Department of Biochemistry

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General Summary

Tumor is 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

The identification of effective treatment for breast cancer with low expression of dual specificity tyrosine phosphorylation-regulated kinase 2

Our recent study revealed that dual specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2) has a tumor-suppressive function through the expression of c-Myc, c-Jun, and Snail and the phosphorylation of p53. The expression of DYRK2 is decreased in advanced breast cancer and serous ovarian cancer. The decreased expression of DYRK2 causes breast cancer to be resistant to cytotoxic chemotherapy and to have a poorer prognosis. However, a therapeutic strategy has not been established for breast cancers with low DYRK2 expression. The mechanistic target of rapamycin (mTOR) complex 1 pathway was shown with microarray analysis to be activated in DYRK2-depleted cells. Treatment with everolimus, an inhibitor of mTOR, was associated with a significant inhibition of tumor growth compared with a vehicle *in vitro* and *in vivo*.

This year, we found that DYRK2 phosphorylates mTOR at 631T to degrade mTOR through the ubiquitin-proteasome pathway by F-box and WD repeat domain-containing protein 7. Moreover, patients with low DYRK2 expression required a longer treatment period and had a higher clinical benefit rate than did patients with high DYRK2 expression.

Elucidation of the regulatory mechanism of breast cancer stem cells

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 (KLF4) was upregulated and resulted in an increased population of the cancer stem cell. This year, we analyzed the clinical samples. Low DYRK2 expression was significantly correlated with an upregulation of $CD44^{+}/CD24^{-}$ and aldehyde dehydrogenase 1-positive cancer stem cell population. We identified androgen receptor as a transcription factor,

binding to the KLF4 promoter region, which is dependent on the activity of DYRK2 kinase. Our findings provide a mechanism of cancer stem cell regulation through the DYRK2–androgen receptor–KLF4 axis in breast cancer. This pathway is thus a potential therapeutic target for the breast cancer stem cell.

Cancer-associated thyroid hormone receptor interacting protein 13

Thyroid hormone receptor interacting protein 13 (TRIP13) is the mammalian ortholog of pachytene checkpoint protein 2 (Pch2), which in yeast regulates several meiotic processes, such as synaptonemal complex formation, interhomologous recombination, and the repair of DNA double-strand breaks. A recent report suggested that TRIP13 promotes error-prone nonhomologous end-joining and induces chemoresistance in head and neck cancer. We attempted to determine TRIP13 subcellular localization under physiological and DNA-damaged conditions using immunofluorescence techniques. We found that TRIP13 was located in cytoplasm and that its distribution did not change after DNA damage. The expression profile of TRIP13 is highly restricted and most abundant in the testis but is aberrantly expressed in multiple types of cancer. Nevertheless, the functional roles of TRIP13 in cancer-cell growth and survival are largely unknown. Through the short hairpin RNA expression system, long-term knockdown of TRIP13 resulted in significant arrests of cell growth in several human cancer cell lines. In particular, in the breast cancer cell line MCF7, TRIP13 knockdown exhibited cell senescence-like phenotypes, such as cytoplasmic enlargement and multinucleated cell formation. Additionally, TRIP13-knockdown MCF7 cells had enhanced expression of p21 (WAF1/CIP1) and phosphorylation of AMP kinase. Transcriptional regulation of TRIP13 expression was not investigated. We characterized the promoter region of the TRIP13 gene (*TRIP13*) by bioluminescence-based reporter assay. A 2.7-kb upstream fragment from the initiation codon of *TRIP13* and its deletion series were cloned into a luciferase reporter vector. Maximum levels of luciferase activity were detected with the 369-bp fragment from the initiation codon.

Polo-like kinase 1 regulates mitotic chromosome condensation

Chromosomal aberration and genomic instability are hallmarks of cancer. A large proportion of cancer cells is aneuploid and contains incorrect numbers of chromosomes. We focused on Polo-like kinase 1 (Plk1), an essential regulator for proper mitotic progressions which is overexpressed in several cancers. To investigate Plk1 functions in mitosis, aneuploid cancer cell lines were treated with a Plk1 inhibitor. We found that inhibition of Plk1 leads to a striking reduction during mitosis of chromosome-associated protein (CAP)-H2, which is a subunit of condensin II that contributes to mitotic chromosome condensation and segregation. We performed further analysis and found that inhibition of Plk1 leads to anaphase-promoting complex/cell division cycle protein 20 (APC/Cdc20)-mediated degradation of CAP-H2 in mitosis. We also demonstrated that Plk1 phosphorylation of CAP-H2 at Ser288 is required for the stabilization of CAP-H2 and accurate chromosomal condensation during mitosis. These findings suggest that Plk1-mediated phosphorylation controls condensin II functions by modulating CAP-H2 expression levels to facilitate proper mitotic chromosome condensation.

Publications

Dashzeveg N, Yogosawa S, Yoshida K. Transcriptional induction of protein kinase C delta by p53 tumor suppressor in the apoptotic response to DNA damage. *Cancer Lett.* 2016; **374:** 167-74. Epub 2016 Feb 13.

Reviews and Books

Dashzeveg N, Yoshida K. Cell death decision by p53 via control of the mitochondrial membrane. *Cancer Lett.* 2015; **367:** 108-12.