Department of Biochemistry

Kiyotsugu Yoshida, *Professor* Tadashi Asakura, *Associate Professor*

General Summary

Tumors are 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 these myriad signals. Normal proliferative cells are endowed with the ability to choose from among growth, 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 from among proliferation, differentiation, and apoptosis.

Research Activities

Induction of amphiregulin by p53 promotes apoptosis via control of microRNA biogenesis in response to DNA damage

Tumor suppressor p53 functions as a transcription factor to induce its target genes modulating cell-cycle arrest, DNA repair, and apoptosis induction. Selective transactivation of p53 target genes is determined by posttranslational modifications of p53. In particular, Ser46 phosphorylation is a requisite for commitment to induce apoptotic cell death. To clarify the mechanism of p53-mediated apoptosis, we explored target genes that are induced in a Ser46 phosphorylation-specific manner. By means of chromatin immunoprecipitation analysis, we demonstrated that amphiregulin (*AREG*) is a novel target gene for p53. To investigate the mechanism of AREG-mediated apoptosis induction, we analyzed AREG-interacting proteins by mass spectrometry. One candidate, DEAD box protein 5 (DDX5), was found to co-localize with AREG in the nucleus. DDX5 plays an essential role in precursor microRNA processing. Intriguingly, AREG regulates microRNA biogenesis (i.e., miR-15a) in response to DNA damage.

Dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 controls the epithelial-mesenchymal transition and metastases in breast cancer by degrading Snail

The epithelial-mesenchymal transition (EMT) plays a fundamental role in the early stages of breast cancer invasion. Snail, a zinc finger transcriptional repressor, is an important regulator of EMT. Snail is phosphorylated by glycogen synthase kinase 3β and is subsequently degraded by β-transducing-repeat-containing protein-mediated ubiquitination. We identified an additional kinase, dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 (DYRK2), which regulates Snail stability. We stably silenced DYRK2 in MCF-7 cells (short hairpin RNA-DYRK2 cells). Stable DYRK2 depletion led to Snail accumulation and decreased E-cadherin in MCF-7 cells. Knockdown of

DYRK2 promoted the EMT and cancer invasion in vitro. In MDA-MB-231 cells, the overexpression of DYRK2 reduced the invasive capacity. Our results suggest that DYRK2 phosphorylates Snail at Ser104 as a priming phosphorylation for glycogen synthase kinase 3β. In a xenograft model, a significant increase in bone and lung metastasis was observed in the DYRK2-shRNA group. Consistent with these results, DYRK2 was found to be down-regulated in human breast cancer tissue. Patients with low DYRK2-expressing tumors had a worse outcome than did patients with high DYRK2-expressing tumors. These findings suggest that DYRK2 regulates cancer invasion and metastasis by degrading Snail.

E-cadherin suppression in epoxomicin-resistant cells may be regulated by expression of ZEB1

Our previous study demonstrated that in endometrial carcinoma Ishikawa cells resistant to the proteasome inhibitor epoxomicin (Ishikawa/EXM cells), E-cadherin was suppressed via expression of the transcriptional repressor gene *ZEB1*. Down-regulation of E-cadherin plays an important role in the EMT. The expression of zinc finger E-box-binding homeobox (ZEB) 1 was concerned with the suppression of dual-specificity protein phophatase 6 (DUSP6) via extracellular signal regulated kinase (ERK) 1/2 signal transduction. Because we found DUSP6/MAP kinase phosphatase (MKP) 3 disappearing in Ishikawa/EXM cells, we studied the participation of DUSP6/MKP3 in E-cadherin expression in Ishikawa/EXM cells. Suppression of DUSP6 and expression of FOS-like antigen 1 (Fra1) were observed in Ishikawa/EXM cells. It was reported that activated ERK2 was upregulated ZEB1/2 following phosphorylation of Fra1. Both knock-down of DUSP6 by treatment of Ishikawa cells with short interfering RNA for DUSP6 and inhibition of DUSP6 activity by treatment of Ishikawa cells with (E)-2-benzylidene-3-(cyclohexylamino)-2,3-dihydro-1H-inden-1-one (BCI), an inhibitor of DUSP6 activity, induced expression of Fra1 by activation of ERK2 and induced repression of E-cadherin following expression of ZEB1. Moreover, overexpression of Fra1 in Ishikawa cells transfected with Fra1/pcDNA3.1 caused ZEB1-induced suppression of E-cadherin. On the other hand, expression of DUSP6 by transfection of Ishikawa/EXM cells with DUSP6/pcDNA3.1 induced expression of E-cadherin following suppression of Fra1 and ZEB1. These results suggest that the disappearance of DUSP6 in Ishikawa/EXM cells causes up-regulation of ZEB1 via the expression of Fra1 and induces the EMT.

Development of an efficient production system of fibrinogen using a hepatocellular carcinoma cell line

With the increasing demand for blood products globally, transfusions are not without risk because blood products that are mostly dependent on blood donations can be associated with infectious disease transmission. Therefore, the development of an effective production system to ensure the safe and plentiful supply of blood products is important. We attempted to develop an effective fibrinogen production system that satisfied these requirements using human functional hepatic cell lines (FLC-7) and serum-free medium (ASF104N and IS-RPMI). Massive fibrinogen production was induced with a

radial flow bioreactor. In the radial flow bioreactor culture, the fibrinogen secretion rate reached 109.5 μg per day during a 42-day cultivation period. The subunit composition and clot formation activity of FLC-7 cell-derived fibrinogen corresponded to those of plasma-derived fibrinogen. Thus, the system we developed is suitable for large-scale fibrinogen production.

Study of deubiquitinating enzyme ubiquitin-specific protease 46 underlying despair behavior in mice

Ubiquitin-specific proteases (USPs) are deubiquitinating enzymes that remove ubiquitin from specific protein substrates and modulate the ubiquitin-proteasome system. Recently, *Usp46* was identified as a quantitative trait gene responsible for decreasing tail suspension test and forced swimming test immobility time in CS mice. The CS mouse has a 3-bp deletion coding for Lys 92 of the protein USP46, but the effect of the deletion mutation on deubiquitinating enzyme function is unclear. To investigate the structural basis of the deletion of Lys 92 in USP46, we used the homology-modelling program SWISS-MODEL and predicted the 3-dimensional structure of USP46 based on the crystal structure of USP21. In the obtained model, Lys 92 was located at the loop structure on the surface of USP46 and was distant from the active site. This model suggests that Lys 92 mediates the interaction of USP46 with the target or partner proteins. To identify the proteins interacting with USP46, we first generated a stable neuroblastoma cell line (SH-SY5Y) expressing Flag-USP46 (wild-type and mutant). Then, we screened the interacting proteins using liquid chromatography/tandem mass spectrometry analysis, combined with the co-immunoprecipitation method. Our analysis identified several endogenous proteins associated with USP46. For example, WD repeat domain 48 and dystrophia myotonica, WD repeat-containing, interacted with either wild-type or mutant Flag-USP46. Detailed studies are currently in progress.

Publications

Taira N, Yamaguchi T, Kimura J, Lu ZG, Fukuda S, Higashiyama S, Ono M, Yoshida K. Induction of amphiregulin by p53 promotes apoptosis via control of microRNA biogenesis in response to DNA damage. *Proc Natl Acad Sci U S A.* 2014; **111:** 717-22.

Mimoto R, Taira N, Takahashi H, Yamaguchi T, Okabe M, Uchida K, Miki Y, Yoshida K. DYRK2 controls epithelial-mesenchymal transition in breast cancer by degrading Snail. *Cancer Lett.* 2013; **339:** 214-25.