that mTOR only partially regulates VEGF under hypoxia. To determine whether VEGF production was controlled by Akt signaling, independent of mTOR, we exposed cells to the Akt-selective inhibitor A443654 for 24 hr and VEGF secreted into medium determined. A443654 completely inhibited signaling through Akt as judged by loss of detectable phospho-S6 protein, and hypophosphorylation of GSK-3b. Of note inhibition of Akt was more effective than rapamycin in blocking hypoxia-driven VEGF in 3/3 RMS and 2/4 NB cell lines. Combination of A443654 with rapamycin was additive or synergistic and completely blocked hypoxia-driven increases in VEGF. Because inhibition of Akt may result in unacceptable toxicity (hyperglycemia and hyperinsuliemia), we have explored the effect of blocking the IGF-1R using an antibody (CP-751871) as an alternative strategy. Administration of 0.25 mg to tumor bearing mice resulted in dramatic downregulation of IGF-1R in 4/5 sarcoma xenograft models, associated with a dramatic decrease in pAkt and pS6 levels.
Conclusions: These preliminary results suggest that direct inhibition of IGF-1R may be an interesting approach to modulating VEGF in pediatric sarcoma, and other solid tumors.

45 POSTER

Focal adhesion kinase is a key signalling intermediate in interleukin-8 promoted chemotaxis and adhesion of prostate cancer cells to bone marrow endothelium

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Purpose: To characterize the biochemical and functional relationship between interleukin-8 (IL-8) signaling and focal adhesion kinase (FAK) activation and to define their importance to prostate cancer metastasis.
Experimental design: Immunohistochemistry (IHC) conducted on human prostate biopsy tissue was used to determine the phosphorylation status of FAK in tumour cells relative to IL-8 expression. Experiments using metastatic prostate cancer PC3 cells established the biochemical, molecular and functional importance of FAK to IL-8 promoted cell motility and adhesion to bone marrow endothelial cells (BMECs).
Results: IHC demonstrated normal prostate epithelium to be devoid of FAK expression/activation but expression and autophosphorylation of FAK was detected in tumour cells of locally-invasive and hormone-independent prostate tissue. Statistical analysis confirmed that IL-8 expression correlated with increased autophosphorylation of FAK on Tyr^{397} in prostate cancer cells ($p < 0.001$). Stimulation of PC3 cells with IL-8 induced cell polarization and promoted the redistribution of FAK to sites of focal adhesion. Immunoblotting confirmed that IL-8 induced time-dependent phosphorylation of FAK on Tyr^{397}, Tyr^{576} and Tyr^{925}, that was mediated by a complex signaling cascade downstream of CXCR1 and CXCR2 receptors. Inhibition of FAK activity, using the dominant-negative FRNK construct or through RNAi-mediated depletion of FAK, attenuated IL-8-promoted activation of Rac-GTPase in pull-down assays, abrogated IL-8 promoted chemotaxis and attenuated IL-8 potentiated adhesion of PC-3 cells to BMECs, respectively.
Conclusions: IL-8 signaling regulates FAK activation in prostate cancer cells and is functionally important in mediating IL-8 promoted cell motility and adhesion, consistent with the metastasis promoting function of this chemokine. Our results describe a novel molecular basis to IL-8 promoted metastasis of prostate cancer and indicate the potential therapeutic significance of attenuating IL-8 expression in prostate cancer.

46 POSTER

Genetically engineered PAI-1 in anti-angiogenic and anti-metastatic therapy

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Abnormal proteolytic activity of urokinase (uPA) is one of the factors causing metastasis and angiogenesis. Thus eradication of uPA activity might result in inhibition of these processes. The small molecular inhibitors of uPA and plasminogen activator inhibitor (PAI-1) have been successfully used to reduce the angiogenesis and tumor growth. While uPA inhibitors were used we have observed reduction of sprout formation of human vascular endothelial cells, lowering blood vessel density in chick embryo chorioallantoic membrane (CAM) assay and reduction of tumor size of xenografts of human prostate LnCAP and DU145 cancer cells.
Plasminogen activator inhibitor (PAI-1) that inhibits the uPA could be used as an inhibitor of angiogenesis. However, wild PAI-1 is not stable and converts into the latent form in t1/2 $\approx$ 2 hours. This conversion is associated with partial insertion of the reactive loop (P4−P10′) into the PAI-1 molecule. In such conformation, P1−P1′ are not accessible for reaction with uPA. By 2 to 6 point mutations that could form disulfide bridges in PAI-1s in proximity of A3, A5 strands, we have extended half-life of this protein up to ~650 h. This PAI-1 is called VLHL PAI-1 and is fully functional as demonstrated by uPA inhibition. Additional, genetically engineered clone was produced by deleting part of this protein and lowering its size to create molecule that is more therapeutically desired. Mutant of Arg346 $\rightarrow$ Ala produced VLHLns PAI-1 that do not react with uPA and will be used as negative control.
Using baculovirus expression system PAI-1s were expressed in Sf9 insect cells and purified using affinity tag (6His). In single step purification we achieve +95% purity. The identity of PAI-1 was conformed by tandem liquid chromatography-mass spectroscopy. Disulfide bridge of VLHL PAI-1 could be reduced by DTT and reduced cysteine can't keep A3 and A5 strands together that is prerequisite of extending PAI-1 activity. Reduced form of VLHL PAI-1s convert into latent form as wPAI-1 does, and do not inhibit uPA. These conform our assumption of importance of the disulfide bridges in extending the half life of PAI-1.
Novel PAI-1 was fully functional against uPA and showed anti-angiogenic activity in the in vitro and in vivo models. Such prolonged serpin activity, which is therapeutically desired in cancer treatment could launch a new class of novel anti-cancer agents based on Cys mutated PAI-1s.

47 POSTER

A phase II study of Sorafenib (BAY 43–9006) in recurrent and/or metastatic squamous cell carcinoma of the head and neck (SCCHN) and nasopharyngeal cancer (NPC): final results

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Background: Sorafenib is an oral multi-kinase inhibitor targeting Raf kinase and VEGFR-2 among others. As the Ras-Raf-MAPK-ERK signaling pathway and angiogenesis are thought to play a significant role in the pathogenesis of head and neck cancers, we conducted a phase II study of sorafenib in recurrent and/or metastatic SCCHN and NPC to determine its efficacy and safety in this patient population.
Patients and Methods: This is a single arm, two-stage phase II trial. Sorafenib was administered orally at 400 mg BID continuously. Patients had $\leqslant$1 line of chemo for recurrent disease, performance status (PS) ECOG 0–2, and adequate organ function. Response was evaluated every 8 weeks according to RECIST criteria. At the end of stage one, efficacy criteria for further accrual were not met but the study was amended to enroll an additional 5 patients for pharmacodynamic evaluations. The biologic effects of sorafenib on tumors were assessed before and 4 weeks after treatment initiation.
Results: We enrolled 28 patients, of whom 27 and 26 were eligible for toxicity and efficacy evaluations. Median age was 53 years (range 37−77); 63% patients were male; 89% had PS 0 or 1; 74% SCCHN and 26% NPC; 70% of patients received prior chemotherapy, 48% had prior first-line chemotherapy for their recurrent and/or metastatic disease. In total, 72 cycles have been administered with a median of 2 cycles per patient (range 1–7). Most common adverse events (AE), at least possibly related to sorafenib, were fatigue in 79%, lymphopenia in 42%, mucositis in 42%, anemia in 35%, hand-foot skin reaction in 29% and hypertension in 28% of cycles. Most common grade 3 AEs were lymphopenia and fatigue in 17% and 7% of cycles. No grade 4 AEs were observed, 2 deaths on study were unlikely related to sorafenib. One patient with SCCHN (3.7%) had a confirmed partial response, 10 (37%) had stable disease ranging from 2 to 6 cycles and 15 patients (55.6%) had progressive disease. Median time to progression was 1.8 months (95% CI: 1.6–3.4) and median overall survival was 4.2 months (95% CI: 3.6–8.7). Results of the PD analysis will be presented at the meeting.
Conclusions: Sorafenib was well tolerated in this group of patients. Although the criteria for the second stage were not met, single-agent sorafenib has modest anti-tumor activity, comparable to single-agent erlotinib and gefitinib. Further evaluation of sorafenib in combination with other agents may be warranted in SCCHN and NPC.

48 POSTER

Distinct gene expression profiles and cell death pathways in clear-cell renal cell carcinoma (CCRCC) and colorectal carcinoma (CRC) cells:r elationship to hypoxia, von Hippel Lindau protein (pVHL) expression and anti-tumor activity of sorafenib

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Background: The interaction between pVHL and hypoxia is important in regulating target genes in CCRCC cells. Sorafenib, a multi-kinase inhibitor