# POSTER ABSTRACTS

## *Angiogenesis Regulatory Proteins*



## *Inhibitors of Angiogenesis*

## *Vascular Extracellular Biology*



## *Emerging Therapeutic Targets*



## *Molecular Genetics*



## *Metastasis*

## ANGIOGENESIS REGULATORY PROTEINS

**P1**
**Functional autocrine and intracrine VEGF/VEGFR2 signaling loop in malignant melanoma: impact on tumor progression**

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Vascular endothelial growth factor-A, or VEGF, produced by tumor cells promotes angiogenesis by binding and activating two tyrosine kinase receptors, VEGFR-1 and VEGFR-2 on endothelial cells. Recent data suggests that VEGF can directly aid in tumor progression by binding and activating VEGFR2 expressed by different types of cancer cells, including malignant melanoma. This autocrine VEGF/VEGFR2 loop stimulates survival of melanoma cancer cells by activation of PI3-kinase pathway. Here we report the expression of VEGFR2 at the protein and mRNA level by human melanoma cells. When compared to endothelial cells, the expression of VEGFR2 receptor was significantly lower in both primary and metastatic melanoma cells. VEGF produced by melanoma cells *in vitro* was able to phosphorylate VEGFR2 receptor in both primary and metastatic cells. However, metastatic melanoma cells produced approximately 13 fold greater amounts of VEGF compared to the primary melanoma cells and had concomitant enhanced levels of phosphorylated VEGFR2. Through western blotting of nuclear and cytosolic protein lysates, we also detected the presence of an activated intracrine VEGF/VEGFR2 loop in primary and metastatic melanoma cells. In addition, the significant increase in VEGF production by metastatic melanoma compared to primary melanoma correlates with a significant decrease in the production of the endogenous anti-angiogenic factor thrombospondin-1 (TSP-1) by metastatic melanoma compared to primary melanoma cells. Taken together, our results support a direct role for VEGF on malignant melanoma cells, and suggest that anti-cancer therapies targeting the VEGF/VEGFR system may act differentially depending on progression of this disease. This abstract was supported through a grant from CIHR.

**P2**
**Oncogenic receptor exchange via microvesicles – a possible role of microvesices in tumor progression, angiogenesis and as a biomarker**

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Our study shows that oncogenic epidermal growth factor receptor vIII (EGFRvIII), wild type EGFR and possibly other active oncoproteins can be transferred between cancer cells and their indolent and normal counterparts, as cargo of microvesicles. Thus, in malignant glioma (GBM) cells the expression of the oncogenic EGFRvIII triggers production of membrane microvesicles, which incorporate this oncoprotein into their cargo, become released into the extracellular space and enter blood of tumour bearing mice and

GBM patients. Exposure of indolent, or normal (e.g. endothelial) cells to microvesicles (oncosomes) containing oncogenic EGFR results in the 'ectopic' activation of the downstream pathways involved in cell proliferation (MAPKs) , survival (AKT) and other functions. Notably, microvesicular EGFRvIII transfer to indolent glioma cells (U373) elicits their morphological transformation, increased capacity to grow in soft agar, and upregulation of vascular endothelial growth factor (VEGF). Moreover, transfer of EGFR from squamous cell carcinoma to endothelial cells switches on autocrine production of VEGF and leads to activation of the VEGF receptor 2(VEGFR-2). This effect is not blocked by bevacizumab (Avastin), but is inhibited by VEGFR-2 inhibitor (SU5416), suggesting that the activation of this pathway by the uptake of microvesicular EGFR occurs juxta- or intracellularly, and is indeed, dependent on the endogenous production of VEGF. Pharmacological blockade of EGFR activity (CI-1033) in microvesicles or their uptake (annexin V, Diannexin) abrogates these effects, while treatment of tumour bearing mice with Diannexin produces an antitumour and antiangiogenic effect in vivo. This suggests a role of oncogenic microvesicles in tumour angiogenesis. We also report that EGFRvIII and EGFR are detectable in the microvesicular fraction recovered from blood of cancer (GBM) patients, and we propose that this property could be used as a prognostic and predictive biomarker, including in the context of antiangiogenic, EGFR-directed and other targeted therapies.

**P3**
**Roles of the GAB1 andGAB2 scaffolding adapters in VEGF-mediated angiogenic responses**

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Vascular endothelial growth factor (VEGF) is a key regulator of normal and pathological angiogenesis. Most of its biological activities mainly result from its interaction with the tyrosine kinase receptor VEGFR2. We have previously identified the scaffolding adapter Gab1 as a substrate of VEGFR2, and as a positive modulator of VEGF-dependent signaling (Akt, Src, Erk1/2), migration, and capillary formation. However, its involvement in endothelial cell survival, as well as the potential contribution of the other family member Gab2 to signaling and biological responses remained unknown. We have observed that like Gab1, Gab2 is tyrosine phosphorylated in a Grb2-dependent manner downstream of activated VEGFR2, and that it associates with signaling proteins including PI3K and SHP2, but apparently not with the receptor. Similarly to Gab1, over-expression of Gab2 induces endothelial cell migration in response to VEGF, whereas it's silencing using siRNAs results in its reduction. Importantly, depletion of both Gab1 and Gab2 leads to an even greater inhibition of VEGF-induced cell migration. However, contrary to Gab1, the silencing of Gab2 results in increased activation of Akt, Src, and Erk1/2, and up-regulation of Gab1 protein levels. Consistent with their opposite roles on Akt, the depletion of Gab1, but not of Gab2, results in reduced FOXO1 phosphorylation and VEGF-mediated endothelial cell survival. Mutation of VEGFR2 Y801 and Y1214, which abrogates the phosphorylation of Gab1, also correlates with inhibition of Akt. Altogether, these results underscore the non-redundant and essential roles of Gab1 and Gab2 in endothelial cells, and suggest major contributions of these proteins during in vivo angiogenesis. *Project supported by the Canadian Institutes of Health Research.*

**P4**
**Annexin 1, a new target of LIM kinase 1, that regulates VEGF-induced endothelial cell migration**

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The pro-angiogenic factor VEGF induces actin remodeling downstream of VEGFR2-mediated activation of p38 MAP kinase/MAPKAP kinase-2 axis (Lamalice et al. Circ. Res 2007). We now obtained evidence indicating that annexin 1 is a target of this axis and that its phosphorylation regulates endothelial cell migration in response to VEGF. Firstly, by 2D-gel electrophoresis and mass spectrometry, we identified annexin 1 as a protein whose phosphorylation is induced by VEGF, and is impaired by inhibiting p38. Secondly, using *in vitro* kinase assays and *in vivo* phosphorylation assays, we found that VEGF-mediated activation of p38/MAPKAP kinase-2 pathway triggers the activation of LIM kinase, which in turn phosphorylates annexin 1 on serine 37. Thirdly, consistent with a role of annexin 1 in cell migration we observed that, in response to VEGF, it quickly colocalises with F-actin in the lamellipodia. Fourthly, VEGF-induced cell migration and tube formation in Matrigel are inhibited following small interfering RNA-mediated knockdown of LIM kinase and annexin 1. Fifthly, both processes are rescued in cell expressing an annexin 1 construct that is insensitive to the siRNA knockdown. We conclude that VEGF-induced LIM kinase-dependent phosphorylation of annexin 1 on serine 37 is a key regulator of actin remodeling and endothelial cell migration associated with the activation of the p38 pathway. *Work supported by CIHR.*

**P5**
**ROLE OF AHA1 (ACTIVATOR OF HSP90 ATPASE) IN VEGF-INDUCED ENDOTHELIAL NO PRODUCTION**

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*Objective:* Vascular endothelial growth factor (VEGF) is essential for both physiological and pathological angiogenesis. Our studies are designed to gain a better understanding of signaling pathways involved in the angiogenic effects of VEGF. Heat shock protein 90 (HSP90) belongs to a family of molecular chaperones and is important for maintaining appropriate folding and conformational maturation of several proteins. HSP90 is also known to interact with signaling molecules and to regulate cell signaling. Indeed, following stimulation of endothelial cells (EC) with agonists known to stimulate nitric oxide (NO) production, HSP90 associates with endothelial NO synthase (eNOS). In addition, it has been suggested that HSP90 acts as a scaffold to recruit the serine/threonine kinase Akt, which leads to increased eNOS activity. Furthermore, many co-chaperones of HSP90 have been identified and shown to modulate the ATPase activity of HSP90. Among them, the activator of HSP90 ATPase1, AHA1, contributes to accelerate the HSP90 cycle. However, the influence of AHA1 on endothelial NO production in response to VEGF is still unknown.

*Methods/Results:* In EC, VEGF stimulation leads to an increase in eNOS phosphorylation on serine 1177 as well as Akt on serine 473. However, when cells are transfected with a siRNA raised against AHA1, eNOS phosphorylation is reduced, but Akt activity is maintained. NO production following VEGF stimulation is also reduced in the presence of siRNA against AHA1. We demonstrate that HSP90 phosphorylation on tyrosine 300 and its association with eNOS in response to VEGF are decreased by a reduction of AHA1 protein expression. We will also evaluate the importance of AHA1 for endothelial cells migration in response to VEGF. Finally, since phosphorylation of tyrosine 300 of HSP90 is essential for its activity, we are studying the role of this residue on AHA1 interaction with HSP90.
*Conclusion:* These results highlight the importance of AHA1 on intracellular VEGF signaling and on endothelial NO production. Furthermore, AHA1 as well as the ATPase activity of HSP90 might represent new antiangiogenic therapeutic targets for the treatment of cancer. *This abstract was supported through grants from FRSQ and CIHR*

**P6**
**CEACAM1 deletion delays cutaneous wound healing: implications for tumor angiogenesis**

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CEACAM1 (CarcinoEmbryonic Antigen-related Cell Adhesion Molecule 1), is expressed at the surface of new blood vessels of tissues undergoing proliferation. In human tumors, CEACAM1 expression is associated with early stages of angiogenesis. CEACAM1 is a known pro-angiogenic factor, increasing VEGF activity *in vivo*; however, the role of CEACAM1 in angiogenesis warrants further investigation. Excisional wounds were used as an experimental model, as many of the processes that occur in healing wounds also take place in tumor growth - epithelial hyperproliferation, inflammation, and angiogenesis. 6-mm diameter skin wounds were inflicted on the dorsal side of *Ceacam1*$^{-/-}$ and wild-type mice. Upon histological examination, wound healing in *Ceacam1*$^{-/-}$ mice is indeed delayed relative to that of their wild-type counterparts. In *Ceacam1*$^{-/-}$ wounds, re-epithelialization is decreased significantly at 3 and 7 days post-injury. Inflammation in *Ceacam1*$^{-/-}$ wounds is also altered: the infiltration of F4/80$^{+}$ macrophages into the wound at 7 and 10 days post-injury is significantly decreased, as is the influx of mast cells at 7 days post-injury. Vascular density in *Ceacam1*$^{-/-}$ wounds is significantly decreased at 7 and 10 days post-injury; however, VEGF expression in those wound is not altered. This work is complemented by *in vitro* adhesion and migration studies of primary *Ceacam1*$^{-/-}$ mouse lung endothelial cells. *Ceacam1*$^{-/-}$ endothelial cells have reduced adhesion to extracellular matrices including fibronectin, collagen I and gelatin, as well as reduced migration in fibronectin-coated Boyden chambers compared to wild-type control cells. Taken together, these results confirm CEACAM1's role as an important factor in angiogenesis, and further expand its role as a mediator of epithelial growth and inflammation. *Funded by the Canadian Institutes of Health Research (CIHR), Fonds de la recherche en santé du Québec, and the Rosalind and Morris Goodman Cancer Centre.*

**P7**

**Angiomotin-like 1 associates with an angiomotin protein complex and induces the remodeling of the actin cytoskeleton**

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Angiogenesis is a term used to describe the formation of new blood vessels from a pre-existing vascular bed (Folkman and Shing 1992). Angiostatin inhibits angiogenesis, in part, by binding to the cell surface protein p80-angiomotin (Troyanovsky et al. 2001). The p80-angiomotin protein promotes angiogenesis by conferring a hypermigratory phenotype to endothelial cells. The identification of two other proteins similar to p80-angiomotin led to the classification of these polypeptides into one protein family known as the motins (Bratt et al. 2002). The members of this protein family include angiomotin, angiomotin-like 1 (Amotl-1) and angiomotin-like 2. Although p80-angiomotin is extensively characterized, less is known about Amotl-1. We report that Amotl-1 forms part of a protein complex containing p80-angiomotin. The protein domain that targets Amotl-1 to the p80-angiomotin-containing complex was identified through structure-function studies. Since p80-angiomotin plays a role in cell migration (Levchenko et al. 2003), a process that involves the remodeling of the actin cytoskeleton, we then addressed the hypothesis that Amotl-1 may interact with the cytoskeleton. Our studies reveal that Amotl-1 not only co-localizes with filamentous actin but also significantly modifies the architecture of the actin cytoskeleton. Regarding migration, Amotl-1 modifies several parameters of cell migration. Together these observations strongly suggest that Amotl-1 is involved in actin-cytoskeleton-based processes, in part, via its interaction with a p80-angiomotin-containing complex and the actin cytoskeleton. These findings have important implications for angiogenesis-driven disease since angiomotin and Amotl-1 are both expressed in capillaries. *This project was funded by the Canadian Arthritis Network, The Arthritis Society and the CIHR.*

**P8**

**The Role of Tissue Factor and the Coagulation System in the Function of Tumour Initiating Cells**

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Oncogenic events affect tumour initiating cancer stem cells (CSCs) and play a significant role in tumour-host interrelationships, including cancer-related coagulopathy, angiogenesis and metastasis. Importantly, these latter events are attributed to the procoagulant cell membrane receptor known as tissue factor (TF). TF binds to the clotting factor FVIIa thereby activating blood coagulation. At the same time, TF also triggers intracellular signaling, gene expression and alters cellular behavior. Collectively, this raises the question as to whether there is a relationship between CSCs and TF in cancer.

Our studies show that cancer cells frequently upregulate TF downstream of several different oncogenic events. In A431 cancer cells, tumourigenicity is attributed to amplification of the epidermal growth factor receptor (EGFR) which also drives TF levels. Conversely, EGFR inhibitors downregulate TF expression,

procoagulant activity and suppress tumour growth and angiogenesis. In a subset of A431 cells, which express high levels of CD133 (a marker of CSCs), we observed several fold higher TF activity than in the CD133-negative cell populations. Importantly, in some of these instances tumour initiation can be inhibited by the administration of TF inhibitors.
Host TF also plays a role in tumour growth. Thus, while TF expressing cancer cells readily form tumours in mice hypomorphic for this receptor, cells rendered TF-deficient fail to grow in such recipients. We therefore postulate that activation of the coagulation system and TF signaling may deliver growth-promoting stimuli (e.g. fibrin, thrombin, platelets) to dormant CSCs that facilitate tumour initiation, growth, angiogenesis and metastasis.
We postulate that TF may be expressed by CSCs and provide them with a provisional (coagulation-dependent) CSC niche due to their capacity to trigger the release of growth factors from activated platelets, stimulatory influences of coagulation proteases (e.g. thrombin) and deposition of the adhesive fibrin matrix, all of which may regulate CSCs expansion and formation of the tumour mass. Similarly, CSCs may also incorporate TF shed as microvesicles or may otherwise utilize TF expression by various components of the host compartment, e.g. blood vessels, inflammatory cells and stroma. Thus, targeting TF may diminish the potential of CSCs to initiate tumour growth, metastasis or recurrence. *This project was supported by grants from the National Cancer Institute of Canada and Canadian Cancer Society.*

**P9**
**S-Nitrosylation of b-catenin contributes to increased endothelial permeability stimulated by VEGF**

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Endothelial barrier function is largely dependent on signalling at the adherens junction (AJ) complex that is composed of VE-cadherins, b-catenin (b-cat), p120-catenin and a-catenin (a-cat), a cytoskeletal actin binding protein. During angiogenesis, VEGF-mediated increase in endothelial permeability is dependent on nitric oxide (NO) produced by endothelial NO synthase (eNOS). Presently, the molecular mechanisms by which NO contributes to increased permeability of the endothelium is not fully understood. S-nitrosylation of cysteine residues, a post-translational modification, is now well recognized as a mean for NO to directly modulate the activity of signalling proteins. In this study, we investigated the possibility that NO-mediated increase in endothelial permeability is dependent on S-nitrosylation of proteins in the AJ complex of endothelial cells (EC).
Using S-nitrosylation specific “biotin switch” procedure, we determined that endothelial b-cat is nitrosylated in the AJ complex. Treatment of EC with VEGF (40 ng/ml) increases S-nitrosylation of b-cat. Pharmacological inhibition and genetic suppression of eNOS abolish S-nitrosylation of b-cat in response to VEGF. Conjugating mass spectrometry and site-directed mutagenesis, we identified Cys 619 as the major S-nitrosylation site on b-cat. We also investigated the effect of S-nitrosylation of b-cat on its association with a-cat. *In vitro* treatment of GST-b-cat with increasing concentrations of NO donor decreases its association with a-cat. Our results suggest that S-nitrosylation of b-cat could decrease its association with a-cat and consequently induce an increase in endothelial permeability. These results suggest that eNOS-dependent S-nitrosylation of b-cat participates in the increased endothelial permeability in response to VEGF. *Supported by the CIHR. (MOP-86464 to JPG).*

**P10**

## IMPLICATION OF THE PROTEIN TYROSINE PHOSPHATASE DEP-1 IN VEGF-MEDIATED SRC KINASE ACTIVATION

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DEP-1 (Density enhanced phosphatase-1/CD148/PTPeta/PTPRJ) is a receptor-type protein tyrosine phosphatase known for its association with VE-cadherin adhesion complexes in endothelial cells and its inhibitory function on cell proliferation and transformation. Accordingly, DEP-1 can dephosphorylate several receptor tyrosine kinases, including the PDGF-b and VEGF receptors. However, in contrast to these negative regulatory roles, we have previously shown that DEP-1 can activate Src in adherens junctions and promote endothelial cell survival in response to VEGF stimulation via the Src-Gab1-PI3K-Akt pathway. Similarly, knockout studies also demonstrated that DEP-1 contributes to the activation of another Src family kinase (SFK), Lyn, and overexpression of DEP-1 in a malignant rat thyroid cell line was shown to lead to Src activation, presumably by dephosphorylating the inhibitory Src Y529. However, the molecular mechanism underlying the ability of DEP-1 to promote activation of Src associated with VE-cadherin remains ill-defined. We have observed that a catalytically inactive DEP-1 mutant (D/A) expressed in HEK 293 cells is highly phosphorylated in a Src-dependent manner on the C-terminal tail Y1311 and Y1320. Since Src binds to these two residues via its SH2 domain, and that DEP-1 D/A Y1311F/Y1320F mutant is unable to associate with Src, our results suggest that DEP-1 could promote the activation of Src by a phosphotyrosine displacement mechanism. Consistently, expression of DEP-1 Y/F mutants in VEGF-stimulated endothelial cells blocks Y529 dephosphorylation of VE-cadherin-associated Src. This work thus uncovers a novel molecular mechanism implicating DEP-1 in VEGF-mediated Src activation. *Project supported by The Cancer Research Society Inc.*

## INHIBITORS OF ANGIOGENESIS

**P11**
**Regulation of $\alpha_1$Collagen IV-derived antiangiogenic factor by p53**

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*Introduction:* Increasing evidence suggests that the *TP53* tumor suppressor gene limits tumor growth partly by increasing the production of collagen-derived antiangiogenic factors (CDAFs), such as Endostatin. We propose that p53 regulates an entire transcriptional program leading to the production of CDAFs by 1) trancriptionally activating the collagen genes parent to CDAFs, 2) upregulating the gene encoding α (II)-prolyl 4-hydroxylase (*P4HA2*), a rate-limiting enzyme in collagen synthesis and assembly, and 3) stimulating cleavage of CDAFs from parent collagens by upregulating the proteases necessary to release them. Interestingly, studies establishing a global map of p53 binding sites in the human genome have found a consensus p53 binding site near the α1collagenIV gene (*COL4A1*) parent to the Arresten CDAF (1). Therefore, the aim of this study is to determine if Arresten is a p53 regulated CDAF.
*Methodology & Results:* The ability of p53 to transcriptionally activate COL4A1 was established using RT-PCR in a series of human tumor cell lines. p53 has been previously reported to mediate the stabilization and result in increased production of other CDAFs via the transcriptional upregulation of P4HA2 (2). The ability of this enzyme to stabilize COL4A1 and lead to increased Arresten production in the extra-cellular matrix (ECM) was also validated in experiments where control and stable P4HA2 expressing cells were transfected with full-length COL4A1. Finally, the ability of p53 to stimulate the processing of COL4A1 in the ECM was also tested. Following activation of p53, an increase in the processing of transfected and endogenous COL4A1 was detected in the conditioned media. This effect was found to be partially inhibited by a global MMP inhibitor, suggesting a role for the MMPs in the p53 mediated processing of COL4A1 into its anti-angiogenic fragment.
*Conclusions:* We have demonstrated the ability of p53 to regulate the production of the $\alpha_1$Collagen IV-derived antiangiogenic factor, Arresten. Thus, this CDAF potentially represents a novel factor involved in mediating the antiangiognenic function of p53 *in vivo*. *This abstract was supported through a grant from CIHR (MOP-86752).*
*References:* 1) Wei, C. L., *et al.* Cell, *124:* 207-219, 2006. 2) Teodoro, J. G., *et al.* Science, *313:* 968-971, 2006.

**P12**
**ARF1 Modulates VEGF induced eNOS activation and NO release**

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Vascular endothelial growth factor (VEGF) induces angiogenesis and regulates endothelial function via production and release of nitric oxide (NO), an important regulator of cell functions. The molecular basis leading to NO production involves phosphatidylinositiol-3 kinase (PI3K), Akt and endothelial nitric oxide synthase (eNOS) activation. We have recently shown that stimulation of the PI3K/Akt pathway, by EGF, requires the presence of ARF1 in invasive breast cancer cells. We therefore hypothesized that in endothelial cells, VEGFR-2 stimulation also leads to the activation of this GTPase, a step required for signaling to the PI3K pathway. Here, we show that VEGF can promote activation of the small GTPase ARF1, and phosphorylation of Akt and of serine 1177 on eNOS in BAECs. Inhibition of ARF1 expression using RNA interference leads to the inhibition of eNOS phosphorylation induced by VEGF stimulation. As a consequence, ARF1 depleted cells are impaired in their ability to produce NO following VEGF stimulation. In order to delineate the underlying mechanism, we examined the signalling events activated by VEGF in ARF1 depleted BAECs. In these cells, we show that VEGF failed to activates Akt, a key regulator of eNOS. Taken together, these findings provide evidence that activation of ARF1 is necessary for activating the signaling cascades responsible for eNOS activation as well as NO release. This small GTP-binding protein therefore represents a potential therapeutic target for the treatment of vascular diseases, where impaired NO production is observed. *This work was supported by CIHR and HSFC.*

**P13**
**Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis**

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The use of VEGF pathway inhibitors to impair angiogenesis now represents a clinically validated anticancer treatment strategy. However the benefits of VEGF-targeted agents in the treatment of late-stage cancers can be transitory resulting in eventual drug resistance, tumor (re)growth, and rapid vascular recovery when therapy is stopped. Herein we report that the VEGFR/PDGFR kinase inhibitor sunitinib/SU11248 can accelerate metastatic tumor growth and decrease overall survival in mice receiving short-term therapy in various metastasis assays, including after intravenous injection of tumor cells or after removal of primary orthotopically grown tumors. Acceleration of metastasis was also observed in mice receiving sunitinib prior to intravenous implantation of tumor cells, suggesting possible 'metastatic conditioning' in multiple organs. Similar findings with additional VEGF RTKIs implicate a class-specific effect for such agents. Importantly, these observations of metastatic acceleration were in contrast to the demonstrable antitumor benefits obtained when the same human breast cancer cells, as well as mouse or human melanoma cells, were grown orthotopically as primary tumors and subjected to identical sunitinib treatments. Our findings demonstrate that angiogenesis inhibition in mice can lead to opposing effects on tumor growth and metastasis depending on tumor stage and treatment duration. These observations could have clinical implications with respect to optimal dose and treatment schedule, therapy in the adjuvant/neoadjuvant setting, and highlight the importance of testing additional drugs in combination as a possible approach to abrogate this effect. *Financial Support: The Terry Fox Foundation (TFF) supports J.M.L.E. through an award from the National Cancer Institute of Canada (NCIC) and R.S.K. is a Canada Research Chair. This work was supported by grants from the Ontario Institute for Cancer Research (OICR) and the NCIC (all to R.S.K.).*

**P14**
**Phosphomimetic mutants of PEDF as antiangiogenic, anticancer agents: an insight into mechanisms of action**

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Pigment epithelium-derived factor (PEDF) is a potent endogenous inhibitor of angiogenesis, which can be used as neovascularization-targeting agent for tumor growth inhibition. A number of reports have shown that PEDF attenuates tumor growth in animal cancer models, though the exact molecular mechanism of its antitumor activity remains to be elucidated. Our group has previously demonstrated that PEDF is a phosphoprotein, and that its phosphomimetic mutants possess significantly increased antiangiogenic activity as compared to their wild-type counterpart. Here we report that intravenous administration of the PEDF mutants to tumor-bearing mice produces much more profound inhibition of tumor growth and intratumoral neovascularization than wild-type PEDF. Importantly, the most potent PEDF mutant exhibits somewhat better antitumor activity than the established antiangiogenic anticancer agent Avastin. We further demonstrate that PEDF mutants operate in a VEGF-independent manner, by directly affecting endothelial cell angiogenicity and exhibiting significantly enhanced proapoptotic activity towards tumor-residing endothelial cells. Detailed examination of the mechanism of action of the PEDF mutants in cultured cells revealed that the most potent PEDF mutant induces caspase-3-dependent apoptosis and inhibits migration of endothelial cells much stronger than wild-type PEDF and these activities are respectively regulated by the JNK and the p38 MAPKs cascades. In cancer cells, PEDF constructs affect only cell migration via the p38 cascade, suggesting a potential inhibitory effect on cancer cell invasiveness. On the other hand, PEDF and its mutants do not affect survival of cultured cancer cells, further implying the antiangiogenic effects of the PEDF constructs as a foremost component of their antitumor activity. These findings encourage the development of the phosphomimetic mutants of PEDF as highly potent and apparently non-toxic antiangiogenic anticancer agents. *This work was supported by grants from the Israeli Ministry of Industry and Trade, the Horowitz foundation, the Gurwin Fund for Scientific Advancement and GROWTHSTOP consortium from the EU 6$^{th}$ Framework Program.*

**P15**
**Thrombospondin-1 mimetic peptides increase the uptake of chemotherapeutics and induce regression of established epithelial ovarian tumours**

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Epithelial ovarian cancer (EOC) is the most common gynaecologic malignancy and current therapies have limited success. Thrombospondin-1 (TSP-1) is an endogenous anti-angiogenic protein and mimetic peptides such as ABT-510 and ABT-898 have been created and are currently in clinical trials. Preliminary experiments performed in our laboratory determined that TSP-1 mimetics decrease tumour volume and reduce abnormal tumour vasculature. We hypothesize that through vasculature normalization, these peptides will enhance tumour uptake of chemotherapeutics in an orthotopic syngeneic mouse model of human EOC.

Our model involves injecting tumourigenic epithelial cells under the bursa of the ovary where they then colonize, grow tumours and eventually form ascites approximately 90 days post-injection. 60 days post-tumour induction animals were treated with ABT-510 to determine whether the peptide could induce regression of established tumours. Prior to sacrifice and tissue collection, some mice were injected intraperitoneally with tritiated paclitaxel to quantify the tumour tissue uptake of the chemotherapy drug following treatment with ABT-510. Animals were also treated 80 days post-tumour induction with ABT-898 which has been modified to be more efficacious than previous peptides. Mice were treated for 10 days and then sacrificed for tissue collection or removed from treatment to determine the length of time for the disease to recur.

TSP-1 mimetics and chemotherapeutics caused a significant regression of established ovarian tumours and inhibited formation of secondary lesions. Animals treated with ABT-510 also had a significantly higher uptake of radiolabeled paclitaxel, suggesting that the peptide facilitated intratumoural delivery of the drug. Results from these studies demonstrate that ABT-510 has the ability to decrease primary tumour growth, inhibit abnormal tumour vascularity and increase the uptake of other cytotoxic agents. The use of this drug in combination with current chemotherapeutic approaches may significantly improve our ability to reduce the morbidity and mortality associated with epithelial ovarian cancer.

## VASCULAR EXTRACELLULAR BIOLOGY

### P16
### CO-OPERATIVITY OF SRC AND EZRIN IN THE ACTVATION OF MET AND INVADOPODIA FORMATION IN BREAST EPITHELIAL CELLS

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The Met receptor tyrosine kinase (RTK) is frequently over-expressed and activated in invasive human breast cancer and correlates with poor patient prognosis. We and others have shown that the tyrosine kinase Src mediates cell adhesion-dependent activation of Met and cell spreading. In addition, we have also shown that Src acts co-operatively with the cytoskeletal crosslinker ezrin, causing increased cell scattering and migration. In the present study, we examined the role of Src and ezrin in promoting Met activation and invasion of breast epithelial cells. We showed that expression of both activated Src and ezrin strongly increases Met phosphorylation over levels resulting from activated Src alone. Interestingly, co-activation of Src and ezrin acts synergistically in the formation of extracellular matrix-degrading invadopodia - characteristic of invading cancer cells - which is abrogated upon introduction of a DN ezrin mutant. Src-ezrin co-operativity also increases tyrosine-phosphorylation of cortactin and MMP2 expression, known markers of invadopodia activity. These findings implicate the Src/ezrin axis in regulating adhesion-dependent Met activation and invadopodia formation and may provide important clues toward treatment strategies for invasive breast tumours which exploit this signalling pathway *(Supported by CBCRA).*

### P17
### Gene Expression Profiling in Breast Cancer Microvasculature Identifies Subtypes Linked to Vessel Maturity and Disease Outcome

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*Background:* Angiogenesis plays an important role in the progression of solid tumors, providing both nutrients required for growth and a way to escape the tumor bed(1). The level of vascularization, as measured by microvessel density, varies greatly between breast cancer patients. A high microvessel density significantly predicts poor survival in breast cancer, but between-study variation is high(2).
It is also known that the tumor vasculature differs significantly from its normal counterpart. Among other changes, it is often leaky and generally less mature, lacking the functional pericytes that help stabilize the vessels. Exploitation of these differences has led to the development of several therapeutic avenues to target the tumor vasculature, most notably anti-VEGF therapy(3). Several studies have helped characterize

the gene expression of tumor endothelial cells in different cancers and identify tumor-specific endothelial markers[4-7]. However, none had sufficient samples to investigate the variations that exist between patients.

*Methods:* To study endothelial gene expression we performed microarray hybridization (N=32) of laser capture microdissected endothelial cells from invasive ductal carcinomas and matched endothelial morphologically normal adjacent tissue. We used various statistical techniques to analyse the data and compare it with the additional datasets from the published literature.

*Results:* We identified two distinct subtypes of tumor endothelial cells in breast cancer patients. They are associated with tumors high and low vascular density but not with recurrence. The gene expression of pericyte markers offers evidence that the subtypes also associated with vessel maturity. Surprisingly, most of the published markers of tumor endothelial cells are specifically associated with the low vascular density group. We also identified differences in the Notch and TGFB signaling pathways. Using the information from the subtypes, we developed a prognostic predictor of recurrence based on tumor vascular gene expression. The genes in this predictor are linked to several pathways linked to DNA repair, apoptosis and energy production.

The identification of distinct tumor endothelial classes will help clarify the complex role that the vasculature plays in tumor progression. Our prognostic predictor reinforces that view and identifies differences in tumor vascular gene expression can be linked to distant recurrences. *This abstract was supported through US Department of Defense Breast Cancer Predoctoral Traineeship Award (BC050298).*

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## EMERGING THERAPEUTIC TARGETS

**P18**
**GPR30: A novel therapeutic target in ER- breast cancers**

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While estrogens are crucial to the development and function of many organs such as the mammary gland, they can contribute to mammary tumorigenesis. Aside from the classical genomic signalling pathway, it has long been known that estrogens (E2) generate very fast cellular response independently of genic transcription, such as RTK and AKT transactivation, in estrogen receptor negative (ER-) breast cancer cell lines[a]. These effects have been associated to an E2-sensitive GPCR: GPR30. Activation of this receptor has recently been linked to increased cellular motility. We propose to study[b] GPR30 activation to develop antagonists that could be used as anti-proliferative and anti-metastatic agents. MDA-MB-231 cells (basal-like mammary tumor cells) and SkBr-3 (HER2+ mammary tumor cells) both ER- cell lines that express endogenous GPR30 were used as a model for GPR30 signaling. These cells respond to E2 with increased motility and full anti-estrogens (AEs) with reduced motility, while partial AEs such as tamoxifen and raloxifen can exert molecule specific effects. Interestingly, ER+ breast cancer cell lines that express GPR30 such as MCF-7 and BT-474 do not respond in that same fashion as ER- cell lines. Moreover, knockdown of the classical estrogen receptors (ERalpha and ERbeta) in MDA-MB-231 and SkBr-3 left the cells unresponsive to E2 or AEs which leads us to believe that residual ERalpha/beta can play an active non-genomic role in ER- cells and tumors such as increased invasiveness and proliferation. Furthermore, it appears that full AEs can be useful as anti-metastatic agents in a subset of breast cancers classically determined as ER-.
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**P19**
**Hyperglycemia and anti-hyperglycemic drugs alter the expression of angiogenic factors in a mouse-derived epithelial ovarian cancer cell line**

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Epithelial ovarian cancer (EOC) is the most lethal gynecologic cancer, and also one of the most poorly understood. Recent epidemiological studies have suggested a role for hyperglycemia in the pathogenesis of a number of cancers. Given this association, we have proposed anti-hyperglycemic drugs as possible treatments for EOC. Preliminary reports suggest that these drugs not only reduce available plasma glucose, but also have cytostatic effects on cancer cells via energy-sensing pathways. Because

hyperglycemia is often associated with changes in vasculature, we hypothesize that it also contributes to the development of EOC by altering the balance of pro- and anti-angiogenic molecules and their receptors. Furthermore, we suggest that anti-hyperglycemic drugs exert some of their therapeutic effects by changing the expression of these angiogenic factors. Using a mouse-derived EOC cell line, we have shown that hyperglycemia increases cell metabolic viability in a dose-dependent manner. In this study, we investigated protein levels of the pro-angiogenic factor VEGF and its receptor Flk-1 in cells grown in media containing 0 mM, 2 mM, 6 mM (normal), 16.7 mM and 25 mM glucose. By Western blot, we found that the expression of both VEGF and Flk-1 were increased at higher glucose concentrations. The anti-hyperglycemic drugs metformin and rosiglitazone decreased the metabolic viability of ID8 cells *in vitro*. Dose-response curves were created for each drug by MTT assay, and an $IC_{50}$ treatment concentration was determined for use in subsequent protein analyses. Rosiglitazone (at $IC_{50}$ 100μM) decreased production of VEGF compared to controls at all glucose concentrations, and there was greater VEGF inhibition with increasing glucose. Metformin (at $IC_{50}$ 4 mM) decreased VEGF, but only at high glucose concentrations. The results of these experiments suggest that elevated glucose levels may contribute to ovarian tumour formation by increasing the expression of pro-angiogenic factors and stimulating ovarian tumour angiogenesis. By reducing pro-angiogenic factor expression, anti-hyperglycemic drugs may reduce tumour size by inducing cell death and inhibiting tumour angiogenesis.
*This abstract was supported through grants from the Canadian Foundation for Women's Health and CIHR.*

**P20**

**A novel chimeric protein borne of the fusion of IL-2 and TGFb receptor ectodomain inhibits tumor growth, metastasis and associated neoangiogenesis**

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Cancer cells secrete elevated levels of active TGFb, which acts as a pro-oncogenic factor promoting tumor growth and metastases. As a pro-metastatic factor, TGFb induces both the degradation of extracellular matrix and epithelial-to-mesenchymal transition of normal and transformed epithelial cells and thus enhanced migratory ability. In addition, TGFb promotes myofibroblast differentiation and angiogenesis. As tumor progresses, tumor derived-TGFb suppresses antitumor immune response by directly inhibiting the activation of cytolytic T cells, NK cells and macrophages, as well as interfering with dendritic cell function. Therefore, the notion of blocking tumor-derived TGFb activity and contemporaneously driving a pro-inflammatory response should effectively be synergistic in eliciting a potent immune rejection of cancer. To test this hypothesis, we successfully generated a chimeric protein consisting in the fusion of IL-2 and the ectodomain of TGFb receptor II (TbRII) (aka FIST). IL2, as part of the fusion, preserves the ability to bind the IL2 receptor and to stimulate the proliferation of IL2 dependent cell line (CTLL-2). FIST induces a potent activation of signalling pathways downstream of IL2 receptor such as Jak/Stat, (PI3K)/Akt and ERK. In addition, FIST acts as dominant negative on TGFb signalling pathway by inhibiting the phosphorylation of Smad2 and Smad3. Interestingly, FIST also induces *de novo* expression of Smad7. As an immunostimulator, FIST primes lymphocytes to produce more than 10 fold superior amounts of pro-inflammatory cytokines than equimolar concentrations of IL-2 and prolongs the survival of lymphocytes by upregulating the expression of c-Myc. The analysis of in vivo host immune response to FIST-transfected tumor cells demonstrates a substantial recruitment of CD8 T cells, NK cells, NKT cells and B cells in the tumor site. In murine cancer models, FIST blocks

tumor metastasis, inhibits tumor growth and prolongs survival of immunocompetent mice implanted with metastatic breast cancer cells (4T1). In conclusion, coupling of proinflammatory IL2 to TGFb signalling inhibition leads to an unheralded and potent multifactorial antitumor effect. *This work was supported by a Canadian Institute for Health Research operating grant MOP-15017. CP is recipient of Montreal Centre for Experimental Therapeutics in Cancer Scholarship and US army Medical Research and Material Command, Breast Cancer Predoctoral Traineeship Award BC061595 and JG is a Fonds de recherché en santé du Québec chercheur-boursier senior.*

**P21**
**GPNMB is Novel Pro-Angiogenic Factor and Therapeutic Target in Breast Cancer**

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GPNMB is a type I transmembrane cell-surface protein commonly expressed in melanoma, glioma and breast cancer. We have shown that GPNMB promotes breast cancer metastasis *in vivo*. Currently, there are very few available therapies that effectively target breast cancer metastases; however, Phase II clinical trials are underway to investigate the efficacy of a GPNMB-targeted, cytotoxin-conjugated antibody (CR011) for the treatment of patients with advanced melanoma or breast cancer. We performed IHC staining for GPNMB on a tissue microarray composed of tumor samples from 161 breast cancer patients. Patients whose tumors displayed epithelial-specific GPNMB staining experienced shorter recurrence-free survival than those with little or no GPNMB expression. These results reveal that GPNMB is expressed in metastatic human breast cancers and may represent an important target for therapeutic intervention. To determine whether GPNMB is a viable target in breast cancer, we performed *in vitro* cell-killing assays with CR011. GPNMB-expressing breast cancer cells, but not those which lack GPNMB expression, were killed by CR011 in a dose-dependent manner. Moreover, preliminary results indicate that CR011 is capable of inducing regression of MDA-MB-468 tumors *in vivo*. To investigate the mechanisms by which GPNMB mediates breast tumor progression we have engineered vector control (VC) and GPNMB-expressing 66cl4 mammary cancer cells. Mice injected with GPNMB-expressing cells developed more tumors, which grew at a faster rate than tumors derived from control cells. IHC characterization of these tumors revealed that GPNMB-expressing tumors displayed increased vascular density suggesting that GPNMB may act as a pro-angiogenic factor. Indeed, this phenotype was recapitulated in an independent *in vivo* model, employing VC or GPNMB-expressing BT549 breast cancer cells. VEGF levels were similar among VC and GPNMB 66cl4 or BT549 cells, suggesting that GPNMB may promote angiogenesis in a VEGF-independent manner. Taken together, our data identify GPNMB as a prognostic indicator of recurrence, a novel pro-angiogenic factor and an exciting therapeutic target in patients with metastatic breast cancer. *This work is supported by grants from the CBCRA (Grant No. MOP-84386 to P.M.S.), the CIHR (Grant No. CTP-79857 to M.P. and P.M.S.), the McGill University Health Centre Foundation (MUHC to M.P.) and the Réseau de la recherche sur le cancer of the Fonds de recherche en santé du Québec (FRSQ to M.P.).*

## MOLECULAR GENETICS

### P22
### Towards the rational design of estrogen receptor mutants with altered DNA-binding specificity

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Estrogen receptor alpha (ER) binds to palindromic response elements composed of PuGGTCA motifs. Our goal has been to examine whether ER can be tailored to recognize novel motifs. We have shown that rational design of ER mutants with altered DNA binding specificity is complicated by the mode of recognition of the two central base pairs of repeated motifs, which involves two charged amino acids (aa) each interacting with two bases on opposite DNA strands. Combined mutagenesis of these residues was required to generate a receptor that recognized a new type of motifs (1). Here we investigated whether ER can be designed to recognize new bases at positions 2 and 5. We used computer assisted modeling to examine whether replacement of aa R211 or K206, which interact with bases through one aa-one base interactions, can yield novel DNA binding specificities. We identified different limitations for each residue. R211 was found to contribute to complex formation with DNA not only due to hydrogen bonding, but also by packing of the terminal end of its side chain both with DNA and with the backbone of the receptor DNA recognition helix. This crucial contribution to DNA binding affinity cannot be maintained with any other aa. This likely explains both the complete loss of DNA binding of ER mutants at position R211 in gel shift and transactivation assays, and the total conservation of this residue within the nuclear receptor family. In the case of K206, either steric conflicts or lack of chemical complementarity was observed with bases at position -5. Accordingly, mutagenesis of K206 indicated that while its contribution to the affinity of the interaction is more minor than that of R211, no switch in specificity could be engineered. Rather, short residues at this position relaxed the ER specificity towards the corresponding base. These results identify specific criteria that must be taken into consideration in the rational design of nuclear receptor mutants with novel DNA binding specificities, and suggest that combined changes in several aa of the DNA binding helix may be necessary to achieve a specificity switch.

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### P23
### DNA hypermethylation of angiogenesis-related genes *RECK* and *uPA* in invasive breast carcinoma: preliminary data

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Breast cancer is a heterogeneous disease that can be classified into at least 4 different groups (luminal A, luminal B, ERBB2 and basal). Identification of new clinical biomarkers for these subgroups is important because they could be used as therapeutic targets. Blood vessel formation, or angiogenesis, is one of the most important steps in the propagation of malignant tumour growth and metastasis. Expression of some angiogenesis-related genes is regulated by DNA methylation, a mechanism often altered in breast cancer. Our objective is to identify markers of breast cancer subgroups by targeting angiogenesis-related genes that are regulated by DNA methylation. First, methylation percentages of the promoters of many genes, including *RECK* and *uPA*, will be evaluated in 335 laser captured microdissected breast cancer tissues using a validated technique developed in our laboratory for small DNA samples. Then, we will determine the associations of these methylation percentages with breast cancer subtypes, tumour characteristics and survival. At this time, we measured the methylation percentage of *uPA* and *RECK* genes from 72 invasive breast carcinomas. For *uPA* gene, a methylation percentage >10% was observed in 15% (6/39) of luminals (A or B) and in 6% (2/33) of cases classified as ERBB2 or basal. For *RECK* gene, a methylation percentage >10% was observed in 23% (9/39) of luminals (A or B) and in 18% (6/33) of cases classified as ERBB2 or basal. Our preliminary results suggest that hypermethylation of *uPA* and *RECK* promoters could be clinical biomarkers of some breast cancer subgroups. *Acknowledgments: This project was supported by grants from Hoffmann-La Roche Limited, Réseau de la Recherche sur le Cancer: Axe Banque de Tissus et de Données pour les Cancer du Sein et de l'Ovaire du Fonds de Recherche en Santé du Québec, La* Fondation *des H*ôpitaux Enfant-Jésus – *Saint-Sacrement and Le Centre des Maladies du Sein Deschênes-Fabia.*

**P24**

**CnABP, a novel modulator of the Calcineurin-NFAT signaling pathway, is overexpressed in Wilms' tumors and promotes cell migration**

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Wilms' tumor (WT) is a classical cancer type that arises from abnormal differentiation of kidney progenitor cells, and occurs at a frequency of 1 in 10,000 live births, accounting for approx. 90% of childhood kidney cancer. Abnormally high levels of Pax2 (paired-box protein 2), a key regulator of kidney development, have been observed in both WT and renal cell carcinoma, which correlate with proliferation and increased invasiveness (1-5). Therefore the misexpression of Pax2 and its target genes may play an important role in tumor initiation and/or progression. To test this, we screened for target genes of Pax2 by cDNA microarray in the embryonic kidney. We identified *CnABP* (Calcineurin A Binding Protein), a novel gene under Pax2 regulation. In situ hybridization indicates that *CnABP* coexpresses with *Pax2* in the condensing mesenchyme, the abnormal differentiation of which gives rise to WT. Furthermore, expression analysis by quantitative PCR indicates that *CnABP* is overexpressed in more than 70% of Wilms' tumors. Interestingly, in the proportion of tumors with upregulated *PAX2* expression, more than 80% also overexpress *CnABP*. We characterized CnABP as a membrane-anchored protein that primarily promotes cell proliferation and migration. Mediators of these activities were investigated by Yeast-two-hybrid and immunoprecipitation, which identified an interaction between CnABP and Calcineurin A, the catalytic subunit of a calcium-responsive serine/threonine phosphatase Calcineurin (6). We showed that CnABP modulates phosphatase activities of Calcineurin, which consequently inhibits calcium/Calcineurin-dependent NFAT nuclear translocation. We further demonstrated that the inhibition of NFAT nuclear localization results in reduced NFAT-specific transcriptional activity. Components of the Calcineurin complex have been reported as upregulated genes

distinguishing recurrent from non-recurrent Wilms' tumors (7). This is in line with the evidence we presented, as CnABP is upregulated in Wilms' tumors and is shown to promote proliferation and migration. *This work was supported by a grant from the National Cancer Institute of Canada (#015121).* *References:* (1) Gnarra JR et al. Cancer Research 1995; 55:4092-8. (2)Muratovska A et al. Oncogene 2003; 22:7989-97. (3)Fonsato V et al. Am J Pathol 2006; 168:706-13. (4)Hueber PA et al. Kidney Int 2006; 69:1139-45. (5)Li CM et al. American Journal of Pathology 2002; 160:2181-90. (6)Wang MG et al. Cytogenet Cell Genet 1996; 72:236-41. (7)Li W et al. Cancer Res 2005; 65:2592-601.

**P25**
**Ischemic conditions induce DNMT disregulation in human colorectal cancer**

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DNA methylation plays a significant role in cancer initiation, progression and aggressiveness. DNA methyltransferases (DNMTs), the enzymes responsible for modulation of methylation patterns, are strictly controlled in normal cells. However, regulation of these enzymes is lost in cancer cells due to unknown reasons. Cancer therapies which target DNMTs have proven promising in treatment of blood cancers, however lack the same level of effectiveness in solid tumors. Solid tumors exhibit areas of hypoxia and hypoglycaemia due to their irregular and insufficient vasculature. When human colorectal carcinoma cells (HCT116) were subjected to ischemia (hypoxia and hypoglycaemia) *in vitro*, a significant decrease in mRNA for DNMT1, DNMT3a and DNMT3b ($p < 0.05$) was seen. Similar reductions in protein levels of DNMT1 and DNMT3a were detected by western blotting. In addition, total activity levels of DNMTs (as measured by an ELISA-based DNMT activity assay) were reduced in hypoxic and hypoglycaemic conditions. HCT116 tumor xenografts analyzed with immunofluorescence showed a clear inverse relationship between hypoxia (as revealed by carbonic anhydrase 9 expression) and DNMT1 protein. These studies provide evidence for the down-regulation of DNMTs in hypoxia and hypoglycaemia, both *in vitro* and *in vivo*. Current research shows that methylation patterns of certain genes in colorectal cancer are reduced between primary and metastatic lesions. Perhaps ischemic conditions are accelerating these methylation changes, resulting in increased metastatic potential. Knowledge of factors modifying DNMT behaviour in solid tumors may lead to improved therapeutic strategies for epigenetic remodeling. *This abstract was supported through a grant from* Cancer Research Society (CRS).

**P26**
**Tumor Cell Autonomous ShcA is a Paracrine Integrator of the Adaptive Immune Response During Breast Cancer Progression**

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To explore the *in vivo* significance of tumor cell autonomous ShcA signaling during Neu/ErbB2-induced mammary tumorigenesis, we interbred floxed-ShcA mice with a unique breast cancer mouse model expressing both Neu/ErbB2 and Cre from the same bicistronic transcript (NIC). We demonstrate that loss

of ShcA expression in mammary epithelial cells severely attenuates tumor development. With parity, we are able to generate NIC/ShcA null breast tumors, albeit with reduced penetrance and a significantly longer latency compared to wild-type NIC animals. We performed gene expression profiling to determine the mechanisms contributing both to the impaired outgrowth of mammary tumors in a ShcA-deficient background and identify those processes that contribute to their eventual emergence. A significant number of chemokines, chemokine receptors and genes associated with the B and T cell response are overexpressed in NIC/ShcA null breast tumors. Furthermore, we observe both infiltrating CD3 positive T cells and a robust humoral immune response in NIC/ShcA-deficient mammary tumors. We demonstrate that subsets of these T cells are proliferating and upregulate expression of ICOS, both of which are indicative of antigen-stimulated T cell activation. We propose that early loss of ShcA signaling may result in Th1-dependent anti-tumor immune response and contribute to impaired tumor formation in ShcA null breast tumors. We further suggest that emerging breast tumors may compensate for the loss a ShcA signaling by switching to a Th2/Treg response to favor an immunosuppressive state and allow cancer progression. Thus, a major role for ShcA signaling in breast cancer cells may be to regulate immune cell recruitment within the local microenvironment to facilitate tumor progression. *This work was supported by a grant from the Canadian Breast Cancer Research Alliance (CBCRA).*

## METASTASIS

**P27**
**Interaction of OPN, thrombin, and integrins in breast cancer malignancy and metastasis**

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Osteopontin (OPN) is a secreted phosphoprotein containing integrin-binding sites and a thrombin cleavage domain and is often overexpressed in the blood and primary tumors of breast cancer patients. We *hypothesized* that the thrombin cleavage domain and integrin binding are necessary for OPN-mediated malignant behavior.
Methods: Breast cancer cell lines transfected with OPN (468-OPN), OPN lacking the thrombin cleavage domain (468-ΔTC) and control vector (468-CON) were compared for differences in adhesion and migration *in vitro,* +/- Argatroban (thrombin inhibitor) or integrin-blocking antibodies. Expression and activity of the pro-invasive protein uPA were also measured. Primary tumor growth and metastasis were measured when the cell lines were injected into the mammary fat pad of nude mice. Lastly, microarray analysis was conducted to identify possible mechanisms for the observed functional differences.
*Results:* We observed that overexpression of OPN resulted in significantly increased cell adhesion and migration ($p<0.001$). These increases were specific to the interaction between OPN, thrombin, and integrins, since treatment with either Argatroban or integrin-blocking antibodies caused significant reduction of 468-OPN cell adhesion and migration ($p<0.05$), but had little effect on 468-ΔTC and 468-CON cells. However, uPA expression and activity were highest in 468-ΔTC cells ($p<0.01$) compared to 468-OPN and 468-CON. 468-ΔTC cells grew larger tumors ($p<0.01$) than the other cell lines and resulted in a significantly increased metastatic burden to the lymph nodes ($p<0.001$). Microarray analyses indicated upwards of 700 genes were significantly differentially regulated in the cell lines.
*Conclusions:* These novel findings indicate that the thrombin cleavage site in OPN is important for mediating adhesion and migration of breast cancer cells *in vitro*. However, tumor growth and metastasis were actually enhanced by the loss of the thrombin cleavage domain suggesting a more complicated relationship between OPN and thrombin *in vivo.* Validation of genes identified in the microarray is ongoing and may shed light onto possible mechanisms for the identified functional differences. *This work was supported by grants from the National Research Council of Canada, the Canada Foundation for Innovation, and the Schulich School of Medicine and Dentistry at the University of Western Ontario. M.S.B. is the recipient of a Canadian Institute of Health Research-Strategic Training Program Scholarship and a Translational Breast Cancer Scholarship through the London Regional Cancer Program. A.L.A. is supported by the Imperial Oil Foundation.*

**P28**
**ARF1 controls the activation of the PI3K pathway to regulate EGF dependent growth and migration of breast cancer cells**

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Stimulation of growth factor receptors leads to the activation of intracellular signalling cascades to tightly regulate cell motility. Recent studies have demonstrated that the small GTPase ARF6 is overexpressed in different invasive cancer cells and acts to control invasiveness of these different cancer cell lines. In this study, we demonstrate that ARF1, the isoform classically associated with the Golgi, is present in dynamic plasma membrane ruffles of invasive breast cancer cells and is an important regulator of the epidermal growth factor (EGF)-dependent biological effects. Using invasive breast cancer cell lines, we demonstrate that both ARF1 and ARF6 are transiently activated following EGF stimulation. Inhibition of endogenous ARF1 expression by using small interfering RNA (siRNA) approach or overexpression of wild type and mutant ARF1 markedly impairs cell migration. The underlying mechanism involves the activation of the phosphatidylinositol-3 kinase (PI3K) pathway, assessed by the phosphorylation of Akt. Our data demonstrate that depletion of ARF1 markedly impairs the recruitment of the PI3K catalytic subunit (p110α) to the plasma membrane and subsequently the recruitment of Akt. Moreover, the ARF1 knockdown blocks the endogenous association between the regulatory subunit p85α to EGFR in an EGF-dependent manner. In addition, depletion of ARF1 does not impair EGF receptor expression nor its ability to be activation by EGF. In ARF1 depleted cells, activation of the MAPK cascade remains intact. Moreover, ARF6 depletion does not affect PI3K signaling but blocks MAPK activation. All together, these data demonstrate that ARF1 and ARF6 act as molecular switches to control the activation of PI3K and MAPK pathways, respectively. These results uncover a novel molecular role of ARF1 by which this ARF isoform regulates breast cancer cell migration during cancer progression.

**P29**
**Type IV collagen promotes liver metastasis**

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The molecular mechanisms that regulate the site-specificity of metastasis are not well understood. Both tumor-dependent factors and the host microenvironment have been implicated. Using the murine Lewis lung carcinoma model, we found that IGF-I receptor overexpression led to the acquisition of a liver-metastasizing phenotype and this was associated with an altered extracellular matrix (ECM) composition, including a significant increase in type IV collagen expression. In cells stably transfected with type IV collagen alpha 1 and alpha 2, a switch from a lung to a liver-colonizing phenotype was observed and this was associated with profound changes to cellular morphology and an increase in anchorage independent growth. Moreover, these cells had an enhanced ability to survive under conditions of anoikis and that was linked to increased □2 integrin expression and focal adhesion kinase (FAK) activation. To assess the clinical relevance of these findings, collagen IV expression was analyzed in primary colorectal carcinoma (CRC) tissue and in multiple hepatic metastases derived from different primary tumors. In uveal melanoma sections, we found that an intense collagen IV staining was associated selectively with hepatic metastases and could not be seen in eye, bone, skin, lymph node or lung metastases of this tumor. In primary CRC tumors, collagen IV staining was variable with the majority of sections showing no positive staining. In contrast, hepatic metastases of CRC were uniformly (40/40) highly positive and a similar high level of staining was seen in hepatic metastases from other primary sites such as breast and renal carcinoma. Taken together, these results implicate type IV collagen in liver metastasis from different

primary sites and suggest that this ECM protein increases tumor cell rescue from anoikis via integrin signaling. These findings may have important implications to the prognosis and therapeutic targeting of liver metastases. *Supported by Canadian Institute for Health Research grant MOP- 81201 (PB) and a McGill University Health Center Research institute fellowship (JVB).*

**P30**

**The SDF-1/CXCR4 ligand/receptor axis: an example of a tumor-host interaction determining risk of metastasis in breast cancer**

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*Background:* Stromal cell-derived factor (SDF)-1, a chemokine, is overexpressed in those organs to which breast cancer metastasizes, and serves to home in cancer cells which express its receptor, CXCR4. We previously reported the role of low plasma SDF-1 levels as a host-derived marker predictive of distant metastasis. We hypothesized that the prognostic value of low plasma SDF-1 levels, imparting an innate metastatic risk, would be enhanced in patients with tumors having high CXCR4 or phosphorylated (p)-CXCR4 expression. CXCR4 has been shown to be an independent prognostic marker, however it is plausible that activated CXCR4, or p-CXCR4 may be able to better discriminate metastatic risk.
*Methods:* Using the same cohort of patients in whom we previously measured plasma SDF-1 levels, we built a tissue microarray containing paraffin-embedded tissue blocks of the primary tumor for 237 patients. There were 212 patients for whom plasma and tissue blocks were both available. Plasma SDF-1 levels were previously measured using an ELISA, and tumor protein expression was detected using immunohistochemistry. Survival analysis was calculated using cox regression analysis.
*Results:* We found that tumor p-CXCR4 was a stronger prognostic marker than CXCR4. Patients with high tumor expression of p-CXCR4 demonstrated a 4-fold increased rate of mortality (hazard ratio (HR) 3.95; $P = 0.004$) compared to a 3-fold increased risk in patients whose tumors highly expressed CXCR4 (HR, 3.20; $P = 0.03$) in univariate analysis. The combination of low plasma SDF-1 and high p-CXCR4 had an even greater prognostic value (HR, 5.96; $P < 0.001$). This remained significant with multivariate analysis (HR, 3.78; $P = 0.01$).
*Conclusion:* We report here for the first time the prognostic value of phosphorylated-CXCR4 in breast cancer, and its superiority over CXCR4 expression. Furthermore, the combination of p-CXCR4 and low-plasma SDF-1 levels was found to be an independent prognostic marker, stronger than either factor alone, suggesting that low plasma SDF-1 may especially favor the metastatic process in patients with tumors containing the activated CXCR4 receptor. *Grant Support: Canadian Breast Cancer Research Alliance (#14598 to Dr. Basik), Fonds de la recherche en santé du Québec Reseau de Recherche sur le Cancer for the tumor bank.*

**P31**
**The role of the GTPase-activating protein CdGAP in cell migration, adhesion, and invasion using a mouse breast cancer metastasis model system**

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RhoA, Rac1, and Cdc42, the best-characterized members of the Rho family of small GTPases, are critical regulators of many cellular activities. CdGAP (Cdc42 GTPase-activating protein) is a serine- and proline-rich RhoGAP protein showing GAP activity against both Cdc42 and Rac1 but not RhoA. We have previously demonstrated that CdGAP is phosphorylated downstream of the MEK-ERK pathway in response to serum and is also required for normal cell spreading, polarized lamellipodia formation, and cell migration. Recently, we have observed a high increase in the protein and mRNA expression levels of endogenous CdGAP in mammary tumors explant arising from NMuMG immortalized mouse mammary cells expressing the activated ErbB-2/Neu receptor. Northey *et al* (1) have shown that, in response to TGF-β stimulation, NMuMG cells expressing activated Neu demonstrate a clear induction in both cell motility and invasion. Here, we show that both mRNA and protein expression levels of CdGAP are increased in response to TGF-β stimulation in NMuMG/activated Neu cells. Furthermore, downregulation of CdGAP expression by siRNA abrogates the ability of TGF-β to induce cell motility and invasion of NMuMG/activated Neu cells. However, it has no effect on TGF-β-mediated cell adhesion on collagen. Rescue analysis using re-expression of various CdGAP deletion mutant proteins reveals that the proline rich domain of CdGAP is essential for its function in TGF-β-mediated cell motility. In addition, downregulation of CdGAP expression decreases cell proliferation of NMuMG/activated Neu cells independently of TGF-β. Taken together, these results suggest that CdGAP is required for TGF-β-induced cell motility and invasion in mammary breast cancer cells and may function as an oncogene in breast cancer. *This work is supported through a grant from CIHR (MOP-84449).*
*References:* (1) Northey, J.J et al. Mol Cell Biol (2008) 28, 3162-76

**P32**
**Sam68 adaptor function in cell polarity and migration**

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The *S*rc-*a*ssociated substrate during *m*itosis with a molecular mass of *68* kDa (Sam68) is predominantly nuclear and is known to associate with proteins containing the Src homology 3 (SH3) and SH2 domains. Although Sam68 is a Src substrate, little was known about the signaling pathway linking them. Src is known to be activated transiently after cell spreading, where it modulates the activity of small Rho GTPases. We found that Sam68-deficient cells exhibit loss of cell polarity, inadequately formed actin stress fibers and increase focal adhesion site. The modulation of both cell morphology and migration point towards a novel signaling/cytoplasmic function of Sam68 and its impact on the cytoskeleton. Interestingly, Sam68-deficient cells exhibited sustained Src activity after cell attachment, resulting in the constitutive tyrosine phosphorylation and activation of p190RhoGAP and its association with p120rasGAP. Furthermore, the increased Src kinase activity in these cells led to a deregulated RhoA and Rac1 activity. We also observed that Sam68 inactivation could inhibit growth factor-induced migration,

suggesting an important function in both EGF and intergrins activation pathway. As such, Sam68 could serve as a complementary treatment for highly metastatic cancers where growth and malignancy is induced by EGF.

**P33**
**C-MET ACTIVATION IN MEDULLOBLASTOMA INDUCES TISSUE FACTOR EXPRESSION AND ACTIVITY: EFFECTS ON CELL MIGRATION**

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Met, the receptor for hepatocyte growth factor (HGF), is a receptor tyrosine kinase that has recently emerged as an important contributor to human neoplasia. In physiological and pathological conditions, Met triggers various cellular functions related to cell proliferation, cell migration and the inhibition of apoptosis, and also regulates a genetic program leading to coagulation[a]. Since medulloblastomas (MB) express high levels of tissue factor (TF), the main initiator of blood coagulation, we therefore examined the link between Met and TF expression in these pediatric tumors[b]. We observed that stimulation of the MB cell line DAOY with HGF led to a marked increase of TF expression and procoagulant activity, in agreement with analysis of clinical MB tumour specimens, in which tumours expressing high levels of Met also showed high levels of TF. The HGF-dependent increase in TF expression and activity required Src family kinases and led to the translocation of TF to actin-rich structures at the cell periphery, suggesting a role of the protein in cell migration. Accordingly, addition of physiological concentrations of the TF activator FVIIa to HGF-stimulated DAOY cells promoted a marked increase in the migratory potential of these cells. Overall, these results suggest that HGF-induced activation of the Met receptor results in TF expression by MB cells and that this event likely contribute to tumour proliferation by enabling the formation of a provisional fibrin matrix[c]. In addition, TF-mediated non-haemostatic functions, such as migration towards FVIIa, may also play a central role in MB aggressiveness. *This work was supported by grants from the NSERC to R.B.*
*References:* [a] Boccaccio et al., *Nature* 2005; 434:396-400. [b] Provençal, M.; Labbé, D. et al., *Carcinogenesis* 2009; in press. [c] Boccaccio et al., *Cell Mol Life Sci* 2006; 63:1024-7.

**P34**
**The invasiveness of breast cancer cell is enhanced by radiation**

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For women with early stage breast cancer, the primary tumour is removed by conservative surgery. However, cancer cells often scattered throughout the breast, malignant microfoci are observed in 39-63% of women[a]. To eliminate these cells radiotherapy is designed to irradiate the whole breast. But the dose is not calculated to eliminate all residual cancer cells but rather to optimize long-term results with minimal complications. Although radiotherapy efficiency is well established, only a moderate increase of survival rate is observed 15 years after treatment[b]. Radiation also enhances matrix metalloproteinases (MMPs)

activity, key proteins in invasion and angiogenesis[c]. The aim of this study was to determine whether the invasiveness of the residual breast cancer cells could be enhanced by radiation.
Balb/c mice were irradiated (30 Gy) on one thigh, while the other thigh was used as control. Non irradiated MC7-L1 mouse mammary carcinoma cells were injected subcutaneous in both thighs and the kinetic and extent of cancer cell invasion were followed for 6 weeks by magnetic resonance imagining. Tissues were processed for histopathology and zymography analysis.
Six weeks following the subcutaneous injection of MC7-L1 cells, large volumes of cancer cell invasion penetrating deeply in the muscle in the irradiated thighs were measured. Conversely, cells injected in the non irradiated thigh grew almost exclusively under the skin. Histological evaluation confirmed these results. Invasiveness was related with an increased activity of MMP-2.
Our results clearly demonstrated that normal tissue irradiation before cancer cells injection substantially increases their invasion ability. This mechanism might contribute to local recurrence and metastases several years after treatments.
*References: a*-Holland et al., 1985, Cancer; 56:979. *b*-Coles et al., 2005, Clin Oncol, 17;16. *c*-Paquette et al., 2007, Br J Cancer, 97;1505.

**P35**
**The IGF-I receptor controls the metastatic properties of tumor cells by regulating their cytokine profiles**

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Metastatic tumor cells that invade the liver initiate a host inflammatory response that promotes metastasis. The objective of this study was to identify molecular mechanism(s) that regulate this inflammatory response. Lung carcinoma M-27 cells overexpressing the IGF-I receptor (IGF-IR) acquire an ability to induce an inflammatory response and metastasize to the liver. Cytokine profiling revealed that IGF-IR overexpression altered the cytokine profile of these cells. Because cytokine production is regulated by IKK/IκB/NF-κB signalling, we undertook to anlayze TNF-a activated, NF-kB signaling in these cells. Western blotting revealed that in M-27$^{IGFIR}$ cells, TNF-α −induced IkB-a phosphorylation and degradation increased relative to wild type cells and this resulted in accelerated nuclear translocation of (p65) NF-kB. An EMSA confirmed these results and revealed a more rapid, TNF-a-induced, nuclear NF-kB binding in these cells. When gene transcription downstream of NF-kB was analyzed, we found that TNF-a induced a rapid and significant increase in IL-6 and IkB-a expression in M-27$^{IGFIR}$, but not in wild type cells. In contrast, IkB kinase (IKKe) expression levels and downstream targets were significantly suppressed in M-27$^{IGFIR}$ cells relative to controls. The results identify the IGF-I receptor both as a positive and a negative regulator of NF-kB signalling and suggest that the cytokine profiles of tumor cells may be determined by the outcome of these opposing effects. Further progress in understanding these signaling events and how they affect tumor cell interaction with the host innate response and metastasis should lead to identification of novel targets and development of strategies for anti-metastatic therapy. *Supported by CIHR grant # MOP-81201 (to PB) and a McGill University Health Center Research Institute fellowship (to SL).*

**P36**
**ACTIVATION OF A NOVEL SRC/EZRIN/MET SIGNALING PATHWAY IN A TRANSGENIC MOUSE MODEL OF BREAST TUMOURIGENESIS**

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Metastasis is the leading cause of mortality in human breast cancer. However, there are few predictive, prognostic, or therapeutic targets of breast cancer metastasis. Our previous findings indicate that the activation of a novel Src/ezrin/Met pathway promotes invasion and metastasis of breast carcinoma cells. Src also acts co-operatively with Stat3 in the activation of an autocrine HGF/Met loop. Thus, inhibition at a number of sites along this pathway may present potential biomarkers and/or therapeutic targets in human breast cancer. As a test of principle, we examined the expression and activation profile of the Src/ezrin/Stat3-HGF/Met pathway in a Polyoma Middle T (PyMT) viral-induced mouse model of breast carcinogenesis. Results showed a marked increase in expression and activation of Src, ezrin, Stat3, HGF and Met in PyMT-induced tumours, compared to nulliparous or lactating mouse mammary tissue. Furthermore, there was a marked shift from an apical expression of Src/ezrin/Met in normal epithelium to either a membranous or an intense, diffuse cytoplasmic expression in tumour tissues. These findings provide *in vivo* evidence for the activation of the Src/ezrin/Stat3 – HGF/Met pathway in breast tumourigenesis and metastasis. Furthermore, this study provides support and validation for the analysis of the expression of this signalling network in a human breast tumour tissue microarray. *(Supported by CBCRA).*

**P37**
**Breast tumor kinase phosphorylates PSF promoting its cytoplasmic localization and cell cycle arrest**

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BReast tumor Kinase (BRK) is a non-receptor tyrosine kinase overexpressed in ~60 % of human breast carcinomas. BRK has been shown to be a positive effector in epidermal growth factor (EGF) signaling, potentiating EGF-induced cell proliferation and migration. Activation of BRK results in the phosphorylation of numerous intracellular targets, but the identity of these targets and their link to BRK signaling have not been adequately defined. To identify novel BRK substrates and interacting proteins, we performed large-scale co-immunoprecipitation assay on the BT-20 breast cancer cell line, followed by mass spectrometry. Herein, we report the identification of RNA-binding protein PSF (polypyrimidine tract-binding (PTB) protein-associated splicing factor) as a BRK-interacting protein and substrate. BRK and PSF co-eluted in a large protein complex that was regulated by EGF stimulation and both proteins were shown to associate directly via SH3 domain-polyproline interaction. PSF was shown to be phosphorylated by BRK and also upon EGF stimulation, consistent with a role of PSF and BRK downstream of the EGF receptor. Tyrosine phosphorylation promoted the cytoplasmic relocalization of PSF, impaired its binding to polypyrimidine RNA, and led to cell cycle arrest. These results identify PSF as a novel BRK-binding protein and substrate, and suggest that the function of BRK in breast cancers may be regulated PSF. *This work was funded by the Cancer Research Society Inc, the Week-end to End*

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**P38**
**Thrombospondin-1 is a transcriptional repression target of PRMT6**

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Protein arginine methyltransferase 6 (PRMT6) is known to catalyze the generation of the mono- and asymmetric di-methylarginines in polypeptides. Although the cellular role of PRMT6 is not well understood, PRMT6 has been implicated in HIV pathogenesis, DNA repair and transcriptional regulation. PRMT6 is known to methylate histone H3 Arg 2 (H3R2) and this negatively regulates the lysine methylation of histone H3 Lys4 (H3K4) resulting in gene repression. To identify novel genes regulated by PRMT6 in a non-biased manner, we performed a microarray analysis on human U2OS osteosarcoma cells transfected with control and PRMT6 siRNAs. Using this method, we identified thrombospondin-1 (TSP-1), a potent natural inhibitor of angiogenesis, as a transcriptional repression target of PRMT6. Moreover, we show that PRMT6-deficient U2OS cells exhibited cell migration and invasion defects that were rescued by blocking the secreted TSP-1 with a neutralizing peptide or with an anti-TSP-1 blocking antibody. PRMT6 associates with the *TSP-1* promoter and regulates the balance of methylation of H3R2 and H3K4 such that, in PRMT6-deficient cells, H3R2 was hypomethylated and H3K4 was trimethylated at the *TSP-1* promoter. Using a *TSP-1* promoter reporter gene, we further show that PRMT6 directly regulates the *TSP-1* promoter activity. These findings show that PRMT6, by mediating H3R2 asymmetric dimethylation at the *TSP-1* promoter, represses the transcription of TSP-1, thus revealing an epigenetic mode of TSP-1 regulation. Altogether, the present study suggests that specific PRMT6 inhibitors that neutralize the activity of PRMT6 may be useful to suppress cancer cell invasion and malignant development and/or progression. Therefore, these results are the first to point out the viability of PRMT6 as a potential cancer therapeutic target.
Acknowlegements: We thank Amber Zabarauskas for assistance with the microarray data analysis. *This research was funded by grant MOP-93811 from the Canadian Institutes of Health Canada. J.M.-L. is a recipient of a postdoctoral fellowship from the Fond de la Recherche en Santé du Québec (FRSQ). S.R. is an FRSQ Chercheur-National.*

**P39**
**TGF-β1 is the predominant isoform required for breast cancer cell outgrowth in bone**

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Interactions between metastatic tumor cells and bone-derived growth factors facilitate the growth of tumor cells in this unique microenvironment.[1] TGF-β signaling is a potent modulator of the invasive and

metastatic behavior of breast cancer cells. Indeed, breast tumor responsiveness to TGF-β is important for the development of osteolytic metastases that associate with excessive bone resorption.[2-8] However, the specific TGF-β isoforms that promote breast cancer outgrowth in bone are unknown. Furthermore, it is unclear whether the primary source of TGF-β required for the development of osteolytic metastases originates from the bone matrix, resident bone cells or the tumor cells themselves. In our study, we utilize the human breast cancer-derived MDA-MB-231 cells that form aggressive osteolytic bone metastases in immunocompromised mice. We demonstrate that expression of a TGF-β1 and TGF-β3 specific ligand trap in MDA-MB-231 cells diminished their osteolytic outgrowth in bone compared to controls. Furthermore, this observation correlated with the reduced expression of TGF-β induced pro-osteolytic factors *PTHrP* and *IL-11*. We further show that specific loss of TGF-β1 expression within the bone microenvironment of TGF-β1+/- and TGF-β1-/- mice reduced the incidence of breast tumor outgrowth compared to wild-type animals. Moreover, the small percentage of tumors that grew in a TGF-β1 depleted microenvironment displayed elevated expression of the TGF-β isoforms specifically within cancer lesions. Finally, breast cancer cells expressing the TGF-β ligand trap are further impaired in their ability to form osteolytic lesions within the bone of TGF-β1+/- mice, when compared to the wild-type animals. Collectively, our studies reveal that both host- and tumor-derived TGF-β expression plays a critical role during the establishment and outgrowth of breast cancer cells in bone. *Funding acknowledgements: A.A.M. acknowledges studentship support from the FRSQ. P.M.S. is a research scientist of the NCIC. Grant support for this project was provided by the CBCRA.*
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**P40**
**α4 integrin-expressing tumor cells transmigrate through fibroblasts**

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*Objective:* Our recent results demonstrated that neutrophils and B lymphocytes can migrate by a transcellular route through fibroblasts (Couture et al. Exp. Cell Res. in Press). This cellular phenomenon is mediated by b2 integrins/ICAM-1 and α4 integrin/VCAM-1. We therefore wondered whether other cell types harboring α4 integrin could also cross fibroblasts. Some tumor cells lines are known to express α4 integrin. The objective of this study was to determine whether tumor cells can interact with fibroblasts and whether this interaction results in the passage of tumor cells through fibroblasts.
*Methods and results:* Several tumor cells lines, expressing or not α4 integrin, were selected (B16F1, B16F10, BLM, MV3, IF6, 530, WM9, 1205Lu, 451Lu, MDA-MB-231, SKOV-3, and HT-29). Primary cultures of fibroblasts were prepared by protease digestion of rat heart. Interaction between tumor cells and fibroblasts was quantified by flow cytometry and visualized by confocal microscopy after intracellular labeling of each cell type with distinct fluorescent tracers. There was a good correlation between the level of α4 integrin expression and the properties of tumor cells to interact with fibroblasts. Analysis of this interaction by confocal microscopy showed that this interaction translates into the passage of tumor cells into fibroblasts. Among the cell lines that showed the strongest interaction were the B16, and MV3. Stimulation of fibroblasts with TNF-α increased both VCAM-1 expression and the

interaction. Interestingly, BLM which does not express α4 integrin was also able to interact, suggesting that other receptors might be involved.
*Conclusion:* Our results demonstrated that tumor cells can transmigrate through fibroblasts. Considering that the microenvironment surrounding tumor cells can affect their invasiveness and the development of metastasis, this active transmigration may facilitate tumors cells to escape from the primary tumor. *This abstract was supported by grants from the NSERC and CIHR.*

**P41**
**Investigating a role for CCN3 in the promotion of breast cancer metastasis to bone**

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Breast cancer is the most frequent and the second most lethal cancer affecting woman in Canada. When the tumor remains confined to the breast, patients have a good prognosis following surgery. The most devastating aspect of this disease is the emergence of tumor cells that are capable of spreading or "metastasizing" to other organs and tissues. The skeleton is a common site for breast cancer metastasis; however, the reasons for this are not fully understood. We have used mouse models to isolate breast cancer cell lines that aggressively metastasize to bone and have compared them to cells that are weakly bone metastatic. Through gene expression profiling, we have identified several candidates, including the protein CCN3 (Nov), which are expressed at higher levels in the aggressively bone metastatic cells versus those that weakly metastasize to bone. We have verified that our bone metastatic cells overexpress *ccn3* mRNA and that elevated levels of CCN3 protein is detected in the conditioned media of the bone metastatic 4T1 sub-populations. CCN3 has been shown to impair osteoblast differentiation using both *in vitro* and *in vivo* systems. Given the osteolytic nature of the bone metastases that develop in our model, we wish to test the hypothesis that CCN3 plays a causal role in promoting the formation of osteolytic breast cancer metastasis to bone through the inhibition of osteoblast differentiation. Finally, we will evaluate CCN3 expression in bone metastases derived from patients with breast cancer. We wish to determine if factors, such as CCN3, which are identified using animal models are relevant to the human disease and could prove to be diagnostic markers or potential therapeutic targets that may improve our ability to manage breast cancer metastasis to bone.

**P42**
**Oncogenic engagement of the Met receptor promotes tumorigenesis and metastates in a model of normal intestinal epithelial cells**

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In 2008, colorectal cancer was the second leading cause of cancer-related death in Canada. An estimated 21 500 new cases were diagnosed and 9000 died due to metastatic spread to the liver. Compelling lines

of evidence imply that deregulation of the Met/HGF receptor tyrosine kinase contributes to the etiology and progression of colorectal cancer. Notably, a recent study has shown that co-expression of the Met receptor and of its ligand, the hepatocyte growth factor, in primary colon cancer specimens predicts tumor stage and clinical outcome. However, the functional significance for the Met receptor in early neoplasic transformation of the intestinal epithelium and its contribution to the transition of colorectal cancers from a non-invasive to a metastatic malignant phenotype remain poorly defined. In this study, we have investigated whether oncogenic engagement of the Met receptor is alone sufficient to induce cancer behaviors in the non-transformed intestinal epithelial crypt cell model, the IEC-6 cells. Our results demonstrate that the expression of a constitutively activated form of the Met receptor, TprMet, in IEC-6 cells is sufficient to induce morphological transformation, epithelial to mesenchymal-like transition, proliferation, loss of growth contact inhibition and anchorage-independent growth. More importantly, we show that oncogenic activation of the Met receptor confers to normal intestinal epithelial cells the capacity to induce angiogenic responses, tumor formation and experimental lung metastases in nude mice. Overall, these results support the concept that deregulation of the Met receptor signaling pathways play fundamental roles in a wide variety of cancer biological processes relevant to both the initiation and malignant progression of colorectal cancer. Therefore, this further validates that the Met receptor and its downstream signaling pathways represent attractive targets for the development of new therapeutic approaches for the treatment of colorectal cancer. *This work was supported by a CIHR operating grant and CFI Leaders Opportunity Fund awarded to C.S.*

**P43**

**E-selectin- and TL1A-induced activation of *Death Receptor 3* on colon cancer cells activates Src family kinases and the PI3K/Akt/p65NFkB survival axis**

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Extravasation of circulating cancer cells is a key event of metastatic spreading. It relies on complementary adhesive interactions between cancer cells and their microenvironment, which determines organ selectivity of metastasis formation. Notably, E-selectin, a specific endothelial adhesion receptor, is involved in the binding of colon cancer cells to the endothelium and promotes metastatic invasion of the liver. Along these lines, we have previously shown that E-selectin interacts with Death Receptor 3 (DR3), a member of the TNF receptor family that is expressed by colon cancer cells (Gout et al. Cancer Res, 2006). This interaction increases the migratory and survival potentials of the cancer cells. The aim of this study is to investigate the mechanisms by which the pathways activated downstream of DR3 by E-selectin or TL1A, its cognate ligand, confer survival advantages to colon cancer cells. We found that E-selectin triggers the tyrosine phosphorylation of DR3 in a Src family member-dependent manner, being inhibited by the pan Src kinase inhibitor PP2. We also obtained evidence indicating that interaction between DR3-expressing HT-29 colon carcinoma cells and either E-selectin or TL1A induces a time-dependent activation of the PI3K/Akt pathway, as shown by phosphorylation of Akt at Ser 473. We further discovered that p65/RelA, the anti-apoptotic subunit of NFkB, is rapidly phosphorylated at Ser 536 in response to E-selectin or TL1A. This phosphorylation is impaired by LY 294002 suggesting that PI3K/Akt pathway contributes to E-selectin or TL1A induced NFkB activation in colon cancer cells. We conclude that E-selectin and TL1A induced-activation of DR3 contributes to confer a metastatic advantage to colon cancer cells by inducing Src familly kinase-dependent tyrosine phosphorylation of

DR3 and by activating survival pathways involving the PI3K/Akt/NFkBp65 axis. *Supported by the Canadian Cancer Society/NCIC.*

**P44**
**Identification of a Stat3-dependent transcription regulatory network involved in metastatic progression**

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High levels of activated Stat3 are often found in human breast cancers and can correlate with poor patient outcome. We employed an activated-ErbB2 mouse model of breast cancer to investigate the *in vivo* role of Stat3 in mammary tumor progression and found that Stat3 does not alter mammary tumor initiation but dramatically affects metastatic progression. Four-fold fewer animals exhibited lung metastases in the absence of Stat3 and a 12-fold reduction in the number of lung lesions was observed in the Stat3-null tumors when compared to tumors from the wild type cohort. The decreased malignancy in Stat3-deficient tumors is attributed to a reduction in both angiogenic and inflammatory responses associated with a Stat3-dependent transcription regulatory network involving C/EBPd. This Stat3-dependent inflammatory cascade may serve as a promising target for the treatment of metastatic breast cancers. *This work was supported by an NCIC/Terry Fox Foundation New Frontiers Program Project Team Grant # 017003. JJR is funded by a CIHR Canada Graduate Scholarships Doctoral Award. WJM is the recipient of a Canadian Research Chair in Molecular Oncology, McGill University.*

**P45**
**Elevated Claudin-2 expression is associated with breast cancer metastasis to the liver**

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Breast cancer is the most commonly diagnosed cancer affecting Canadian women and is the second leading cause of cancer deaths in these patients. The acquisition of metastatic abilities by breast cancer cells is the most deadly aspect of disease progression. Upon dissemination from the primary tumor, breast cancer cells display preferences for specific metastatic sites. The liver represents the third most frequent site for breast cancer metastasis, following the bone and lung. Despite the evidence that hepatic metastases are associated with poor clinical outcome in breast cancer patients, little is known about the molecular mechanisms governing the spread and growth of breast cancer cells in the liver. We have utilized 4T1 breast cancer cells to identify genes that confer the ability of breast cancer cells to metastasize to the liver. *In vivo* selection of parental cells resulted in the isolation of independent, aggressively liver metastatic breast cancer populations. The expression of genes encoding tight-junctional proteins were elevated (Claudin-2) or lost (Claudin-3, -4, -5 and -7) in highly liver aggressive *in vivo*

selected cell populations. We demonstrate that loss of claudin expression, in conjunction with high levels of Claudin-2, is associated with migratory and invasive phenotypes of breast cancer cells. Furthermore, overexpression of Claudin-2 is sufficient to promote the ability of breast cancer cells to colonize and grow out in the liver. Finally, examination of clinical samples revealed that Claudin-2 expression is evident in liver metastases from patients with breast cancer. The identification and functional validation of candidate genes important for the ability of breast cancer cells will provide basic insights into the pathways required for breast cancer cells to metastasize to the liver. Our results suggest that claudin-2 may play an important role in enabling breast cancer cells to metastasize to the liver.