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 between 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

Carbonic anhydrase 13 suppresses bone metastasis of breast cancer cells
Metastatic progression is the leading cause of mortality in breast cancer. However, the molecular mechanisms that govern this process remains unclear. A line of breast cancer stem cells—induced cancer stem cell-like 10A (iCSCL-10A) cells—was established by introducing reprogramming factors (octamer-binding transcription factor 4 [OCT4], sex determining region Y-box 2 [SOX2], Kruppel-like factor 4 [Klf4], and c-Myc) into MCF-10A nontumorigenic mammary epithelial cells. The iCSCL-10A cells display a malignant phenotype and form tumors when injected into immunodeficient mice and possess the hallmarks of cancer stem cells. However, the metastatic ability of iCSCL-10A cells and a potential metastatic model for breast cancer has not yet been reported. Here, we found that carbonic anhydrase 13 (CA13) has the potential to suppress bone metastasis of iCSCL-10A breast cancer stem cells. The iCSCL-10A cells possess the hallmarks of cancer stem cells and indeed exerted the ability of bone metastasis in the hind limbs of mice in 5 weeks after injection, whereas no metastasis was observed in those of mice injected with control MCF-10A cells. Transcriptome analysis indicate that the expression of several genes involved in cell adhesion, signaling, and metabolism was reduced in bone metastatic iCSCL-10A cells. In-vitro and in-vivo analysis determined that overexpression of CA13 in iCSCL-10A cells suppressed migration, invasion, and bone metastasis. Furthermore, we found that breast cancer patients with low CA13 expression had a significantly shorter overall survival. These findings indicate that CA13 might act as a novel prognostic biomarker and therapeutic candidate for the prevention of bone metastasis of breast cancer.

DYRK2-null mouse recapitulates VATER/VACTERL association with lung hypoplasia
Congenital malformations are a major issue in pediatric healthcare and the leading cause

of infant mortality in the United States. Most rare congenital malformations, such as VATER (vertebral anomalies, anal atresia, tracheoesophageal fistula and/or esophageal atresia, and radial dysplasia)/VACTERL (VATER plus cardiac defects and limb defects) association, Alagille syndrome, and CHARGE (coloboma, heart defect, atresia of the choanae, retardation, and genital and ear abnormalities) syndrome, have multiple component features, and some are known to involve genetic mutations. The genetic knockout of causal genes in mice often reproduces congenital malformations, providing extremely valuable models for the study of rare pediatric diseases. However, there is a lack of adequate animal models recapitulating rare congenital diseases. We have previously shown that dual-specificity tyrosine-phosphorylation-regulated kinase 2 (DYRK2) exerts antitumor effects in various cancer cells. However, the effect of DYRK2 gene ablation during embryogenesis has not been previously investigated. In this study, we report the generation of DYRK2-deficient mice using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) nickase system. We found that the phenotypes of DYRK2-deficient mice recapitulated those of VATER/VACTERL human congenital malformations. Transcriptome analysis indicated close similarities between the molecular phenotypes of VATER/VACTERL association and DYRK2-deficient mice, particularly with respect to *Foxf1* reduction. Mutant pups died soon after birth owing to respiratory failure, a feature secondary to VATER/VACTERL components. Detailed analyses of primordial lungs during early development demonstrated that DYRK2 deficiency leads to altered airway branching and insufficient alveolar development. Furthermore, the Foxf1 expression gradient in mutant lung mesenchyme was disrupted, reducing Foxf1 target genes, which are necessary for airway and alveolar development. Taken together, our results confirm the establishment of a novel DYRK2-deficient mouse model that recapitulates the pathological and molecular phenotypes of VATER/VACTERL association with lung hypoplasia. Collectively, these findings provide new insights into VATER/VACTERL association.

Molecular functions of DYRK2 during mammalian tissue development

In this study, we aimed to elucidate the function of dual-specificity tyrosine-regulated kinase 2 gene (*Dyrk2*), which is a key regulator of p53 in response to DNA damage. In 2019, we researched the following issues: (1) the functions of DYRK2 during mammalian tissue development, (2) the functions of DYRK2 in tissue/cancer stem cells, and (3) the antitumor effect of DYRK2 in colorectal cancer.

1. The 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. However, little is known regarding the molecular functions of DYRK2 during mammalian tissue development. In this study, to identify molecular functions of DYRK2 in tissue development, we analyzed *Dyrk2* knockout ($Dyrk2^{-/-}$) mice. In 2019, we identified a candidate signaling by analyzing $Dyrk2^{-/-}$ mice and mouse embryonic fibroblasts. We would like to analyze the molecular functions of DYRK2 in the identified signaling.

2. The functions of DYRK2 in tissue/cancer stem cells:
In the process of tumorigenesis, tissue stem cells are known to transform into cancer-initiating cells. We have demonstrated DYRK2 localized in tissue stem cells in several tissues. In 2019, to analyze the functions of DYRK2 in cancer-initiating cells, we developed conditional *Dyrk2*-knockout mice specifically in leucine rich repeat containing G protein coupled receptor 5 gene (*Lgr5*)-expressing cells (*Dyrk2*^{flox}; *Lgr5*-CreERT2-IRES-EGFP mice). We would like to analyze these conditional *Dyrk2*-knockout mice in the process of tumorigenesis.
3. The antitumor effect of DYRK2 in colorectal cancer:
In colorectal cancer cell lines, we recently reported that knockdown of the dual-specificity tyrosine-phosphorylation-regulated kinase 2 gene (*DYRK2*) induces proliferation in vitro. In this study, we aimed to examine whether a forced expression of DYRK2 has a potential for novel gene therapy against cancer. We developed a xenograft model of colorectal cancer cell lines and are analyzing the effects of DYRK2-overexpression by adenovirus-infection in proliferation and apoptosis.

Subcellular localization of Ser/Thr kinases
Liver cancer has a high mortality rate. Although surgical resection has been recognized as the only curative treatment when liver cancer is at an early stage, this cancer is diagnosed in a majority of patients at an advanced stage, when present therapies are ineffective. Until now, few tumor markers can be used to discriminate liver cancer with high sensitivity and specificity. Therefore, novel biomarkers should be developed to predict initiation or progression of liver cancer. Recently, we found that novel localization machinery by which several nuclear trafficking proteins were translocated into the extracellular space. We established a proteomics approach to detect nuclear trafficking proteins from extracellular fluid of liver cancer and identified protein kinase C delta (PKCδ), which is known to be involved in various signaling events. We detected PKCδ in the culture media of several liver cancer cell lines and the sera of tumor model mice. Furthermore, serum levels of PKCδ were significantly higher in patients with liver cancers than in either healthy donors or high-risk group patients for liver cancers (chronic hepatitis and hepatic cirrhosis). We have also found that the extracellular secretion of PKCδ was induced by distinct secretion mechanism from that of other liver cancer markers (alpha-fetoprotein and proteins induced by vitamin k antagonism or absence [PIVKA] II) in living liver cancer cells. Furthermore, we revealed that extracellular PKCδ augmented phosphorylation of the growth signaling, such as extracellular signal-regulated protein kinase (ERK) 1/2 and signal transducer and activator of transcription (STAT) 3, which results in increased tumor growth. These results demonstrate a novel function of PKCδ as a secretory growth factor and suggest that serum PKCδ might be an active biomarker for liver cancer.

Gene silencing with CRISPR/Cas9 method
Gene silencing is the useful method for elucidating the function of a particular gene. Conventional methods using RNA interference are insufficient as experimental systems because (1) complete repression of expression is not possible and (2) recovery of expression is observed in many cases. Recently, genome editing methods have been used to sup-

press the expression of specific genes, and these methods are now being used in various fields. Therefore, we have also used the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) gene-editing system in an attempt to disrupt specific genes in cancer cell lines. Although several methods can be used for gene disruption with the CRISPR/Cas9 method, we estimated the efficiency of gene disruption in lentiviral vector systems and plasmid vector systems. First, the lentiCRISPR v2 plasmid was transfected into 293T cells along with the packaging vectors to obtain particles of the lentiviral vector. Next, these particles were infected with various cancer cell lines at a multiplicity of 1, and puromycin-resistant cells were cloned. Whereas these clones were analyzed, many of them did not undergo genome editing and were unable to disrupt their genes. In contrast, when the lentiCRISPR v2 plasmid was transfected into cells by lipofection and subjected to strong puromycin selection, genome editing occurred in a majority of surviving cells. These differences in the efficiency of genome editing might be caused by differences in the amount of Cas9 protein expressed in the cell.

Publications

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Reviews and Books

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