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 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

Cancer research

1. Dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 phosphorylation of c-Jun/c-Myc controls tumor progression by monitoring the G1/S transition

Transcription factors c-Jun and c-Myc are indispensable regulators of the G1/S transition, and their expression is tightly regulated at transcriptional and posttranslational levels. Dysregulation of this expression leads to tumor development and progression. Degradation of c-Jun/c-Myc is triggered by sequential phosphorylation by unknown priming kinase(s) and glycogen synthase kinase 3 beta. This year, we clarified that dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 2 (DYRK2) functions as a priming kinase for c-Jun/c-Myc whose phosphorylation is required for subsequent glycogen synthase kinase 3 beta phosphorylation and proteasomal degradation. Stable knockdown of DYRK2 results in aberrant expression of c-Jun and c-Myc and facilitates cell proliferation and tumor progression *in vivo*. Furthermore, DYRK2 expression is down-regulated in invasive breast cancer tissues. Notably, expression of c-Jun and c-Myc is elevated in tissues with low DYRK2 expression. Taken together, these results suggest that depletion of DYRK2 expression contributes to tumorigenesis.

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

E-Cadherin was suppressed via expression of transcriptional repressor gene *ZEB1* in endometrial carcinoma Ishikawa cells resistant to the proteasome inhibitor epoxomicin. This finding suggests that the expression of *ZEB1* is involved in the suppression of dual-specificity protein phosphatase 6.

3. Targeted chemotherapy with polymeric micelles against CD147-expressing carcinoma cells

Because a specific accumulation of cytotoxicity was observed in CD147-expressing cells treated with glutathione-doxorubicin conjugate-encapsulated anti-CD147 antibody-labeled micelles, we prepared tumor-bearing mice for *in vivo* investigation of the chemotherapeutic effect.

Other research

1. Mechanistic elucidation of cadmium-induced cytotoxicity

We analyzed the cadmium-induced cytotoxicity with proximal tubular HK-2 cells by using reagents that modulate the selective proteolytic system. The results strongly suggest that the ubiquitin-proteasome system is responsible for resistance to this toxicity.

2. Production of fibrinogen by a well-differentiated human hepatoma cell line

We developed an efficient and economical system for fibrinogen production by optimization of the FLC-7 cell culture system comprising 2 serum-free media (ASF104N and IS-RPMI) and a radial-flow bioreactor. We expect this system will be used to develop methods of risk-free fibrinogen preparation.

3. 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 immobility time in the tail suspension test of the CS mouse. 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 activity is not clear. To construct a measurement system for wild-type and mutant USP46 activity, we prepared an expression system using an episomal vector in mammalian cells. An episomal vector bearing USP46 complementary DNA was transfected into HeLa cells and stably expressed the proteins (wild-type and mutant). However, the USP46 levels in the transfected HeLa cells were lower than in nontransfected HEK293 cells. Hence, we assumed that USP46 forms deubiquitinating enzyme complexes with partner proteins and that USP46 protein is unstable by itself. A search for known proteins interacting with USP46 identified a WD-domain repeat (WDR) 20, which is a protein containing WD40-repeat motifs and a subunit for USP12 deubiquitinating enzyme complexes, as stabilizing factor for USP46 proteins.

4. Primary structural analysis of host-defense peptides of *Xenopus*

We determined amino acid sequences of host-defense peptides separated from skin secretions of several species of *Xenopus* and elucidated the primary structures of these peptide groups.

Publications

Suzuki K, Dashzeveg N, Lu ZG, Taira N, Miki Y, Yoshida K. Programmed cell death 6, a novel p53-responsive gene, targets to the nucleus in the apoptotic response to DNA damage. *Cancer Sci.* 2012; **103:** 1788-94.

Ueda K, Yamada K, Kiyokawa T, Iida Y, Nagata C, Hamada T, Saito M, Aoki K, Yanaihara N, Takakura S, Okamoto A, Ochiai K, Ohkawa K, Tanaka T. Pilot study of CD147 protein expression in epithelial ovarian cancer

using monoclonal antibody 12C3. *J Obstet Gynaecol Res.* 2012; **38:** 1211-9.

Reviews and Books

Taira N, Yoshida K. Post-translational modifications of p53 tumor suppressor: determinants of its functional targets. *Histol Histopathol.* 2012; **27:** 437-43.

Taira N, Yoshida K. A linkage between the tumor suppressive kinase and tumor progression (in Japanese). *Jikken Igaku.* 2012; **30**: 1786-9.