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.

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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.

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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.

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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.