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

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 near the femur and tibia after 4 weeks in mice to with 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 microarray. Consequentially, we obtained several genes involved in cell adhesion, signalling, and metabolism. At the present time, we have examined whether these genes function as novel regulators of bone metastasis.

Identification of critical residues required for DYRK2 activity

DYRK2 is an evolutionarily conserved eukaryotic protein kinase that belongs to CMGC protein kinase group. This implies that important structural and functional features are associated with evolutionarily conserved amino acid residues. Among the CMGC kinases, phosphorylation of a regulatory region termed the activation loop is critical to exert their kinase activity. Thus, we attempted to make the series of GFP-fused expression vectors bearing the activation loop mutants of DYRK2. It is known that the kinase activity of

DYRK2 depends on the autophosphorylation of a tyrosine residue (Y382) in activation loop of catalytic domain. Overexpression of wild-type DYRK2 in COS7 cells induced change of its morphology from an elongated epithelial-like shape to a round shape. On the other hand, overexpression of non-phosphorylated mutant, Y382F, remained the epithelial-like morphology and was localized in cytoplasm, but not in nucleus. Predictions using consensus sequences identified the potential phosphorylation sites (Y380, T381 and S385) in DYRK2 activation loop. We also expressed non-phosphorylated mutants (Y380F, T381A and S385A) in COS7 cells. Overexpression of Y380F and T381A exhibited a similar phenotype as the wild-type DYRK2. It was suggested that these residue (Y380 and T381) are dispensable for kinase activity of DYRK2. However, S385A presented the similar phenotype, which was observed in the overexpression of Y382F that has no kinase activity. Furthermore, overexpression of phosphoserine mimic mutants (S385D and S385E) also indicated the similar phenotype that observed in expression of non-phosphorylated mutants, Y382F and S385A. These results may support that the S385 is critical residue required for DYRK2 activity.

Plk1 regulates mitotic chromosome condensation

The chromosomal aberration and genomic instability are hallmarks of cancer. A large proportion of cancer cells is aneuploidy, which contain incorrect number of chromosomes. We have focused on Plk1 that is an essential regulator for proper mitotic progressions and is overexpressed in several cancers. To investigate Plk1 functions in mitosis, aneuploid cancer cell lines were treated with Plk1 inhibitor. Immunoblot analysis revealed that inhibition of Plk1 leads to a reduction of CAP-H2 at mitosis. CAP-H2 is a subunit of condensin II that contributes to mitotic chromosome condensation and segregation. We performed further analysis and revealed that inhibition of Plk1 leads to Cdc20-mediated degradation of CAP-H2. We also demonstrated that Plk1 phosphorylation of CAP-H2 at Ser288 contributes to the stabilization of CAP-H2 and is required for accurate chromosomal condensation during prophase and subsequent chromosomal segregation. These findings suggest that Plk1-mediated phosphorylation controls condensin II functions by modulating CAP-H2 expression levels to control mitotic chromosomal organization.

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 cancers. Accumulating evidence has 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 which including self renewal ability. Although CSCs are associated with the maintenance and growth of tumors, the cellular signaling pathways by which regulates 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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