a professional antigen-processing enzyme in cell-mediated immunity, the thymoproteasome is involved in the development of CD8+T cells in the thymus; i.e., it has a key role in the generation of the MHC class I-restricted CD8+T cell repertoire during thymic selection called “positive selection”. Currently, we are conducting a deep proteomic screen to validate the positive selection model.



Keiji Tanaka
(The chairperson of TMIMS)

Murata S, Takahama Y, Kasahara M, and Tanaka K. (2018) “The immunoproteasome and thymoproteasome: functions, evolution and human disease.” ***Nature Immunol.*** 19, 923-931.

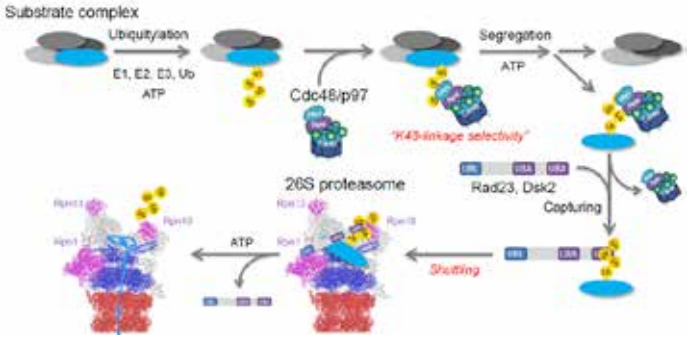
Murata S, Sasaki K, Kishimoto T, Niwa S, Hayashi H, Takahama Y, and Tanaka K. (2007) “Regulation of CD8+ T cell development by thymus-specific proteasomes.” ***Science*** 316, 1349-1353.

3. Deciphering the Ubiquitin Code

Different polyubiquitin chain linkages direct substrates to distinct pathways. This is known as the ‘ubiquitin code’. We have developed a highly sensitive MS/MS-based quantification method for ubiquitin chains. The method allows us to analyze linkage-type selectivity of ubiquitin decoder proteins at endogenous experimental settings. We recently identified the main pathway targeting K48-linked ubiquitylated substrates for proteasomal degradation. We also identified more complexed ubiquitin chains branched at K48 and K63, which act as a unique coding signal to enhance NF-κB signaling. We are further analyzing the decoder proteins throughout the ubiquitin-mediated pathways to reveal the ubiquitin network.

Fumiaki Ohtake

Hikaru Tsuchiya



Members

Keiji Tanaka	Ai Kaiho
Yasushi Saeki	Naoko Arai
Fumiaki Ohtake	Yuko Okamoto
Hikaru Tsuchiya	Arisa Kawano
Sayaka Yasuda	Marcel Diallo

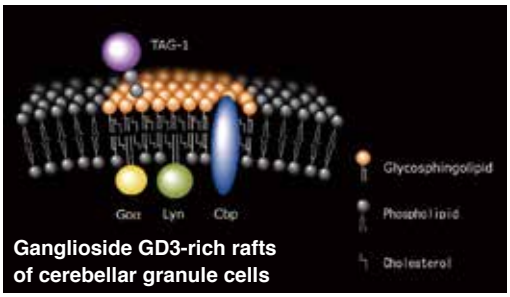
Protein Metabolism

Unit Leader **Kohji Kasahara** Biomembrane Unit

Physiological Functions of Lipid Rafts / Glycosphingolipid Microdomains in Transmembrane Signaling

Lipid rafts are dynamic assemblies of glycosphingolipids, sphingomyelin, cholesterol, and proteins that can be stabilized into microdomains involved in the regulation of a number of cellular processes. We have been investigating the function of lipid rafts by studying the interactions of glycosphingolipids in the nervous system and in blood platelets. We have found that anti-ganglioside GD3 antibodies co-precipitate the GPI-anchored neural cell adhesion molecule TAG-1, src-family kinase Lyn, its substrate Cbp, and the trimeric G protein Goα in cerebellar granule cells, suggesting that these proteins are all found in lipid rafts.

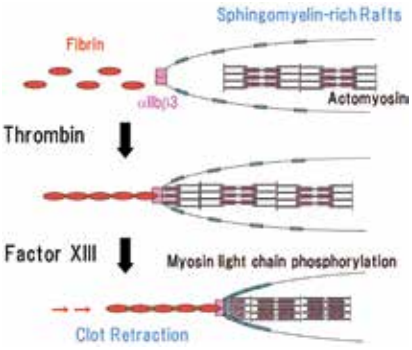
TAG-1 plays roles in axonal guidance and cellular migration, suggesting that it required for transmembrane signal transduction. However, TAG-1 is a GPI-anchored protein and GPI anchors do not directly contact the cytoplasm. We found that TAG-1 transduces signals via interactions with Lyn/Cbp in ganglioside GD3-rich rafts of cerebellar granule cells. The chemokine SDF-1α triggers the chemoattraction of cerebellar granule cells during cerebellar development. We demonstrated that SDF-1α stimulates GTPγS binding to Goα, and causes Goα translocation to lipid rafts, leading to growth cone collapse in cerebellar granule cells.



Ganglioside GD3-rich rafts of cerebellar granule cells

"We found that glycosphingolipids function as platforms in transmembrane signaling for the attachment of various signaling molecules to neurons and platelets."

Fibrin associates with lipid rafts on platelets and raft integrity is required for clot retraction. We propose that clot retraction is mediated by factor XIII-dependent fibrin-integrin αIIbβ3-myosin axis in sphingomyelin-rich membrane rafts.



Sphingomyelin-rich rafts of platelets

Members: Ikuo Kawashima, Kiyoshi Ogura, Tetsuya Hirabayashi
Keisuke Komatsuya, Kei Kaneko

Kasahara K, Kaneda M, Miki T, Iida K, Sekino-Suzuki N, Kawashima I, Suzuki H, Shimonaka M, Arai M, Ohno-Iwashita Y, Kojima S, Abe M, Kobayashi T, Okazaki T, Souri M, Ichinose A, and Yamamoto N. (2013) "Clot retraction is mediated by factor XIII-dependent fibrin-αIIbβ3-myosin axis in platelet sphingomyelin-rich membrane rafts." ***Blood*** 122, 3340-3348.

Sekino-Suzuki N, Yuyama K, Miki T, Kaneda M, Suzuki H, Yamamoto N, Yamamoto T, Oneyama C, Okada M, and Kasahara K. (2013) "Involvement of gangliosides in the process of Cbp/PAG phosphoryla-tion by Lyn in developing cerebellar growth cones." ***J.Neurochem.*** 124, 514-522.

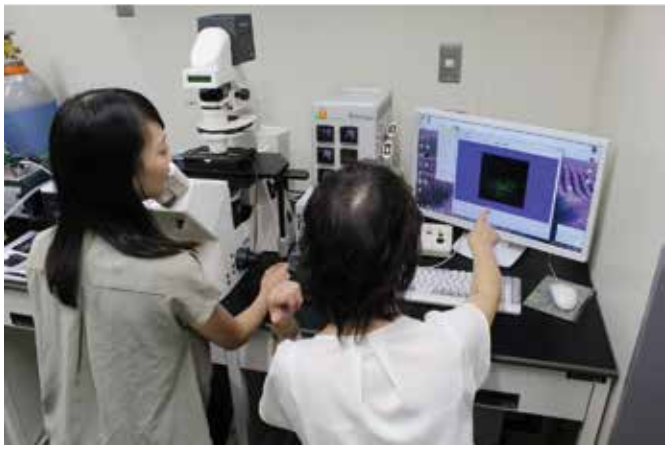
Kasahara K, Souri M, Kaneda M, Miki T, Yamamoto N, and Ichinose A. (2010) "Impaired clot retraction in factor XIII A subunit-deficient mice." ***Blood*** 115, 1277-1279.

Yuyama K, Sekino-Suzuki N, Sanai Y, and Kasahara K. (2007) "Translocation of activated heterotrimeric G protein Gαo to ganglioside-enriched detergent-resistant membrane rafts in developing cerebellum." ***J.Biol. Chem.*** 282, 26392-26400.

Kasahara K, Watanabe K, Takeuchi K, Kaneko H, Oohira A, Yamamoto T, and Sanai Y. (2000) "Involvement of gangliosides in GPI-anchored neuronal cell adhesion molecule TAG-1 signaling in lipid rafts." ***J.Biol. Chem.*** 275, 34701-34709.

Kasahara K, Watanabe Y, Yamamoto T, and Sanai Y. (1997) "Association of src family tyrosine kinase Lyn with ganglioside GD3 in rat brain. Possible regulation of Lyn by glycosphingolipid in caveolae-like domains." ***J.Biol. Chem.*** 272, 29947-29953.

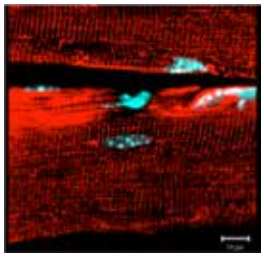
Biomembrane



Research Support

Center for Basic Technology Research

General Manager
Minoru Saitoe



The Basic Technology Research Center provides multiple resources to assist scientists in the institute in conducting research with maximum efficiency. The services provided include state-of-the art technologies required for biomedical and life science research, and maintenance of various facilities which are routinely used by the researchers. Details of the services provided and specific activities of related facilities are given below.

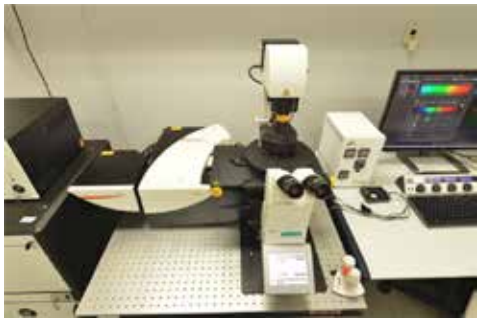
1. The Animal Research Division maintains the animal facility that is used by scientists who use animals for their research. This facility assists researchers in generating knock-out or transgenic animals and maintains sperm and eggs of various mutant animal. It also provides maximal care for the welfare of animals.

2. The Advanced Technical Support Department consists of facilities for Protein Analyses, FACS, Microarrays, Confocal Microscopy, Electron Microscopy, Histology, etc. and offers state-of-the-art technology to researchers.

3. The Information Support Department consists of the Library, Information Technology Section, Media Technology Laboratory and Public Relation Office. It assists researchers search for references, provides support for daily use of computer systems, and deals with the media.

4. The Authorized and General Core Facility Department consists of the Radioisotope Laboratory, Hazardous Chemical Control Room and General Common Facility, and provides the researchers with various special and common facilities. It also maintains safety for accident-free daily operation of the institute.

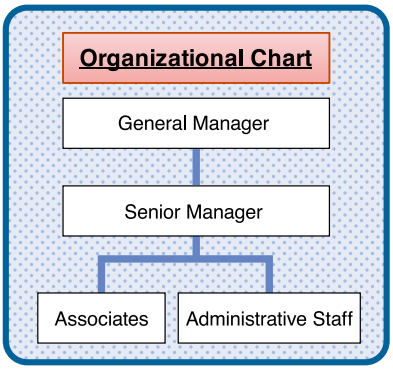
- Center for Basic Technology Research
  - Animal Research Division
    - Animal Research Control Laboratory
    - Laboratory for Transgenic Technology
  - Technology Research Division
    - Advanced Technical Support Department
      - Protein Analyses, FACS, Microarray, Confocal Microscopy, Electron Microscopy, and Histology Laboratories.
    - Scientific Advisory Office
    - Information Support Department
      - Library, Information Technology Section, Media Technology Laboratory, Graphic Design Unit.
    - Authorized and General Core Facility Department
      - Radioisotope Laboratory, Hazardous Chemical Control Room, General Common Facility.



Technology Licencing Office (TLO)

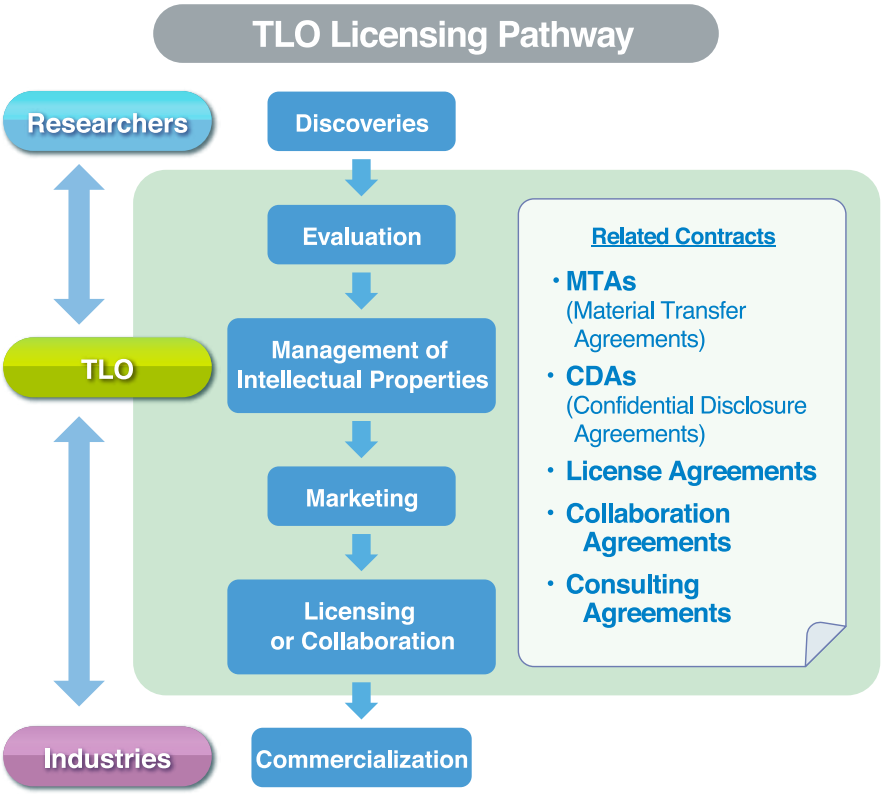
General Manager
Futoshi Shibasaki, MD, PhD

Senior Manager
Kazumasa Aoki, PhD



Who we are

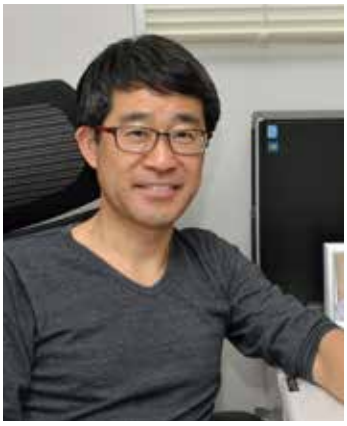
- The Technology Licensing Office (TLO) facilitates the conversion of scientific discoveries to innovative technologies with the ultimate goal of improving public health and welfare.
- We evaluate basic research findings (seeds) as intellectual property assets, and license promising candidates to industries for development as medicines, diagnostics, medical devices, foods, cosmetics and research tools.



What we do

- We manage intellectual properties from our institute including patents, copyrights and materials in order to develop them for commercialization.
- To promote technology transfer, we introduce seeds and intellectual properties with potential commercial value to pharmaceutical, medical device, and startup companies.
- We attend business meetings such as the BIO international convention in the US, BIO-EUROPE, and BioJapan, to develop Public Private Partnership opportunities between our institute and industries.
- We support collaborative research projects with industries by arranging Joint Research Agreements, Material Transfer Agreements (MTA), and other contracts to protect and develop a wide range of research discoveries.

Center for Medical Research Cooperation

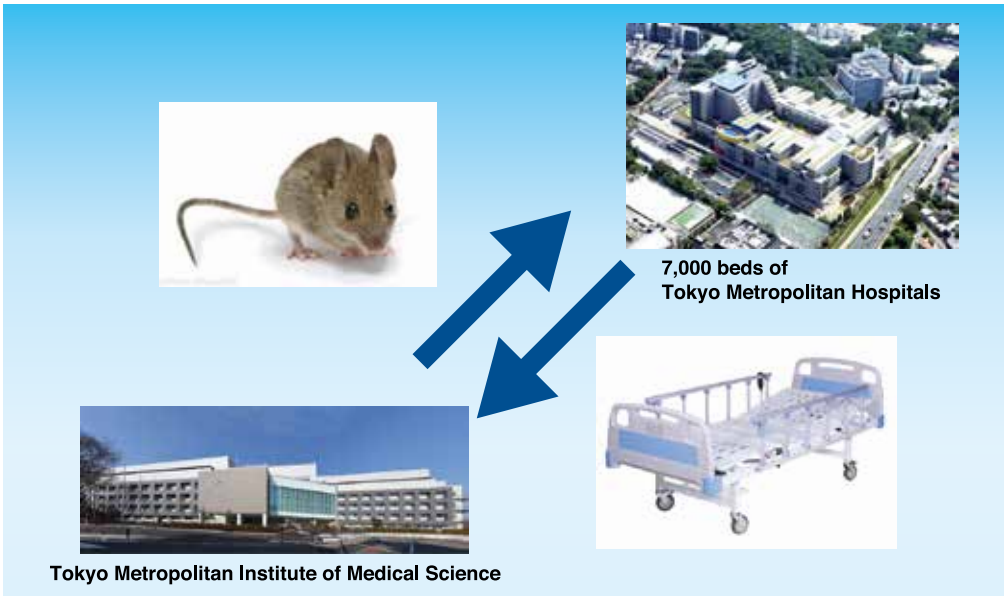


Head of Office
Masanari Itokawa MD, PhD

Making the Dreams of Young Scientists Come True

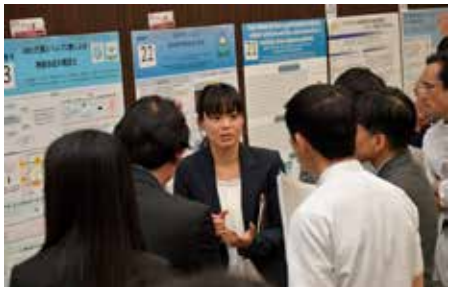
– from bench to bed and back again –

We provide---
-Consultation on statistical analyses
-Advice on ethical issues related to human specimens
-A bridge between basic scientists and medical doctors
-We promote collaborations between the institute and hospitals. We have a supporting budget of 500,000 yen for collaborative clinical studies with medical doctors at Tokyo Metropolitan Hospitals.



Conference with researchers and medical doctors

We provide tools for developing medical technology from simple findings at the bench; the process similar to making a brilliantly sparkling jewelry by cutting a piece of crude stone. We provide consultation on statistical analyses of raw data. We manage ethical issues related to human specimens. We facilitate the communication between the basic scientists and medical doctors.



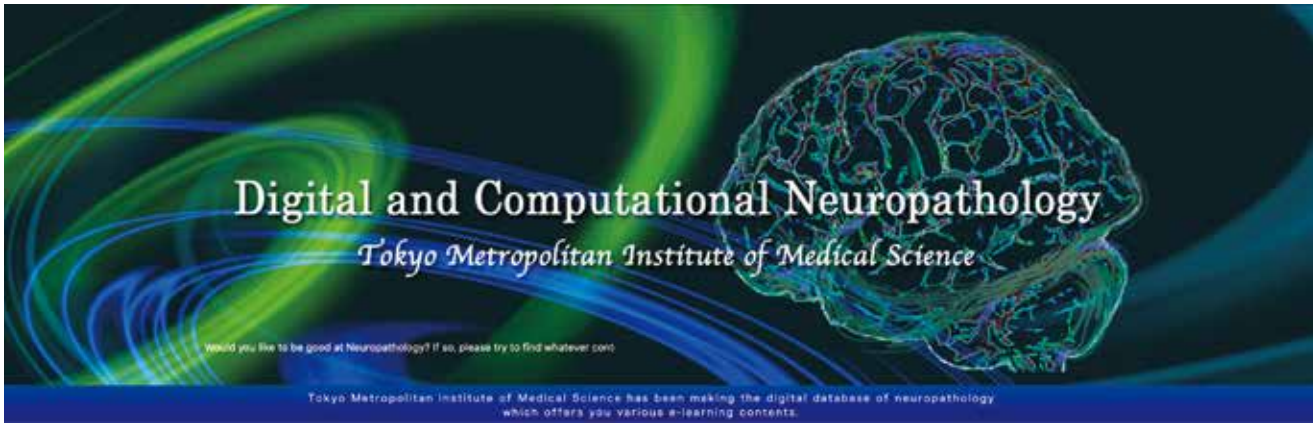
A young scientist discussing with medical doctors in a conference

Most discoveries in scientific research are unexpectedly made by scientists who still have very “flexible” scientific minds, a particular asset of young people.

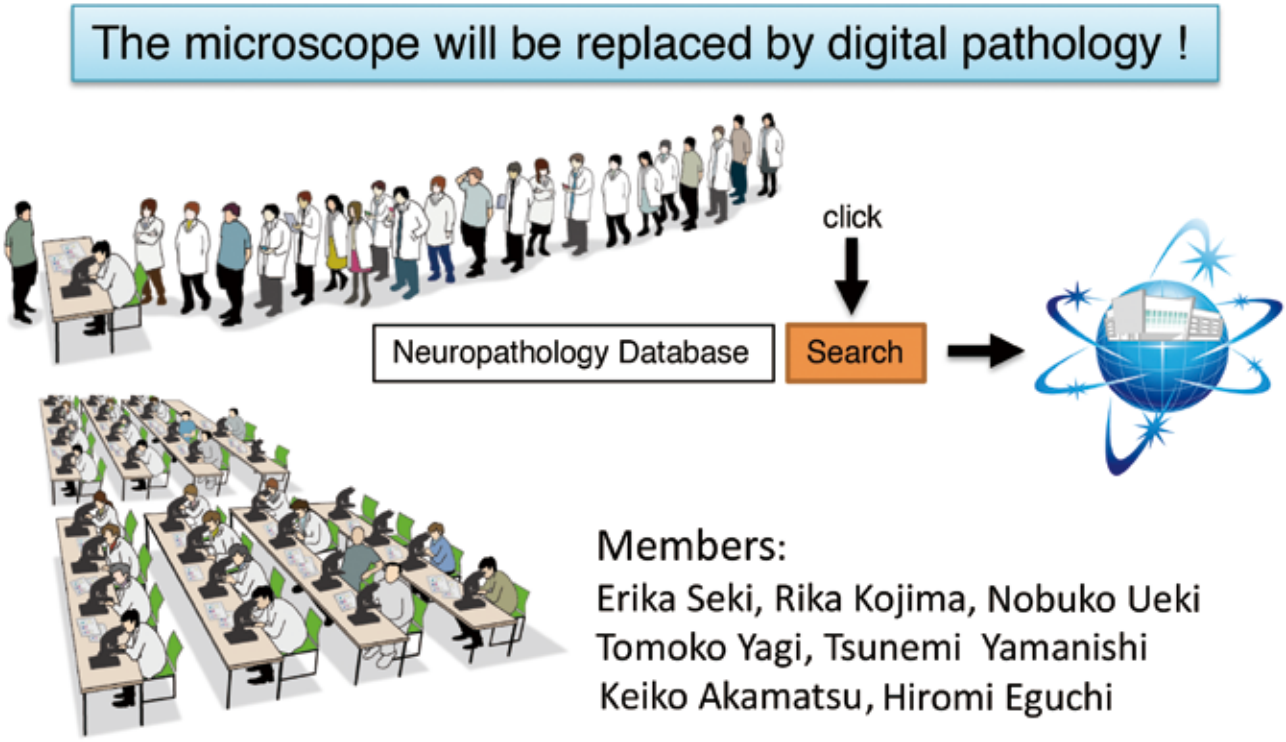
Laboratory of Neuropathology

Laboratory Head
Nobutaka Arai

Translational Research using human materials and Management of Database for Essential Brain Anatomy & Neuropathology



The Laboratory of Neuropathology has more than 5,000 sets of human autopsied brain slides with a wide variety of human neurological diseases. In recent years, we have been scanning these slides with virtual slide instruments. Using this digital data and its derivatives, we are constructing a digital neuropathology library.



Members:
Erika Seki, Rika Kojima, Nobuko Ueki
Tomoko Yagi, Tsunemi Yamanishi
Keiko Akamatsu, Hiromi Eguchi

Neuropathology

TMIMS Programs

Seminars/ lectures and related activities

Scientific seminars:
We have scientific seminars by renowned scientists from Japan and overseas almost every week.

International Symposium:
We have two annual international symposia attended by top tier scientists from all over the world.

Science forum with industry:
We have annual forums hosted by our TLO office where scientists at our institute introduce our discoveries and discuss potential applications with scientists from industry.

Lectures to high school students:
We give lectures to visiting high-school students and visit high schools to present lectures as a part of our effort to educate the younger generation and introduce them into research.

Joint graduate school programme:
Many scientists at our institute have joint appointments as lecturers or visiting professors at various universities. Many undergraduate and graduate students are conducting their masters/Ph.D. research at our institute under the guidance of our scientists.

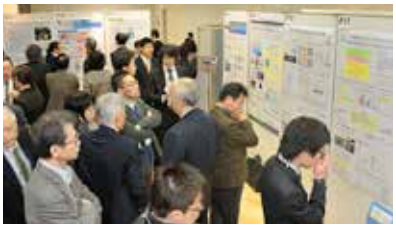
Open laboratory to students:
Once a year we invite students to our institute, where we present our research and give students opportunities to experience the laboratory.

Summer training courses:
We have annual summer training courses to provide in-house training on various experimental techniques.

Lectures to public:
We conduct public lectures (8 times a year) to let the public know our scientific progress and the various activities available at our institute.

Science Café:
We have Café-style meetings (3 times a year) where we give the public the opportunity to learn, discuss and experience science, and let them know the joy of science in a very casual setting.







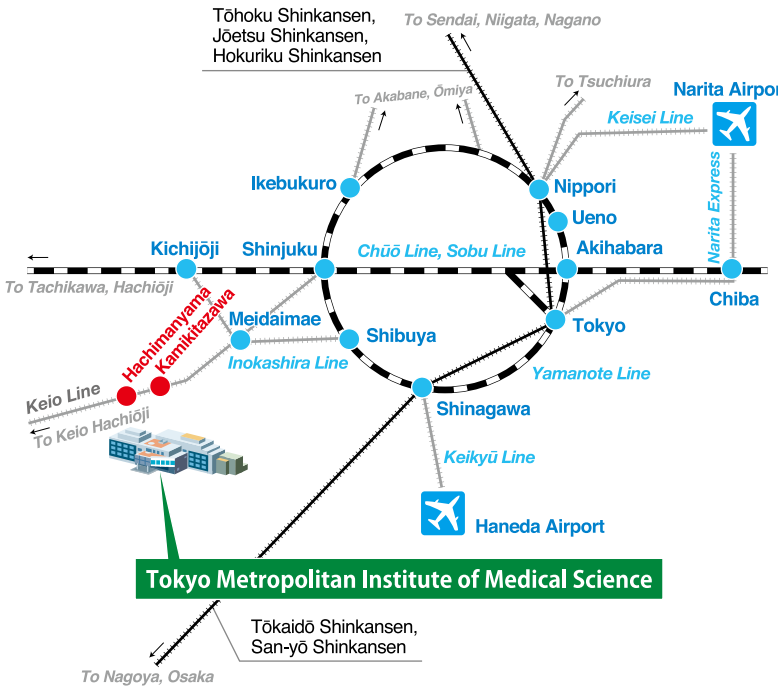

Support for young scientists

Research Associate Fellowship:
We provide graduate students who conduct their masters/Ph.D. thesis research at our institute with research associate fellowships for financial support.

Travel support for young scientists attending international meetings:
We provide students and young scientists at our institute with travel fellowships with which they can attend international meetings where they can present their latest findings.

Access Map

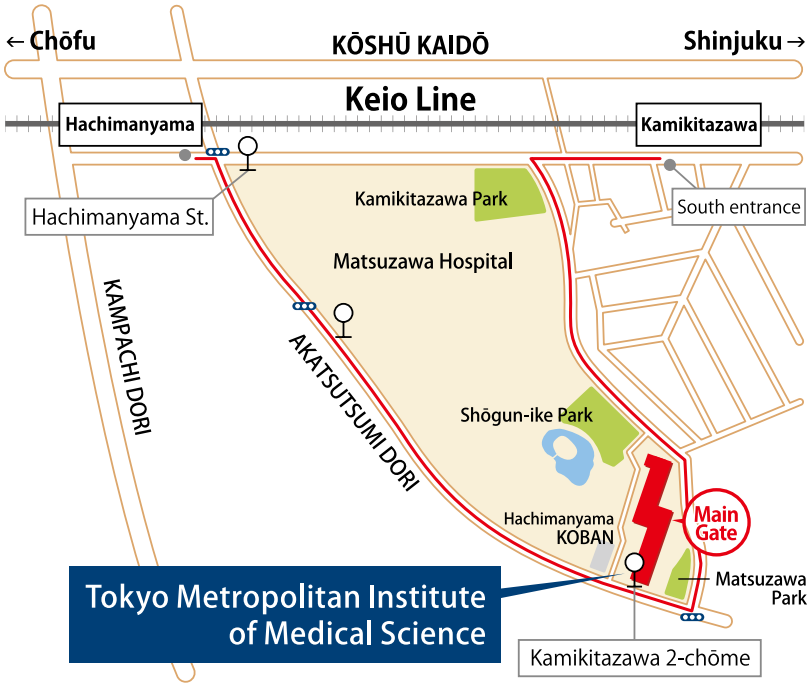
Tokyo Metropolitan Institute of Medical Science	
Address	2-1-6 Kamikitazawa, Setagaya-ku, Tokyo, 156-8506, Japan
Tel	+81-3-5316-3100
Fax	+81-3-5316-3150



AIRPORT to INSTITUTE

From Narita Airport to Kamikitazawa Station / Hachimanyama Station	
Narita Airport - Shinjuku Station	JR Narita Express
Shinjuku Station - Kamikitazawa Station / Hachimanyama Station	Keio Line

From Haneda Airport to Kamikitazawa Station / Hachimanyama Station	
Haneda Airport - Shinagawa Station	Keikyū Line
Shinagawa Station - Shinjuku Station	JR Yamanote Line
Shinjuku Station - Kamikitazawa Station / Hachimanyama Station	Keio Line



- **From Kamikitazawa Station to Institute**
  Walk (approx. 10 min From South entrance of Station).

- **From Hachimanyama Station to Institute**

Hachimanyama Station - Kamikitazawa 2-chōme	Keio bus / Odakyū bus
Kamikitazawa 2-chōme - Institute	Walk

http://www.igakuken.or.jp/english/

As of December 1, 2019