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.

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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.