423 MULTIMERIN2 effects on tumoural vessel development

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Background: MULTIMERIN2 (MMRN2) is a secreted protein belonging to a family of ECM molecules termed EMILINs. Given its specific localization in tight contact with the endothelial cell surface, we have hypothesized that MMRN2 may affect angiogenesis.
Material and Methods: For these investigations we have employed human umbilical vein endothelial cells (HUVEC) and over-expressed or down-regulated MMRN2 by means of adenoviral vectors carrying the MMRN2 coding sequence or siRNA sequences, respectively. Following the alteration of MMRN2 endogenous expression we have thus analyzed different parameters of the angiogenic process *in vitro* as well as verified tumour angiogenesis *in vivo*.
Results: We have found that endothelial cells specifically adhere to MMRN2, the interaction though does not influence HUVECs proliferation and viability. On the contrary the treatment of endothelial cells with recombinant MMRN2 induces a significant reduction of cell motility as well as an impairment of tubules formation in Matrigel. This negative effect on the angiogenic process has been confirmed *in vivo* by means of the Matrigel plug assays. At the molecular level these findings are supported by a phosphoproteomic analysis following treatment with MMRN2 which highlighted a significant reduction of the phosphorilation levels of different Tyrosine Kinase Receptors (RTKs) and other molecules regulating endothelial cell migration.
To verify whether the angiogenetic impairment induced by MMRN2 could lead to a decreased tumour growth we have injected tumour cells overexpressing or not MMRN2 in nude mice. A striking reduction of tumour growth was observed in xenografts overexpressing MMRN2 and this effect was accompanied by a significant decrease of blood vessels.
Conclusions: These preliminary data indicate that MMRN2 exerts a profound effect on endothelial cell function by affecting the activation of different RTKs on the cell surface. The inhibition of RTK functions leads to an inhibitory effect on blood vessel development that results in an impairment of tumour growth *in vivo*. For this reason MMRN2 may represent a promising novel tool for the development of new antiangiogenic drugs for cancer treatment.

424 Aberrant retinoic acid signaling in astrocytic gliomas

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Background: In glioma immature cell populations influence tumour growth and seem to be irresponsive to physiological differentiation stimuli, persisting in an undifferentiated developmental state. Here we investigated potential disruptions in the retinoic acid (RA) differentiation pathway that could lead to a loss of differentiation capacity and impact on patient prognosis.
Materials and Methods: Expression of key-molecules belonging to the RA differentiation pathway was analyzed on a tissue microarray comprising tumour samples from 283 astrocytic gliomas WHO II-IV and further studied in primary glioma cell lines.
Results: Contrary to previous findings in other tumour entities expression of RA signaling molecules increased with tumour malignancy. This included tumour grade-dependent expression of (1) the intracellular RA-binding protein CRBP1 ($p < 0.001$) catalyzing cellular retinoid up-take, (2) ALDH1A1 ($p = 0.012$) capable of activating RA precursors, (3) the RA-degrading enzyme CYP26B1 ($p < 0.001$) and (4) the intracellular RA-binding protein FABP5 ($p < 0.001$) which can hinder RA-induced differentiation diverting RA into an alternative signaling pathway. On the other hand, expression of the RA-binding protein CRABP2 which fosters differentiation decreased with tumour malignancy ($p < 0.001$). In WHO IV high expression of CRBP1 was associated with increased tumour cell proliferation ($p < 0.001$) and elevated FABP5 levels correlated with an undifferentiated tumour phenotype ($p = 0.003$). Finally, ALDH1A1, discussed as potential (cancer) stem-cell marker besides its involvement in RA signaling, proved to be an independent marker for poor patient survival ($p = 0.016$).
Conclusions: Our data indicate that a complex deregulation of RA signaling exerts an unfavorable influence on patient prognosis and seems to be involved in the loss of differentiation capacity in glioma.

425 IGFBP-3 knockout mice develop earlier mammary tumours following dimethylbenz[a]anthracene treatment

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Background: Insulin-like growth factor binding protein-3 (IGFBP-3) is the main carrier protein for IGFs in the circulation. IGFBP-3 antagonizes IGF-I growth-promoting and anti-apoptotic activities in several experimental systems. It has been shown that recombinant human IGFBP-3 can slow the growth of breast cancer and other tumour cells in culture by sequestration of IGFs thus reducing their binding, and blocking their anti-apoptotic activity. It has also been suggested that IGFBP-3 could act independently of IGF signaling. The goal of this study is to determine the role of IGFBP-3 in breast cancer development.
Material and Methods: To study breast carcinogenesis, we used medroxyprogesterone acetate (MPA) and dimethylbenz[*a*]anthracene (DMBA) protocol. Fourteen wild-type and 15 IGFBP-3 knock-out female mice were treated with MPA and DMBA while 3 mice of both genotypes were treated only with MPA as controls. Mice were followed for up to 13 months for breast tumour appearance, including measurement of tumour size weekly. Mice were also monitored for behavioral changes, weight loss (>20%), and dehydration according to the established guidelines and protocols approved by McGill University's Animal Ethics Committee. At the time of euthanasia, blood, tumours and tissues were collected and frozen until use. Downstream signaling was analyzed by western blot and hormone levels by ELISA.
Results: In general, IGFBP-3 knockout mice were slightly smaller than wild type mice. They also developed breast tumours significantly earlier than the wild type (mean: 13.9 ± 1.1 vs. 24.1 ± 3.4 weeks, range: 9 to 26 weeks vs. 9 to 45 weeks, respectively, $p = 0.0207$). The number of tumours was not influenced by the presence of IGFBP-3. No significant differences between the tumours in wild type and IGFBP-3 knockout mice were observed in levels of phospho-AKT ^{Ser473}, or total insulin or IGF-1 receptors.
Conclusion: These data show that IGFBP-3 has an important role in delaying mammary gland carcinogenesis. However, by the time tumours became macroscopic, signaling downstream of IGF-1 receptor is not increased in the absence of IGFBP-3, and underlying mechanisms are under study.

426 Impact of hypoxia on furin trafficking and the formation of invadopodia

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The ability of cancer cells to invade and metastazize is the major cause of death in cancer patients. Recent studies indicate that tumoural invasion and metastasis, triggered by the hypoxic microenvironment, involves strategic relocalization of convertases, adhesion molecules, and metalloproteases. Furin, a proprotein convertase, is well known to be implicated in cancer invasion and progression by its ability to activate tumourigenic substrates. Recently, initiation of cancer invasion has been linked to the formation of actin-rich protusions, invadopodia. The purpose of this study was to assess the impact of hypoxia on furin relocalization and its implication in invadopodia formation and cancer cell invasion.
We used the invasive fibrosarcoma cell line, HT-1080, stably transfected with eGFP-tagged furin to determine the influence of hypoxia on furin cellular localization. Our results indicated that in hypoxic cells, furin is relocalized at the plasma membrane and is internalized via both clathrin- and caveolin/raft dependent endocytosis. Using furin trafficking mutants, we demonstrated that filamin-A, a cytoskeletal tethering protein, is essential for the membrane localization of furin under hypoxia. We further demonstrated that in hypoxic cells, furin and its substrate MT1-MMP relocalized to specific pericellular compartments and this relocalization was associated with an increased cell ability to convert pro-MT1-MMP into its active form. Because MT1-MMP is known to be involved in ECM degradation at site of invadopodia, we further looked at the implication of cell-surface furin in the formation and functions of these structures. Using a matrix degradation assay, we found that furin colocalized at invadopodia sites under hypoxic conditions. This was associated with an increase in both formation and functions of invadopodia. Such event was linked to the ability of the cell to migrate in a 3D invasion assay. Using furin trafficking mutants, we also showed that furin redistribution to the plasma membrane under hypoxia was essential for the increase in both invadopodia production and cell invasion.
Our results suggest that hypoxia promotes the formation of a peripheral processing compartment in which furin is concentrated for enhanced processing of substrate involved in the formation of invadopodia leading to cell invasion.

427 Array CGH analysis of matched patient samples from primary breast tumour tissue and immunomagnetically isolated cancer cells from sentinel lymph nodes and bone marrow

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Background: Metastasis is the leading cause of death in patients with solid epithelial tumours, and circulating tumour cells are thought to represent the origin of metastatic disease. In some cancers, the sentinel lymph node (SLN) is the hypothetical first lymph node reached by metastasizing cancer cells from the primary tumour, and the bone marrow (BM) is another compartment known to harbor disseminated cells. In this study, we have

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,