histology to determine the effect of MMP-9 and SHH signalling in the growth of prostate tumour cells in mice.
Results: PN-1 inhibits signalling in the Hedgehog (HH) pathway in PC3 cells and acts to decrease cell proliferation and viability. These read-outs correspond to decreases in a trio of important HH proteins (GLI1, PTCH1, and Cyclin D1) as well as one ligand (Sonic) following PN-1 over-expression. The results were verified by the use of mutations devised to interrupt functionality of PN1. MMP-9 by affecting PN-1 is thus able to alter cellular hedgehog signalling. MMP-9 deficient mice have increased levels of PN1 in the prostate. Furthermore, ablation of MMP-9 correlated with decreased formation and growth of prostate tumours in an orthotopic *in vivo* prostate tumour model. Future research will identify the compartments responsible for regulating PN1 and MMP-9 during prostate tumourigenesis and progression.
Conclusion: PN1 levels may influence prostate tumour cell proliferation, viability, and cell invasion *in vitro* and *in vivo* by regulating sonic hedgehog and its downstream targets. MMP-9 is now shown to regulate hedgehog signalling by controlling the levels of PN1. Regulation of this pathway could constitute a significant therapeutic advance in treating cancers that exhibit high expression of HH markers.

476 POSTER

The selective alpha5beta1 integrin antagonist, CLT-28643, inhibits tumors angiogenesis and growth

P. Caldirola[1], U. Bäckman[1], Y. Cao[2]. *[1]Clanotech, Karolinska Development, Solna, Sweden; [2]Microbiology Tumor and Cell Biology, Karolinska Institute, Solna, Sweden*

Background: Extracellular matrix is crucial for endothelial cells survival, proliferation and motility during new vessels formation. Integrins are cell adhesion receptors involved in pathological angiogenesis. Particularly, abnormal expression of a5b1-integrin and its ligand, fibronectin, is a hallmark of cancer and angiogenic endothelial cells. Clanotech's proprietary compound, CLT-28643, is a selective $\alpha 5\beta 1$ antagonist as it was confirmed in several in vitro assays competing for the RGD fibronectin binding site. We have evaluated the in vivo effect of CLT-28643 by oral once daily administration in xenografted human tumor mouse models (lung, glioma, VEGF-resistant, and renal) and obtained significant inhibition of both tumor growth and tumor angiogenesis. CLT-28643 was well tolerated with no signs of toxicity.
Material and Methods: The test compound was evaluated in vitro for the capacity of inhibiting cell migration (chemotaxis), cell adhesion and binding of recombinant a5b1-integrin to fibronectin. In vivo efficacy studies were performed in xenograft and synograft mice models. Tumor cells were implanted subcutaneously in the hind leg of SCID mice. Oral administration of the test compound started at tumor volume of 250 mm^3. Changes in tumor volume were monitored every two days and calculated according to $0.52 \times \text{length} \times \text{width}^2$. Tumor angiogenesis was determined by 3 D technique.
Results: CLT-28643 inhibited cell migration by 53 % at 10 uM, adhesion to fibronectin by 50 % at 100 uM and binding to fibronectin by 90 % at 10 uM.

Xenograft models	Oral dose (mg/kg/day)	Inhibition of tumor growth (%)	Inhibition of angiogenesis (%)
Lung tumor	25–50	52–72	30–50
Glioma	50	30	14
VEGF-resistant tumor‡	50	43	24
Renal Cell Carcinoma	50	18–28	11

‡Synograft.

Conclusion: We demonstrated that the a5b1-integrin antagonist, CLT-28643 inhibits tumor angiogenesis, thereby resulting in regression of several human tumors in animal models when given orally. CLT-28643 has also shown to reduce both tumor growth and metastasis in the metastatic VEGF resistant tumor. These results suggest CLT-28643 as a good candidate for cancer therapy.

477 POSTER

The high invasive activity of S-adenosylmethionine decarboxylase-transformed fibrosarcoma cells is regulated by hepatocyte growth factor–Met signaling

M. Yin[1], E. Hölttä[1]. *[1]Haartman Institute, Department of Pathology, Helsinki, Finland*

Background: Tumor cell invasion and metastasis are the main cause of death of cancer patients. Understanding of the molecular mechanisms of these complex processes would therefore be of utmost importance. The signaling driven by the interaction of hepatocyte growth factor (HGF) with its tyrosine kinase receptor, Met, is thought to play an important role in the regulation of proliferation, survival and migration of various tumor cells, particularly the epithelial cancer cells.
Material and Methods: Here, we performed comparative DNA microarray (Affymetrix MOE430 Set), RT-PCR, Western blot, and ELISA analyses of normal and S-adenosylmethionine decarboxylase (AdoMetDC)-transformed fibroblasts, which are highly invasive in nude mice [1,2], to identify the invasion-related genes and proteins in fibrosarcoma cells. The candidate genes/proteins identified were further functionally tested by three-dimensional Matrigel assays.
Results: We found the AdoMetDC-transformants to show highly increased expression of HGF both at the mRNA and protein level relative to the normal fibroblasts. Most importantly, knock-down of HGF by short hairpin RNA expression or addition of neutralizing antibodies to the Met receptor was found to effectively inhibit the invasion of AdoMetDC-transformants in Matrigel. Further, we found the downregulation of HGF to block the attachment and spreading of the cells on laminin.
Conclusions: The results suggest an important role for the HGF/Met signaling axis in the regulation of the invasiveness of AdoMetDC-transformed fibrosarcoma cells. HGF and its receptor Met may thus offer good therapeutic targets for the prevention of fibrosarcoma cell invasion and metastasis.

References

[1] Paasinen-Sohns et al. J Cell Biol.151:801–10, 2000.
[2] Nummela et al. Cancer Res 66:701–12, 2006.

478 POSTER

Bcl-2 regulates HIF-1a protein stabilization in hypoxic melanoma cells via the molecular chaperone HSP90β

D. Trisciuoglio[1], C. Gabellini[1], M. Desideri[1], E. Ziparo[2], D. Del Bufalo[1]. *[1]Regina Elena Cancer Institute, Experimental Chemotherapy Laboratory, Rome, Italy; [2]La Sapienza University, Department of Histology and Medical Embryology, Rome, Italy*

Background: Hypoxia-Inducible Factor 1 (HIF-1) is a transcription factor that is a critical mediator of the cellular response to hypoxia. Enhanced levels of HIF-1α, the oxygen-regulated subunit of HIF-1, are often associated with increased tumour metastasis, therapeutic resistance and poor prognosis. In this context we previously demonstrated that the anti-apoptotic protein bcl-2 cooperates with hypoxia to promote HIF-1/Vascular Endothelial Growth Factor (VEGF)-mediated tumour angiogenesis.
Material and Methods: Expression vectors encoding human bcl-2, *wild type* or hydroxylation resistant HIF-1α were used for stable and transient transfections of M14 human melanoma line. The effect of bcl-2 stable transfection will be evaluated in cells under hypoxic conditions in terms of bcl-2 and HIF-1α protein expression and localization (*Pulse-chase, western blot and confocal microscopy analyses*) HIF-1α protein stability and ubiquitination (*Western blot and immunoprecipitation analyses*) and HIF-1 transcriptional activity (*reporter assay*). The role of Heat Shock Proteins (HSPs) in the bcl-2-mediated regulation of HIF-1α expression and transcriptional activity (*Western blot analysis and reporter assay*) was evaluated by using chemical or genetical inhibition. Immunoprecipitation experiments were also performed to investigate the possible effect of bcl-2 protein on the interaction of HIF-1α and HSPs.
Results: By using M14 human melanoma cell line and its derivative bcl-2 overexpressing clones, we demonstrated that bcl-2-induced accumulation of HIF-1α protein during hypoxia was not due to an increased gene transcription or protein synthesis. In fact, it was related to a modulation of HIF-1α protein expression at a post-translational level, indeed its degradation rate was faster in the control lines than in bcl-2 transfectants. The bcl-2-induced HIF-1α stabilization in response to low oxygen tension conditions was achieved through the impairment of ubiquitin-dependent HIF-1α degradation involving the molecular chaperone HSP90 but it was not dependent on the prolyl hydroxylation of HIF-1α protein. We also showed that bcl-2, HIF-1α and HSP90 proteins form a tri-complex that may contribute to enhancing the stability of the HIF-1α protein in bcl-2 overexpressing clones under hypoxic conditions. Finally, by using genetic and pharmacological approaches we proved that HSP90 is involved in bcl-2-dependent stabilization of HIF-1α during hypoxia, and in particular the isoform HSP90β is the main player in this phenomenon.
Conclusions: We identified the stabilization of HIF-1α protein as a mechanism through which bcl-2 induces the activation of HIF-1 in hypoxic tumour cells involving the β isoform of molecular chaperone HSP90.