Results: Cell cycle analysis after treatment with CPT-11 in G0/G1 synchronized cells demonstrated the activation of transfected wild-type p53 and a consequent $p21^{WAF1/CIP1}$-dependent cell cycle blockage in S phase. Activated wt-p53 also increased apoptosis, leading to enhanced sensitivity to CPT-11. DNA microarray analysis showed that, in p53-deficient cells, the cell cycle regulatory machinery did not respond to CPT-11, leading to the accumulation of the G2/M cdk1/cyclin B complex. We found subsequent p53-independent activation of the cdk-inhibitor $p21^{WAF1/CIP1}$, which prevented cell cycle progression. We further exploited cdk1 induction in p53-deficient cells to improve the sensitivity to CPT-11 by additional treatment with the cdk-inhibitor roscovitine.

Conclusions: We demonstrate a gain of sensitivity to CPT-11 in a p53 mutated colon cancer cell line both by restoring wild-type p53 function or by additional treatment with a cdk-inhibitor. Considering that mutations in p53 are among the most common genetic alterations in colorectal cancer, a therapeutic approach that specifically targets tumors with mutated p53 could greatly improve the treatment outcomes.

965 POSTER

Molecular therapy for peritoneal dissemination of gastric cancer with adenovirus-mediated Bax gene transfer

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Gene therapy is one of the candidates for an innovative therapeutic approach against cancer. An adenoviral vector expressing the tumor suppressor p53 gene (Ad/p53) is currently under clinical evaluation in various cancers. We have recently developed a binary adenoviral vector system that can express the strong apoptotic Bax gene (Ad/PGK-GV16+Ad/GT-Bax: Ad/Bax). To evaluate the potential of Bax gene therapy for gastric cancer, we assessed the antitumor effect of the Bax gene in comparison with the p53 gene. The responses of human gastric cancer cell lines, MKN-1, MKN-7, MKN-28 and MKN-45, to recombinant adenoviruses, Ad/Bax or Ad/p53 were assessed in vitro. Cell viability was measured by XTT assay; transgene expression and caspase activation were analyzed by western blotting; and morphological feature of apoptosis was observed by Hoechst staining. The treatment with Ad/Bax or Ad/p53 resulted in the marked Bax or p53 protein expression and effective apoptosis induction in MKN-1, MKN-7, and MKN-28 cells. In contrast, MKN-45 cells showed resistance to Ad/p53 and only the treatment with Ad/Bax resulted in massive apoptosis. To compare antitumor effects between the Ad/Bax and Ad/p53 treatment in vivo, MKN-45 subcutaneous tumors were generated by inoculation of 2×10^6 MKN-45 cells into the dorsal flank of nude mice. When tumor had reached a diameter of about 3-5mm, each mouse was given intratumoral injection of 100 μl of 2×10^{10} particles of each virus. Mean tumor volume of the Ad/p53 group was 515.2± 151.9 mm^3, while that of Ad/Bax was only 236.5± 83.8 mm^3, as of 35 days after inoculation. Furthermore, peritoneal dissemination of MKN-45 cells were generated in nude mice, and each mouse was treated by intraperitoneal injection of 200 μl of 2×10^{10} particles of each virus. Disseminated tumor numbers and weights were assessed 24 days after inoculation. Similarly, mean total tumor weight of the Ad/p53 group was 371.8± 44.0 μg, while that of Ad/Bax was 161.9± 96.9 μg. The treatment with Ad/Bax significantly inhibited the growth of p53-resistant gastric cancer in vitro and in vivo. Therefore, our results suggest that Adenovirus-mediated Bax gene transfer may be useful in gene therapy for gastric cancers.

966 POSTER

Activation of a plasma membrane-cationic channel and apoptosis in prostate cancer cells overexpressing Bax

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Background: Our group has previously identified and characterized a 23 pS non selective-cation channel (NSC channel) in prostatic cancer-LNCaP cells undergoing apoptosis (J Physiol, 1999, 517:95-107). The activation of the channel was only induced by pro-apoptotic stimuli including ionomycin, thapsigargin, staurosporine or serum depletion. Accordingly, channel activity was never registered in intact cells. Further studies using an anti-Bax antibody suggested that the channel opening could be mediated by Bax proteins (Biophys J 2001, 80:2764). To test this hypothesis, we have induced cell death in prostatic cancer LNCaP cells using an inducible Bax adenoviral vector generated by a Cre/loxp system.

Material and Methods: Adenoviruses were replicated, purified and tittered by plaque assay, as described elsewhere (). For overexpressing Bax protein, cells were coinfected with a Bax recombinant adenovirus (Ad/Bax) and the inducing adenovirus Ad/Cre in a 5:1 relation (kind gifts of Dr D. Curiel's group, Mol Ther 2000, 1:545-554). LNCaP cells (2×10^5/ well) were infected at an m.o.i. of 5. The transgene encodes a fusion protein containing Bax and a hemaglutinin (HA) tag. Patch clamp in the cell-attached configuration was used to evaluate the opening of the 23 pS NSC channel at 20 to 24 hours post infection. Protein extraction and Western Blot analysis were carried out at 24 hours for evaluating the expression of endogenous Bax and Bax-HA proteins. Cell death was studied by fluorescence activated cell sorter analysis of annexin 5/ iodide propidium and by crystal violet staining at 72 hrs.

Results: The 23 pS NSC channel was only registered in cells co-infected with AdBax/Ad Cre but neither in control cells nor in cells co-infected with AdBax/AdTK. Overexpression of the Bax-HA fusion protein (27 kDa) was confirmed by WB in the former cells. Cell death occurred in over 90% of Ad Bax/AdCre cells at 72 hours.

Conclusions: The sole overexpression of Bax induces apoptosis and activates a 23 pS non selective-cation channel in LNCaP cells. The significance of the opening of this channel in early stages of apoptosis is under study.

967 POSTER

The detection of metastatic cancer cells in peripheral blood using reverse transcriptase polymerase chain reaction for CK 19

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Metastatic cancer cells spread is not detectable by conventional staging methods, but the combination of immunomagnetic cell enrichment and reverse transcription (RT-PCR) is an efficient method to identify small numbers of disseminating tumour cells in blood or bone marrow of patients with cancer. Our aim is to determine whether RT-PCR for cytokeratin 19 (CK19) provides a sensitive method for the detection of a single metastatic cell in peripheral blood.

In several spiking experiments, cell-line (ZR-75) derived tumour cells (2-100 cells) were added to 5ml of human peripheral blood of a healthy donor. We designed a semi-junctional CK19 specific primer set. Human peripheral blood without added tumour cells was used as our specificity control. After enrichment, mRNA was extracted using Oligobead mRNA extraction and a silica-based total mRNA extraction method. Products were amplified by a single-enzyme RT-PCR for CK-19 mRNA. Amplicon were visualised on 2% agarose gel.

Total mRNA extraction from unspiked blood samples followed by RT-PCR yielded in falsepositive amplification due to circulating hemaptopoietic elements. Immunomagnetic enrichment drastically improved the specificity for the CK-19 RT-PCR assay. Both mRNA extraction methods demonstrated similar sensitivity levels. Our detection sensitivity for the combined method including cell enrichment, RNA extraction and subsequent RT-PCR is less than 3 cells.

Immunomagnetic enrichment combined with CK19 RT-PCR is a very sensitive and specific method to detect disseminating tumour cell in peripheral blood of breast cancer patients. Further evaluation by real time quantitative PCR and using other specific breast markers is needed.

968 POSTER

FCU1: a highly potent suicide gene therapy based on 5-FU

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Background: Direct transfer of pro-drug activation systems into tumours was demonstrated to be an attractive method for the selective in vivo elimination of tumour cells. Besides its local cytotoxic impact, this strategy was further demonstrated to enhance the host anti-tumour immune response through the local release of cellular debris that can be presented by the antigen presenting cells.

Material and methods: We describe a novel and highly potent suicide gene derived from the Saccharomyces cerevisiae cytosine deaminase (FCY1) and uracil phosphoribosyltransferase genes (FUR1). This suicide