Results: Rab27B-upregulation promoted G1/S phase cell cycle transition and increased proliferation, F-actin reorganization and invasion in cell culture, and invasive tumour growth and haemorrhagic ascites in a xenograft mouse model (at 10 weeks, survival of MCF-7 GFP vs GFP-Rab27B injected mice was 100% vs 62.5%, P = 0.0307). Proteomic analysis of purified Rab27B-secretory vesicles and the secretome of exogenous Rab27B-expressing breast cancer cells identified heat shock protein (HSP)90α as key pro-invasive growth regulator. HSP90α secretion occurred in a Rab27B-dependent manner and was required for matrix metalloproteinase(MMP)-2 activation. All Rab27B-mediated functional responses were GTP- and geranylgeranyl-dependent. Endogenous Rab27B mRNA and protein, but not Rab3D and Rab27A mRNA, associated with lymph node metastasis (P = 0.0002) and differentiation grade (P = 0.0014) in ER-positive human breast tumours.
Conclusion: Rab27B regulates invasive growth and metastasis in ER-positive breast cancer.

364 JAM-A is over-expressed in aggressive Her2 positive breast cancers

K. Brennan[1], T. Roche[2], E.A. McSherry[1], T. O'Grady[2], E.W. Kay[2], A.M. Hopkins[1]. *[1]Royal College of Surgeons, Surgery, Dublin, Ireland, [2]Royal College of Surgeons, Pathology, Dublin, Ireland*

Background: Each year breast cancer is diagnosed in an estimated 1 million women worldwide, and is the cause of death of over 400,000. Most breast cancers originate from previously normal breast epithelial cells. During their transformation into cancer cells, the epithelial cells often lose the expression of cell adhesion proteins, which may also confer a motile or migratory advantage to breast cancer cells. Among the cell adhesion proteins potentially affected by this transformation is Junctional Adhesion Molecule A (JAM-A) a membranous cell-cell adhesion protein involved in tight junction formation in epithelial cells. Previous data by McSherry et al. (2009) demonstrated a novel association between JAM-A gene and protein upregulation and aggressive tumours in breast cancer patients.
Objective: To investigate a potential association between JAM-A and aggressive breast cancers.
Methods: A breast cancer tissue microarray (TMA) consisting of benign and invasive cancers was analysed for JAM-A protein expression (n = 48). A second TMA in which Her2 staining had been performed was also analysed for JAM-A expression (n = 166). JAM-A and HER2 protein expression levels were also compared in a range of breast carcinoma cells by western blotting. The effects of functional antagonism of JAM-A (using an inhibitory antibody) on cell proliferation was examined using an MTT proliferation assay. Alterations in the phosphorylation of downstream PI3K and JNK pathway components were investigated by western blotting.
Results: We have observed a significant association between JAM-A protein upregulation and invasive cancers (p = 0.023), particularly those of the HER2 subtype (p = 0.023). In our second TMA, high JAM-A expression was significantly associated with HER2 protein expression (p = 0.019), lower patient age (p = 0.02) and poorly differentiated breast cancers (p = 0.001). Moderate to high JAM-A expression also significantly associated with the more aggressive luminal B, HER2 and basal subtypes of breast cancer (p = 0.005). Furthermore, high JAM-A expression was noted in invasive breast cancer cells which over-express HER2 (UACC-893). In contrast, JAM-A expression was low in invasive MDA-MB-231 cells which express very low levels of HER2. Additionally, functional antagonism of JAM-A reduced cell proliferation and altered the phosphorylation of PI3K and JNK pathway components in MCF-7 breast cancer cells.
Conclusion: Our data reveals that JAM-A is up-regulated in HER2-expressing breast cancers, associating with the more aggressive breast cancers affecting a younger portion of the population. Since JAM-A is highly expressed at the membrane we speculate it may offer promise as a future diagnostic and therapeutic target.

365 Cell delivery of the Met docking site peptide inhibits angiogenesis and vascular tumour growth

A.R. Cantelmo[1], R. Cammarota[1], D.M. Noonan[2], C. Focaccetti[1], P.M. Comoglio[3], M. Prat[4], A. Albini[1]. *[1]Casa di Cura Multimedica – IRCCS, Oncology Research, Milan, Italy, [2]University of Insubria, Department of Experimental Medicine, Varese, Italy, [3]Institute for Cancer Research and Treatment (IRCC), University of Turin Medical School, Candiolo, Italy, [4]Università del Piemonte Orientale "A. Avogadro", Department of Medical Sciences, Novara, Italy*

Background: Hepatocyte Growth Factor (HGF) and its receptor Met are responsible for different cell responses involved in developmental processes and pathological conditions, including cancer. In cancer, Met can act as an oncogene in tumour cells as well as a pro-angiogenic factor activating endothelial cells and inducing new vessels formation. Angiogenesis is a necessary step for tumour growth and metastatic dissemination. Current studies suggest that tumour growth would be hampered by blocking the neovascularisation process. Molecules interfering with the activity of Met could be valuable therapeutic agents that could interfere with the tumourigenic and metastatic processes triggered by Met in human cancer. Here we investigated the anti-angiogenic properties of a synthetic peptide mimicking the docking site of the Met C-terminal tail, which was delivered into cells by fusion with the internalization sequences of two cell penetrating peptides.
Material and Methods: *In vitro* we treated human endothelial cells with three different peptides containing Met docking site fused to the internalization sequences of Antennapedia homeodomain or of Tat peptides and we quantified proliferation. We then evaluated the ability of peptides to interfere with HGF-induced migration, invasion and morphogenesis of endothelial cells. By western blotting analysis we investigated whether these peptides affect Met activation and downstream signaling. *In vivo* we performed matrigel sponge assay and Kaposi's sarcoma xenograft to test the peptides efficacy on angiogenesis and tumour growth.
Results: We observed that in endothelial cells internalized peptides inhibited ligand-dependent cell proliferation, motility, invasiveness and morphogenesis *in vitro,* which correlated with interference of HGF-dependent downstream signaling, as shown by reduction in erk 1/2 activation. *In vivo,* the peptides inhibited HGF-induced angiogenesis when subcutaneously co-injected with HGF in mice in the matrigel sponge assay and significantly impaired Kaposi sarcoma xenograft tumour growth and vascularization.
Conclusions: these data show that the carboxyl-terminal sequence of Met receptor impaired angiogenesis triggered by HGF/Met interaction, suggesting the feasibility of using anti-docking site compounds as therapeutic agents to interfere with tumour progression and angiogenesis-impaired diseases.

366 Screen for inhibitors of cell migration in cancer metastasis using adenoviral knock-down

R. de Pril[1], A. Lekkerkerker[1], E. Frische[1], D. van Steenhoven[1], I. Maghrani[1], T. Perera[2], J. Arts[2], M. Page[2], D. Fischer[1], R. Janssen[1]. *[1]Galapagos B.V., Target Discovery, Leiden, The Netherlands, [2]Janssen Pharmaceutica, Ortho Biotech Oncology R&D, Beerse, Belgium*

Enhanced cell migration is a hallmark of metastatic cancer cells. The propensity of cancer cells to close an open wound in a cell monolayer is thought to predict this ability. Using our adenoviral shRNA knock-down library we have established a high-throughput wound healing assay to identify novel genes involved in cell migration. Therefore, a 96-pin scratch tool was designed to apply a constant mechanical scratch-wound in the cellular monolayer. Cells were fixed at a preset time period after scratching and the plates were imaged on an InCell Analyzer1000. We used transmitted light imaging for segmentation and quantification of the scratch wound that remained open. Accordingly, we developed an algorithm to measure the open space, which comprises the area of the wound as well as open spaces between cells. Genes whose knock-down inhibit cell migration can be identified by their effect on the open wound. In addition, genes that affect either proliferation or viability can thereby be counter screened as these disrupt the monolayer. Using this approach we demonstrated that two knock-down constructs targeting a known player in motility, CXCR4, inhibit wound healing, validating our set-up.
Using this wound healing assay we have identified a number of novel genes associated with cancer cell motility. These targets are currently validated for their effect in 3D invasion using Boyden chambers. We use confocal imaging on a Pathway 435, of both the seeded cells in the top compartment and the invaded cells on the bottom of the filter. In this way, we have identified a number of control genes which inhibit invasion through matrigel. Using this assay we will validate the hits that inhibit migration, for their ability to additionally inhibit 3D invasion. As our adenoviral knock-down libraries focus on drugable targets, these validated targets can quickly be employed to generate small molecule compounds or antibody therapeutics targeting cancer metastasis.

367 Genomic profiles of single tumour cells in metastatic breast cancer patients

R.R. Mathiesen[1], R. Fjelldal[2], E.U. Due[1], J.B. Geigl[3], S. Riethdorf[4], A.L. Borresen-Dale[1], K. Pantel[4], M.R. Speicher[5], L.O. Baumbusch[1], B. Naume[6]. *[1]Institute for Cancer Research Oslo University Hospital Radiumhospitalet, Department of Genetics, Oslo, Norway, [2]Institute for Cancer Research Oslo University Hospital Radiumhospitalet, Department of Pathology, Oslo, Norway, [3]Institute of Human Genetics Medical University of Graz, Center for Applied Biomedicine, Graz, Austria, [4]Institute of Tumour Biology Center for Experimental Medicine University Medical Center Hamburg Eppendorf, Hamburg, Germany, [5]Institute of Human Genetics Medical University of Graz, Center for Applied Biomedicine, Graz, Austria, [6]Division of Surgery and Cancer Medicine Oslo University Hospital Radiumhospitalet, Department of Oncology, Oslo, Norway*

Background: In breast cancer progression the critical step is the dissemination of tumour cells to distant organs. An early marker for tumour dissemination is the presence of circulating tumour cells (CTC) in blood and disseminated tumour (DTC) in the bone marrow. DTC has been shown to be an independent prognostic factor for systemic relapse and breast cancer related