processes that involve ion exchange. Of the 13 α-CA isozymes, the membrane bound CA IX and CA XII are regulated by the hypoxia inducible transcription factor (HIF). CA IX is over-expressed in a number of solid tumours and its expression has been linked to poor prognosis and is implicated in tumorigenesis.
CA IX is involved in the acidification of extracellular pH (pHe), and several selective CA IX inhibitors are able to reduce extracellular acidification in MDCK-CA IX cells under hypoxic conditions. Recent studies have suggested that CA IX selective inhibitors may enhance the action of some chemotherapeutic agents (e.g. Doxorubicin) when used in a combination therapy.
HT-29 colon carcinoma cells express high basal CA activity, which is induced under hypoxic conditions (5-fold). Using a novel series of selective CA IX inhibitors and the clinically used broad range CA inhibitor acetazolomide (AZM) the effects of modulating CA activity in HT-29 cells was evaluated. The ability of selective CA inhibitors to enhance the uptake and cytotoxicity of the anticancer agent doxorubicin (DOX) was also investigated. The clonogenic assay was used to assess changes in cytotoxicity and FACS to investigate DOX uptake under aerobic and hypoxic conditions.
When used in combination with DOX (5 μM) at a non-toxic concentration of 100 μM, the CA IX selective inhibitor WRM-34 was able to enhance the cytotoxicity of DOX under hypoxic conditions (2.3-fold). A 2-fold increase in DOX (5 μM) uptake was observed in HT-29 cells when combined with WRM-34 (100 μM) under hypoxic conditions. No enhancement was seen under aerobic conditions.
The non-selective CA inhibitor AZM (100 μM) was able to enhance the cytotoxicity of DOX (5 μM) (1.8-fold) and uptake (1.4-fold) when used in combination under hypoxic conditions, with no enhancement observed under aerobic conditions.
The combination of a potent and specific CA IX inhibitor increased the cytotoxicity and uptake of the anticancer agent DOX in HT-29 cells under hypoxic conditions. This suggests that CA IX selective inhibitors may be used to enhance the uptake and efficacy of weakly basic chemotherapeutic agents, possibly via a mechanism, which decreases the pHe microenvironment surrounding tumour cells. In conclusion, selective CA IX inhibitors may be useful diagnostics and therapeutic agents in the treatment of solid tumours.

436 POSTER

Single nucleotide polymorphisms of Akt1 in colorectal cancer

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Background: Various molecular pathways are altered by cancer cells to confer a survival advantage. By molecularly characterizing prosurvival and apoptotic pathways, more effective pharmacologic interventions can be used to target cancer cells. For example, the presence of a tumor-specific mutation at a known drug target site may better predict positive response to therapy than an alternative drug target site. The three major signal transduction pathways that have critical roles in sensing and integrating important signals in the cell are the p53 pathway, the Akt pathway, and the mTOR pathway. There is extensive cross-talk between these pathways in the regulation of cellular responses. In addition, p53, PTEN, PI3K, Akt1, and mTOR are some the most frequently altered tumor suppressor genes and oncogenes in cancers. Akt1 is an important prosurvival protein functionally involved in antiapoptosis in various cancers. Single nucleotide polymorphisms (SNP) are single base changes in the gene that are stable, heritable and occurs in greater than 1% of the population. Furthermore, approximately 30 million SNPs occur in the human genome. SNPs found in intron 3 (C/T) and exon 9 (A/G) of Akt1 affect apoptosis with the homozygous SNP haplotype (CC/GG) having decreased apoptosis.
Materials and Methods: The Trizol protocol was used for DNA extraction from HT-29 cells. Primers designed for Akt1 were used to produce amplicons that were then sequenced using the CEQ DNA Analyzer.
Results: The HT-29 colorectal cancer cell line was used to determine whether SNP 3 and SNP 4 in Akt1 can be detected. Both SNPs were detected in HT-29 cells. The haplotype predominant in the HT-29 cell line is the (CC/GG) haplotype which confers decrease apoptotic signals.
Conclusions: By characterizing cell lines in a pathway specific manner, a genetic profile for colorectal cancer can be constructed to make drug discovery more efficient. The data obtained indicate at least one molecular event conferring a survival advantage to cancer cells in HT-29 cell line, the (CC/GG) haplotype in Akt1. Finally, with the ability to identify molecular biomarkers predictive of drug response will help clinicians to better select the combinations of cancer treatments that maximize benefits for their patients and minimize their side effects.

437 POSTER

In vivo evaluation of sorafenib (Nexavar®) and sunitinib (Sutent®) alone and in combination with Rapamycin in two human renal tumor xenograft models

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Sorafenib (Nexavar®) and sunitinib (Sutent®) are recently approved anticancer agents targeting vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Rapamycin is an immunosuppressive agent found to demonstrate potent anticancer activity by modifying cellular signaling through specific pathways, resulting in apoptosis. Recent preclinical and clinical studies have demonstrated some single agent treatment benefit; however, whether sorafenib or sunitinib ± rapamycin would demonstrate additive or synergistic combination effects was unclear. To test this hypothesis, we examined tumor growth inhibition (TGI) potential of these agents both alone and in combination in two human renal tumor xenograft models, G401 and Caki-1; sorafenib was dosed per os (PO) via oral gavage at 60 mg/kg (QD × 10), sunitinib PO at 40 mg/kg (QDx21), and rapamycin PO at 8 mg/kg (QDx14). Significant endpoints included tumor growth inhibition (TGI), tumor regression, weight loss, and agent toxicity. Differential single agent activity was noted in these studies, with rapamycin demonstrating significant TGI in G401 ($p < 0.05$) compared with sorafenib and sunitinib alone. Some benefit was also reported in combination groups compared with control in G401 and some selective activity was noted between sorafenib and sunitinib in these studies. Overall, these results demonstrate single agent activity of rapamycin towards renal cancer and suggest a benefit with combination therapy.

438 POSTER

Organic and inorganic arsenics operate by different biochemical pathways to induce apoptosis in cancer cells

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Background: Arsenics are potent anti-cancer drugs. ZIO-101 (S-dimethyl-arsino-glutathione), a new organic arsenic, is active against diverse cancers in experimental models in vitro and in vivo. ZIO-101 is also unassociated with many of the toxicities of inorganic arsenics, like arsenic trioxide (As_2O_3). At equimolar extra-cellular arsenic concentrations, ZIO-101 is 5–10-fold more efficient than As_2O_3 in entering cancer cells resulting in more mitochondrial damage and apoptosis than As_2O_3. Furthermore, ZIO-101 induces apoptosis in leukemia cells from subjects resistant to therapy with As_2O_3.
Methods: NB4 (leukemia) and U937 (lymphoma) cells were cultured alone or with microM of As_2O_3 or ZIO-101 for 24 h. Aliquots of treated cells were tested in parallel for viability (MTT) and gene-expression profile using the Affymetrix U133_Plus_2 arrays. Differentially expressed genes were identified by fitting a linear model for each gene, making contrast for each of the comparisons based on linear model estimates, using a Beta-Uniform Mixture model (BUM) to analyze p-values of linear model F-test and t-test of each contrast and estimating cutoffs of p-values of F-test and t-test by setting FDR 0.05 for BUM results to identify differentially expressed⩽ genes.
Results: Data are summarized in the Table.

Comparison	Cutoff for F-test	Cutoff for t-test	N differential genes
U937 + As_2O_3 vs U937 + control	P < 0.03	P < 6.5e-011	0
U937 + ZIO 101 vs U937 + control	P < 0.03	P < 0.006	2534
NB4 + As_2O_3 vs NB4 + control	P < 0.03	P < 0.0005	190
NB4 + ZIO-101 vs NB4 + control	P < 0.03	P < 0.00008	14

Conclusions: These data indicate the biochemical pathways by which ZIO-101 and As_2O_3 induce apoptosis in cancer cells differ. This is consistent with discordant effects of modulators of reactive oxygen species (ROS) on apoptosis-induction and by the differential patterns of gene-expression in cancer cells treated with these drugs. The data support the notion that ZIO-101 may be active in cancer cells resistant to As_2O_3.