Department of Biochemistry

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Research Activities

Cancer research

1. To establish methods for the early diagnosis and treatment of cancer, several molecular properties of CD147 for cancer-cell — targeting has been evaluated. CD147, as an extracellular matrix metalloprotease inducer or basigin, is a transmembrane glycoprotein with 2 immunoglobulin-like domains. Using a murine monoclonal antibody against CD147 (MAb12C3) developed by Ohkawa, et al. in 1995, several studies were performed with clinical tumor specimens and demonstrated the significant correlations between prognosis and the expression levels of the CD147 protein in tumors and in patients with gynecologic malignancies (130 cases) or early hepatocellular carcinoma (22 cases). The expression levels of CD147 on the surfaces of tumor cells were significantly correlated with prognosis and with the malignant behavior of tumors, such as metastasis or invasion by tumor cells, even if small specimens had been obtained with fine-needle biopsy. The tumor-targeting ability *via* CD147 molecules on the surfaces of tumor cells was determined with tumor cells expressing CD147 at high levels and their CD147-knockdown sublines. The effects of an anti-CD147 antibody — labeled liposome (MAb12C3-liposome) encapsulating a glutathione-doxorubicin conjugate (GSH-DXR) on specific accumulation and cytotoxicity against CD147-expressing human carcinoma cells were studied. After treatment of the cells with MAb12C3-liposome — encapsulated GSH-DXR for 2 hours, specific accumulation and cytotoxicity were observed in CD147-expressing cells but not in CD147-knockdown cells, suggesting that GSH-DXR-encapsulated MAb12C3-liposome would be an effective chemotherapeutic agent for CD147-expressing carcinoma cells. For the early detection, diagnosis, and treatment of cancer with ultrasound technology, the MAb12C3 antibody and its active Fab' fragments were coupled to ultrasound contrast agents, nano/micro bubbles. In hepatoma cells grown in an *in-vitro* 3-dimensional culture, CD147-specific bubbles can be detected with ultrasound devices and with infrared immunofluorescent devices.

2. The resistance of tumor cells to chemotherapeutic agents is a serious obstacle in cancer therapy. The GSH-DXR conjugate 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). Treatment of cells with GSH-DXR induced cytochrome c release from the mitochondria to the cytosol, followed by potent activation of caspase-3 and -9 with typical DNA fragmentation. JNK signaling is thought to be regulated by GST P1-1 via interaction with the C-terminals. In the present experiment, we found the C-terminal region of GST P1-1 binds to the JNK molecule and

that the active center of GST P1-1 plays important roles in the regulation of JNK enzyme activity. The findings suggest that inhibition of GST P1-1 activity by the binding of GSH-DXR to the active center of the enzyme activates JNK and induces apoptosis via the mitochondrial pathway in the cells. This study revealed a novel mechanism by which the enzyme activity of GST controls JNK activity.

3. Six epoxomicin-resistant cell lines 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. This year, the relation between expression of matrix metalloproteinases 14 and 2 and proteasomal inhibition was analyzed.

Other Research

1. With methods to purify and identify ubiquitinated proteins in biological materials, several ubiquitin-protein conjugates in Tris-saline—soluble and Tris-saline—insoluble 2% sodium dodecylsulfate (SDS)-soluble fractions were analyzed from cadmium-exposed human proximal tubular HK-2 cells and the brains of Niemann-Pick type C (NPC) disease (lipid storage disease with progressive neuronal death) model mice. The amino acid sequences of some of the purified ubiquitinated proteins were determined. The HK-2 cells exposed to cadmium at a concentration of 70 μM (median lethal dose) showed a marked increase in ubiquitinated signal transducer and activator of transcription 6, interleukin-4-induced. In NPC mice brains, mean levels of the SDS-soluble ubiquitin-protein conjugates in the cerebrums of NPC (-/-) mice (aged 4 and 9 weeks) were significantly higher (increased 200%) than those of wild-type or heterozygous mice.

2. Regulatory mechanisms of transcriptional co-activator with PDZ-binding motif (TAZ) linked to the fibroblast growth factor (FGF)/receptor signaling, which plays an essential role in ossification, were determined with osteoblast-like MC3T3-E1 cells. We found that FGF-2, which inhibits bone mineralization and stimulates cell proliferation, reduced the TAZ protein expression level in MC3T3-E1 cells. The removal of FGF-2 from the culture medium reversed this reduction and restored the osteoblastic features of MC3T3-E1 cells. Furthermore, FGF-2-induced reduction of TAZ was blocked by an inhibitor specific for stress-activated protein kinase/JNK. These findings suggest that the expression of TAZ protein is involved in osteoblast proliferation and differentiation.

3. The radial-flow bioreactor (RFB) is a high-functioned 3-dimensional culture system that can be used for high-density culture that both maintains original cellular functions and mimics the architecture of human tissues. Several human cancer cell lines that were cultured in this system, rather than tumor tissues transplanted into nude mice, were used to prepare a well-organized artificial tumor tissue model *in vitro*. These results suggest that the RFB culture method is a useful and powerful system for improving and maintaining the conditions in acute liver failure and for assessing the efficacy and safety of newly synthesized drugs and biomaterials before application for clinical use. To evaluate the reduction of efficacy of anticancer agents against tumor

cells cultured in the RFB, gene and protein expressions in A431 tumor cells during culture were compared under physically different environments of 3-dimensional culture in the RFB, in 2-dimensional culture in a monolayer, and in nude mice.

Publications

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