common eliminated region (CER) at 3p21.3 and an eliminated region (ER2) at 3p21.1-p14.2 (Kholodnyuk et al., 1997). ER2 borders at but does not include the FHIT gene, considered as a putativeTSG. We have found that FHIT was deleted at the DNA level in 17 of 21 tumors. The remaining 4 of 21 had no FHIT transcript. Later we have generated new SCID tumors that remained PCR-positive for all of the chr3-markers tested ("PCR+" tumors). FISH chromosome painting showed normal intact chr3 in 65-98% of MCHs cells and in 16-75% of 'PCR+' tumor cells. FHIT was expressed in vitro in 5 out of 7 MCH lines. All 'PCR+' tumors had no FHIT transcript. Our compiled data have shown that FHIT was either physically or functionally impaired in all 34 of the 34 analyzed tumors (Kholodnyuk et al., 2000).

The purpose of the present work was to examine the "PCR+" tumors by RT-PCR for the expression of 30 human chr3p genes located within CER1, ER2, and the regions that were homozygously deleted (HD) in a variety of carcinomas.

Results: FISH-RP has indicated losses over 3p26-3p25, 3p24, 3p22, 3p21 and 3p14 in 6 'PCR+'tumors derived from two MCHs, but not in 3 'PCR+' tumors derived from the third MCH. We have examined the expression of 30 human chr3p genes: among them 6 genes located within CER1 (LIMD1, CCR1, CCR2, CCR3, CCR5, LTF), and 12 genes located within regions that were homozygously deleted in a variety of carcinomas. We have found that the majority of the genes analyzed, including VHL, TGFBR2, MLH1, ITGA4L, SEMA4, SEMA5, BLU, LUCA1, PTPRG and DUTT1, were expressed in the MCH lines in vitro and in the derived SCID tumors. No transcripts of the four CCR genes, MYL3 or TGM4 have been detected in any of the MCH lines. Comparative duplex RT-PCR revealed the significant reduction of the amount of GNAI2 (3p21.3) transcript and DLEC1 (3p22) transcript in SCID tumors versus the MCH lines in vitro. In the addition to the FHIT gene, LTF (CER1), DRR1 (ER2) and LUCA2 (SCLC HD) have lost their expression in SCID tumors. These genes may function as tumor suppressor genes. Further studies of the biological activity of these genes are needed to clarify the role of these genes in tumorigenesis.

1025 ORAL

A new variant of cystein-rich FGF receptor (CFR-1) as target for mitotic antibody with possible diagnostic value in gastric cancer

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Purpose: In several human diseases autoantibodies are discussed to play a crucial role in inition and maintenance. In development of gastric carcinoma there is evidence that they are involved in inducing or enhancing proliferative changes of epithelial cells in the stomach mucosa. Here we describe the identification of the receptor of the mitogenic antibody 103/51 as a new variant of CFR-1 (cystein-rich fibroblast growth factor receptor). Expression of the CFR-1 variant was tested by immunochemistry on various tissues and cancerous lesions.

Method: The receptor of antibody 103/51 was purified by chromatographic methods and idenfied by MALDI-analysis. Binding of antibody 103/51 to CFR-1 was proved by transfection of stomach carcinoma cell lines with a CFR-1-antisense vector and the antigenic site to be a carbohydrate residue by specific protein deglycosylation. A murine monoclonal antibody against CFR-1 was prepared, which has identical immunohistochemical and stimulating properties. Expression pattern of CFR-1 was shown by immunohistochemical staining. Physiological in vitro effects were investigated by Western blot analysis and MTT-proliferation assay

Results: The antibody 103/51 enhances profileration of stomach cancer cell lines in vitro by binding to a carbohydrate residue of a CFR-1 isoform. The stimulation is dose-dependent and results in the phosphorylation of various proteins. The expression pattern of the described CFR-1 isoform is very restricted on normal tissues while it is widely expressed on cancerous tissues. Most interestingly, the receptor is also present in Helicobacter pylori gastritis and gastric dysplasia, where it is expressed on proliferating cells, while it is absent on non inflammed stomach mucosa.

Conclusion: Autoantibodies like antibody 103/51 can serve as ligands for receptors and can influence cell cycle control of the epithelial cell, and might be involved in the cancerogenesis of stomach carcinoma. The antibody 103/51 is also of diagnostic value, since the tissue distribution shows that the CFR-1 molecule is correlated with cellular activation and proliferation demonstrated by staining with antibody Ki67. The data show that human antibodies are a helpful tool for understanding of tumor-related mechanisms and for the discovery of new diagnostic targets.

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Role of the tyrosine kinase lck for the induction of apoptosis in response to ionizing radiation

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Introduction: Preliminary studies revealed that the tyrosine kinase p56/lck is involved in caspase-8 activation and apoptosis in response to ionizing radiation. However, the definite role of tyrosine kinases for apoptosis regulation remains unclear. Our purpose was to define the relative position of p56/lck within the apoptotic signalling pathway.

Materials/Methods: The induction of apoptosis 12, 24, 36, 48 and 72h after irradiation with 10 Gy was quantified in Jurkat T cells, JCaM1.6(without lck), JCaM1.6lck+ and Jurkat/Bcl-2 employing FACS analysis and fluorescence microscopy (Hoe33342). In parallel, apoptosis-induction via the CD95 Death-receptor was determined after 6, 12, 24, 36 and 48 h. Activation of caspase-9, -8, -3 and PARP-cleavage was analysed by western blotting. The integrity of the mitochondrial function (DYm) was determined by TMRE-staining and flow cytometry.

Results: Induction of apoptosis after irradiation was almost completely blocked in p56/lck-negative JCaM1.6 cells, retransfection of lck restored the capacity to undergo apoptosis. The kinetics of CD95-receptor-mediated apoptosis was delayed. There was no difference in the baseline expression of CD95, FADD, caspase-8,caspase-3, Bcl-2, MCL-1 and Bcl-x, as determined by western blotting. Analysis of DYm revealed an delayed breakdown of DYm after CD95-stimulation; after irradiation, no breakdown of DYm occurred. The comparison with Bcl-2-overexpressing cells showed marked differences regarding CD95-mediated apoptosis which remains nearly unaffected by Bcl-2.

Conclusion: The lack of p56/lck causes a generalized apoptosis defect. The absence of lck most probably influences the signal transduction at the level of the mitochondria.

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Expression, function and clinical implications of the estrogen receptor (ER) beta in human lung cancers

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Purpose: The higher frequency of human lung adenocarcinoma in females than in males, strongly suggests the involvement of gender dependent factors such as sex hormones in the etiology of this disease. Although there have been several reports of expression of ER-alpha in lung cancer, the results are inconsistent and controversial. Here, we assessed the expression of ER-beta in various human lung tissues and address the question of its physiological functions.

Methods & Results: Immunohistochemistry using an ER-beta polyclonal antibody revealed ER-beta protein expression in normal bronchiolar epithelium and all foci of atypical adenomatous hyperplasia (AAH), considered as a pre-cancerous lesion for adenocarcinomas. Adenocarcinomas showed significantly higher expression of ER-beta than squamous cell carcinomas, and especially the hobnail type, which tends to occur in females, was consistently positive. On the other hand, ER-alpha expression was not detected in any of the cases we tested. The functional integrity of ER-beta in lung cancer cells was confirmed using a RERF-LC-OK human lung cancer cell line, in which ER-beta protein expression was the highest of all 16 lung cancer cell lines examined. Binding ability to estrogen responsive elements (ERE) was observed in electrophoretic mobility shift assay. Transcriptional activity was assessed by transient transfection of an ERE-luciferase reporter plasmid, shown to be slightly but significantly stimulated by 17beta-estradiol, and suppressed by a pure antiestrogen, ICI 182,780 (ICI). Colony formation was significantly reduced in the presence of ICI, both anchorage dependent and independent growth.

Conclusion: ER-beta but not ER-alpha is present in lung tissues with an important physiological function in normal lung. Furthermore, ER-beta may play a role in growth and development of adenocarcinoma. Finally, we propose that pure antiestrogen may be useful for hormone therapy of certain types of lung cancer.