macroscopically infiltrative type of esophageal carcinoma from the viewpoint of tumor angiogenesis.

Methods and Materials: A total of 40 surgically resected esophageal carcinoma tissues without preoperative treatment were selected at random from macroscopically localized type (n=20) and infiltrative type of esophageal carcinoma (n=20). The highest intra-tumoral microvascular density, Ki67 labeling index, and expression of VEGF in each section were estimated. The highest microvascular density was estimated in a magnification of x200 field where showed the most developed neovascularization in the tumor.

Results: The highest microvascular density was significantly (p=0.0006) greater in the infiltrative type than in the localized type, and Ki67 labeling index (p=0.022) were significantly lower in the infiltrative type than in the localized type. The expression level of VEGF was significantly (p

994 POSTER

The Casein kinase 1 delta (CK1 delta) specific inhibitor IC261 impinges growth of pancreatic tumor cells and the expression of CK1delta in healthy young adult BALB/c mice

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Background: During the development of pancreatic tumors mutations in oncogenes and tumor suppressor genes and alterations in signal pathway occur. CK1 delta, especially CK1delta, mediated signals seem to play an important role in insuring genome integrity. Alterations in CKIδ mediated signals may play an important role in pancreatic tumorigenesis.

Methods and animals: CK1delta expression levels were analyzed in ASPC1, BXPC3, Capan1, Colo357, Panc1, Panc89, and PancTu1 by Western blotting. Cells treated with the CKIδ specific inhibitor IC261 were analyzed by FACS analysis at different time points (12, 24, 36, and 60 hours). In addition, immunofluorescence (IF) analyses were performed using a polyclonal rabbit anti pericentrin serum. Tissue specific distribution of CK1delta in perfusion fixed, paraffin embedded pancreatic tissue of 4 to 6 weeks old BALB/c control and IC261 treated (1mM) mice were detected by the CK1delta specific polyclonal antiserum NC10.

Results: Different time courses indicated good response between 0.4 and 1.6μM IC261 in PancTu1 cells. Therefore, different pancreatic tumor cells were treated with IC261 (1 μM). Our FACS analysis revealed a cell line specific sensibility towards IC261 which lead to cell death or to cell cycle arrest in a G1 like status. Furthermore, structural changes and amplifications of centrosomes could be detected by IF. Immunohistochemistry of CKIδ in the pancreas of young adult BALB/c mice revealed a finely granulated staining in the exocrine part in the cytoplasm of the acini cells, the intralobular, and interlobular ducts. The cytoplasm of cell types in the endocrine part was strongly positive. Inhibition of CKIδ activity by IC261 was accompanied with a reduced CK1' staining in the whole pancreas.

Discussion: Our results show that inhibition of CKIδ by IC261 differentially effects the growth the pancreatic tumor cells and reduces CKIδ levels in the pancreas of mice. Therefore, down regulation of CK1' could be used as a new approach in the treatment of pancreatic cancer.

995 POSTER

A novel adenoviral vector encoding angiogenin cDNA from cancerous liver tissue

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Background: Angiogenin is a 14 kDa protein with a potent angiogenic effect and a poor ribonuclease activity. In previous reports, these activities have been studied with the use of recombinant proteins. To further characterize these functions, we have constructed a replication-defective adenoviral vector with an angiogenin cDNA isolated from a hepatoma library (Neznanov N *et al.*, Mol.Biol Moscow 1990), which differs in its 5'UTR from the original cDNA reported in non-cancerous liver tissue (AV646980).

Material and Methods: 1) Construction of the adenoviral vector: the human angiogenin cDNA was isolated from the pBluescript SK-Angio plasmid with BamH1 / Xho1 restriction. This fragment was cloned into the pcDNA1 plasmid, further isolated by restriction with Not1/EcoRV and sublconed into the pAdTrack-CMV plasmid. All plasmids were evaluated by restriction analysis, PCR and/ or sequencing. The construction of E1a-, partially E1-b, and partially E3-deleted vectors based on human adenovirus type 5 Ad vectors was carried out as previously described (He, 1998). The resultant viruses were purified by ViraPrep columns© and quantitated by OD 260/280 and plaque assay. 2) Human fibroblasts and HeLa cells were infected at different m.o.i. (1 to 20) in serum free media for two hours; 3) Flow cytometry analysis was used to evaluate the transfection efficiency and survival rates; 4) RNA and protein extractions were carried out at 24 and 48 hours after infection, respectively; 5) RT PCR and further amplification of the 5'UTR and coding regions was achieved by two different sets of primers; 6) Western Blot analysis was carried out as described elsewhere using a polyclonal antibody against human angiogenin.

Results: An adenoviral vector containing the Green Fluorescence Protein and angiogenin genes under the transcriptional control of the CMV promoter has been constructed (Ad-Angio-GFP). The transfection efficiency of this virus was over 90% in fibroblasts and HeLa cells. No cytopathic effects were observed even at the highest dose tested (i.e. 20 m.o.i). The expression of the exogenous angiogenin gene was dose dependent in both cell lines. Interestingly, we could also amplify the 5'UTR of our angiogenin transgene in cDNA from non infected-HeLa cells, but not from intact fibroblasts' cDNA. The angiogenin protein could be tracked down by WB in both, cellular extracts and culture media. Thus, it seems that the angiogenin protein can be secreted by infected cells. The concomitant expression of GFP allowed us to monitor the expression of the transgenes in all conditions.

Conclusion: a novel adenoviral vector that expresses an angiogenin transcript found in cancerous liver tissue, has been constructed. The functional activity of the encoded protein is under study.

996 POSTER

Effect of retinoic acid analogue on tumor growth and angiogenesis

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Background: Retinoic acid (RA) can be regarded as a pharmacological agent that is commonly used for its ability to affect growth and differentiation of a variety of cell types, such as several tumor and endothelial cells. In the present work, we studied the effect of all-trans RA (ATRA) and its analogue EA4 on the growth of several human prostate normal epithelial and tumor cell lines in vitro, as well as the formation of new capillaries, in the in vivo chicken embryo chorioallantoic membrane (CAM). Methods ATRA was purchased from Sigma Chemical Co. and the modified steroid EA16 was prepared as described in literature. For the synthesis of the final esteric derivative EA4 the method of esterification with unsymmetrical anhydrite was applied. At first step the unsymmetrical anhydrite of ATRA with 2,4,6-trichlorobenzoylchloride was prepared and then by adding the EA16, under the appropriate conditions, the desired product EA4 was obtained. The biological evaluation of ATRA, EA16 and EA4 was performed on the human prostate cell lines PC3, LnCap and PNT1. The MTT assay was used to measure the number of cells after treatment with different concentrations of the various agents for several time periods.The effect of the agents on angiogenesis in the chicken embryo CAM, as well as on the morphology of the tissue, was estimated in tissue paraffin sections stained with haematoxylin and eosin.

Results: ATRA caused a slight decrease in the number of prostate cells only at the concentration of 10-5 M. Higher concentrations could not be tested because of solubility problems. The analogue EA4 significantly decreased the number of tumor but not normal prostate cells, in a dose-dependent manner. This decrease was significant even at concentrations lower than 10-7M of EA4 and was not due to the steroid component (EA16) of the molecule. ATRA and EA16 induced angiogenesis in the CAM and moreover, ATRA increased the layer of CAM keratinocytes and induced the deposition of fibrin matrix. EA4 had no effect on either angiogenesis or tissue structure in general.

Conclusions: The retinoid EA4 seems to be a promising agent for the inhibition of tumor prostate cell growth.

997 POSTER

Activity of trastuzumab plus vinorelbine in patients with erb-B2 overexpressing metastatic breast cancer

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Introduction: Trastuzumab (T) is an anti-erb-B2 humanized monoclonal antibody with activity in patients with erb-B2 overexpressing metastatic breast