Department of Biochemistry

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Research Activities

Cancer research

1. Earlier diagnosis and therapy are the most effective means to control cancer. To establish a noninvasive and effective *in vivo* system for detecting small tumors through molecular targeting/imaging with ultrasound or infrared fluorescence, both CD147 (extracellular matrix metalloprotease inducer, basigin) and tenascin-C (TN-C), molecules that function as promoters of tumor-cell motility and metastasis and as prognostic markers, were selected as targeting molecules for cancer detection. The final goal of our study was to realize antibody-directed early cancer diagnosis with a noninvasive *in vivo* detection method. The usefulness of antibodies against human CD147 and TN-C was first determined biochemically. CD147 is expressed by almost all malignant tumors with the exception of several of those with a neurogenic nature, and the ability of antibodies to recognize CD147 was consistent and effective both *in vitro* and *in vivo*. In contrast, TN-C was detectable in only a few human cancer cell lines. Immunocytologic studies with Alexa 488—labeled rat anti-human TN-C antibodies revealed the distinct but focal and patchy distribution of intracellular TN-C fluorescence with fine intercellular deposits. Further study confirmed that TN-C expression was localized in piled-up cells rather than in flat-growing cells. Spheroid culture, a conventional 3-dimensional (3D) culture method, showed TN-C expression was higher in A431 cells than in monolayer culture. In spheroid-cultured cells TN-C was overexpressed at both the messenger (m) RNA and protein levels with simultaneous downregulation of E-cadherin and upregulation of vimentin, as in the epithelial-mesenchymal transition. Effects of microenvironmental factors on TN-C expression were further examined with a radial flow bioreactor (RFB) for a 3D culture system, which provides tissue architecture and molecular functions mimicking the *in vivo* environment. It is of interest that A431 cells growing in an RFB secreted and accumulated larger amounts of transforming growth factor (TGF) beta 1 in conditioned media than did cells in a 2-dimensional monolayer culture. A431 cells in RFB showed increased expression of the mRNAs of TGF beta 1 and TGF beta receptors 1 and 2. The A431 cells also overexpressed matrix metalloproteinase 7, a downstream molecule of the TGF beta1 signaling pathway, and showed increased release of soluble 80-kDa fragments of E-cadherin in conditioned media time-dependently, resulting in a decrease in the E-cadherin molecule at the cell surface without downregulation of its mRNA expression. This decrease in E-cadherin release promoted the liberation of beta-catenin and its nuclear partner, upregulation of lymphoid enhancer factor (LEF) 1, and accelerated secretion of Wnt protein. Additional upregulation of a transcriptional factor, high mobility group A2 (HMGA2), and the downstream protein Slug was noted. The TGF beta 1-dependent, matrix metallo-

proteinase 7-mediated upregulation of beta-catenin/LEF1 and the TGF beta 1-activated HMGA2 pathways converged to cause Slug overexpression due to the disassembly and further repression of E-cadherin. Goosecoid, a transcriptional repressor of E-cadherin, was also upregulated. Taken all the results described above together, A431 tumor cells cultured in RFB induced the epithelial-mesenchymal transition *via* the TGF beta 1 autocrine loop and overexpressed TN-C both at the mRNA and protein levels, as were molecules related to the epithelial-mesenchymal transition. Other transcriptional factors, Notch/HEY1 and nuclear factor kappa B, were also upregulated. These signals recruited the ECM-cell remodeling and angiogenetic molecules. 3D culture in an RFB is a useful tool for cancer biology, as are nude mice.

2. Glucose metabolism is another target for cancer chemotherapy. CD147 is an accessory subunit of a heteromeric lactate transporter, monocarboxylate transporter (MCT), a member of the SLC16 family of solute transporters. The MCTs transport lactate across the plasma membrane, and CD147-MCT interaction is required for MCT activity, as well for trafficking to the plasma membrane. Three-bromopyruvate (3-BrPA), a pyruvate/lactate analog, is a potent glycolytic inhibitor and candidate anticancer agent. To clarify which transporters are involved in the cellular influx of 3-BrPA, the role of MCTs was examined. The mRNAs of MCT1, MCT2, MCT4, MCT8, MCT9, and MCT14 were expressed at various levelvs in a 3-BrPA-sensitive prostate cancer cell line, PC-3. To determine which MCT molecule was involved in 3-BrPA transport, the expression of MCTs was inhibited by small interfering (si) RNAs. Resistance to 3-BrPA was found only when the PC-3 cells were transfected with the MCT1 siRNA. PC-3 cells pretreated with MCT1 inhibitors were also resistant to 3-BrPA. Furthermore, short hairpin RNA expression vectors specific for CD147 reduced the sensitivity of PC-3 cells to 3-BrPA. These results suggest that the MCT1-CD147 complex is essential for 3-BrPA uptake.

3. Resistance of tumor cells to chemotherapeutic agents is a serious problem in cancer therapy. A conjugate of doxorubicin and glutathione *via* glutaraldehyde (GSH-DXR) strongly inhibited glutathione *S*-transferase (GST) activity in rat hepatoma AH66 cells. Treatment of the cells with GSH-DXR induced apoptosis *via* activation of c-Jun N-terminal kinase (JNK) by the binding of GSH-DXR to the active center of the GSTP1-1 enzyme, including cytochrome c release from mitochondria to cytosol, caspase-3 activation, and DNA fragmentation. Through the treatment of the cells with GSH-DXR in a recent study, another possible cytotoxic mechanism after the activation of JNK was demonstrated: the induction of apoptosis *via* deamidation of B-cell lymphoma 2, extra large, followed by the translocation of Bax to mitochondria.

4. Six cell lines with resistance to epoxomicin were established. The epoxomicin-resistant cell lines are reliable tools for the 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 E-cadherin expression and proteasomal inhibition was analyzed.

Other research

1. Regulatory mechanisms of transcriptional co-activator with PDZ-binding motif (TAZ) protein linked to the fibroblast growth factor (FGF)/receptor signaling, which plays an essential role in ossification, were determined with osteoblast-like MC3T3-E1 cells. It was been found that FGF-2, which inhibits bone mineralization and stimulates cell proliferation, reversibly reduced TAZ protein expression in MC3T3-E1 cells. Recent studies have shown that FGF-2 and adipogenic differentiation are related and that the TAZ protein acts as a transcriptional regulator during the differentiation. When preadipocyte-like cells were cultured with FGF-2, expression of the mRNA of aP2, an adipocytic differentiation maker transcribed by peroxisomal proliferator-activated receptor (PPAR) gamma, was increased. In contrast, FGF-2 significantly decreased the expression of TAZ, which is a corepressor of PPAR gamma. These results suggest that the adipogenic differentiation involved in FGF-2 results from the reduction of the TAZ level.

2. With 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 and brains of Niemann-Pick type C (NPC) disease (lipid storage disease with progressive neuronal death) model mice. Some of purified ubiquitinated proteins were determined with amino acid sequencing analysis. The HK-2 cells exposed to cadmium at a concentration of 0.07 mM (median lethal dose) showed a marked increase in levels of ubiquitinated signal transducer and activator of transcription 6. Mean levels of sodium dodecylsulfate—soluble ubiquitin-protein conjugates in cerebrums of NPC (−/−) mice (4 and 9 weeks old) were significantly higher (up to twice as high) than in wild-type or heterozygous mice.

Publications

Shimada Y, Fukuda T, Aoki K, Yukawa T, Iwamuro S, Ohkawa K, Takada K. A protocol for immunoaffinity separation of the accumulated ubiquitin-protein conjugates solubilized with sodium dodecyl sulfate. *Anal Biochem* 2008; **377:** 77–82.

Asakura T, Maeda K, Omi H, Matsudaira H, Ohkawa K. The association of deamidation of Bcl-xL and translocation of Bax to the mitochondria through activation of JNK in the induction of apoptosis by treatment with GSH-conjugated DXR. *Int J Oncol* 2008; **33:** 389–95.