often spidery-shape appearance. The expression of markers commonly used to identify pericytes in situ was confirmed by flow cytometry: desmin, alpha smooth muscle actin, VCAM, and fibronectin. Pericytes contribute to vasculature and tube formation is a fundamental process in angiogenesis. The ability of the pericytes isolated from clinical lung samples to form tubes was evaluated in vitro. Pericytes were cultured onto Matrigel, a mixture of basement membrane proteins purified from murine tumors. Pericytes from both normal and diseased tissues formed linear tubes when 20–30,000 cells were seeded into a well of a 48-well plate. In summary, the cells isolated from several human lung tumors possess the characteristics typically associated with pericytes. The ability to propagate pericytes directly from the tumors of cancer patients is a valuable resource that will enhance our understanding of the contributions pericytes make during angiogenesis in malignant phenotypes. Incorporation of such pericytes into drug development programs may lead to more effective cancer therapies that can destabilize tumor vasculature and cause tumor regression.

63 POSTER

Identification of important genes for recurrence of gastric cancer by gene expression profiling

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Background: Recent progress in diagnostic and treatment technologies have enabled us to offer excellent long-term survival results for early gastric cancer, but the prognosis of advanced gastric cancer is still unfavorable. Even after curative resection, 40% of patients with advanced gastric cancer die of recurrence. Cancer is a genetic malady, which progresses through the continuous accumulation of genetic and epigenetic changes. These aberrations may affect the expression of large number of genes. Hence, systematic analysis of gene expression profiling might be beneficial for searching important genes.
Purpose: To search important genes associated with recurrence, we performed gene expression profiling in 60 advanced gastric cancer tissues using a PCR-array system: a high-throughput quantitative RT-PCR technique based on adaptor-tagged competitive PCR (ATAC-PCR).
Materials and Methods: To select only genes actually expressed in gastric tissues, we constructed two cDNA libraries from gastric cancer and normal gastric mucosa. From these two cDNA libraries and literatures described the carcinogenesis or development of gastric cancer, we designed 2304 PCR primers for the ATAC-PCR reaction. We obtained gene expression profiling data from 40 advanced gastric cancer patients (21 recurrence-free cases and 19 recurred cases). To search important genes associated with recurrence, we obtained signal-to-noise ratio and ranked genes. We selected 20 top ranked genes and confirmed the reliability of these selected genes by constructing a molecular-based diagnostic system with these genes. Briefly, by calculating 'prediction strength' ('PS') each case is assigned to 'PS>0' or 'PS<0' groups; in our system, 'PS>0' means a recurrence-free case and 'PS<0' means a recurred case, respectively. We prepared other 20 (11 recurrence-free cases and 9 recurred cases) advanced gastric cancer cases as a validation set and predicted the recurrence. Furthermore, Kaplan-Meier analysis with recurrence was performed.
Results: Selected 20 genes involved the genes reported to be concerned with the development and malignancy of gastric or other cancer, such as ERBB2 and HSP40. These genes revealed distinct expression patterns between recurrence-free and recurred cases. Our diagnostic system correctly predicted recurrence in 15 of 20 cases in the validation set and Kaplan-Meier analysis revealed significant difference between 'PS>0' and 'PS<0' groups.
Conclusions: We selected 20 important genes for recurrence of advanced gastric cancer. These 20 genes might be the potential therapeutic targets for gastric cancer. Our molecular-based diagnostic system is clinically useful to predict recurrence of gastric cancer.

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Assessment of antitumor effects of erlotinib prior to first-line surgical treatment of head and neck squamous cell carcinoma

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Epidermal growth factor receptor (HER1/EGFR) overexpression is implicated in tumourigenesis, including head and neck squamous cell carcinoma (HNSCC). Erlotinib (Tarceva™), a small molecule inhibitor of HER1/EGFR tyrosine kinase (TK), has antitumor activity in several tumour types. However, there is a clinical need to identify markers that predict response, and to further understand pharmacokinetic (PK) and pharmacodynamic properties of this agent class. Patients with HNSCC (diagnosed by pan-endoscopy) requiring cervical lymph node dissection or likely to benefit from curative surgery were studied. Oral erlotinib was administered (150mg/day) for 3–4 weeks prior to surgery. Pre- and post-surgery tumour samples were assessed. The primary endpoint was tumour HER1/EGFR TK inhibition. Secondary objectives included: correlation of PKs with effective biological dose; relationship of biological effect to tumour site and morphology; effects on protein effectors of cell cycle arrest (e.g. P-MAP kinases, cyclins and cyclin-dependent kinases, cycle-progression inhibitors, Ki-67, AKT) and molecular comparison of the HER1/EGFR catalytic domain in normal and tumour tissue. To date, eleven patients have been recruited into this study. A comparative immunohistochemistry (IHC) analysis has been performed on tumour samples collected pre- and post-treatment with erlotinib. In patients responding to erlotinib treatment, dramatic changes in cell proliferation (MAP kinases) and apoptotic pathways (AKT and cell cycle inhibitors p21 and p27) were observed. These changes were not correlated with the initial expression levels of HER1/EGFR. Molecular analysis of HER1/EGFR catalytic domain is currently underway. In conclusion, the available preliminary data suggest that it may be possible to define potential tumour markers to assist in the selection of patients likely to benefit from treatment with erlotinib. Further results from this ongoing trial will be presented.

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Kinomic profiling identifies PKC/Akt and beta-catenin/TCF mediated signal transduction as important targets of celecoxib in colon cancer

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Introduction: NSAIDs and selective cyclooxygenase-2 (Cox-2) inhibitors possess anti-carcinogenic potential against colorectal cancer (CRC). However the molecular targets in cancer of these drugs remain to be elucidated.
Aims & Methods: We hypothesized that celecoxib, a selective cox-2 inhibitor directly targets the activity of kinase proteins resulting in apoptosis and downregulation of Wnt signaling. A new kinase substrate peptide array consisting of 1200 peptides with specific phosphorylation sites was used to comprehensively evaluate the effects celecoxib on the entire kinome in the colon cancer cell lines HT29 and DLD-1. Results of the kinome array were validated with Western blot analysis. To study β-catenin/TCF dependent transcription, a luciferase reporter assay was used. We evaluated the expression of oncogenes with quantitative PCR. Apoptosis was measured by the level of cleaved caspase 3.
Results: Celecoxib has important anticancer mechanism since apoptosis and cell cycle arrest was induced at low levels of celecoxib, 25 μM. The kinase array analysis revealed inhibition of the kinases IGF-r, Act, PKC and upregulation of GSK3 in the presence of celecoxib, independent of Cox-2 expression. Moreover an increase phosphorylated β-catenin was observed within 60 minutes. This effect of celecoxib was accompanied by a downregulation of the β-catenin/TCF dependent transcription. Subsequently expression levels of the oncogenes cyclin d-1, c-Myc and c-Met were reduced.
Conclusion: Celecoxib directly inhibits the activity of the IGF-r, PDK1/2, Akt and PKC. In addition GSK3 activity was enhanced which can explain the increase of phosphorylated β-catenin. Celecoxib caused a downregulation of beta-catenin–TCF dependent transcription of oncogenes at relatively low levels independent of tumour-Cox-2. Hence we have identified a possible link between Akt and Wnt signal transduction which can explain the chemopreventive and anticarcinogenic properties of celecoxib in colon cancer. This study provides a novel mechanism of action of celecoxib in colon cancer.

66 POSTER

Anti-proliferative activity of a PPAR gamma agonist is associated with changes in the expression of cell cycle and apoptosis related genes in human ovarian cancer cells

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Ovarian cancer is the most common cause of cancer-related death in women. Current therapies for advanced ovarian cancer are clearly inadequate and new molecular-targeted agents need to be evaluated for treatment of this disease. The peroxisome proliferator-activated

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.

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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.

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.