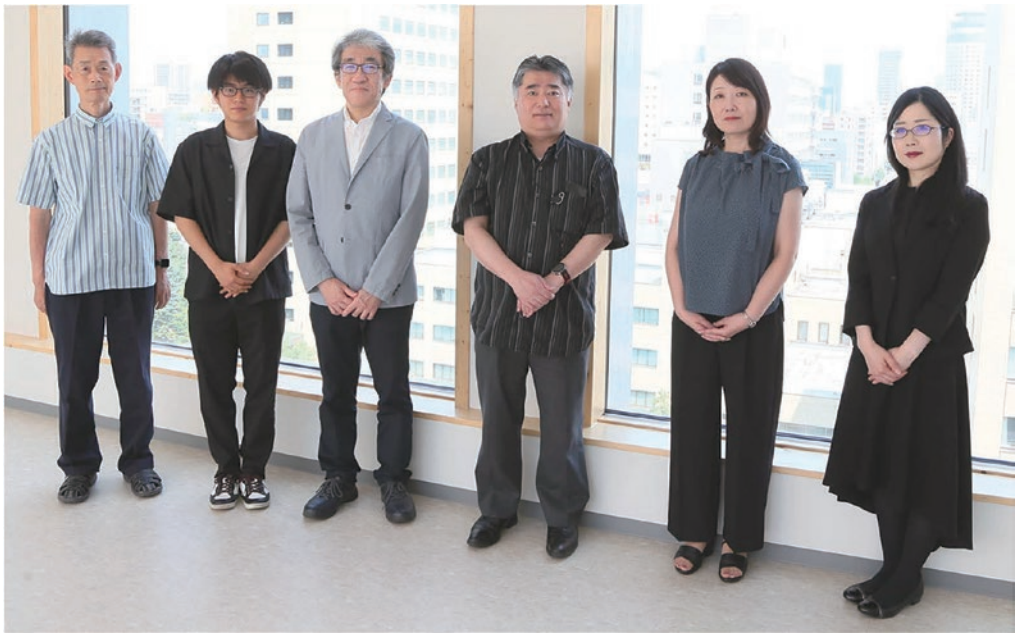
Biology

The Biology Division has been focusing on a variety of themes as follows: characterization and identification of novel molecular targets for the diagnosis and treatment of cancer, transcriptional regulation of cancer-related genes, molecular and cellular biology of immune synapse, live-cell imaging analysis of signaling molecules, and methodology for molecular biological microscopy. We also offer a Molecular and Cellular Biosciences course for graduate students.



Division members:
Yukiharu Sawada, Ph.D. (Visiting researcher), **Yojiro Shimada** (M.D., Ph.D. student), **Yasushi Sasaki**, M.D., Ph.D. (Professor), **Takeshi Suzuki**, M.S., Ph.D. (Associate Professor), **Miwa Suzuki** (Secretary), **Asami Matsuda**, M.S. (Research student)

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(Third from the left)
Interests:
Molecular mechanisms of human carcinogenesis, functional analysis of p53 family

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Interests:
Cell biology of signaling molecules, molecular and cellular immunology

1. Molecular genetics of human cancer

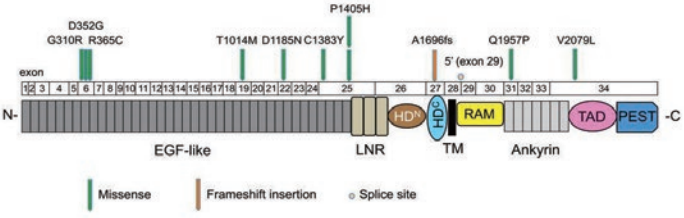
To identify novel molecular targets for the diagnosis and treatment of human cancer, we have been analyzing genetic characterization in human oral, head and neck, esophageal, gastric, colorectal, non-ampullary duodenal, pancreatic, hepatic and cervical cancers, primary central nervous system lymphoma (PCNSL) and multiple myelomas as well as normal human tissues using next-generation sequencing (NGS) technologies. We have also designed several tumor- and tissue-type-specific panels of genes that are frequently altered in human cancers, including esophageal squamous cell, colorectal, pancreatic, and cervical cancers.

Through NGS data, we have identified several candidate driver genes as novel molecular targets for therapeutic drugs.

a) The most frequent mutations in Japanese oral squamous cell carcinoma (OSCC) tissues were in *TP53*, *NOTCH1*, *CDKN2A*, *SYNE1*, and *PIK3CA*. Pathway assessment showed that the somatic aberrations within OSCC genomes are mainly involved in several important pathways, including cell cycle regulation and RTK–MAPK-PI3K. In addition, mutations in *NOTCH1* and *PIK3CA* were found to be associated with worse overall survival in OSCC patients.

b) The most frequent mutations in PCNSL tissues were in *PIM1, MYD88, CD79B, DST, IRF4, ERBB3, MYH11, DCC,* and *KMT2D*. Furthermore, somatic mutations of *MYH11* were related to poor prognosis in PCNSL patients.

Mutation distribution in the exons and functional domains of NOTCH1 (oral squamous cell carcinoma, OSCC)



c) Early circulating tumor DNA (ctDNA) changes before and after an initial cycle of chemotherapy predict later responses

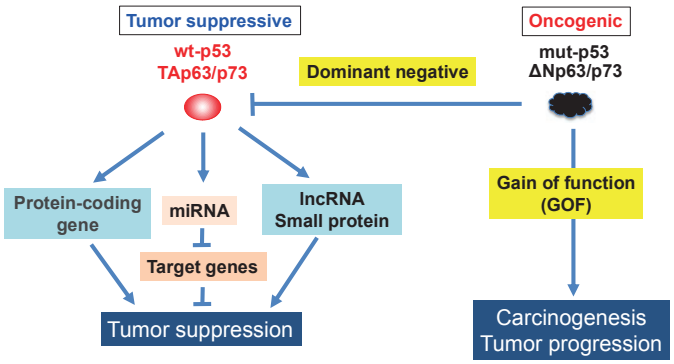
at the end of chemotherapy with high accuracy in esophageal squamous cell carcinoma (ESCC) patients.
d) Mutations in genes associated with Wnt signaling play a greater role in the colorectal carcinogenesis of traditional serrated adenomas (TSAs) than sessile serrated adenomas (SSAs).
e) *FGFR* mutations are associated with worse progression-free survival in uterine cervical cancer patients treated with definitive radiotherapy.
f) We also published reports of interesting rare cases with genetic analysis, including malignant paraganglioma of posterior mediastinum, familial cases with urothelial carcinoma, renal angiomyolipoma, mosaic neurofibromatosis type 1, splenic marginal zone lymphoma, intrahepatic mucinous cholangiocarcinoma, and ESCC with epidermalization.

This targeted NGS had significant advantages over classical molecular methods used to perform high-throughput sequencing in clinical laboratories.

2. Functional analysis of p53 family genes

Genome sequencing studies of cancer have revealed the genomic landscapes of human cancer and shown that the *p53* tumor suppressor gene is most frequently mutated in cancers among human genes. The p53 family is composed of a group of transcription factors, p53, p73, and p63. The p53 family protein is activated by DNA damage or other cellular stresses and the activated p53 exerts its tumor suppression function mainly through the transactivation of a large number of downstream target genes. We recently isolated several p53 family target genes, including *breast cancer metastasis suppressor 1-like (BRMS1L), LIMA1/EPLIN, Armadillo Repeat gene deleted in Velo-Cardio-Facial syndrome (ARVCF)*, and lncRNA *NEAT1*.

Role of p53 family in cancer



3. Cell biological analysis of fenestra formation in the fenestrated endothelial cells

Endothelial fenestrae are transcellular pores divided by diaphragms formed by plasmalemma vesicle-associated proteins (PLVAP), and they function as channels for peptide hormones and other substances. Caveola, a key regulator of clathrin-independent endocytosis, may be involved in the invagination and fusion of plasma membranes, which are essential for fenestra formation. As we observed caveolae in fenestrated endothelial cells in the anterior lobe of the rat pituitary by transmission electron microscopy, we studied the relationship between the caveolae-mediated endocytosis pathway and fenestrae formation in cultured endothelial cells isolated from the anterior lobe of the rat pituitary (CECAL) using immunofluorescence and scanning electron microscopy. The inhibition of caveolae-mediated endocytosis by genistein enlarged the PLVAP-positive oval-shaped structure that represented the sieve plate and induced the formation of a doughnut-shaped bulge around the fenestra in CECAL. In contrast, the acceleration of caveolae-mediated endocytosis by okadaic acid induced the diffusion of PLVAP-positive signals in the cytoplasm and reduced the number of fenestrae in CECAL. As we found that caveolin-1 and -2, the major components of caveolae, were expressed and localized near the PLVAP-positive sieve plates in CECAL, we examined the effect of okadaic acid on the intracellular positional relationship of these caveolin isoforms with the PLVAP-positive sieve plates in CECAL. Okadaic acid treatment dispersed the PLVAP-positive sieve plates and induced the colocalization of PLVAP with caveolin-1 and -2. These results indicate that the caveolae-mediated endocytosis pathway, regulated by caveolin isoforms, is essential for fenestra formation in the fenestrated endothelial cells of the rat pituitary.

4. Live-cell imaging analysis on the self-heating mechanism of immune cells in a cold environment

Immunity against infections is reduced by a drop in body temperature. In this study, we investigated how tissue temperature affects the motility of T cells and their ability to form immune synapses by live-cell imaging. We found that T-cell motility decreases with decreasing temperature, but up to a certain temperature (22°C), contact with antigen-presenting cells (APC) increases the cell temperature of T cells and reactivates their motility. Then, we investigated the effect of Genipin, a specific inhibitor for uncoupling protein 2 (UCP2), on T cells to test the possibility that reactivation of T cells is due to increasing cell temperature caused by mitochondrial uncoupling. As a result, UCP2 had little or no effect on T-cell motility above about 30°C, but completely inhibited APC-mediated immune synapse formation under cold conditions below 30°C. These results suggest that mitochondrial uncoupling is associated with reactivation of T-cell motility in cold environments.

Keywords:
next-generation sequencing, cancer genetics, p53 family, immune synapse, live-cell imaging, endothelial fenestrae formation

List of Main Publications (September 2018 to August 2023)

See 2D Barcodes below

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