We recently discovered novel and crucial roles of non-standard DNA structures in regulation 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 the genome by elucidating the fundamental and universal functions of G-quadruplex and other non-B type DNA structures in regulation of various genome functions. Through these efforts, 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 but somehow have been disregarded in the past.
Cdc7 kinase as a modulator of chromosome transactions

- Mcm: Replication initiation
- Claapin/Mrc1: Replication Checkpoint
- Rad18: Trans-lesion DNA synthesis/repair
- Mer2: Meiotic recombination
- Rec8: Ls/r4: Meiotic cell division
- HP1: Heterochromatin formation
- H3 T45: Histone modification
- Cstf1: Chromatin reconstruction
- Mrc1, Ame2, Eso1: Protein degradation
- Top2A: Centromere regulation
- TDP43: Protein aggregation
- Mus14-Mus81: Homologous recombination

Rif1 and G4 as organizers of chromatin architecture

With Rif1:
- Chromatin loops
- Late-replicating domain
- G4, G4
- Nuclear matrix

Without Rif1:
- Dynamic movement
- Loosened chromatin
- More interaction with other domains
- Loss of suppression
- Early-replication

Diverse biological functions of G-quadruplex

Table:
- Transcription
- DNA replication
- Recombination
- Chromatin architecture

Cellular responses to replication stress
- Unusual DNA structures (G-quadruplex etc.)
- DNA replication and development
- Novel drugs and therapies for cancer

Genome Dynamics
“We are trying to identify genes associated with human diseases using mutant mice and are aiming to develop new mouse models for human disease.”
Main project: Genetics of deafness

Hearing loss is the most common sensory disease in humans, which 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.

Members

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Mammalian Genetics
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 pathogenesis by these virus infections. To overcome the problems, we have been developing various animal models including transgenic mice, humanized mice with human liver cells, monkeys and tree shews. We also investigate the precise mechanisms by which host factors regulate viral growth.

“We are studying to clarify 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 infecting 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.
**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 virus (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 led to increased levels of matrix metalloproteinase (MMP)-8 mRNA in the liver, along with elevated levels of intrahepatic neutrophils and macrophages/monocytes. These results suggest that inhibition of hepatic stellate cells activation and induction of fibrolytic cells expressing MMP-8 contribute to the anti-fibrotic effects of PRI-724.

![Figure. PRI-724 ameliorates hepatitis C virus-induced liver fibrosis.](image1)

**Transmission of HBV DNA Mediated by Ceramide-Triggered Extracellular Vesicles**

Extracellular vesicle is a nanovesicle that shuttles proteins, nucleic acids, and lipids, thereby influencing cell behavior. We showed that ceramide-triggered extracellular vesicles work as DNA cargo for hepatitis B virus-DNA and are capable of transmitting to naive hepatocytes. Further, we demonstrated that the transmission of hepatitis B virus-DNA via these extracellular vesicles is resistant to antibody neutralization.

![Diagram](image2)

**Members**

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Enterovirus 71 (EV71) is a human enterovirus species A of the genus *Enterovirus* within the *Picornaviridae* family, and it is known to be one of the causative agents of hand-foot-and-mouth disease (HFMD). HFMD is considered to be a mild and self-limiting disease in general. 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.

“...The development of vaccine and anti-viral drugs and that of experimental models for the evaluation of these agents are important for controlling emerging and re-emerging viral infections. We will study the basic principles of neurotropic enterovirus infection and provide knowledge and technologies to control infectious diseases.”

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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

The transgenic mouse expressing human SCARB2 is susceptible to EV71. It is a useful model for the study of EV71 pathogenesis and vaccine efficacy test.
Japanese cedar pollen allergy is the major allergic disease in Japan, and the approximately 35% of Japanese people are affected. In recent years, sublingual immunotherapy has been recognized as an effective curative treatment for the allergic diseases. However, the molecular mechanisms of mucosal tolerance still remain unclear. In our laboratory, we focus on the following subjects.

1. Search for effective biomarkers of SLIT
   - CNVs
   - SNPs
   - Epigenome
   - Proteome, etc.

2. Elucidation of molecular mechanisms to induce immunological tolerance by SLIT
   - iTregs
   - Apoptosis
   - CTLA-4
   - TGF-β
   - IL-10 etc.

“We are developing new diagnostics and treatments for allergies.”


Current Topics of Another 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 will call for reconsideration of Treg/CTLA4-based immunological modulation to suppress or treat inflammatory diseases.

(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.

Recent discoveries of biomarkers and novel technologies have opened the new aspects of the mechanisms and drug developments especially in cancer and infectious diseases. In basic research, we have been focusing on the mechanisms of cancer angiogenesis and the drug development using siRNA, and on malignant transformation and metastasis caused by cell fusion. In addition, the novel mechanisms for H5 influenza virus entrance into cell surface would be a drug target.


In clinical and translational research, we focus on the establishment of platform to perform “Precision Medicine” by Whole genome analysis with next generation sequence in collaboration with Metropolitan Hospitals. For Private Public Partnership (3P), we have already established the Bio-Consortium “Tokyo Biomarker Innovation Research Association” (TOBIRA).

Our specific aims are to perform the basic science and be to develop the new findings to the translational research.
Malignant cancer progression after cell fusion with stem cells

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

Drug development of Int6-siRNA

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.

Diagnostics and device development through Private Public Patient Partnership

Development of drugs for highly pathogenic H5N1 influenza viruses

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.