tosis is due to up-regulation of the receptors and downstream sensitization of the respective pathways.

983 POSTER

The impact on human renal cell carcinoma cell line by transfection of Cox-2

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Background: Cyclooxygenase (Cox)-2 is an inducible isoenzyme of Cox that catalyzes the rate-limiting step in arachidonate metabolism. Many studies have indicated that Cox-2 plays an important role in carcinogenesis in several neoplastic diseases. We had reported there is Cox-2 expression in some human renal cell carcinoma (RCC). In present study, the impact on human RCC cell line by transfection of Cox-2 were investigated.

Materials and Methods: The expression vectors containing full-length sense and antisense cDNA of Cox-2 were constructed using the mammalian expression vector, pTargeT (Promega). Transfection of OS-RC-2, the human RCC cell line which overexpress Cox-2, with pTargeT/*Cox-2 sense*, pTargeT/*Cox-2 antisense* or vector control was done using LIPOFECTAMINE PLUS. The expression of Cox-2 in transfectants were detected by Western Blot and the production of PGE2 and VEGF by transfectants were examined by ELISA. Sensitivity of transfectants to apoptosis inducer, butyric acid, was observed by Fluorescence-activated Cell Sorting (FACS). The expression of CD44 in transfectant were detected by FACS, and the expression of MMP2 and MMP9 in transfectant were detected by FACS and zymography. The tumorigenicity of the transfectants were observed in nude mouse.

Results: The expression of Cox-2, PGE2 and VEGF were increased in sense transfectant and remarkable decreased in antisense transfectant. The sensitivity of antisense transfectants to apoptosis inducer was significantly higher than sense transfectant and the parental OS-RC-2. Although no difference of the expression of MMP2 and MMP9 observed between sense and antisense transfectant, there was significant difference of the expression of CD44 between sense and antisense transfectant. The result of tumorigenicity showed that overexpression of Cox-2 in sense transfectant can enhance tumorigenicity contrast to blocking Cox-2 expression in antisense transfectant.

Conclusions: Cox-2 expression may be related with some RCC carcinogenesis. Blocking Cox-2 expression in RCC cell line through anti-sense strategy suppressed growth of the cells in vitro and in vivo, as well as increased sensitivity of the cells to apoptosis inducer. These findings are suggestive of a new therapeutic strategy for some RCC through targeting Cox-2 expression.

984 POSTER

Interaction between Interferon-beta and cellular DNA-repair

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Background: Pretreatment of tumor cells with Interferon-beta (IFN) has reported radiosensitizing activity, associated with a decline of the shoulder and an increase of the alpha-component of the survival curve. We tested the possibility of an interaction between IFN-treatment and the regulation of the repair of radiation-induced damage.

Materials and Methods: The following cell lines were used: MCF-7, WiDr, ZMK-1, A549 expressing either wild type or mutated TP53, and DNA-PK-proficient M059K and DNA-PK-deficient M059J cells. Cells were incubated with IFN 24 h before irradiation, and cycling or confluent cultures were used. HDR irradiation was either given as single dose between 1 and 6 Gy or as split dose. LDR irradiation was given at total doses of 5.45 Gy and 12.5 Gy. DNA rejoining was measured by constant gel electrophoresis. The repair capacity of M059-K cells was inhibited by wortmannin treatment. Cytotoxicity was evaluated by a standard colony-forming assay; and survival curves were fitted by the linear-quadratic equation. Sensitizer enhancement ratios were calculated, and isobologram analysis was applied to test the IFN-radiation interactions. Apoptosis was determined morphologically.

Results: Sublethal damage repair was strongly inhibited after IFN treatment, with recovery ratios decreasing form 1.14 to 0.96 in cycling cells and from 1.59 to 1.00 in confluent cells. LDR irradiation of WiDr cells resulted in an inverse dose rate effect, which, after IFN-treatment, increased dramatically to a sparing ratio of 0.222 for cycling cells. There was no increase in initial DSBs and no alteration of DNA rejoining after IFN treatment. M059J cells showed a supraadditive, M059K cells an additive IFN-radiation interaction. In repair-inhibited M059K cells, we found an increase in IFN-induced radiation cytotoxicity. The TP53-status did not influence IFN-induced radiosensitization of A549 cells. Incubation with 3000 I.U./ml IFN enhanced the radiation-induced apoptosis in MCF-7 and ZMK-1 cells, but not in A549 cells.

Conclusions: In general, the sensitizing ability of IFN was higher in cycling cells compared to confluent cells and did not depend on the TP53-status. Increased radiation-induced apoptosis may play a role at high IFN concentrations. All results are pointing towards an interaction between IFN, or IFN-induced proteins and the regulation of the repair of radiation-induced damage as the predominant mechanism of IFN-related radiosensitization.

985 POSTER

Molecular mechanisms of G1 arrest of Antineoplaston AS2-1 against colon cancer.

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Introduction: Antineoplastons are naturally occurring peptides and amino acid derivatives found in human blood and urine, were first described by Burzynski in 1976. The small peptides reportedly control neoplastic growth and have minimum adverse effects. However, the molecular mechanisms by which Antineoplastons exert antitumor effects are not known. Characteristics of Antineoplaston were consider now that antagonism against l-glutamine and cell growth arrest to intercalation between DNA base pair, interaction of oncogenes and activation of suppressor genes to normalization of methylation status. In the present study, we have investigated the antiproliferative effect of Antineoplaston AS2-1 against colon cancer, and its influence to cell cycle.

Methods: We tested effects of Antineoplaston AS2-1 on *in vitro* and *in vivo* cell growth activity using human colon cancer cells (KM12SM, SW620, SW1417, Colo205). And we analyzed cell cycle of the Antineoplaston treated cells by FACS and investigated expression of cell cycle related factors by Western Blot.

Results: Antineoplaston AS2-1 inhibited the proliferation of all human colon cancer cells in a dose and a time dependent manner *in vitro*. Antineoplaston AS2-1 also inhibited the growth of implanted human colon cancer (KM12SM, SW620) in nude mouse in a dose and a time dependent manner *in vivo*. The cell cycle analysis demonstrated cell arrest at the G1 phase by treatment with Antineoplaston. The protein levels of cyclin-dependent kinase (cdk)-2, cyclin E, cdk-4, and cyclin D in the cells decreased and the levels of p16 and p21 increased in a time and dose dependent manner by Antineoplaston treatment. Antineoplaston AS2-1 also down-regulated the levels of the phosphorylated Rb protein.

Conclusion: Antineoplaston AS2-1 shows antiproliferative effect through the G1 cell arrest in colon carcinoma.

986 POSTER

Immunization with mutant p53- and K-ras-derived peptides in cancer patients: immune responses and clinical outcome

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Purpose: Many human cancers are associated with mutations in dominant and recessive oncogenes including *K-ras* and *p53* and frequently express mutant K-ras and p53 that are uniquely present in a patient's cancer cells but not in the normal tissue. Immunization with individual patient tumor-specific mutant peptides was evaluated for clinical use to induce tumor-specific cytotoxic T lymphocytes (CTL) against the tumor while avoiding immune reaction against normal organs. The aim was to assess 1) cellular immunity specific to an individual patient's tumor, 2) to assess whether such immunity can be induced or boosted by immunization with a synthetic peptide specific to the mutation in K-ras or p53, 3) to assess the toxicity of oncopeptide immunization, and 4) to monitor clinical outcome.

Experimental Design: Patients with common human cancers underwent genetic analysis for suitable mutations in *ras* and p53. 39 patients were enrolled and vaccinated at least once. Patients had 17-mer peptides custom synthesized to their corresponding mutations. Baseline *in-vitro* immunological assays were performed to assess CTL response against mutant peptide-pulsed target cells and interferon-γ(IFN-γ) release from lymphocytes primed with mutant-peptide. Peripheral blood mononuclear cells were harvested and pulsed with patient's corresponding mutant-specific peptides, irradiated, and used for intravenous immunization schedule on days 0, 21, 77, and 133. Patients were followed for CTL, interferon-γ, IL-2, IL-5 and GM-CSF responses. They were also followed for signs of treatment-related toxicity and tumor response or progression.

Results: Toxicity potentially attributed to vaccination did not exceed grade I. Ten of 35 (29%) patients evaluated had a detectable cellular immune response against mutant p53 or ras and two patients had a positive CTL assay at baseline. Positive responses to IFN-γoccurred in twelve patients (34%) after vaccination, whereas six patients had positive IFN-γreaction to mutant peptides prior to vaccination. Of 27 patients with evident disease five had a period of stable disease, but all progressed eventually. Detectable cytotoxic lymphocytes or a positive IFN-γ release, but not IL-5 release, after peptide stimulation with mutant p53- or ras were associated with prolonged median survival. A positive IFN-γ response was associated with longer survival in multivariate analysis.

Conclusions: Custom made peptide vaccination is feasible without relevant acute or delayed toxicity. Pre-vaccination cellular immunity may exist and CTL and cytokine responses specific to a given mutation can be induced or enhanced. Evidence of cellular immunity to mutant p53 and K-ras oncopeptides was associated with longer survival.

987 POSTER

DNA encoding a Pan-MHC class II peptide analogue augmented antigen-specific cellular immunity and suppressive effects on tumor growth elicited by DNA vaccine immunotherapy

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Background: Activation of both helper and cytotoxic T cell function are required for eliciting a potent anti-tumor immunity capable of suppressing tumor progression by cancer immnotherapy. We previously reported that a sixteen-amino acid peptide analogue derived from pigeon cytochrome c (Pan-IA peptide) can bind a broad range of MHC class II types and activate the helper T function in mice. We addressed whether DNA encoding Pan-IA peptide can enhance the efficacy of tumor suppression by DNA vaccine targeting tumor antigens.

Material and methods: Pan-IA DNA was injected with DNA encoding a model antigen, ovalbumin (OVA) into C57BL/6 mice intramuscularly, thereafter spleen cells from these mice were examined for their proliferative responses and cytotoxic activities in response to OVA-expressing targets. To test therapeutic efficacy of the combined vaccines, E.G7 (OVA-expressing) tumor-bearing mice were treated with OVA and Pan-IA DNA vaccination.

Results: The specific proliferative response and cytotoxic activity were induced in mice vaccinated with both OVA and Pan-IA DNA, but not in those vaccinated with OVA DNA alone or control DNA plus Pan-IA DNA. Tumor growth of E.G7 cells was suppressed only by combined vaccination with OVA and Pan-IA DNA, and 5 of the 9 mice that received this combination completely eradicated the tumors. Both CD4- and CD8-positive splenic T cells from the mice receiving the combined vaccination showed proliferative responses to OVA-expressing cells, but only CD8-positive T cells were responsible for killing the targets in the effector phase. An immunofluorescent study showed that CD8-positive T cells commonly infiltrated the tumors of vaccinated mice. A distinctive feature of the co-vaccinated mice was that an increased number of CD4-positive T cells infiltrated the tumors.

Conclusions: The data suggest that Pan-IA DNA can augment antigen-specific cytotoxic activity of CD8-positive T lymphocytes via activation of the helper T function in DNA-vaccinated hosts. This animal model may contribute to the development of therapeutic DNA vaccines against cancer.

988 POSTER

Preventive vaccination of mice with human melanoma cells injected subcutaneously into encapsulated polyacrylamide gel

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Background: High degree of some melanocytic human and mouse antigens homology, presence of minor xenoantigens and species specificity are the main features in favour of xenogenic vaccination. In our studies, the vaccinating material was injected subcutaneous (s/c) into preliminary implanted encapsulated polyacrylamide gel (PAAG). The use of this approach affords effective protection of the xenogenic vaccine from rapid elimination by NK-cells.

Experimental goal: Elaboration of a method for xenogenic vaccination using a model of mouse melanoma -16.

Materials and methods: Intact mice (BDF) were injected s/c with 0.5 ml PAAG. After 3 to 6 weeks, melanoma cells (SKMEL-28 or cells isolated from surgical material) ($0.25E10^6$ $4,0E10^6$ cells/animal) were introduced into the gel. Six weeks thereafter melanoma -16 cells ($0.062E10^6$-$1.5E10^6$ cells/animal) were inoculated s/c into these mice. The melanoma B16 growth parameters and the cytotoxic activity of mouse splenocytes against B-16 cells were examined. Continuous control over the state of the gel, tumor nodes and animal lungs was made.

Results: It was found that the vaccination ($2E10^6$ cells/animal) inhibited melanoma B16 growth (inoculating B16 dose - $0.5E10^6$ cells/animal) by more than 70% for 2.0-2.5 weeks. An increase in the vaccinating dose enhanced this effect at later stages of B-16 development. For inoculating dose of B16 $0.062E10^6$ cells/animal, the vaccination prevents the melanoma growth in 72% of animals. The cytotoxic activity of splenocytes begins to increase from 2nd week following the vaccination, and remained at 50% level during 12 weeks.

Conclusion: Preventive xenogenic vaccination with subcutaneously injected and naturally encapsulated PAAG proved to be highly effective in melanoma growth prevention in mouse model.

989 POSTER

Identification of highly active, cancer specific promoters for use in targeted gene therapy of small cell lung cancer

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To identify highly and specifically expressed genes in small cell lung cancer (SCLC) we have used the DNA microarray technology. We analysed the expression of 12.000 genes in 21 SCLC cell lines and 6 xenografted tumors thereof and compared to the expression in 17 normal representative tissues. We have identified a number of genes with high expression in SCLC and low or no expression in all or most normal tissues. Only a few of these genes were previously known to be highly expressed in SCLC. Most of these genes are also expressed at similar levels in the published array data of 6 ressected SCLC tumors (Bhattacharjee et al., 2001, *PNAS, USA* 98:13790) demonstrating that the high expression is not an artefact due to establishment or propagation of cell lines.

Some of the expressed genes are known to be highly expressed in cancer cells due to hypo- or hyper methylation, such as the MAGEs or due to amplification, such as the myc family and are therefore not candidates for cancer specific regulation of expression. Several of the genes identified as highly expressed reflect the neuroendocrine origin of the cancers, such as INSM1, PTTG1, NCAM1, UCHL1 and ASCL1, which are known to be regulated in tissue and developmental manner by regulatory elements. These are therefore the most potential candidates to clone and test for cancer specific expression. However, there is a number of other genes known to be highly and specifically expressed in a variety of cancers such as EHZ2, STK12, KIAA0101, KIF14 and HSU79266. These genes are therefore candidates, but need further characterization.