Department of Biochemistry

Kiyoshi Ohkawa, *Professor*
Koji Takada, *Associate Professor*
Tadashi Asakura, *Associate Professor*

Research Activities

Cancer research

1. Glucose metabolism is another target for cancer chemoprevention. CD147 is known as the accessory subunit of heteromeric lactate transporters, monocarboxylate transporters (MCTs), known as the SLC16 family of solute transporters. The MCTs transport lactate across the plasma membrane, and CD147-MCT interaction is required for expression of MCT activity, as well as for trafficking the MCT molecules to the plasma membrane. 3-Bromopyruvate (3-BrPA), a pyruvate/lactate analog, is a potent glycolytic inhibitor and a candidate anticancer agent. Last year, 3-BrPA was shown to be transported into PC-3 cells through the CD147-MCT1 heteromeric lactate transporter complex and to promote cell death using the MCT1/small interfering RNA technique and the small molecular MCT1 inhibitor. The cytocidal activity of 3-BrPA against several cancer cell lines was enhanced under hypoxic conditions, because the expression of CD147 and MCT1 was higher than that under normoxic conditions. To confirm the molecular chaperon function of CD147, interacting proteins were screened with the co-immunoprecipitation method. Results showed the existence of novel endogenous CD147-associated proteins, matrix metalloproteinase (MMP) 3 and carbonic anhydrases (CA9 and CA12), in addition to previously identified proteins, such as MMP1, MCT1, MCT4, and PDZ and LIM domain 7.
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 many tumor cells tested. The mechanism of action of GSH-DXR is induction of apoptosis *via* activation of c-Jun N-terminal kinase by the binding of GSH-DXR to the active center of the GSTP1-1 enzyme. This year, the cytotoxic effects of GSH-DXR-encapsulated polymeric micelles were examined. The GSH-DXR-encapsulated micelles exhibited potent cytotoxicity against cancer cells. Further study will be performed to prepare a polymeric micelle labeled with an anti-CD147 antibody for tumor targeting chemotherapy.
3. Six cell lines with epoxomicin resistance were established. The epoxomicin-resistant cell lines are a reliable tool 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. In an epoxomicin-resistant human endometrial carcinoma cell line, Ishikawa variant, E-cadherin gene (*CDH1*) expression was suppressed *via* overexpression of zinc finger E-box-binding homeobox (ZEB) 1, a transcriptional repressor of E-cadherin. Treatment of parental Ishikawa cells with epoximicin immediately induced ZEB1, followed by transient suppression of E-cadherin expression.

4. The incidence of ovarian clear cell adenocarcinoma is higher in Japan than in North America or Europe. To develop new therapeutic strategies and to more effectively administer current treatments, HAC2 cells were used to examine the relation between intracellular glycogen accumulation, a conspicuous feature of ovarian clear cell adenocarcinoma; the expression of hypoxia-inducible factor (HIF) 1, the main regulator of cellular metabolism in hypoxia; and the exhibition of chemoresistance under such conditions. Expression of several proteins induced by hypoxia, such as HIF1alpha and HIF1 downstream targets, containing glucose transporter isoform1, hexokinase II, pyruvate dehydrogenase kinase 1, and MCT4, were significantly elevated. Hypoxia also induced glycogen accumulation in the cells.

Other research

1. Pharmaceutical plasma products play important roles in controlling many disorders in clinical medicine. However, plasma products are associated with many risks, such as known and unknown infections, because they are usually produced from donated blood. It is an important to supply such pharmaceutical plasma products safely and in sufficient quantities. Our project to produce a large quantity of high-quality human albumin and fibrinogen using a well-defined human hepatocyte cell line cultivated in a radial flow bioreactor has been successful. Concanavalin A affinity-crossed immunoelectrophoresis showed the microheterogeneity of N-linked sugar chains in fibrinogen.
2. Using methods to purify and identify ubiquitinated proteins in biological materials, several ubiquitin-protein conjugates in Tris-saline-soluble and Tris-saline-insoluble but 2% sodium dodecylsulfate-soluble fractions were analyzed from cadmium-exposed human proximal tubular HK-2 cells. Treatment of HK-2 cells with a sublethal concentration of cadmium induced augmentation of water-insoluble but sodium dodecylsulfate-soluble ubiquitin-protein conjugates and insolubilization of the transcription factor signal transducer and activator of transcription (STAT) 6 and, thus, a decrease in normal molecules. To clarify the molecular mechanism of cadmium toxicity to renal tissue, a mouse model of cadmium nephropathy was established in which dose-dependent pathological lesions develop in the renal cortex, and the ubiquitin-protein conjugates in renal tissues were analyzed. Large amounts of the weakly soluble ubiquitin-protein conjugates were produced by cadmium treatment in a dose-dependent manner. Cadmium-induced structural changes in renal STAT6 molecules were also observed. These results support the findings of previous cell culture studies, and the structural damage of cellular proteins, which is caused by cadmium exposure, seem to be involved in the expression of renal cellular toxicity *in vivo*.

Publications

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