for unresectable HCC is even worse as chemotherapy response rate is low (less than 20%) with median survival duration less than a year. As HCC is highly malignant, there is an urgent need for an alternative novel therapeutic approach in addition to conventional clinical management.
Targeted cancer therapy is promising to limit non-specific toxicity and to improve therapeutic efficiency compared to conventional chemotherapy. Clinically approved therapeutic antibodies include trastuzumab (Herceptin) for metastatic breast cancer, bevacizumab (Avastin) for colorectal/lung cancer, and cetuximab (Erbitux) for colorectal cancer. However, no therapeutic antibody has been approved for HCC, and the research literatures on the molecular targets in HCC are limited. To address this issue, we have systematically examined the global gene expression profiles of various liver tissues by cDNA microarray to better understand the molecular signatures of liver cancers (Cancer Res 2002; Mol Biol Cell 2002). More than 200 liver samples have been examined, and genes differentially expressed between HCCs and their adjacent non-tumor liver tissues (chronic hepatitis and cirrhosis), and normal liver tissues have been identified. Differential expression of a number of genes was shown to associate with aggressive tumor features, including GPAA1, CLDN-10, AA454543, GEP and CYP2E1. Down-regulation of expression in some of these genes by anti-sense approach revealed inhibition of growth and invasion, and these genes would be promising novel therapeutic target for HCC.

417 POSTER

Membrane Type 1-Matrix Metalloproteinase (MT1-MMP) is overexpressed in lung cancer and can cleave peptide-conjugates

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Matrix metalloproteinase (MMP) activity is required for tumour growth and metastasis. This study assessed the expression of membrane-type 1 MMP (MT1-MMP) in human Non Small Cell Lung Cancer (NSCLC) specimens and paired histologically normal lung tissue. Analysis of cell lines, xenografts and NSCLC specimens (representative of all stages and grades), as well as corresponding histologically normal lung tissue, was undertaken by quantitative Real Time PCR (qRT-PCR). A statistically higher level of MT1-MMP expression was observed in tumour tissue relative to histologically normal lung samples. MT1-MMP activity, as measured in cell lines and xenografts by ELISA assay, demonstrated a strong correlation between MT1-MMP activity and gene expression levels. This indicates that qRT-PCR data gives a realistic indication of MT1-MMP activity in NSCLC. Following demonstration of selective expression, an MT1-MMP targeted peptide-conjugate was synthesised using solid-phase peptide synthesis. This targeted peptide conjugate is shown by liquid chromatography mass spectrometry techniques to be preferentially cleaved in MT1-MMP expressing tumour homogenates relative to mouse plasma and liver homogenates. Cell lines and xenografts expressing MT1-MMP (as determined by qRT-PCR and western blotting) efficiently cleave the peptide-conjugate to release the active agent, whilst those negative for MT1-MMP do not. Clinically derived NSCLC tumours expressing MT1-MMP are also able to release the active agent whereas the peptide-conjugate was stable in serum from the same patients. This study shows that MMPs are potential therapeutic targets in NSCLC.

418 POSTER

H_2O_2-associated DNA-damage induces acetylation-dependent upregulation of p21WAF1 expression in colorectal cancer cells

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Background: Tumor cells are frequently subjected to oxyradicals generated by immune cells or after treatment with anticancer drugs. It is only poorly understood how oxidative stress contributes to histone modifications and associated alterations of gene expression
Material and Methods: To address this question, we studied the p53 target gene and cell cycle regulator $p21^{WAF1}$ after H_2O_2 treatment (30mM, 3min) with and without pre-treatment with the histone deacetylase inhibitor trichostatin A (TSA) in HCT116 colorectal cancer cells. The MTT and cytotoxicity assay was used to measure cell viability and cytotoxicity. mRNA expression was determined by *real-time* RT-PCR on a LightCycler, and protein expression was detected by Western Blotting. Promoter status of the $p21^{WAF1}$ gene was analyzed by chromatin immunoprecipitation (ChIP). HDAC activity was determined using a HDAC fluorimetric assay.
Results: In HCT116 cells, H_2O_2 caused G_2/M arrest that was accompanied by a strong increase in p53 and $p21^{WAF1}$ expression. Chromatin immunoprecipitation experiments demonstrated that the oxidative stress induced the recruitment of p53 to the $p21^{WAF1}$ promoter and concomitant histone H4 acetylation. Pretreatment of the cells with TSA reinforced these effects through several pathways. Firstly, TSA prevented H4 deacetylation. Secondly, it caused the dissociation of HDAC1 from the $p21^{WAF1}$ promoter, thus allowing for higher p53 binding efficiency. Finally, TSA enhanced acetylation of p53, increasing its binding efficiency at the $p21^{WAF1}$ promoter. All these mechanisms contributed to the increase in $p21^{WAF1}$ expression and to the ensuing G_2/M arrest.
Conclusions: These results suggest that the acetylation-dependent up-regulation of $p21^{WAF1}$ seems to be a common principle after H_2O_2-based DNA damage. TSA in combination with a H_2O_2-based anticancer drug might have remarkable antiproliferative activity in colorectal cancer cells.

419 POSTER

Defining Hsp90 as inhibitor of apoptosis in small cell lung cancer

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Apoptosis plays an essential role in the elimination of mutated or transformed cells from the body. In order to survive, cancer cells and their precursors must develop highly efficient, and usually multiple, mechanisms to avoid apoptosis. The complexity of apoptosis resistance in lung cancer is especially apparent; in many such cancers there is not only loss of proapoptotic proteins, but also activation or overexpression of anti-apoptotic molecules. Among these, several caspases including caspase-1, -4, -8 and -10 are either not expressed or are inactivated in small cell lung cancer (SCLC) cell lines and tumors, suggesting that major perturbations in the death receptor pathway and other aspects of apoptosis characterize this tumor type. These defects ultimately result in resistance to routine chemotherapy accounting for the poor prognosis of SCLC. Whereas this disease often initially responds well to chemotherapy, relapses occur almost without exception, and these are usually resistant to cytotoxic treatment. It is thus of major importance for SCLC treatment to identify novel targets whose sensitivity is not perturbed in chemotherapy-resistant tumors. We identify Hsp90 as one such target in SCLC. Probing selective Hsp90 inhibition in SCLC cells by pharmacological means, we show that both chemotherapy naive and resistant SCLC cells exhibit a strong apoptotic response when challenged with an Hsp90 inhibitor. Apoptosis in SCLC cells is independent of upstream caspase activity and occurs through a mitochondrion-mediated pathway, via caspase-9 activation and employing caspase-3 as effector caspase. Induction of apoptosis is restricted to SCLC cells, as normal lung fibroblasts are unaffected by Hsp90 inhibition. These effects of Hsp90 inhibitors are maintained in animal models of SCLC. Further, treatment of mice bearing xenografted tumors established from SCLC cells harvested from a patient whom had failed several lines of chemotherapy, resulted in both tumor growth inhibition and reduction of metastasis. With several Hsp90 inhibitors, such as 17AAG, 17DMAG and the purine-scaffold CNF2024 currently in clinic in Phase I and II evaluations, and with more novel scaffold small molecules to soon follow, these findings provide a strong platform for the introduction of Hsp90 in clinic as a novel target in the treatment of patients with SCLC.

420 POSTER

Measuring alpha-folate receptor expression levels on ascites tumour cells may help to identify patients that are more likely to respond to alpha-FR targeted therapy

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The alpha-folate receptor (α-FR) is a folate transporter with very restricted expression levels in normal tissues but is overexpressed in several cancers, particularly epithelial carcinomas. This offers a novel therapeutic target for new selective imaging and cytotoxic agents including BGC 945, an α-FR targeted TS inhibitor. Tumour specimens from >90% of patients with non-mucinous ovarian cancer homogenously overexpress α-FR. However, tumour samples are often unavailable if patients subsequently relapse. A number of these patients develop ascites that is often rich in tumour cells. A novel three antibody flow cytometric method to assess α-FR expression on tumour cells from ascites has been developed. An antibody to BerEP4, an epithelial cell marker expressed on >90% of ovarian cancers, and an α-FR

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