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Research Activities

Cancer research

1. To establish methods for early cancer diagnosis and therapy, we evaluated several molecular properties of CD147 for cancer-cell-targeting. CD147, known as an extracellular matrix metalloprotease inducer (EMMRPIN) or basigin, is a transmembrane glycoprotein with two immunoglobulin-like domains. A murine monoclonal antibody against CD147 (MAb12C3) was generated by Ohkawa, et al. in 1995. Several studies of clinical tumor specimens have demonstrated significant correlations between patents' prognoses and expression levels of the CD147 protein in the tumors. To confirm these results, we performed immunohistopathologic studies in patients with endometrial carcinoma (112 cases) or early hepatocellular carcinoma (22 cases). Expression levels of CD147 on the surfaces of tumor cells were significantly correlated with both prognosis and malignant behavior, such as metastasis and invasion of tumor cells. In particular, CD147 expression in early hepatoma was useful pathodiagnostic information, even if small specimens had been obtained with a fine-needle aspiration biopsy. The tumor-targeting ability *via* CD147 molecules localized on the surfaces of tumor cells was examined with tumor cells expressing CD147 at high levels and their CD147-knockdown sublines. An anticancer drug directed against CD147, MAb12C3-antibody-conjugated doxorubicin, showed specific cytotoxicity against CD147-expressing tumor cells both in *in vitro* and *in vivo* experiments. For early detection, diagnosis, and treatment of cancer by means of ultrasound technology, the MAb12C3 antibody and its active Fab′ fragments were coupled to ultrasonographic contrast agents containing nanobubbles or microbubbles. Methods for effectively detecting accumulated bubbles *in vitro* and *in vivo* are now being investigated.

2. Resistance of tumor cells to chemotherapeutic agents is a serious obstacle in cancer therapy. A conjugate of doxorubicin and glutathione via glutaraldehyde (GSH-DXR) strongly inhibited the glutathione S-transferase (GST) activity of rat hepatoma AH66 cells. Treatment of the cells with GSH-DXR induced apoptosis, including caspase-3 activation, DNA fragmentation, and activation of c-Jun N-terminal kinase (JNK). The stress-induced apoptosis pathway *via* JNK activation was further evaluated by means of a JNK inhibitor, SP600125, and a dominant negative expression of mutant molecules with site-directed mutagenesis (K55R) in the ATP-binding domain of JNK in cells over-expressing GSTP1-1. Phosphorylation of JNK induced by GSH-DXR also resulted in apoptosis with translocation of cytoplasmic Bax to mitochondrial membranes, which was suppressed by GSTP1-1. Additionally, treatment of AH66 cells with GSH-DXR caused deamidation of BcL-xL (N52D and N66D), which was suppressed by GSTP1-1, followed by the induction of apoptosis. Further studies are necessary to

clarify the mechanism.
3. Six cell lines resistant to epoxomicin were established. The epoxomicin-resistant cell lines are reliable tools for therapeutic evaluation of proteasome inhibitors in preclinical trials. Moreover, these cell lines may also be useful for clarifying mechanisms of resistance to proteasome inhibitors and examining a wide variety of proteasomal functions.

Other Research

1. Using methods to purify and identify ubiquitinated proteins in biological materials, we analyzed several ubiquitin-protein conjugates in Tris-saline soluble and Tris-saline-insoluble but 2% sodium dodecylsulfate (SDS)-soluble fractions in cadmium-exposed human proximal tubular HK-2 cells and brains from a model mouse of Niemann-Pick type C (NPC) disease (lipid storage disease with progressive neuronal death). Immunofluorescent-antibody studies demonstrated that HK-2 cells exposed to cadmium at a concentration of 70 μM (median lethal dose) showed a marked but diffuse increase in ubiquitin-protein conjugates without aggresome formation. Mean levels of the SDS-soluble ubiquitin-protein conjugates in the cerebrums of NPC(−/−) mice (4 and 9 weeks old) were significantly higher (up to two fold) than those in wild-type or heterozygous mice.
2. The regulatory mechanisms of transcriptional co-activator with PDZ-binding motif (TAZ)-linked fibroblast growth factor (FGF)/receptor signaling, which plays an essential role in ossification, were investigated with MC3T3-E1 cells. Although TAZ functions as a transcriptional coactivator for RUNX2 and a co-repressor of peroxisome proliferator-activated receptor (PPAR)γ, the regulatory mechanisms of TAZ protein expression are largely unknown. Our findings suggest that FGF2 regulates osteoblast differentiation through TAZ protein expression *via* several signal-transduction cascades.
3. The radial flow bioreactor (RFB) is a highly functioned 3-dimensional culture system that can be used for high-density culture maintaining the original cellular functions and mimicking the architecture of human tissues. Several human cancer cell lines, which were also cultured in this system, were prepared for a well-organized artificial tumor tissue model in vitro, rather than for tumor tissue xenotransplantation into nude mice. These results suggest that the RFB culture method is a useful and powerful system for improving and maintaining conditions during acute liver failure in clinical situations and for studying the safety and efficacy of newly synthesized drugs or biomaterials before clinical use. To evaluate the efficacy of anticancer agents, a detection and evaluation system for reliable cell number in RFB *in situ* was established using a ^{13}C-glucose/$^{13}CO_2$ assay system.

Publications

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Minami J, Takada K, Aoki K, Shimada Y, Okawa Y, Usui N, Ohkawa K. Purification and characterization of C-terminal truncated forms of histone H2A in monocytic THP-1 cells. *Int J Biochem Cell Biol* 2006; **39:** 171–80.