antibody have been used to label tumour cells, with a CD45 (pan-leucocyte marker) antibody used to exclude white blood cells from the analysis. The individual antibodies were optimised using 3 cell lines with increasing levels of α-FR expression (JEG-3, IGROV-1 and KB cells). BGC 945 causes increasingly high levels of α-FR mediated growth inhibition in these cell lines. The three antibody protocol successfully measured α-FR expression levels in cell line samples spiked with blood. CellQuant calibrator beads were used to semi-quantify antigen sites/cell. KB cells expressed around 1×10^6 antigen sites/cell and IGROV-1 and JEG-3 cells around 5.5 and 0.7×10^5 sites/cell respectively (~50% and 7% of KB cells). Tumour cells were obtained from ascites in 19 patients with relapsed ovarian cancer. In each case sufficient cells were harvested to isolate a tumour cell population by this method in order to estimate the number of binding sites/cell. The majority of samples (13/19) had expression levels between 0.6×10^5 and 4.9×10^5 binding sites/cell, which lie between the JEG-3 and the IGROV-1 cell lines. A smaller number (4/19) formed a population of lower expressors with $<1\times10^4$ binding sites/cell. The final 2 samples lie in between these groups. These data may be useful in identifying a cohort of patients more likely to respond to α-FR targeted therapy.

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421 POSTER

New low-toxic analogs of vitamin D in the treatment mice bearing lung carcinoma

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A proper level of the steroid hormone 1,25-(OH)2D3 (1,25-dihydroxy-vitamin D3 (calcitriol) – the most potent metabolite of vitamin D3) is important not only in regulating calcium homeostasis and bone metabolism, but also in protecting against the development of cancer. Calcitriol and several synthetic vitamin D derivatives showing reduced calcemic activity inhibit the growth of a number of different cancer cells (epithelial, melanoma, soft tissue sarcoma, and leukemic) by inducing cell cycle arrest or apoptosis. Calcipotriol is a synthetic vitamin D3 analog that binds to vitamin D receptors. In vitro studies have shown that calcipotriol exerts similar effects on cell proliferation and differentiation to those of calcitriol, but has less effect on calcium metabolism.

The aim of our study was to examine the toxicity and antitumor activity of new vitamin D analogues selected during in vitro experiments, i.e. PRI-2202 (24R calcipotriol) and PRI-2205 (5,6-trans calcipotriol).

Subacute toxicity after 5 subcutaneous (s.c.) administrations was determined. We also compared antitumor activity (LLC tumor model) of calcitriol (in the dose 2 μg/kg/day) and PRI-2201 with PRI-2202 and PRI-2205 (20 μg/kg/day) injected s.c. or applied s.c. in various doses (1, 10, 50 i 100 μg/kg/day).

The toxicity studies showed, that PRI-2202 and PRI-2205 were very low-toxic analogs. Even in doses of 2.5–5.0 mg/kg (in 5 daily doses), no changes in body weight were observed. Calcitriol and tacalcitol showed toxicity in the same model system at 100-times lower doses. LD50 for calcitriol was 7.4 and for tacalcitol 21.0 μg/kg/day (total: 37 and 105 μg/kg, respectively). Also, cacipotriol caused death of all mice (mean life-span ± SD: 7.4±1.1 days) when the total dose of 5.0 mg/kg was administered.

Next we tested the antitumor activity of these analogs in the LLC mice tumor model. We show that the analog PRI-2205 is more active than both calcitriol and calcipotriol as well as PRI-2202. It revealed no calcemic activity in the doses which inhibit tumor growth nor at higher doses.

These data demonstrate that the analogs PRI-2202 and PRI-2205 are non-toxic and potent inhibitors of cancer growth. In particular, their role in combined treatment with cytostatics is considered for further study.

422 POSTER

Endosialin/TEM 1 a tumor stromal target in stem cells, progenitor cells and pericytes

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Background: Endosialin was originally identified as a cell surface protein expressed by reactive tumor stroma. Later, TEM 1 (Tumor Endothelial Marker 1) was described as a cell surface protein expressed by tumor endothelial cells (EC). Endosialin and TEM 1 are the same protein. The investigation of TEM 1 and other TEM expression has expanded to several distinct tumor stromal cells types including endothelial precursor cells (EPC), mesenchymal stem cells (MSC) and pericytes as well as tumor cells of mesenchymal origin.

Materials and Methods: Cells from various tissues were analyzed for TEM 1 expression prior to use in experiments. TEM 1 was abundantly expressed by EPC and MSC derived from human bone marrow. By RT-PCR, the message for TEM 1 was present at negligible levels in CD133+/CD34+ precursor cells, was abundantly expressed when these cell differentiated to EPC and is expressed at very low levels by fully differentiated EC such as HUVEC/HMVEC. Immunohistochemistry (IHC) was employed to evaluate TEM 1 expression in clinical samples.

Results: Exposure to rabbit polyclonal anti-TEM 1 inhibited EPC migration and tube formation in culture. In an in vivo Matrigel™ plug assay, EPC continued to express TEM 1 abundantly. TEM 1 protein expression was determined by IHC in human normal tissues and in frozen and paraffin-embedded human tumors. TEM 1 was expressed primarily in the vasculature of many tumor types especially bladder, sarcomas, colon, breast and non-small cell lung cancer. In most specimens it appeared that pericytes had the most intense expression of TEM 1 with additional expression in EC and reactive stroma which may be carcinoma-associated fibroblasts. Pericytes isolated from fresh human non-small cell lung cancer specimens also express TEM 1 as determined by flow cytometry. Some malignant cells of mesenchymal origin express TEM 1. For specific tumor-types TEM 1 was expressed in 90–100% of the specimens examined. In some normal tissues TEM 1 expression was observed in occasional cells that had a spindloid appearance.

Conclusions: TEM 1/endosialin is a potentially interesting therapeutic target that is selectively expressed in tumor vasculature. The development of new therapies directed toward the non-malignant cellular components of the disease process such as EC, pericytes and cancer-associated fibroblasts may yield may yield therapeutics with a high degree of tumor selectivity and limited normal tissue effects.

423 POSTER

Metformin is an AMP-kinase dependent growth inhibitor for breast cancer cells

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Background: Recent population studies provide clues that the use of metformin may be associated with reduced incidence and improved prognosis of certain cancers. This drug is widely used in the treatment of type 2 diabetes, where it is often referred to as an 'insulin sensitizer' because it not only lowers blood glucose but also reduces the hyperinsulinemia associated with insulin resistance. As insulin and insulin-like growth factors stimulate proliferation of many normal and transformed cell types, agents that facilitate signalling through these receptors would be expected to enhance proliferation.

Methods: Breast cell lines were treated with metformin for 3 days and/or AMP kinase siRNA. Proliferation assays were performed using Alamar reducing dye. AMP kinase downstream signalling pathway protein levels and phosphorylation were evaluated by Western blots.

Results: We demonstrate here that metformin acts as a growth inhibitor rather than an insulin sensitizer for epithelial cells. Breast cancer cells can be protected against metformin-induced growth inhibition by siRNA against AMP kinase. This demonstrates that AMP kinase pathway activation by metformin, recently shown to be necessary for metformin inhibition of gluconeogenesis in hepatocytes, is also involved in metformin-induced growth inhibition of epithelial cells. The growth inhibition was associated with decreased mTOR and S6 Kinase activation, and a general decrease in mRNA translation.

Conclusion: These results provide evidence for a mechanism that may contribute to the antineoplastic effects of metformin suggested by recent population studies, and justify further work to explore potential roles for activators of AMP kinase in cancer prevention and treatment.

424 POSTER

Effects of statins on IGF-IR signaling in normal and transformed breast epithelial cells

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Background: The 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are widely used cholesterol lowering drugs. Some epidemiologic studies imply that individuals taking statin decrease their cancer risk. Statins disrupt cellular processes such as (iso)-prenylation (required for the activity of proteins such as Ras and Rho) or dolichol synthesis (required for correct N-glycosylation of proteins such as insulin

receptor and IGF-1 receptor). Here we evaluated the effects of statins on IGF-1R signaling, and growth of normal and transformed breast epithelial cells.
Methods: Breast cancer cell lines (MCF-7, MDA-231), non-transformed cell line (MCF-10A), and normal mammary epithelial cells (HMEC) were treated for 3 days with fluvastatin or atorvastatin in presence or absence of mevalonic acid. Proliferation assay was performed by MTT. IGF-IR levels were determined in different treatment conditions by Western blot and flow cytometry and compared with the effect of IGF-1R siRNA. IGF-IR downstream signaling pathway was also analyzed.
Results: Statins reduced proliferation of all cell lines in a dose-dependent manner. In general, this growth inhibition could be rescued by exogenous mevalonic acid, which would be expected to compensate for HMG-CoA inhibition. We observed a reduction IGF-IR expression when cells were treated with statins, but for all cell lines, IGF-IR siRNA treatment had a stronger effect on IGF-1R levels than statins. IGF-1R expression reduction by statins was accompanied by a decrease in phosphorylation of AKT, p70S6K, and MAPK. Despite this, for most cell lines, maximal growth inhibition achieved by statins was greater than that achieved by IGF-1R siRNA. This suggests that either (a) IGF-1R siRNA was insufficient to completely abolish IGF-1R signaling or (b) effects of statin on growth inhibition involve important additional IGF-1R-independent mechanisms. We observed that fluvastatin repressed the farnesylation of $p21^{WAF1}$ in the cancer cell lines but not in the normal breast epithelial cells.
Conclusion: These data provide evidence for an effect of statins on IGF-1R levels, but suggest that additional mechanisms are involved in statin induced growth inhibition.

425 POSTER

Biological role of the CREB/COX-2 pathway in the regulation of human pancreatic cancer proliferation and survival.

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Cyclooxygenase-2 (COX-2) is a pro-inflammatory enzyme expressed in the majority of human primary pancreatic carcinomas. Its expression is regulated by the cyclic AMP response element binding protein (CREB) and a large body of evidence has implicated the CREB/COX-2 pathway in the suppression of apoptosis, induction of proliferation, angiogenesis and tumor metastasis. The L3.6pl human pancreatic cancer cell line, which displays constitutive CREB phosphorylation and COX-2 overexpression, was exposed for 24 hours to increasing concentrations of H-89 (a selective PKA inhibitor), AH-6809 (an EP_2 receptor inhibitor), and a small interfering RNA against CREB (siRNA CREB). Both H-89 and AH-6809 inhibited DNA synthesis in a dose-dependent manner as measured by ^{3}[H]-thymidine incorporation. DNA synthesis was completely abrogated with 25 μM H-89, whereas a 50% reduction was observed with 50 μM AH-6809. A dose-dependent increase in apoptosis-associated DNA fragmentation as measured by propidium iodide staining and FACS analysis was observed only in cells treated with H-89 (50% apoptosis at 30 μM), whereas no apoptosis was observed with AH-6908 at all concentrations tested. Transient transfection of L3.6pl cells with a CREB-specific siRNA construct (siRNA CREB) under conditions that induced $\geqslant$ 80% reduction in CREB protein expression minimally affected apoptosis induction, as compared with a non-specific siRNA construct (siRNA NS). However, the levels of DNA fragmentation were consistently higher in the siRNA CREB-transfected L3.6pl cells exposed to stimuli that activate CREB via a Ca2+-dependent mechanism, such as the endoplasmic reticular Ca2+ ATPase inhibitor thapsigargin (5 μM, 38% vs 19% apoptosis) or the Ca2+ ionophore A23187 (0.5 μM, 27% vs 17% apoptosis). In contrast, no differences in apoptosis induction were observed in the siRNA CREB- and siRNA NS-transfectants exposed to 250 nM staurosporine, 5 μg/ml brefeldin A, or 5 μg/ml tunicamycin, which do not activate CREB. Our results clearly demonstrate that constitutively active CREB suppresses apoptosis, possibly via COX-2 upregulation, in human L3.6pl pancreatic cancer cells, suggesting that it might be an important new target in the treatment of such a deadly, and currently incurable, disease.

426 POSTER

A high throughput screen and secondary assays for the identification and evaluation of histone methyltransferase inhibitors

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Chromatin is a complex between DNA and histone proteins allowing the compaction of DNA into the nucleus. Chromatin modifications such as acetylation, phosphorylation and methylation on histone N-terminal tails constitute part of the histone code and can determine whether chromatin conformation is heterochromatic (transcriptionally repressive) or euchromatic (transcriptionally active). Chromatin modifying enzymes are emerging as interesting therapeutic targets in cancer. It is hypothesised that in the same way that inhibition of chaperone proteins such as HSP90 causes multiple signalling blockades, inhibition of chromatin modifying enzymes can simultaneously affect many pathways by changing chromatin structure and thus gene transcription. Histone lysine and arginine methylation can signify both heterochromatic and euchromatic domains depending on the residue modified and the combinations of modifications in the local chromatin environment. Histone lysine methyltransferases (HKMTs) are mostly SET domain containing proteins which are divided into four families SET1, SET2, EZH and RIZ and several HKMTs have been shown to be deregulated in cancer. Due to the increasing evidence implicating these enzymes in tumourgenicity, we have run a high throughput screen against SET7/9. This enzyme is a SET1 HKMT, homologous to other cancer-related enzymes, that mono-methylates histone H3 K4 *in vitro* and also p53 *in vivo*. A FlashPlate® assay measuring incorporation of tritiated acetyl coenzyme A into histones was used. In total 64,000 compounds were screened ($Z' = 0.6$, CV% = 9.1). S-adenosylornithine was used as a positive control (7.3 ± 1.4 μM). In addition, a virtual high throughput screen of >100,000 compounds has been carried out against the substrate and co-factor binding site using the published crystal structure. For downstream compound evaluation, two secondary cell-based assays have been developed to confirm HKMT inhibition by measuring changes in mono-methylation at specific sites (i.e. H3 K4). A time-resolved fluorescence immunoassay (TRF-Cellisa) has been validated for this purpose and also provides a format suitable for phenotypic screening of compound libraries. An electro-chemiluminescent assay using the Meso Scale Discovery platform has also been developed. This will be a useful assay to identify changes in mono-methylation as a mechanistic and pharmacodynamic marker in the later stages of drug discovery.
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427 POSTER

Preclinical development of xiapuradamib therapy for lung cancer

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Evasion of apoptosis allows malignant cells to proliferate, and resist response to chemotherapy, radiotherapy or immune surveillance. The ultimate effectors of apoptosis are a family of intracellular cysteine proteases termed caspases, which are activated by various cell death stimuli. X-linked inhibitor of apoptosis protein (XIAP) is one of eight IAPs that selectively inhibit caspase-3, -7, and -9, preventing apoptosis. The observations that overexpression of XIAP confers resistance to chemotherapy, and that suppression of XIAP with siRNA or antisense oligonucleotide restores chemosensitivity in pre-clinical models has heightened interest in XIAP as a potential therapeutic target. Notably, temporary inhibition of XIAP does not appear toxic to normal cells. We are evaluating a small molecule inhibitor of XIAP, xiapuradamib, on lung cancer cell lines *in vitro*. Using a short-term cell viability assay (SRB) we have determined ED50 values for xiapuradamib, in comparison with clinically relevant chemotherapeutic agents. Results from our studies of the combinational effects of xiapuradamib with standard cytotoxics have demonstrated synergism that is most marked with vinorelbine over 24, 48 and 72 hour time courses, and with cisplatin over 48 and 72 hours, in non-small-cell lung cancer cells using the Chou-Talalay and Pritchard & Shipman methods. Synergism is less marked with taxanes. Exposure of cells to xiapuradamib prior to cisplatin or vinorelbine treatment results in greater synergy compared with exposure to the cytotoxic followed by xiapuradamib. Experiments to determine the timing of apoptosis in relation to xiapuradamib treatment as a single agent and in combination will be reported.