respectively. Both were prepared in saline immediately before each experiment and given either as a single intraperitoneal injection (CA4DP; 250 mg/kg), or subcutaneously every second day for two weeks (TNP-470; 100 mg/kg). Radiation (1×10 Gy; 240 kV x-rays) was administered locally to the tumour bearing foot of restrained non-anaesthetised mice. Response to treatment involved measuring tumour volume 5 times/week and calculating the tumour growth time (TGT; time to reach 3 times the treatment volume). Statistical analysis was performed using the Student's t-test, with the significance level being $p < 0.05$.

Results: The mean (±SE) TGT for untreated control tumours was 4.4 days (4.1–4.7). This TGT was increased to 5.5 (5.1–5.9), 5.5 (4.8–6.2) and 6.0 (5.4–6.6) days, by CA4DP, TNP-470 and CA4DP + TNP-470, respectively. However, only in the CA4DP treated groups were these increases significant. A significant increase to 12.6 days (12.0−13.2) was found following irradiation. Injecting CA4DP within 1-hour after irradiating non-significantly increased the radiation TGT to 14.4 days (13.1−15.7), but a further significant increase was observed when radiation was given with either TNP-470 alone or the combination of CA4DP + TNP-470; the respective TGTs being 36.2 days (33.6−38.8) and 50.3 days (46.1−54.5). This response to TNP-470 + CA4DP with radiation was significantly greater than that for TNP-470 and radiation. Additional studies are ongoing to produce full radiation dose response curves with each combination treatment.

Conclusions: VTAs had very little effect on the growth of this C3H mouse mammary carcinoma when used alone or in combination. However, they significantly improved the tumour response to radiation. The greatest affect was obtained when both the AIA and VDA were combined, resulting in a 4-fold increase in radiation response.

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85 POSTER

Inhibition of tumor cell invasion by a heparanase inhibitor and expression of a dominant negative mutant of heparanase

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Background: Overexpression of heparanase has been observed in many human tumors, such as head and neck tumors, suggesting an involvement of heparanase in tumor progression. Thus, heparanase may be considered as a molecular target for the development of cancer therapy. We have reported some heparanase inhibitors, including RK-682, which inhibited tumor cell migration and invasion. In this report, we demonstrated that treatment with a heparanase inhibitor, 4-Bn-RK-682 and expression of an active site-deficient heparanase (HP/E225A) suppressed tumor cell invasion.

Materials and Methods: Homology Modeling of Heparanase: The amino acid sequence alignment of human heparanase and 1,4-beta-xylanase from *Penicillium simplicissimum* was carried out manually using the Homology module of the Discover/Insight II Programs.

Establishment of Heparanase-overexpressing Stable Cell Lines: We established the clones expressing high levels of heparanase protein or HP/E225A protein were designated HT1080-HP cells and HT1080-HP/E225A, respectively.

Results: To develop selective heparanase inhibitors, we synthesized several RK-682 derivatives based on the rational drug design. Among them, 4-Bn-RK-682 has been found to possess a selective inhibitory activity for heparanase. 4-Bn-RK-682 also inhibited the invasion and migration of HT1080 cells. To evaluate antimetastatic potencial of 4-Bn-RK-682 *in vivo*, 4-Bn-RK-682 (50 mg/kg) was administered p.o. in mice received B16ML6 melanoma cells intravenously. After 15 days, about 40% of the number of B16BL6 metastases in their lungs were suppressed.

Moreover, we found that overexpression of heparanase stimulated tumor cell migration, on the other hand, expression of HP/E225A reduced the migration of HT1080 cells. The sample incubated with the extract from HT1080-HP/E225A cells possessed weaker heparanase activity than that from HT1080-Neo cells, indicating that expression of HP/E225A protein suppressed endogenous heparanase activity. Furthermore, the amount of cell surface HS level was dramatically increased in HP/E225A-expressing cells. Therefore, it is suggested that HP/E225A functioned as dominant negative manner, thereby suppressing tumor cell migration.

Conclusions: Strategies that inhibition of heparanase by treatment with heparanase inhibitor and gene transfer of HP/E225A may be effective therapies for those human cancers that are expressing heparanase.

86 POSTER

Additive action of a novel Cathepsin K inhibitor and zoledronic acid (Zometa) in a model of osteolytic human breast cancer metastasis

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Bisphosphonates are a class of drugs that inhibit the breakdown of bone, and are proving useful in the management of breast cancer patients with bone metastasis, where tumour cells activate osteoclasts to degrade bone. However, many breast-bone metastases do not completely benefit from bisphosphonate therapies, and there is very little impact on overall survival, emphasizing the great need for additional therapies. Cathepsin K is a cysteine peptidase secreted by osteoclasts that degrades collagen in the acidic lacunal space, and thus contributes to the bone destruction associated with bone metastasis.

Our study was designed to examine whether a newly derived Cathepsin K inhibitor from Novartis (CK-1) may work synergistically in conjunction with bisphosphonate. Female SCID mice were inoculated intratibially with T47D human breast cancer cells, and treated with a single i.v. bolus of bisphosphonate (zoledronic acid [Zometa]: 10, 50 or 100 ug/kg) two weeks after inoculation. Mice were then randomised to additionally receive either CK-1 (50 mg/kg) or vehicle i.p. twice daily from two weeks post inoculation, to provide combinations of each dose of Zometa with or without CK-1 ($n = 8$ per group).

Although only a slight inhibition of tumour-induced bone degradation was observed with the highest dose of bisphosphonate at 7 weeks post-inoculation, co-treatment with cathepsin K inhibitor significantly reduced bone loss ($p = 0.0472$ compared to Zometa alone).

These data clearly demonstrate the potential of CK-1 to complement bisphosphonate treatment of lytic bone metastasis associated with breast cancer.

87 POSTER

Ascorbate (vitamin C): friend or foe in the fight against cancer

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Ascorbic acid (Vitamin C, AA) is an essential dietary factor for humans and other primates due to a genetic absence of the key synthetic enzyme l-gulono-γ-lactone oxidase. The role of AA in enhancing immunity against cancer is widely accepted, although the precise mechanisms of this effect are not well understood. Recently, AA was identified as a cofactor in the hydroxylation and subsequent targeting for proteolysis of Hypoxia Inducible Factor-1α, a transcriptional regulator of the neoplastic response to hypoxia. The importance of AA in augmenting immunity and HIF-1 degradation has led several investigators to conclude that physiological or mega-physiological plasma levels should suppress tumor growth. AA however may also be critical for angiogenesis which is a prerequisite for tumor growth *in vivo*. Effective angiogenesis requires the deposition of type IV collagen into the basement membrane of blood vessels by endothelial cells. Type IV collagen production is dependent on the hydroxylation of proline by prolyl hydroxylase which requires AA as a co-factor.

We hypothesize that the requirement of AA for angiogenesis supercedes the requirements for anti-tumor immunity and HIF-1 degradation, and that dietary AA restriction will cause decreased angiogenesis and tumor growth. We measured type IV collagen produced by human umbilical vein endothelial cells (HUVECs) exposed to 0–200 μM AA (physiological [AA] = 50–100 μM) and found that a minimum of 25 μM AA is needed for immunoreactive type IV collagen production. We surmised that decreased type IV collagen deposition by HUVECs would cause poor tube formation on Matrigel *in vitro*. We observed intact tubes in 50–100 μM AA but disorganized tubes at lower (0−25 μM) and higher (200 μM) [AA]. Mice containing a homozygous genomic deletion for the last enzyme in AA synthesis l-gulono-γ-lactone oxidase (Gulo −/−) die of scurvy within 50 days of dietary AA restriction. We implanted Lewis lung carcinoma cells s.c. into Gulo −/− mice depleted of AA for 28 days, and then continued to restrict (0 mg/day) or fully supplemented the mice with AA (1.6 mg/day). After 12 days, we observed markedly decreased tumor growth in restricted mice (tumor mass: 1124±208.6 mm^3 [1.6 mg/day]; 231±68 mm^3 [0 mg/day] *p-value* <0.005). Microscopy of tumor sections from AA restricted mice showed greatly reduced capillaries (5±2/HPF) compared with repleted mice (19±3/HPF) and, in addition, substantially less collagen staining. Surprisingly, we found no significant differences in

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