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

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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

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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

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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

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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,