Department of Biochemistry

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General Summary

Tumors are genetic diseases. 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 from growth to 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

Forced expression of dual-specificity tyrosine-regulated kinase 2 exerts antitumor effects via apoptotic induction in liver cancer

Liver cancer is highly aggressive and globally exhibits a poor prognosis. Recently, tyrosine kinase inhibitors, including sorafenib, lenvatinib, and regorafenib, have been developed and approved for clinical use against advanced cancers but have limited effects. Therefore, novel molecules that can become targets for future therapies should be quickly identified.

We have reported that dual-specificity tyrosine phosphorylation-regulated kinase 2 (DYRK2) functions as a tumor suppressor by regulating cell survival, differentiation, proliferation, and apoptosis. However, the precise functional roles of DYRK2 in liver cancer remain obscure. Moreover, research into the clinical application of DYRK2 as a molecular target remains to be performed.

We assessed the effects of DYRK2 on tumor growth and apoptosis in liver cancer cells. We showed that DYRK2 knockdown enhances cell proliferation and tumor growth through the expression of c-Myc and cyclin D in liver cancer cells. Conversely and more importantly, adenovirus-mediated overexpression of DYRK2 inhibits cell proliferation, tumor growth, and the induction of apoptosis both in vitro and in vivo. Furthermore, we found that patients with liver cancer and low DYRK2 expression survived for a significantly shorter time. The findings that DYRK2 regulates proliferation and apoptosis of cancer cells suggest that DYRK2 expression is a promising marker for prognosis in liver cancer. Stabilized or forced expression of DYRK2 might thus become a target for novel gene therapy against liver cancer.

Study of DYRK2 expression and stability

Our previous studies have suggested that DYRK2 has a tumor-suppressive function. For

example, immunostaining analysis has shown that DYRK2 is down-regulated in several tumor tissues compared to adjacent normal controls. Therefore, if DYRK2 expression can be restored, it might be used for cancer therapy, but the mechanism of DYRK2 expression in cells is poorly understood.

The promoter region of the *DYRK2* gene containing of high guanine-cytosine (GC) content suggests an expression control mechanism of messenger (m) RNA by DNA methylation. On the other hand, within DYRK2 mRNA is a 5′-untranslated region (5′-UTR) with high GC content over 300 bases upstream of the start codon, and the upstream open reading frame (uORF) is present there. These features suggest the existence of a translational regulatory mechanism via the 5′-UTR of mRNA. Therefore, the influence of this 5′-UTR on the expression of firefly luciferase was analyzed with a simian virus 40 (SV40) promoter-driven reporter assay system. The 5′-UTR starting from the transcription start site of the *DYRK2* gene was amplified by the polymerase chain reaction and inserted between the SV40 promoter and the luciferase reporter gene. As a result, the activity of luciferase was reduced 80%. When the uORF region was deleted from the sequence inserted into the reporter vector, the luciferase activity was restored to half of its original level. These results suggest that translation regulation via 5′-UTR exists as a regulation mechanism of DYRK2 expression.

The expression level of DYRK2 in cells is also regulated by a protein degradation system. The relation of its domain structure to intracellular stability has been investigated in *Drosophila* DYRK2 but not in human DYRK2. On the other hand, the crystal structure of the kinase domain of human DYRK2 has been elucidated, which is consistent with the region reported previously. Here, we forced expression of the human DYRK2 kinase domain in human cell lines and assessed its expression level by immunoblotting. We found that the level of expression of the kinase domain of human DYRK2 in cells was much lower than that of the full-length DYRK2. When several deletion mutants were made and their expression levels were compared, the N-terminus was found to greatly contribute to intracellular stability and to be conserved from human to zebrafish. In contrast, the C-terminal region following the kinase domain contributed little to the stability of DYRK2.

Molecular functions of DYRK2 during mammalian tissue development

Tissue development proceeds via spatiotemporal patterning of several signaling molecules. These signaling molecules are regulated by posttranslational modifications, such as phosphorylation, in addition to gene expression. We have reported that among these posttranslational modification-related factors that the dual-specificity tyrosine-regulated kinase 2 gene (*Dyrk2*) is a key regulator of p53, which it phosphorylates at Ser46 to induce apoptosis in response to DNA damage. However, little is known about the molecular functions of DYRK2 when mammalian tissue develops. In this study, we aimed to identify the molecular functions of DYRK2 in tissue development. For this purpose, we have established methods related to immunohistochemistry, immunocytochemistry, *in situ* hybridization, and *in vitro* cultivation. With these methods, we would like to identify target-signaling molecules of DYRK2.

Subcellular localization of serine/threonine kinases

Intracellular kinases are key factors involved in the intracellular signaling pathway. Our laboratory has paid attention to some serine/threonine kinases. In this study, we analyzed the association between kinases and cancers.

After performing intracellular localization analysis of kinases, we found that a novel kinase X was located both inside and outside of cells. For our experiment systems, we have used biochemical, cellular biological, and immunologic techniques. Furthermore, our research, which was done in collaboration with the Department of Internal Medicine of The Jikei University Hospital, detected kinase X at a high level in the serum of patients with cancer. In addition, we found that kinase X bound to the surface of cancer cells. Thus, from this study, we have clarified the novel localization of kinase X and its association with cancer. We are planning future studies to elucidate the basic biological and clinical significance of this kinase.

Provirus integration site for Moloney murine leukemia virus 1 regulates self-renewal property of colorectal cancer cells by regulating Akt/mechanistic target of rapamycin pathways

Provirus integration site for Moloney murine leukemia virus 1 (Pim-1) is a proto-oncogenic kinase involved in several cellular processes, including cell survival, cell proliferation, and apoptosis. Increased Pim-1 expression is frequently observed in cancer cells and is correlated with a poor prognosis in various types of cancer. Accumulating evidence has demonstrated that cancer stem cells (CSCs) are a small subpopulation of cancer cells that possess stem-like properties. To enrich CSCs, a functional approach is the sphere culture system, which provides self-renewal ability. Although CSCs are associated with the maintenance and growth of tumors, the cellular signaling pathways that regulates the capacity of CSCs has not been fully understood. In this study, we have shown that Pim-1 function is required for self-renewal capacity in colorectal cancer cells. Our results demonstrated that Pim-1 expression is elevated in sphere-forming cells. Depletion of Pim-1 or treatment with the Pim inhibitor SGI-1776 prevented sphere formation. Furthermore, inhibition of Pim-1 prevented phosphorylation of Akt and ribosomal protein S6 in sphere-forming cells. These findings suggest that Pim-1 contributes to the self-renewal property of colorectal CSCs by maintaining Akt and mechanistic target of rapamycin (mTOR) signaling.

Publications

Okabe H, Aoki K, Yogosawa S, Saito M, Marumo K, Yoshida K. Downregulation of CD24 suppresses bone metastasis of lung cancer. *Cancer Sci.* 2018; **109:** 112-20.

Imawari Y, Mimoto R, Hirooka S, Morikawa T, Takeyama H, Yoshida K. Downregulation of DYRK2 promotes tumor cell proliferation and invasion by enhancing CDK14 expression in breast cancer. *Cancer Sci.* 2018; **109:** 363-72.

Reviews and Books

Yogosawa S, Yoshida K. Tumor suppressive role for kinases phosphorylating p53 in the DNA damage-induced apoptosis. *Cancer Sci.* 2018; **109:** 3376-82.

Yoshida K. Tumor suppressive functions of protein kinase C δ in the DNA damage response. In: Pierce DJ, editor. *Protein Kinase C: Emerging Roles and Therapeutic Potential*. Nova Science Publishers; 2018. p. 49-64.