

Department of Biochemistry

Kiyotsugu Yoshida, *Professor*

Tadashi Asakura, *Associate Professor*

General Summary

Tumors are a genetic disease. The fundamental defect of tumor cells is a deregulated proliferation that results from the progressive accumulation of genetic and epigenetic alterations. These alterations invariably affect the regulatory pathways that govern the proper cellular responses to this myriad of signals. Normal proliferative cells are endowed with the abilities to choose between growth and quiescence, differentiation, and apoptosis. The execution of these alternative choices is influenced by physiological factors and stress to achieve a controlled and balanced proliferation. Our research is directed at elucidating signaling pathways that allow normal cells to distinguish between proliferation, differentiation, and apoptosis.

Research Activities

Investigation of new regulators that function in the development and maintenance of the breast cancer stem cells

Cancer stem cells have been suggested to compel tumor development and progression. Although breast cancer stem cells were discovered in 2003, the molecular mechanism of their development and maintenance remains poorly understood. To explore the new regulators that function in the development and maintenance of breast cancer stem cells, we first used flow cytometry to examine cancer stem cells population (markers: CD44⁺/CD24⁻ and aldehyde dehydrogenase [ALDH]) in triple-negative breast cancer cell lines. Unexpectedly, the percentages of cells marked with CD44⁺/CD24⁻ and ALDH were inconsistent in the different cell lines we used. Therefore, cancer stem cell populations are difficult to separate with known markers, such as CD44⁺/CD24⁻ and ALDH. Thus, for the further experiments we have made cancer stem cell-enriched populations with the sphere culture method.

Discovery of the molecular mechanism of metastasis in breast cancer stem cells

A line of breast cancer stem cells, iCSCL-10A, was established in 2014 by introducing defined reprogramming factors (OCT4, SOX2, Klf4, and c-Myc) into MCF-10A nontumorigenic mammary epithelial cells. The iCSCL-10A cells possess the hallmarks of cancer stem cells and develop tumors in immunosuppressed mice. However, the metastatic ability of iCSCL-10A cells is unknown. We examined, with an in-vivo imaging system, the metastatic ability of iCSCL-10A cells that overexpressed red fluorescent protein E2-Crimson in immunosuppressed mice. Whereas no metastasis developed in mice to which control MCF-10A cells had been injected, bone metastasis developed near the femur, tibia, and spine after 6 weeks in mice to which iCSCL-10A cells had been injected. In a further experiment, the molecular mechanism of metastasis will be examined by

comparing those metastasized cells with iCSCL-10A cells.

Generation of DYRK2 knockout mice

We have previously shown that dual specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2) phosphorylates tumor suppressor p53 and transcription factors, c-Jun and c-Myc. The dysfunction of DYRK2 promotes tumor-formation activity in a xenograft model. However, the in-vivo role of DYRK2 is unknown. To investigate the in-vivo function of DYRK2, we generated knockout mice lacking the DYRK2 gene (*DYRK2*) by clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) genome-editing system. Synthetic RNA targeting *DYRK2* and Cas9 nuclease were coinjected into fertilized eggs. As a result, efficient production of mice that carry biallelic mutations in a given gene were created. To determine the mutations generated by CRISPR/Cas9, we examined the sequence of exon 3 of *DYRK2* from 13 pups. Despite no biallelic frameshift mutations being found in all founders, we observed single allelic frameshift and nonsense mutations in 5 founders. At the present time, *DYRK2*^{+/-} heterozygotes have been crossed to obtain *DYRK2*^{-/-} homozygotes, *DYRK2*^{+/-} heterozygotes, and wild-type littermates.

Production of DYRK2 gene-targeted embryonic stem cells

Insights from cell-based research suggest that DYRK2 has tumor suppressive functions. To study the physiological and pathological roles of DYRK2, we attempted to conditional knockout of the gene in mouse. The targeting vectors, obtained from the Knock-out Mouse Project, were checked by restriction enzyme digestion and the polymerase chain reaction. Linearized targeting vector was introduced into embryonic stem (ES) cells by electroporation. After extracting the genome DNA from drug-resistant ES clones, we screened for correctly targeted events with the long-range polymerase chain reaction and Southern blotting. Selected ES cell clones show the correctly recombined *DYRK2* allele, which is the knockout-first allele (tm1a) that can be used for the generation of reporter knockouts, conditional knockouts, and lacZ-tagged null alleles following exposure to site-specific recombinases Cre and Flp.

DYRK2 and cancer stem cells

Our recent study revealed that DYRK2 has a tumor-suppressive function through expression of c-Myc, c-Jun, and Snail and phosphorylation of p53. The expression of DYRK2 is decreased in advanced breast cancer and serous ovarian cancer. Diminished expression of DYRK2 confers drug-resistance to cytotoxic chemotherapy and poor prognosis in these cancers. However, the therapeutic strategy has not been established for patients with breast cancer and low DYRK2 expression. Through microarray analysis, a mechanistic target of rapamycin (mTOR) complex 1 pathway was detected as the activated pathway in DYRK2-depleted cells. Treatment with everolimus, an mTOR inhibitor, was associated with a significant inhibition of tumor growth compared with a vehicle in vitro and in vivo.

Cancer stem cells have tumorigenic potential. Breast cancer stem cells are detected by CD44 high/CD24 low. In DYRK2-depleted cells, the expression of Krüppel-like factor 4

was upregulated and resulted in an increased production of cancer stem cells. We will next analyze the association between DYRK2 and cancer stem cells in clinical samples.

Monopolar spindle 1 kinase regulates mitotic chromosome condensation

In all organisms, the control of cell-cycle progression is a fundamental process for proliferation, development, and survival. During each of phase the cell cycle, the expression of numerous proteins and the status of their posttranscriptional modifications are dramatically changed. An important modification during mitosis is that of phosphorylation. We focused on monopolar spindle 1 (Mps1), a well-conserved protein kinase that is an essential regulator for proper mitotic progressions. To identify proteins associated with Mps1 during mitosis, we performed mass spectrometry analysis. We identified condensin II as a novel Mps1-associated protein. Condensin is a highly conserved complex that contributes to mitotic chromosome condensation and segregation. The condensin complex is pentamer, which are composed of structural maintenance of chromosomes (SMC) heterodimers (SMC2 and SMC4) and non-SMC subunits (chromosome-associated protein [CAP]-H2, CAP-G2, and CAP-D3). We performed further analysis and revealed that Mps1 phosphorylates Ser492 of CAP-H2 during mitosis. Silencing of Mps1 inhibits Ser492 phosphorylation and the chromosomal recruitment of condensin II at mitosis. Furthermore, knock-down of Mps1 disrupts the chromosome condensation in prophase. These results suggest that Mps1 affects chromosomal condensation in the early phase of mitosis by regulating condensin II.

Novel cancer-associated protein thyroid hormone receptor interacting protein 13

In yeast, pachytene checkpoint 2 (Pch2) regulates several meiotic processes, such as synaptonemal complex formation, interhomologous recombination, and DNA double-strand breaks repair. Thyroid hormone receptor interacting protein 13 (TRIP13) is the mammalian ortholog of yeast Pch2. The expression profile of TRIP13 is highly restricted and most abundant in the testis but is aberrantly expressed in several types of cancers. We performed Western blotting to detect the expression levels of TRIP13 among 12 human cancer cell lines and 2 immortalized cell lines. This analysis revealed that all of the cell lines expressed high levels of TRIP13. Interestingly, TRIP13 expression was upregulated in an immortalized lung fibroblast cell line, WI-38 VA13 subline 2RA, as compared with parental normal fibroblast cells. In addition, TRIP13 was phosphorylated during the M phase in synchronized HeLa cells. These finding suggests that TRIP13 has potentially important roles in cell proliferation and tumor growth.

Publications

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