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.

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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).

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

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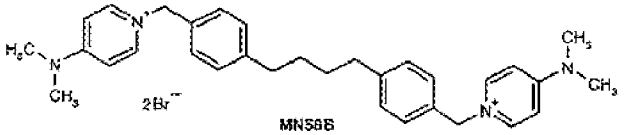
Choline kinase (ChoK), is responsible for the generation of phosphorylcholine, a proposed second messenger required for DNA synthesis induced by growth factors. ChoK levels are increased in different tumor-derived cell lines and in several human tumors when compared to their corresponding normal tissues (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.