HIF-1α protein expression (by immunohistochemistry) between restricted and repleted tumors.
We conclude that during tumor growth the requirements for ascorbate in immunity and the degradation of HIF-1 are superceded by the requirement for ascorbate in angiogenesis. Furthermore, we speculate that ascorbate and prolyl hydroxylases may prove to be useful targets for the development of anti-angiogenic compounds.

88 POSTER

Identification of a small molecule drug that post transcriptionally inhibits production of VEGF protein by targeting 5′ UTR-dependent translation

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Background: Using a phenotypic high-throughput screening platform, termed GEMS (Gene Expression Modulation by Small molecules) we have identified small molecules that modulate the expression of target proteins through the regulatory functions of the untranslated region (UTR) of their mRNAs. Utilizing a reporter gene under the control of the 5′ and 3′ UTRs from the mRNA encoding vascular endothelial growth factor A (VEGF), the development candidate PTC299 was identified. PTC299 is an orally bioavailable small molecule that post transcriptionally inhibits the production of VEGF, thus inhibiting tumor angiogenesis. PTC299 inhibits the expression of all major isoforms of VEGF in cell culture, with EC_{50} values in the low nanomolar range and is highly selective for inhibition of VEGF expression when compared to a number of other growth factors, cytokines, and intracellular proteins.
Materials and Methods: The abundance of VEGF mRNA was assessed via RT-PCR. Polysome-associated VEGF message was isolated via sucrose gradient centrifugation followed by northern blot analysis. The dependence on the VEGF 5′UTR for PTC299 activity was demonstrated by transfecting cells with reporter constructs containing the wild type 5′UTR or various deletions thereof. Pulse-chase studies were conducted with ^{35}S-methionine and experimental conditions included inhibitors of translation, secretion, and proteosome activity.
Results: Studies to elucidate the mechanism of VEGF inhibition demonstrated that PTC299 does not alter the steady-state levels of VEGF mRNA, nor does it measurably affect polyribosome association with VEGF message, strongly suggesting that PTC299 affects neither VEGF mRNA stability nor the initiation of VEGF translation. Utilizing both reporter gene (luciferase) expression and epitope-tagged VEGF, we have demonstrated that the VEGF 5′ UTR is critical for PTC299 activity. In pulse-chase immunoprecipitation studies, PTC299 does not cause intracellular accumulation of VEGF, either in the presence or absence of proteasome inhibitors, suggesting that PTC299 does not inhibit VEGF expression by accelerating the degradation of the protein.
Conclusions: Taken together, these results demonstrate that PTC299 inhibits the 5′ UTR-dependent translation of VEGF. Future studies will focus on identifying the cis-acting elements within the 5′ UTR that are necessary and sufficient for PTC299 activity and the trans-acting factor(s) that bind to the cognate region in the UTR.

89 POSTER

Contribution of nitric oxide and epidermal growth factor receptor in antimetastatic potential of paclitaxel in human liver cancer cell (HebG2)

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Background: Paclitaxel is a general antineoplastic drug used against different types of experimental and human tumors. Several anti-cancer drugs have been shown to stimulate nitric oxide (NO) production, which has been shown to affect many aspects of tumor biology.
Objective: This study was initiated to determine if paclitaxel stimulates NO production in HebG2 cells, and if so, whether NO interferes with the metastatic potential of HebG2 cells and contributes to paclitaxel cytotoxicity. In addition, we sought to determine the relationship between NO production and the expression of epidermal growth factor receptor (EGFR) and matrix metalloproteinases (MMPs) in HebG2 cells.
Materials and Methods: The effects of paclitaxel (0.1000 nM) on surviving fraction, NO production and the expression of EGFR, MMP-2 and MMP-9 were studied in human cancer cells (HebG2).
Results: Paclitaxel resulted in a significant dose-dependent decrease in the surviving fraction of HebG2 cells. A 62% and 86% decrease in the surviving fraction was attained at 10 nM and 100 nM paclitaxel, respectively. Paclitaxel produced a significant increase in NO production, starting from 1 nM. A 64% and 111% increase in NO production was attained after exposure to 10 nm and 100 nM of paclitaxel, respectively. In all of the HebG2 cells treated with paclitaxel (1−1000 nM), mRNA specific for EGFR, MMP-2 and MMP-9 were undetectable. However, untreated HebG2 cells and those treated with paclitaxel (0.1 nM) expressed mRNA specific for these markers.
Conclusion: This study suggests that: (1) increased production of NO may contribute to the toxicity of paclitaxel aganist HebG2 cells, (2) paclitaxel may inhibit metastasis via inhibition of the expression of EGFR and MMPs and (3) an inverse correlation exists between NO production and expression of EGFR and MMPs.

90 POSTER

Inhibition of VEGF (vascular endothelial growth factor)/VEGF receptor system activation and in vivo tumor-induced angiogenesis by an anti-angiogenic small molecule epoxyquinol B

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Angiogenesis is recognized as critical process in the growth and metastasis of tumor cells and many pathological conditions. Effective inhibition of this process should be a promising strategy to cure angiogenesis-related diseases, including cancer. In this regard, we have discovered several novel angiogenesis inhibitors using cell-based screening systems from microbial metabolites; i.e. epoxyquinols A and B, epoxytwinol A, azaspirene, and RK-805. Here, we present the biological activities of the highly functionalized pentaketide dimer epoxyquinol B on VEGF (vascular endothelial growth factor)-induced signaling pathway in HUVECs (human umbilical vein endothelial cells) as well as in vivo tumor angiogenesis. Epoxyquinol B inhibited endothelial-cells migration induced by VEGF-A at ED_{100} value of 3 μM without significant cell toxicity. Epoxyquinol B also inhibited capillary tube formation in 3-D-cultures system of HUVECs in a dose-dependent manner. Moreover epoxyquinol B blocked activation of VEGFR2-PLCγ signaling pathway through the inhibition of VEGFR2 activation by VEGF-A in HUVECs. Next, the ability of epoxyquinol B to inhibit the in vivo tumor-induced angiogenesis was examined using mouse renal carcinoma xenograft cells. As a result, numbers of blood vessels oriented towards the tumor was significantly decreased by treatment of epoxyquinol B. VEGFR2 is the major mediator of the mitogenic, angiogenic, survival and permeability-enhancing effects of VEGF-A in endothelial cells. Taken together, our results demonstrated that epoxyquinol B would provide a new insight into development of a promising angiogenesis inhibitor with a unique structure different from other drugs currently under clinical trial.

91 POSTER

Impact of tumor VEGF expression level on the in situ efficacy of the VEGFR2 associated tyrosine kinase inhibitor ZD6474

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Background: Since it is widely accepted that tumors must elicit an angiogenic response for survival, growth, and metastasis, there has been a great deal of interest in targeting this process therapeutically and lead agents developed for this purpose have entered clinical trials. Given its key role in tumor angiogenesis, vascular endothelial growth factor (VEGF) inhibition represents a prime strategy in angiogenesis inhibition. The present studies were conducted to examine how a tumor's inherent level of VEGF expression influences its response to such therapy.
Material and Methods: Clonal cell lines of a human colorectal carcinoma (HT29) were created via infection with a recombinant adeno-associated virus (rAAV) that contained the human gene for VEGF. Three clones were chosen: the first expressed VEGF at a level comparable to the parental (non-infected) cell line, the second expressed VEGF at an intermediate level (20-fold greater), and the third expressed VEGF at ~60-fold higher concentration.
Results: The response of the clones expressing various VEGF levels in vitro and when grown as xenografts in nude mice to the VEGFR2 associated tyrosine kinase inhibitor ZD6474 was then assessed. In vitro neither the growth kinetics nor their inherent sensitivity to ZD6474 differed significantly between the clonal and the parental cell lines. In situ the tumor vascularity and growth rate increased significantly with increasing VEGF expression. To determine the effect of ZD6474 treatment on angiogenesis in vivo an intradermal assay was used. The results showed that ZD6474 effectively inhibited the number of blood vessels that could be induced by both parental and high expressing VEGF tumor cell inoculates. Therapeutically, ZD6474 treatment led to growth delays in xenografts irrespective of the VEGF expression level of the tumor cells. However, the anti-tumor effect was significantly greater in tumors arising from the high expressing VEGF clonal cell line. For example, a 2-week ZD6474

treatment (25 mg/kg/day, Monday-Friday) resulted in a 10 day growth delay as opposed to a 4 day delay in tumors derived from the parental cells.
Conclusions: These findings suggest that ZD6474 may have particular utility in therapeutic settings involving aggressive tumors.

92 POSTER

Effects of AZD2171 on pharmacokinetics (PK) of carboplatin (C) and paclitaxel (P) in patients with advanced non-small cell lung cancer (NSCLC): a study of the National Cancer Institute of Canada Clinical Trials Group

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Introduction: AZD2171, a potent oral inhibitor of the tyrosine kinase activity of all VEGFR subtypes, is currently in clinical development. Effects of AZD2171 on PK of C and P were evaluated in a phase I study of AZD2171 in combination with standard doses of C and P in patients with advanced NSCLC.
Methods: C was administered at AUC = 6, and P at 200 mg/m^2 over 3 hours, q 3 weekly. AZD2171 was administered daily starting on day 2 cycle 1 at either 30 mg or 45 mg. Blood sampling for PK was performed during day 1 of cycles 1 and 2. Plasma concentrations of C and P were quantitated with high pressure liquid chromatograph (HPLC). PK analysis was conducted using non-compartmental analysis. Effects of the presence of AZD2171 and its dose on PK parameters were analyzed using 2-way ANOVA with interaction.
Results: Cycle 1 and cycle 2 data were available for 18 patients. PK parameters are summarized in the table.

Parameter	AZD1271 dose		P value
	30 mg (n = 8)	45 mg (n = 10)	
Paclitaxel CL (L/hr)			
No AZD2171	19.5±2.9	22.4±4.6	p < 0.0001 for
With AZD2171	14.3±4.3	18.2±6.0	presence of AZD2171
Carboplatin CL (L/hr)			
No AZD2171	7.0±0.9	9.9±3.5	p = 0.04 for dose effect
With AZD2171	6.4±1.3	8.8±3.9	

P clearance was significantly reduced in cycle 2. C clearance was significantly increased at the higher AZD2171 dose level. There was no correlation between pharmacokinetic parameters and toxicity such as neutropenia or GI toxicity.
Conclusions: P Clearance was reduced by approximately 20% in cycle 2, while C clearance was increased at the higher AZD2171 dose level. Further investigations are needed to determine the clinical significance of the role of AZD2171 on these observations.

93 POSTER

Phase I study of daily oral AZD2171, an inhibitor of the vascular endothelial growth factor receptors (VEGFR), in combination with oxaliplatin and infusional 5-FU (mFOLFOX6) in patients with advanced colorectal cancer (CRC): a study of the National Cancer Institute of Canada Clinical Trials Group

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Background: AZD2171 is a potent oral inhibitor of the tyrosine kinase activity of all VEGFR subtypes. Purposes of this study were to determine the recommended phase II dose of AZD2171 in conjunction with standard doses of mFOLFOX6, and the tolerability, safety, pharmacokinetic (PK) profile and anti-tumor activity of this combination in patients with previously untreated advanced CRC.
Methods: Patients (pts) eligibility criteria included: locally advanced or metastatic CRC; PS 0−2; no prior chemotherapy for advanced disease; adequate hematological, liver and renal functions. AZD2171 was administered daily orally starting Day 3 cycle 1 at a starting dose of 30 mg/d. Modified FOLFOX 6 consisted of oxaliplatin 85 mg/m^2 (2 hour infusion) day 1; leucovorin 400 mg/m^2 (2 hour infusion); and 5-FU bolus 400 mg/m^2 day 1 followed by continuous 5-FU infusion at 2400 mg/m^2 over 46 hours. Cycles were repeated every 14 days. Blood sampling for PK was performed during cycles 1 and 2 for oxaliplatin and 5-FU, and cycle 2 only for AZD2171. Response was assessed by RECIST every four cycles.
Results: To date, 9 patients received 16 cycles of treatment. Of the first 3 pts enrolled at the 30 mg dose level, one grade 3 diarrhea was observed in a patient who was not compliant with anti-diarrhea therapy. The cohort was cautiously expanded to enroll 6 additional pt. One DLT of grade 3 diarrhea was observed in the expanded cohort while grade 3 diarrhea was seen in a patient who was not compliant with therapy. Other common toxicities observed so far included hypertension, fatigue and nausea. Hematologic toxicity was similar to that expected with mFOLFOX6 alone. The study continues at the AZD2171 45 mg/d dose level with enhanced guidelines for early detection and treatment of diarrhea.
Conclusions: Toxicities of this combination appear manageable and predictable. Common side effects included diarrhea, fatigue and hypertension.

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Angiogenesis in human cutaneous tumors

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Background: Angiogenesis has become one of the most widely studied topics. Normal adult vasculature is generally quiescent in nature. The induction of new blood vessel growth from a pre-existing vascular bed is a characteristic of virtually all malignant tumors. The crucial regulators of the process of angiogenesis associated with tumor development and metastasis are vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs). VEGFs are a family of endothelial cell-specific cytokines that act as endothelial cell mitogens and regulate vascular permeability. The aim of this study was to analyze changes in the VEGFs and VEGFRs expression in primary cutaneous malignant melanomas in comparison with protein expression in benign melanocytic and trichogenic tumors and with microvascular density.
Material and Methods: The study included 68 malignant melanomas, 39 pigment nevi and 27 benign trichogenic tumors. Angiogenesis was evaluated using alpha-smooth muscle actin (ASMA) and expression. VEGF, VEGF-C, VEGFR-1, VEGFR-2, ASMA and nestin detection was performed on formalin-fixed, paraffin-embedded tissue sections by indirect immunohistochemistry.
Results: Malignant melanoma cells expressed VEGFs and VEGFRs cytoplasmatically in high levels. Their remarkable overexpression accompanied mainly advanced stages (Breslow III, IV, V). On the contrary, both pigment nevi and benign trichogenic tumors revealed less intensive protein staining. Protein expression correlated with microvascular density. An increased amount of capillaries stained by ASMA and nestin was found within malignant melanomas and in the adjacent dermis, where nestin expression demonstrated new blood vessels formation. Benign tumors exhibited sparse network of blood vessels.
Conclusions: Our results indicate that VEGFs and VEGFRs expression can be involved in skin vessel formation under benign and malignant conditions. The up-regulation of the analyzed factors is associated with significantly enhanced angiogenesis and can contribute to the growth and progression of malignant cells.
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95 POSTER

Invasion knock down of human colon cancer cells by siRNA specific for S100A4, a newly identified target gene of beta-catenin/TCF signaling

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Background: It has previously been shown that high expression of S100A4 is associated with cancer metastasis. Our aim was to elucidate the impact of gain-of-function beta-catenin on the metastasis-associated gene S100A4 in human colon cancer cell lines and tumors.
Material and Methods: We analyzed cell lines heterozygous for gain-of-function and wild-type beta-catenin, and variants homozygous for gain- or loss-of-function mutation in beta-catenin, for S100A4 expression, cell migration and invasion. beta-catenin-mediated S100A4 promoter activation