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

Discovery of the molecular mechanism of metastasis in breast cancer stem cells, iCSCL-10A cells

Breast cancer is the most frequently diagnosed malignancy and is the leading cause of cancer death among females. The majority of breast cancer-related deaths is attributed to metastasis to distant organs such as lung and bone. Although the development of effective treatment for breast cancer has been continued, metastatic disease is still considered to be incurable. Therefore, it is important to develop an effective treatment strategy for patients with metastasis of breast cancer.

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. Here, we generated a mouse model of breast cancer bone metastasis. First, we examined, with an in-vivo imaging system, the metastatic ability of iCSCL-10A cells that overexpressed near-infrared fluorescence protein iRFP713 in immunosuppressed mice. Whereas no metastasis developed in mice to which control MCF-10A cells had been injected, bone metastasis developed near the femur and tibia after 4 weeks in mice to which iCSCL-10A cells had been injected. Furthermore, to investigate the new molecules involved in bone metastasis of iCSCL-10A cells, we isolated metastatic iRFP713-positive iCSCL-10A cells in bone-marrow cell population and analyzed gene expression by the microarray. Consequentially, we obtained several candidate genes which are related to bone metastasis involved in cell adhesion, signaling, and metabolism. Overexpression of candidate genes in iCSCL-10A cells resulted in reduced in vitro migration and invasion. At the present time, we have examined whether these genes function as novel regulators of bone metastasis.

Study of DYRK2 expression and stability

Based on the current studies of our laboratory, dual specificity tyrosine-phosphorylation regulated kinase 2 (DYRK2) is predicted to have tumor suppressive functions. In fact, DYRK2 is down-regulated in several tumor tissues compared to adjacent normal controls. However, little is known about how the expression of DYRK2 is regulated by upstream signal transduction processes. Therefore, we have attempted to evaluate the evidence for associations out between DYRK2 expression and other tumor suppressive pathways using cancer cell lines.

Hippo signaling pathway represents an organ-size control through the contact inhibition of cell growth. Mammalian sterile 20-like kinase 1 (Mst1) is a critical component of its pathway and has tumor suppressive functions. By accessing out unified datasets of protein interactions, MST1 could be selected as a candidate molecule associated with DYRK2. Then, we tested the cell lines grown at varying density conditions and evaluated the expression of DYRK2. At low-density condition, DYRK2 protein expression levels were low, whereas at high-density condition led to high expression levels of DYRK2. Results from quantitative-PCR study at high-density condition indicated that mRNA expression of DYRK2 gene increased about 200% compared to low-density. High-density condition induced the Hippo signaling activation, which result in cell growth arrest. These findings suggested that Hippo signaling pathway regulates DYRK2 expression. Detailed studies are currently in progress.

It is known that glycogen synthase kinase 3 beta (GSK3β) is highly inactivated in various tumor types and is known to inhibit tumor migration and invasion. To test the role of GSK3β activity on the DYRK2 expression, we treated colorectal cancer cells (HCT116) with lithium chloride (LiCl), an inhibitor of GSK3β, and assessing the expression of DYRK2 by immunoblotting. As a result, the protein level of DYRK2 appeared to decrease with LiCl treatment for 5 hours. Similar results were obtained from lung carcinoma cells (NCI-H460) and immortalized retinal pigment epithelial cells (RPE1). It may be suggested that GSK3β-mediated phosphorylation of DYRK2 is necessary for stabilization of its protein structure. It is known that maturation of most protein kinases depends on the chaperone activity of HSP90. Short exposure (5 h) of geldanamycin, a drug which destabilizes Hsp90-associated proteins, led to decreased protein levels of DYRK2 in above cell lines. We will clarify the relevance of GSK3β signaling pathway for DYRK2 stability and activity.

Subcellular localization of Ser/Thr kinases

Intracellular kinases have been known to be key factors involved in the intracellular signaling pathway. Our laboratory has paid the attention to some serine threonine kinases. In this study, we push forward analysis about the association between kinases and cancers.

After performing the intracellular localization analysis in the cell among the kinase, we found that a novel kinase X was located outside a cell as well as in the cell. As experimental systems we used, we have done biochemical, cellular biological, and immunologic techniques. Furthermore, we collaborated the research with department of internal medicine in The Jikei university hospital, and found that the kinase X was detected in the serum of patients with cancer at a high level. In addition, we found that this kinase X

bound to the cell surface of cancer cells. Thus, we were able to clarify the novel localization of kinase X, and association with cancer of the kinase. We are now going to push forward studies for elucidation of the basic biological and clinical significance in future.

Pim-1 regulates self-renewal property of colorectal cancer cells by regulating Akt/mTOR pathways

Pim-1 is a proto-oncogenic kinase and involved in several cellular processes including cell survival, cell proliferation and apoptosis. Increased Pim-1 expression is frequently observed in cancer cells and that is correlated with a poor prognosis in various types of cancer. Accumulating evidences have demonstrated that the cancer stem cells (CSCs) are small subpopulation of cancer cells and possess stem-like properties. The sphere culture system is a functional approach to enrich CSCs including self renewal ability. Although CSCs are associated with the maintenance and growth of tumors, the cellular signaling pathways which regulate CSCs capacity have not been fully understood. In this study, we show 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 could contribute to self-renewal property in colorectal CSCs by maintaining Akt and mTOR signaling.

Publications

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Reviews and Books

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