

Biology

Biology Division has been occupied with 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 have a course of "Molecular and Cellular Biosciences" for graduate students.

Professor

Yasushi Sasaki, M.D., Ph.D.

Interests:

Molecular mechanisms of human carcinogenesis,
Functional analysis of p53 family

Associate Professor

Takeshi Suzuki, M.S., Ph.D.

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 the genetic characterization in human oral, esophageal, gastric, colorectal, pancreatic, and cervical cancers, primary central nervous system lymphomas and multiple myelomas as well as normal human tissues by means of next-generation sequencing (NGS) technologies (1, 4-6).

Through NGS data, we have identified several candidate driver genes as novel molecular targets for therapeutic drugs. Surgically resected OSCC tissues from 127 Japanese patients were sequenced for mutations in the coding regions of cancer-related genes using a semiconductor-based sequencing platform. The most frequent mutations were in *TP53* (61.7%), *NOTCH1* (25.5%), *CDKN2A* (19.1%), *SYNE1* (14.9%), and *PIK3CA* (10.6%). We also detected copy number variations in the segments of the genome that could be duplicated or deleted from deep sequencing data. 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 (1). In addition, TP53 mutations tended to be more frequent in HPV-negative OSCCs compared to HPV-positive cases (5).

We investigated the mutation signatures of a unique set of clinical specimens from a uterine cervical cancer that repeatedly locally recurred after multiple rounds of radiotherapy. Exon sequencing of 409 cancer-related genes in treatment-naïve and multiply-recurrent tumors revealed rare simultaneous mutations in *KRAS* and *SMAD4*. In addition, we validated the association between this mutation signature and radioresistance by performing meta-analysis of published *in vitro* data and isogenic cell-based experiments (6). This targeted next-generation sequencing had significant advantages over the 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 have shown that the *p53* tumor suppressor gene is most frequently mutated in cancers among the 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 have recently isolated several p53 family target genes, including *BRMS1L*, *LIMA1*, and lincRNA *NEAT1* (2, 3).

3. Rapid attenuation of DAG in immune synapses during productive T cell activation

Membrane diacylglycerol (DAG) generated during sustained receptor engagement activates productively T cells and prevents their anergy. DAG binds conserved cysteine rich C1 domains in signaling proteins. Remarkably, little is known about the spatial-temporal regulation of DAG in immune synapses and the differential synaptic involvement of select DAG-binding proteins remains puzzling. We found that, contrary to its expected accumulation, specific biosensors detected synaptic DAG just at the onset of intercellular antigen recognition by T cells. DAG produced by PLC γ 1 was consumed by DGK α . Residual DAG released instantly PKC δ but not the essential PKC θ , located in central Supramolecular Activation Clusters (cSMACs). A unique membrane interaction site in θ C1 that was PI3-K dependent supported binding of PKC θ to depleted DAG. However, θ v3, a novel protein domain of previously unknown function, was necessary and sufficient for stable cSMAC targeting of PKC. Single or tandem θ v3 probes remained cytoplasmic, but θ v3 coupled to C1 labeled instantly the cSMAC membrane. In addition to differentially activating PKC θ , depleted

DAG was sufficient for other essential DAG-dependent responses. Thus, physiological T cell activation is coordinated by robust regulated attenuation of DAG that directs essential signaling in functional immune synapses.

4. Bio-imaging analysis in living mammalian cells

Mec17/ α TAT1 is a key enzyme for tubulin acetylation in mammalian cells, where it plays an important role for stabilization of microtubule cytoskeleton. We made a visible fluorescent probe for Mec17/ α TAT1 by linking with EGFP (GFP-Mec17/ α TAT1) and induced it into the rat fibroblast 3Y1-B cells, and analyzed by live cell imaging with fluorescent deconvolution microscopy. The probe restricted to the centrosome in resting cells of confluent cell sheet. On the other hand, the probe localized at leading edges in migrating 3Y1-B cells. These results suggest that the probe visualizes the site of tubulin acetylation in living mammalian cells, and that the tubulin acetylation is occurred at the centrosome in resting cells and leading edge in migrating cells. We also visualized the various membrane proteins such as aquaporin and caveolin, and are investigating the methods for molecular biological microscopy (7-12).

List of Main Publications from 2013 to 2018

- 1) Nakagaki T, Tamura M, Kobashi K, Koyama R, Fukushima H, Ohashi T, Idogawa M, Ogi K, Hiratsuka H, Tokino T, Sasaki Y. Profiling cancer-related gene mutations in oral squamous cell carcinoma from Japanese patients by targeted amplicon sequencing. *Oncotarget*, 8: 59113-22, 2017.
- 2) Koyama R, Tamura M, Nakagaki T, Ohashi T, Idogawa M, Suzuki H, Tokino T, Sasaki Y. Identification and characterization of a metastatic suppressor BRMS1L as a target gene of p53. *Cancer Sci*. 108: 2413-21, 2017.
- 3) Ohashi T, Idogawa M, Sasaki Y, Tokino T. p53 mediates the suppression of cancer cell invasion by inducing LIMA1/EPLIN. *Cancer Lett*. 390: 58-66, 2017.
- 4) Takashima Y, Sasaki Y, Hayano A, Homma J, Fukai J, Iwadate Y, Kajiwara K, Ishizawa S, Hondoh H, Tokino T, Yamanaka R. Target amplicon exome-sequencing identifies promising diagnosis and prognostic markers involved in RTK-RAS and PI3K-AKT signaling as central oncopathways in primary central nervous system lymphoma. *Oncotarget* 9: 27471-86, 2018.
- 5) Nakagaki T, Tamura M, Kobashi K, Omori A, Koyama R, Idogawa M, Ogi K, Hiratsuka H, Tokino T, Sasaki Y. Targeted next-generation sequencing of 50 cancer-related genes in Japanese patients with oral squamous cell carcinoma. *Tumor Biol*. 40: 1010428318800180, 2018.
- 6) Nuryadi E, Sasaki Y, Hagiwara Y, Bunga Mayang Permata T, Sato H, Komatsu S, Yoshimoto Y, Murata K, Ando K, Kubo N, Okonogi N, Takakusagi Y, Adachi A, Iwanaga M, Tsuchida K, Tamaki T, Noda S, Hirota Y, Shibata A, Ohno T, Tokino T, Oike T, Nakano T. Mutational analysis of uterine cervical cancer that survived multiple rounds of radiotherapy. *Oncotarget* 9: 32642-52, 2018.
- 7) Nakakura T, Asano-Hoshino A, Suzuki T, Arisawa K, Tanaka H, Sekino Y, Kiuchi Y, Kawai K, Hagiwara H. The elongation of primary cilia via the acetylation of α -tubulin by the treatment with lithium chloride in human fibroblast KD cells. *Med Mol Morphol*, 48:44-53, 2015.
- 8) Nakakura T, Suzuki T, Nemoto T, Tanaka H, Asano-Hoshino A, Arisawa K, Nishijima Y, Kiuchi Y, Hagiwara H. Intracellular localization of α -tubulin acetyltransferase ATAT1 in rat ciliated cells. *Med Mol Morphol*, 49:133-143, 2016.
- 9) Nakakura T, Nemoto T, Suzuki T, Asano-Hoshino A, Tanaka H, Arisawa K, Nishijima Y, Kiuchi Y, Hagiwara H. Adrenalectomy facilitates the ATAT1 expression and α -tubulin acetylation in ACTH-producing corticotrophs. *Cell Tissue and Res*. 366:363-370, 2016.
- 10) Horibe R, Hirohashi R, Asano T, Mariya T, Suzuki T, Takaya A, Saijo H, Shionoya Y, Kubo T, Nakatsugawa M, Kanaseki T, Tsukahara T, Watanabe K, Torigoe T. Brother of the regulator of the imprinted site (BORIS) variant subfamily 6 is a novel target of lung cancer stem-like cell immunotherapy. *PLoS ONE*. 12(3): e0171460, 2017.
- 11) Nakakura T, Suzuki T, Horiguchi K, Fujiwara K, Tsukada T, Asano-Hoshino A, Tanaka H, Arisawa K, Nishijima Y, Nekooki-Machida Y, Kiuchi Y, Haruo Hagiwara. Expression and localization of forkhead box protein FOXJ1 in S100 β -positive multiciliated cells of the rat pituitary. *Med Mol Morphol*, 50:59-67, 2017.
- 12) Nakakura T, Suzuki T, Torii S, Asano-Hoshino A, Nekooki-Machida Y, Tanaka H, Arisawa K, Nishijima Y, Susa T, Okazaki T, Kiuchi Y, Hagiwara H. ATAT1 is essential to regulate the homeostasis-retaining cellular responses in corticotrophs along the hypothalamic-pituitary-adrenal axis. *Cell Tissue and Res*, 370:169-178, 2017.

Keywords:

next-generation sequencer, TP53, immune synapse, live cell imaging