receptor γ (PPARγ) is a member of the nuclear receptor super-family of ligand-activated transcription factors. PPARγ agonists, such as the antidiabetic thiazolidinedione drugs, inhibit growth and induce apoptosis in several cancer cell types and are seen as potentially useful therapeutic and chemopreventive agents in oncology. This study was designed to investigate cellular and molecular consequences of PPARγ activation in a panel of human ovarian cancer cell lines expressing PPARγ. The PPARγ agonist ciglitazone induced a dose-dependent inhibition of growth as determined by colorimetric and colony forming assays with the A2780 cells being the most sensitive cell line. Treatment of A2780 cells with GW9662 prevented the anti-proliferative effects of ciglitazone, indicating that this effect was a consequence of PPARγ activation. Cell cycle analysis by flow cytometry indicated that ciglitazone induced G1/S phase cell cycle arrest and the appearance of a sub-G1 peak indicative of apoptotic cell death. To determine the mechanisms by which PPARγ activation induced growth arrest and apoptosis, we evaluated changes in gene expression induced by ciglitazone in A2780 cells using Affymetrix U133A GeneChips, RT-PCR and Western blotting. Expression of several genes was found to be affected by ciglitazone with a prevalence of up-regulated genes. Multiple genes involved in growth arrest and apoptosis, such as Bax, p21 and PTEN, were up-regulated in ciglitazone-treated cells. In addition, a number of genes involved in cell proliferation and survival, including survivin, c-myc and cyclin D1, were down-regulated upon treatment with ciglitazone. Collectively, these data suggest that selective PPARγ agonists alone or in combination with other anticancer drugs should be considered for treatment of ovarian cancer.

67 POSTER

Assessment of treatment efficacy in preclinical drug testing using magnetic resonance imaging

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Introduction: New imaging approaches are emerging for the clinical assessment of therapeutic efficacy. One of the most promising methods, diffusion MRI (dMRI), has been shown to be an early surrogate marker of treatment response in human gliomas. The application of dMRI to preclinical in vivo testing may allow more efficient evaluation of drug candidates and reduced study durations, as well as provide a clinically relevant bio-marker for therapeutic outcome.
Methods: Xenografts of four human tumor types were grown subcutaneously in nude mice to 100 mg. Mice with tumors of each type were divided into vehicle control groups, and 1–2 treatment groups per tumor type, which received standard, commercially available chemotherapies (n=4–5). Entire tumor ADC was serially quantified over 60 days using a motion corrected, isotropic dMRI sequence. Tumor volume was determined using both MRI and calipers, and tumor growth delay, log cell kill and estimated surviving fraction were calculated for each treatment group. dMRI data were analyzed according to a recently developed clinical method to predict treatment outcome on the basis of changes in the apparent diffusion coefficient (ADC).
Results: MRI-based volume measurement showed close agreement with caliper measurements when tumor masses were greater than 50 mg. However, accurate volumes below this size could only be determined using MRI. In all treatment groups which exhibited anticancer activity, a concomitant rise in MR-measured ADC was also observed. In all cases, an ADC increase was measured on the first day of MRI following the start of treatment, in most cases reaching a peak value within a few days of the end of treatment. In most cases, the MRIs showed some degree of heterogeneity due to localized necrotic regions with high water mobility and high signal. Despite this, well defined ADC histograms were obtained over the entire tumor.
Conclusion: This study has demonstrated the unique ability of dMRI to characterize early treatment response in pre-clinical drug testing, potentially enabling reduced animal numbers, and decreased study duration, compared with traditional testing methodologies. Other advantages of dMRI include accurate measurement of small tumors, characterization of tumor heterogeneity and its clinical relevance as a bio-marker of therapeutic outcome.

68 POSTER

Spermine/spermidine N1-acetyltransferase: a new target for prevention and/or therapy of colorectal cancer

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Background: The natural polyamines (putrescine, spermidine, and spermine) are critical in cell growth and proliferation. Polyamine pools are tightly regulated, which occurs through modulation of both biosynthetic and catabolic pathways. Spermidine/spermine N^1-acetyltransferase (SSAT), a rate-limiting enzyme in the catabolism of polyamines, has been implicated in cellular stress responses and apoptosis. We have postulated that expression of the enzyme, which is encoded by the X-linked *Sat1* gene, is critical to the development and progression of cancer. To test the role of SSAT in development of colorectal cancer, we have utilized the $Apc^{Min/+}$ mouse, which carries a truncated allele of the *adenomatous polyposis coli* (*Apc*) gene and is therefore predisposed to intestinal tumorigenesis.
Methods and Results: A series of $Apc^{-/+}$ strains with varying levels of SSAT expression were generated. Animals completely lacking SSAT were produced by introducing a targeted mutant allele of the *Sat1* gene ($Sat1^{-/-}$) into the $Apc^{-/+}$ background, while mice expressing high levels of SSAT were produced by introducing an *Sat1* transgene (*Sat1-tg*). Tumor multiplicities in the $Sat1^{-/-}$ and in the *Sat1-tg* mice were determined, and compared to those in normal $Apc^{-/+}$ mice. In the small intestine, tumor numbers were directly correlated with levels of SSAT expression, i.e., $Sat1^{-/-}$ < normal < *Sat1-tg*. In the colon, which typically develops far fewer tumors than the small intestine, the *Sat1-tg* mice had higher numbers of tumors than the normal or $Sat1^{-/-}$ mice; tumor multiplicities in the latter two strains were similar. In order to gain insight into the mechanisms of the SSAT effect, we measured expression of other enzymes of polyamine metabolism, and determined polyamine pool levels in tumors and in normal tissues. In *Sat1-tg* mice, the levels of ornithine decarboxylase and S-adenosylmethionine decarboxylase were significantly increased relative to the other two strains; in addition, putrescine and N^1-acetylspermidine pools were higher in these mice. Spermidine and spermine pools were unchanged among the three strains.
Conclusions: Overall, our results indicate that SSAT promotes tumor development in the $Apc^{-/+}$ model, suggesting that pharmacological inhibition of the enzyme could be an effective means of colorectal cancer prevention and/or therapy. The fact that $Sat1^{-/-}$ mice are healthy and fertile suggests that complete inhibition of the enzyme will have little, if any, toxic effects.

69 POSTER

Essential role of inducible 6-phosphofructo-2-kinase in ras transformation

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Background: Increased uptake of glucose as an anaerobic source of energy and biosynthetic precursors is a common feature of growing tumors. Oncogenic mutations of the *ras* gene have been detected in a wide spectrum of human cancers and oncogenic *ras* causes a marked increase in glucose uptake in immortalized cells. Glycolytic flux in primary cells is normally controlled by the inhibitory effects of ATP on 6-phosphofructo-1-kinase (PFK-1), the rate-limiting step of glycolysis. Fructose-2,6-bisphosphate (F2,6BP) is a potent allosteric activator of PFK-1 and overrides the inhibitory effects of ATP on PFK-1. Transfection of oncogenic *ras* into immortalized fibroblasts rapidly induces the synthesis of F2,6BP and activates PFK-1. The steady-state concentration of F2,6BP depends on the activity of 6-phosphofructo-2-kinase (PFK-2), which is expressed in several tissue-specific isoforms. We recently identified an inducible isozyme of PFK-2 (iPFK-2) that is over-expressed by a majority of human solid tumors in situ, required for K562 leukemia growth in vivo, and upregulated by hypoxic exposure via HIF1α. We hypothesize that oncogenic ras activates iPFK-2 catalyzed synthesis of F2,6BP in order to enable the flux of glucose carbons into anabolic pathways required for growth and invasiveness.
Methods: We silenced iPFK-2 protein expression in immortalized (hT/LT) and H-*ras*V12-transformed (hT/LT/*Ras*) bronchial epithelial cells and examined the consequence on intracellular F2,6BP, glycolytic flux of ^{13}C-labeled glucose into biosynthetic pathways (using 2-dimensional NMR), and anchorage-independent growth. We also transduced pulmonary fibroblasts isolated from iPFK-$2^{+/-}$ and iPFK-$2^{+/+}$ mice with large T antigen and oncogenic ras and examined the ability of the resultant cells to grow as soft agar colonies in vitro and tumors in athymic mice.

Results: We found that oncogenic *ras* induces the PKCι-dependent activation of iPFK-2 in bronchial epithelial cells causing an increase in intracellular F2,6BP (+18.4±2.1 pmol/mg protein). Additionally we found that siRNA-silencing of iPFK-2 expression completely abrogated the formation of soft agar colonies by ras-transformed bronchial epithelial cells (control siRNA 123.4±23.1; anti-iPFK-2 siRNA 3.3±3.5) and attenuated the flux of glucose carbons into de novo nucleic acids and amino acids. Although iPFK-$2^{+/-}$ mice display a normal phenotype, isolated iPFK-$2^{+/-}$ lung fibroblasts were not able to be transformed with T antigen and oncogenic ras as evidenced by zero growth in soft agar or athymic mice. Conversely, 10^3 ras-transformed iPFK-$2^{+/+}$ lung fibroblasts grew as soft agar colonies (172.6±38.3) and as tumors in athymic mice.
Conclusions: iPFK-2 should prove useful as a novel molecular target for the development of anti-neoplastic agents that target the downstream metabolic effects of oncogenic ras.

70 POSTER

Therapeutic human monoclonal antibody targeting VEGFR-1 suppresses growth of human breast cancers

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Vascular endothelial growth factor receptor 1 (VEGFR-1) is activated by the ligands VEGF-A, VEGF-B and placental growth factor (PlGF) and has been shown to be a potential therapeutic target for treatment of tumors and angiogenesis-associated diseases. Studies have shown that VEGFR-1 plays not only an important role in regulating pathological angiogenesis for tumor growth but also a functional role in directly promoting growth of certain cancer cells. IMC-18F1 was generated from the KM strain of human Ig transgenic mice (Medarex). The variable regions of the antibody were engineered into a high expression vector for production of fully human IgG1κ ε antibody. IMC-18F1 binds human VEGFR-1 with a high affinity (K_D = 54 pM) and efficiently blocks the binding of PlGF, VEGF-A and VEGF-B to VEGFR-1 with an IC50 of 0.5, 0.6 and 0.8 nM, respectively. IMC-18F1 inhibited ligand-induced phosphorylation of VEGFR-1 and activation of MAP kinase and Akt downstream signaling pathways in VEGFR-1 expressing endothelial and human breast cancer cell lines. The antibody also inhibited VEGF and PlGF-stimulated growth of breast carcinoma cells *in vitro*. Pharmacokinetic analysis indicates thatIMC-18F1 has plasma T1/2 of 4.8 days. Pharmacodynamic studies showed that a threshold dose of IMC-18F1 for maximal inhibition of VEGFR-1-related tumor growth was 20 mg/kg twice a week and average steady state plasma 18F1 concentration was 454 μg/ml. Treatment of mice with IMC-18F1 significantly suppressed the growth of human breast tumors in several xenograft models. Histology analysis showed that IMC-18F1 treatment inhibited MAPK and /or Akt signaling in breast tumor xenograft. These results indicate that IMC-18F1 is a potent VEGFR-1 antagonist and warrant further investigation.

71 POSTER

In vivo efficacy of STI571 in xenografted human small cell cancer alone or combined with chemotherapy

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STI571 or imatinib selectively inhibits BCR/ABL, PDGFR and c-kit kinase activity. It has been reported that a large proportion of small cell lung cancer (SCLC) cell lines and tumors express the c-kit receptor and that STI571 inhibits tumor cell growth. We therefore investigated the therapeutic efficacy of STI571, alone or combined with chemotherapy, in human SCLC cells or tumors xenografted into *nude* mice. The level of c-kit mRNA expression was variable in SCLC tumors (positive for 2/4 xenografts), and C-kit protein was not detected by immunohistochemistry. STI571 induced inhibition of proliferation of the SCLC6 cell line without inducing apoptosis; in contrast, in combination with etoposide or topotecan, the growth inhibition of SCLC6 cells induced by STI571 was increased, with apoptotic DNA fragmentation. Four human SCLC xenografts (SCLC6, SCLC61, SCLC74, and SCLC108) were transplanted into mice. After intraperitoneal injection of STI571, we observed 80%, 40%, and 78% growth inhibition of SCLC6, SCLC61, and SCLC108 tumors, respectively, without any significant inhibition of SCLC74 tumor growth. In mice bearing responsive SCLC tumors, we observed an increase of growth inhibition induced by chemotherapy (etoposide + ifosfamide or topotecan) by concomitant and continuous administration of STI571, associated with an increase of toxic deaths. In SCLC6-bearing mice receiving sequential treatments, we observed a reduction of toxic deaths, but a decrease of synergistic anti-tumor efficacy. In conclusion, the efficacy of STI571 alone in SCLC xenografted tumors was variable and did not depend on c-kit expression. Moreover, a significant increase of chemotherapy-induced growth inhibition was obtained by concomitant administration of STI571 that should be carefully investigated in SCLC patients.

72 POSTER

Restoration of wild-type p53 in malignant melanoma

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The binding of S100B to p53 down-regulates wild-type p53 tumor suppressor activity in cancer cells such as malignant melanoma, so a search for small molecules that bind S100B and prevent S100B-p53 complex formation was undertaken. Chemical databases were computationally searched for potential inhibitors of S100B, and 60 compounds were selected for testing based upon energy scoring, commercial availability, and chemical similarity clustering. Seven of these compounds bound to S100B as determined by steady state fluorescence spectroscopy (1.0 uM = KD = 120 uM) and five inhibited the growth of primary malignant melanoma cells (C8146A) at comparable concentrations (1.0 uM = IC50 = 50 uM). Additionally, Saturation Transfer Difference (STD) NMR experiments confirmed binding and qualitatively identified protons from the small molecule at the small molecule-S100B interface. Heteronuclear Single Quantum Coherence (HSQC) NMR titrations indicate that these compounds interact with the p53 binding site on S100B. A model of one such inhibitor, pentamidine, bound to calium-loaded S100B was calculated using intermolecular NOE data between S100B and the drug, and indicates that pentamidine binds into the p53 binding site on S100B defined by helices 3, 4, and loop 2 (termed the hinge region).

73 POSTER

Inhibition of choline kinase is a highly specific and selective cytotoxic antitumoral strategy

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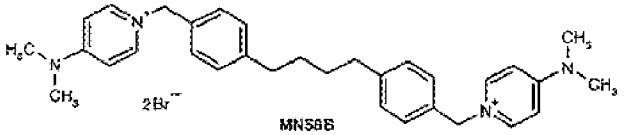
Choline kinase (ChoK), is responsible for the generation of phosphorylcholine, a proposed second messenger required for DNA synthesis induced by growth factors. ChoK levels are increased in different tumor-derived cell lines and in several human tumors when compared to their corresponding normal tissues (1). Moreover, ChoK inhibition has drastic inhibitory effects on cell proliferation and prevents tumor growth in mice (2). The aim of this work was to assess the specificity of the ChoK inhibitor MN58b and to provide a rational understanding for its antitumoral activity.
We have analysed the effects of a previously described ChoK inhibitor, MN58b (3) on different human tumor-derived cell lines compared to their appropriate primary, non transformed, counterparts. The effects on cell growth, cell cycle and the differential response in terms of cell signalling and lipid metabolism have been evaluated.



MN58B

A dramatic difference in the response of primary, non transformed human cells when compared to tumor-derived cell lines was observed. In normal cells, blockage of de novo phosphorylcholine synthesis by inhibition of ChoK promotes the dephosphorylation of pRb, resulting in a reversible cell cycle arrest at G0/G1 phase. In contrast, ChoK inhibition in tumoral cells renders cells unable to arrest at G0/G1 as manifested by a lack of pRb dephosphorylation. Furthermore, tumors cells specifically suffered a drastic wobble in the metabolism of main membrane lipids phosphatidylcholine (PC) and sphingomelin (SM). This lipid disruption results in the enlargement of the intracellular levels of ceramides. As a consequence, human tumor-derived cells are promoted to apoptosis while their normal counterparts remain unaffected. These results provide the evidence that MN58b is a specific and selective antitumoral strategy that works by specifically inducing apoptosis and killing tumoral cells without affecting normal cells.