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