harvested single micrometastastic cells from sentinel node and bone marrow samples from early stage breast cancer patients and analyzed the cells by array CGH. When available, DNA from the primary tumour was analyzed the same way. The ability to compare genomic changes present in cells from different compartments will yield valuable information to better understand the mechanisms of cancer progression and help uncover the steps of the metastatic process.
Patients and Methods: SLN and BM samples taken from patients operated for primary breast cancer are examined for micrometastatic disease by use of magnetic beads coated with antibodies targeting EpCAM. Positive cells with beads bound to the surface are identified in a microscope as bead-rosetted cells. By use of a semi-automated micromanipulator system, the CellEctor, the bead-rosetted cells can be selected and individually picked by a glass capillary. Ten to twenty positive cells are collected from each specimen, and the selected cells are further processed by use of the GenomePlex single cell whole genome amplification kit from Sigma. The resulting amplified genomic DNA is applied on to Agilent 105k CGH arrays for analysis of genomic aberrations.
Results: Preliminary results indicate that the method has high reproducibility; cells picked from SLN of the same patients and individually processed yield highly similar profiles in separate hybridizations. Also cells picked from the same patient, but selected with different antibodies (anti-EpCAM and −Muc1), show identical genomic profiles. Cells taken from different compartments have common as well as unique alterations, with cells disseminated to the BM typically having fewer aberrations than those selected from the sentinel node. The primary tumour shares many aberrations with cells disseminated to the lymph node.
Conclusion: We present a method that allows for direct isolation and genomic characterization of pure populations of disseminated tumour cells. Metastatic spread is the most life threatening aspect of cancer. To understand the nature of the metastatic process it is mandatory to examine the specific characteristics of the "metastatic precursor" cells found in lymphatic or hematopoietic tissue. Such data will be of great value in the treatment of patients in an adjuvant setting where the therapy is aiming at eradicating minimal residual disease.

428 Heparanase powers a chronic inflammatory circuit that promotes colitis-associated tumourigenesis

M. Elkin[1], I. Lerner[1], E. Bensoussan[1], V. Doviner[2], I. Vlodavsky[3]. [1]*Hadassah – Hebrew University Medical Center, Oncology, Jerusalem, Israel*, [2]*Hadassah – Hebrew University Medical Center, Pathology, Jerusalem, Israel*, [3]*Technion, Faculty of Medicine, Haifa, Israel*

Background: Ulcerative colitis (UC) is a chronic inflammatory condition that is closely associated with colon cancer. Here we report a previously unrecognized function of heparanase enzyme in generation of a mechanistic link between colitis and the associated tumourigenesis. Heparanase is the predominant mammalian endoglycosidase that cleaves heparan sulfate, the major polysaccharide of the extracellular matrix, and plays multiple roles in inflammation and cancer progression.
Material and Methods: We applied immunohistochemical analysis of human UC tissue samples, in vitro and ex vivo cell systems, as well as mouse models of of dextran sulfate sodium (DSS)-induced colitis and colitis-associated cancer induced by the carcinogen azoxymethane (AOM) followed by repeated DSS administration.
Results: We found that heparanase is constantly overexpressed and activated during the course of the UC and DSS colitis, both in the active and inactive phases of disease. Employing heparanase-overexpressing transgenic mice in the AOM-DSS model of colitis-associated cancer, we demonstrated that heparanase overexpression markedly increased the incidence and severity of colitis-associated colonic tumours, enabling faster tumour take, angiogenic switch and enhanced tumour progression (via enhanced NFkappaB signaling, augmented levels of COX-2, and STAT 3 induction). Notably, DSS-induced colitis (without AOM pretreatment) lead to formation of colonic tumours in heparanase-transgenic, but not wild type mice, positioning heparanase as important mechanistic determinant in inflammation-driven colon carcinoma. Investigating molecular mechanisms underlying heparanase induction in colitis, we found that macrophage-derived TNFalfa is responsible for continuous overexpression of heparanase by chronically-inflamed colonic epithelium. Moreover, our results suggest the occurrence of heparanase-driven vicious cycle that powers colitis and the associated tumourigenesis: heparanase activity in inflamed colon, acting synergistically with the intestinal flora, stimulates macrophage activation, and the activated macrophages secrete TNFalfa which stimulates further production of heparanase by the colonic epithelium. In addition, activated macrophages secrete cathepsin L – a cysteine protease responsible for proteolytic activation of latent heparanase.
Conclusions: Altogether, our results suggest that heparanase, acting in concert with the innate immune cells, preserves chronic inflammation in the colon and fosters colonic cancer development. Thus, disruption of the heparanase-driven chronic inflammatory circuit might be highly relevant to the design of therapeutic interventions in UC and the associated cancer.

429 Early stage inhibition of autophagy by verteporfin

E. Donohue[1], M. Roberge[1]. [1]*UBC, Biochemistry and Molecular Biology, Vancouver, Canada*

Background: Autophagy, a cellular self-eating process that is activated by several cancer drugs and appears to function as a protective mechanism, is a promising therapeutic target; however, few pharmacological inhibitors suitable for testing the therapeutic potential of autophagy inhibition *in vivo* are known.
Methods: An automated cell-based assay was used to screen >3,500 drugs and pharmacological agents for inhibitors of autophagosome formation. Biochemical and microscopy assays were used to analyze autophagic degradation, LC3/Atg8 processing, sequestration, and cell viability.
Results: Verteporfin, a drug used in photodynamic therapy, was identified as an early stage autophagy inhibitor. Verteporfin did not inhibit LC3/Atg8 processing in response to autophagic stimuli but it inhibited drug- and starvation-induced autophagic degradation and the sequestration of cytoplasmic materials into autophagosomes. Transient exposure to verteporfin selectively reduced cell viability in starvation conditions while cells in nutrient-rich medium were unaffected by drug treatment. Verteporfin inhibited autophagy in the absence of light showing its effect is not photodynamic.
Conclusions: The existence of an autophagy inhibitor among drugs approved for humans should facilitate the investigation of the therapeutic potential of autophagy inhibition *in vivo*.

430 Gelsolin modulates the expression of invasion-associated genes in colorectal cancer

C. Yap[1], J.L. Zhuo[1], E.H. Tan[2], B. Yan[3], H.K. Tay[4], M. Salto-Tellez[5], A.J. Melendez[4], S.K. Maciver[6]. [1]*National University of Singapore, Physiology, Singapore, Singapore,* [2]*Beatson Institute for Cancer Research, Beatson, Glasgow, United Kingdom,* [3]*National University of Singapore, Pathology, Singapore, Singapore,* [4]*University of Glasgow, Division of Immunology Infection and Inflammation, Glasgow, United Kingdom,* [5]*University of Singapore, Pathology, Singapore, Singapore,* [6]*University of Edinburgh, Biomedical Sciences, Edinburgh, United Kingdom*

Background: Gelsolin, an actin-capping and severing protein, is frequently silenced in many carcinomas including colon tumours, but upregulated in the later stages of progression. We postulate that gelsolin acts to promote the progression of tumours by converting non-invasive tumours to invasive ones.
Materials and Methods: We investigated the oncogenic roles of gelsolin in colorectal cancer by overexpression and siRNA knockdown of gelsolin in colorectal tumour cell lines. Stable transfectants that overexpress cytoplasmic gelsolin were generated in the HCT116 cell line. We also investigated the expression of gelsolin in the liver metastatic nodules of human colorectal tumours by immunohistochemistry.
Results: *In vitro* functional studies demonstrated the oncogenic properties of gelsolin through its ability to increase invasion and migration, with little or no effect on cell proliferation. Overexpression of gelsolin also induced scattering in HCT116 – cells became more spindle-like and some exhibited prominent lamellipodia. Conversely, knockdown of gelsolin in tumour cells reduced their invasive potential, and this is consistent with previous observations in other cell types. We also compared the gene expression profiles of gelsolin-overexpressing HCT116 and wild-type HCT116 using microarray and real-time PCR studies. Notably, genes involved in matrix degradation such as MMP7 and uPA were upregulated in gelsolin overexpressors. The upregulation of these genes correlated with increased matrix-degrading activity in gelsolin-overexpressing cells. In liver metastatic nodules, we observed increased gelsolin expression at the invasive front of the tumours.
Conclusion: Gelsolin has been reported to be important for invasion of several cell types, but the mechanisms by which it induces invasion are unclear. Our data suggests that gelsolin can regulate the expression of genes essential for invasion, and thus contribute to tumour progression.

431 Siah2 regulates tumour progression and neo-angiogenesis in a mouse model of breast cancer

C.S.F. Wong[1], C.M. House[1], M.C.P. Liu[1], I. Haviv[2], D.D.L. Bowtell[1], A. Möller[1]. [1]*Peter MacCallum Cancer Centre, Cancer Genomics and Biochemistry Laboratory, Melbourne Victoria, Australia,* [2]*Baker IDI Heart and Diabetes Institute, Human Epigenetics Laboratory, Melbourne Victoria, Australia*

The ubiquitin ligase Siah2 has been demonstrated to regulate cellular responses to hypoxia, a condition commonly observed in solid tumours like breast cancer. Knocking out Siah2 in the Polyoma Middle T (PyMT) oncogene-driven breast cancer mouse model caused a significant delay in breast cancer onset. This was caused by a delayed 'angiogenic switch' in these tumours, a hypoxia-signalling dependent process. Correlating with this observation, blood vessels in endstage tumours of Siah2 knockout mice have a more 'normalised' phenotype, resulting in increased perfusion. In comparison, the wildtype tumours had dilated, tortuous and leaky blood vessels. One probable reason identified was the different cytokine secretion profile of Siah2 knockout breast cancer epithelial cells. These cells secrete higher levels of cytokines,

including GM-CSF, validated by both cytokine array and quantitative real-time PCR.
In addition, almost all endstage Siah2 knockout tumours lacked stromal infiltration. Transplant experiments demonstrated that this was caused by the inability of Siah2 knockout stroma to respond to signals secreted by advanced-stage tumour cells. These *in vivo* results, in agreement with *in vitro* results, show that stromal cells from Siah2 knockout mice cannot or minimally react to tumour cell-derived signals. Thus, these observations suggest that Siah2 could potentially regulate tumour onset and progression in a multi-faceted manner (stromal infiltration and tumour vascularisation). Siah2 displays tumour cell autonomous and stroma cell autonomous functions, suggesting the possibility of developing Siah2 as a target for anti-angiogenic therapy in breast cancer.

432 MicroRNAs in the miR-200 family differentially regulate cell cycle progression and EGF-driven invasion by modulating p27/Kip1, CDK6 and PLC-gamma1 in breast cancer

O. Sahin[1], S. Uhlmann[1], J.D. Zhang[1], A. Schwaeger[1], H. Mannsperger[1], S. Burmester[1], A. Ward[1], U. Korf[1], S. Wiemann[1]. [1]*German Cancer Research Center, Division of Molecular Genome Analysis, Heidelberg, Germany*

MicroRNAs (miRNAs) in the miR-200 family are located in the fragile chromosomal regions and downregulated with tumour progression. Although members of the miR-200 family have been reported to regulate epithelial-to-mesenchymal transition (EMT) and TGF-β-driven cell invasion, there are no studies until now showing the role of individual members of the miR-200 family, especially of the miR-200bc/429 cluster, on breast cancer cell cycle progression, proliferation and EGF-driven invasion. Here, we demonstrate that miR-200 family members differentially regulate viability, cell cycle progression and EGF-driven invasion of breast cancer cells. While the miR-200a/141 cluster results in G1 arrest by increasing p27/Kip1 and downregulating CDK6 levels, the 200bc/429 cluster decreases G1 population by reducing p27/kip1 level and increasing G2/M phase potentially by reducing CDK2 and increasing Cdc2 levels. Furthermore, we have demonstrated for the first time that all miR-200 family members regulate also EGF-driven invasion, but miR-200bc/429 cluster had stronger effect compared to the miR-200a/141 cluster. Genome-wide microarray profiling in combination with gain-of-function studies identified PLCG1, which was downregulated only by the miR-200bc/429 cluster, as a potential candidate contributing to this difference. Downregulation of PLC-gamma1, whose enzymatic activity is required for EGF-induced cell motility, introduces a new role of miR-200bc/429 regulation of cell invasion besides the known TGF-β-dependent pathway. Overall, our results suggest that the miR-200 family has a tumour-suppressor function by inhibiting cell cycle progression and EGF-driven cell invasion in breast cancer.

433 Up-regulation of thymosin beta4, integrin alpha6, and cathepsin L is critical for the high invasiveness of fibrosarcoma cells

P. Nummela[1], M. Kielosto[1], K. Ravanko[1], K. Järvinen[1], M. Yin[1], E. Hölttä[1]. [1]*Haartman Institute and Helsinki University Central Hospital, Department of Pathology, Helsinki, Finland*

Background: To combat against cancer-related deaths, understanding of the mechanisms behind cancer cell invasion and metastasis is of utmost importance. Mouse fibroblasts transformed by S-adenosylmethionine decarboxylase overexpression (Amdc cells) are highly invasive *in vivo* and *in vitro*, thereby providing a valuable model to study the mechanisms of cell invasion.
Materials and Methods: Gene expression changes in Amdc cells, as compared to normal NIH3T3 cells, were analyzed by DNA microarrays. Most interesting changes were confirmed by RT-PCR and Western blotting or immunofluorescence staining, and the functions of the identified molecules were then studied *in vitro* in three-dimensional cell cultures using function-blocking antibodies and specific inhibitors. Finally, the expression patterns of the identified molecules were studied in human sarcoma specimens by immunohistochemistry.
Results: We found the actin sequestering molecule thymosin β4 (Tβ4), the adhesion regulator integrin α6 (ITGα6), and the protease cathepsin L (CTSL) to be markedly overexpressed in Amdc cells. By using a sponge toxin latrunculin A (inhibiting Tβ4), function-blocking ITGα6 antibody, or CTSL inhibitor, we could block the invasion of Amdc cells in three-dimensional Matrigel. Further, we found human high-grade sarcomas to show strong ITGα6 immunostaining, especially in the invasion fronts. Tβ4 and CTSL also showed elevated immunostaining in these tissue specimens.
Conclusions: The up-regulated molecules Tβ4, ITGα6, and CTSL are important in three steps of Amdc cell invasion: migration, adhesion, and proteolysis, respectively. Inhibition of either of them suffices to block the invasion of Amdc cells, but targeting them all at the same time could give the cancer cells less chance for adaptation. Combination of Tβ4, ITGα6, and CTSL antagonists may thus show promise for the treatment of highly invasive fibrosarcomas overexpressing these molecules.

434 A role for Gsdmb in invasion and motility of Her2+ breast carcinoma cell lines

M. Hergueta-Redondo[1], A. Rojo[2], M.A. Castilla[3], J. Palacios[3], A. Cano[1], G. Moreno-Bueno[1]. [1]*Universidad Autonoma de Madrid, Biochemistry-Biology of Cancer, Madrid, Spain,* [2]*MD Anderson Internacional, Pathology, Madrid, Spain,* [3]*Hospital Virgen del Rocio, Pathology, Sevilla, Spain*

Background: One of the molecular markers related to the aggressiveness of breast tumours is increased expression of oncogene Her2neu (ErbB2) (caused in most cases by genetic amplification). The over-expression of this oncogene occurs in around 15−30% of the most aggressive and worst prognosis primary breast cancer tumours. The co-amplification and/or co-expression of certain genes located in the same chromosomic region as Her2neu (17q12-q21) have been studied, and suggest an effect on response to treatment, or even on recurrence, of this type of tumour [1]. *Gsdmb* is a novel gene located on human chromosome 17q21 near to the Her2 amplicon. To date, Gsdmb expression has been described in gastric tumour epithelium [2]; however the functional significance of Gsdmb in cancer biology is still unknown.
Material and Methods: To analyse the hypothetical role of Gsdmb we used different approaches using two different breast tumour series and also in Her2+ breast carcinoma cell lines.
Results: Our work describe that Gsdmb amplification/over-expression occurs in a subgroup of Her+ breast carcinoma. Additionally, our data show Gsdmb cytosolic localization in breast tumour samples correlated to Her2 amplifification and local tumour recurrence. We have identified two different isoforms (named Gsdmb1 and Gsdmb2) that differ only in nine aminoacids and are mostly detected in Her2+ breast carcinoma cell lines. From the molecular point of view, we found that Gsdmb1 promotes increased phosphorylation status of ERK1/2 while Gsdmb2 increases Her2 receptor phosphorylation in specific residues, suggesting a differential role for these isoforms. Moreover, Gsdmb1 and Gsdmb2 over-expression enhances the migration and invasion of SKBR3 breast carcinoma cell line. This phenotype seems to be correlated to Rac1 activation and MMP1/14 mRNA expression.
Conclusions: Our data strongly suggest that Gsdmb1 and Gsdmb2 over-expression in Her2+ breast carcinomas increase migration and invasion of tumour cells. These results together with our data human breast tumours demonstrate that Gsdm1 and Gsdmb2 could be considered as important targets for cancer therapy.
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435 LOXL2 as a new marker of basal-like phenotype in breast cancer

G. Moreno-Bueno[1], A. Martin[1], R. Moreno[1], A. Montes[1], H. Peinado[2], F. Portillo[1], A. Cano[1]. [1]*Universidad Autonoma de Madrid, Biochemistry-Biology of Cancer, Madrid, Spain,* [2]*Cornell University, Paediatrics Cell and Developmental Biology, New York, USA*

Background: Lysyl oxidase-like 2 (Loxl2) interacts with and stabilizes Snai1 transcription factor promoting epithelial-mesenchymal transition (EMT) [1]. Our recent studies showed that human LOXL2 is as a new poor prognosis indicator in human squamous cell carcinomas promoting malignant transformation by both Snai1-dependent and independent mechanisms [2]. In addition, expression profiling meta-analysis showed that high levels of LOXL2 mRNA correlated with poor prognosis in lung squamous cell carcinoma and lymph node negative (N0) breast adenocarcinomas [2], thus suggesting that LOXL2 could be involved in tumour progression.
Material and Methods: Using a high-throughput platform the expression profiling of breast carcinomas tumours (n = 59) was analyzed. Additionally, stable silencing of LOXL2 in MDA-MB-231 basal breast cancer cells was performed using sh-RNA. Cells were characterized at the morphological and behavioral levels.
Results: LOLX2 expression was correlated with basal-like breast tumours subtype, at both mRNA and protein level. Basal-like breast carcinomas are a subtype of breast tumours characterized by the negative expression of ER, PR, and Her2neu and the re-expression of different basal markers (CK5, Vimentin, FN, etc). Silencing of LOXL2 in MDA-MB-231 cancer cells leads to re-expression of epithelial markers such as E-cadherin and promotes reduced cell invasion and motility. In addition, the growth of primary tumours induced by LOXL2-silenced cells in nude mice was also reduced.
Conclusions: These results suggest that LOXL2 is involved in basal-like breast tumours progression and/or dissemination.
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