Invited Presentations

Thursday, June 11

Phil Gold "Scientist in training" Awardees

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Dr. Gerald Batist is Chairman of the Department of Oncology, McGill and Director, Segal Cancer Centre. In 1995, he founded the McGill Centre for Translational Research in Cancer, to stimulate rapid translation of lab discoveries into clinical benefits for patients. A major award from the Canadian Foundation for Innovation led to the expansion of the Centre, which is a major component of the Segal Cancer Centre at the Jewish General Hospital. He graduated from McGill, Medical School. This was followed by post-doctoral training in New York, Boston and at the National Cancer Institute in Washington; he trained in medical oncology and molecular pharmacology. Dr. Batist joined McGill, Faculty of Medicine in 1985, and has developed research programs in novel therapeutics. As Chairman of Oncology, he has nurtured the development of a number of multidisciplinary programs that have been highly innovative and amongst the first of their kind in Canada.

Overview of breast cancer therapeutics: deconstructing a tissue diagnosis into a molecular signature, and the therapeutic implications

Gerald Batist

Department of Oncology, McGill University; Segal Cancer Centre, Jewish General Hospital, Montreal, Quebec, Canada

Breast cancer is rapidly coming into focus as a compilation of a number of sub-groups that can be identified by specific and distinct molecular signatures, which also have distinct biological behaviors and clinical natural histories. Some have already been shown to benefit from distinct and targeted treatments, including hormone antagonists and selective tyrosine kinase inhibitors such as Trastuzumab (Herceptin). Some sub-groups are still seeking an effective therapy, and there are likely to be further molecular signatures yet to be identified. This talk will review the deconstruction of breast cancer into molecularly defined sub-groups, and review what is known and is being examined with regards to effective therapies that target each group. This will include the implications for anti-angiogenesis therapy.

DR. MORAG PARK is a Professor in the Departments of Oncology, Biochemistry and Medicine at McGill University. She serves as the Scientific Director of the CIHR Institute of Cancer Research. She has held investigator awards from the National Cancer Institute of Canada and the Canadian Institutes of Health Research, and currently holds the Diane and Sal Guerrera Chair in Cancer Genetics at McGill University. She has a long standing interest in the molecular mechanisms of cancer. She served as director of the Molecular Oncology Group and joint head of the Cancer Axis at the McGill University Health C entre, and as a member of the Fonds de la Recherche en Santé du Québec, Réseau Cancer. Dr. Park has published over 100 papers in peer-reviewed journals. A major thrust of her work now focuses on human breast cancer and the importance of the tumour microenvironment to the outcome of this disease. Her work has been recognized with numerous awards including becoming a Fellow of the Royal Society of Canada.

Breast Cancer Microenvironment: Friends or Foes

Morag Park

Departments of Oncology, Biochemistry and Medicine, Goodman Cancer Centre, McGill University, Montreal, Quebec, Canada

There is increasing evidence to support that signals provided by tumor stroma influence breast tumor outcome. However, little is known about changes in stromal tissue or how these impact upon disease progression. We have addressed changes in stroma by analyzing changes in gene expression in stromal tissue associated with breast tumors when compared to normal breast tissue. We have integrated gene expression data from laser capture microdissected breast tumor stroma with matched normal stroma. Using this approach we have identified changes in gene expression that reflect different biological processes and are predictive of poor prognosis in breast cancer. A trained predictor of 23 genes was developed and contains new information to stratify breast cancer subtypes. This is independent of clinical parameters and published predictors of outcome and identifies patients with poor outcome in multiple breast cancer expression data generated using whole tissue. Our stromal predictor selects poor outcome patients from multiple clinical subtypes of breast cancer and contains genes representing distinct biological features, including differential immune response, angiogenic response, as well as a hypoxic response. These results highlight the complex relationship between the tumor and its microenvironment, and underline the role that the stroma plays in tumor progression.

HAROLD J. BURSTEIN, M.D., Ph.D. is an Associate Professor of Medicine at Harvard Medical School, and a medical oncologist at Dana-Farber Cancer Institute and Brigham & Women's Hospital. He is a clinician and clinical investigator specializing in breast cancer. Dr. Burstein attended Harvard College, and earned his MD at Harvard Medical School where he also earned a PhD in immunology. In addition, he holds a master's degree in history of science from Harvard. He trained in internal medicine at Massachusetts General Hospital, and was a fellow in medical oncology at Dana-Farber. His clinical research interests include novel treatments for early- and advanced-stage breast cancer. Representative publications can be found in the New England Journal of Medicine, the Journal of Clinical Oncology, and other leading medical journals. With Dr. Gary Lyman, he is co-editor of the book *Translational Therapy for Breast Cancer*, published in 2007. He serves on the NCCN Breast Cancer Panel, The St. Gallen Breast Cancer Panel, the CALGB Breast Cancer Committee, the ASCO Health Services Research and Clinical Research Committees, and is editor-in-chief of the Journal of the National Comprehensive Cancer Network.

Update on Angiogenesis Inhibitor Therapy for Breast Cancer

Harold J. Burstein

Dana Farber Cancer Institute, Harvard University, Boston, Massachusetts, USA

Anti-angiogenesis drugs are emerging as important therapies for advanced breast cancer. In first line treatment of metastatic breast cancer, the addition of bevacizumab to chemotherapy improves progression-free survival and response rates, if not overall survival. Benefits of bevacizumab treatment in more refractory breast cancer remain unclear. Multiple trials are evaluating bevacizumab in early stage breast cancer as adjuvant therapy. Other agents, targeting VEGFR and related tyrosine kinases, are being vigorously studied. To date, there is only limited information on which patients might selectively benefit from angiogenesis inhibition. An update on clinical uses of angiogenesis inhibitors in breast cancer, including recent presentations from ASCO 2009, will be provided.

DR. WILLIAM J. MULLER is currently Professor in Departments of Biochemistry and Medicine at McGill University. Dr. Muller is recognized as one of the leaders in the development of transgenic mouse model s of human breast cancer. In recognition of his important contributions to the development and characterization of the transgenic mouse models of human breast cancer, Dr. Muller was recently awarded a CRC chair in Molecular Oncology at McGill University. Dr Muller extensive collaborations with numerous laboratories around the world have already provided important insight into the molecular basis for breast cancer progression.

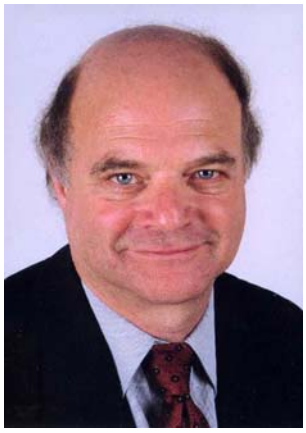
ShcA signaling is essential for tumor progression in transgenic mouse models of human breast cancer

Josie Ursini-Siegel[1], W. Rod Hardy[2], Dongmei Zuo[1], Sonya H.L. Lam[1], Virginie Sanguin-Gendreau[1], Robert D. Cardiff[3], Tony Pawson[2], and William J. Muller[1]

Departments of Medicine and Biochemistry[1], McGill University, Montreal, Quebec; Samuel Lunenfeld Research Institute[2], Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada; Center for Comparative Medicine[3], University of California Davis, Davis, California, USA

To explore the *in vivo* significance of known ShcA tyrosine phosphorylation sites during mammary tumorigenesis, we utilized mice expressing several phosphotyrosine-deficient ShcA alleles under the control of its endogenous promoter. We show that all three ShcA tyrosine phosphorylation sites are involved in the early stages of mammary tumor progression, including loss of the myoepithelial cell layer surrounding the hyperplasias, and also during metastatic mammary carcinoma development. However, signals emanating from Y313 are important for tumor cell survival while Y239/240 transduce signals that promote tumor vascularization. We further demonstrate that loss of ShcA expression in mammary epithelial cells completely abrogates mammary tumor development. These observations argue that ShcA signaling is essential during numerous stages of mammary tumor progression.

KEYNOTE SPEAKER



ROBERT KERBEL, PH.D.
SUNNYBROOK HEALTH SCIENCES CENTRE, UNIVERSITY OF TORONTO

Dr. Robert Kerbel is a Senior Scientist at Sunnybrook Health Sciences Centre in Toronto, and Professor, Department of Medical Biophysics, University of Toronto. He holds a Canada Research Chair in Tumour Biology, Angiogenesis and Antiangiogenic Therapy. A former recipient of Robert Noble Award for Excellence in Cancer Research from the Canadian Cancer Society, he has worked in many areas of tumour biology research since 1975. These include tumour immunology, biology of metastasis, drug resistance, and experimental therapeutics. Since 1990 the main focus of his research has been the study of tumour angiogenesis, and the design of new and innovative therapeutic antiangiogenic treatment strategies. His most noteworthy contributions include co-pioneering the concept of metronomic low-dose chemotherapy, development of biomarker strategies, elucidating mechanism of action of VEGF-pathway targeting drugs in fostering the anti-tumour effects of chemotherapy, and developing new models in mice of advanced metastatic disease for drug therapy testing.

Translational Research Studies of Antiangiogenic Therapy for Advanced Metastatic Disease

Robert S. Kerbel
Molecular & Cellular Biology Research, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada

A recent significant development in medical oncology has been the approval of antiangiogenic drugs, including bevacizumab, sunitinib and sorafenib. However, the progression free or overall survival clinical benefits induced by these drugs are modest which has stimulated considerable

interest in the mechanisms by which resistance to them develops. However, another way in which their potential clinical benefits can be reduced is by drug-induced increases in tumour growth, invasion, and metastasis, subsequent to causing an initial anti-tumour benefit. One way in which this can occur is by the induced increases in tumour hypoxia thereby resulting in the adaptive upregulation of a number of genes which induce angiogenesis, invasion and metastasis, via enhanced HIF-1 expression. In addition, we have been studying a different mechanism which, in theory, may also contribute to both drug resistance and malignant progression. It involves the systemic induction in host tissues of multiple circulating growth factors by antiangiogenic drugs such as sunitinib. These factors, in mice, include not only VEGF and PlGF but also 'off target' changes such as SDF-1, G-CSF and osteopontin. Since all these factors can potentially promote tumour growth/angiogenesis, we reasoned that there may be circumstances where antiangiogenic drug activity may not only be lost but increases in tumour aggressiveness might be induced as well. This stimulated us to assess the effects of short-term sunitinib treatment in preclinical models of neoadjuvant or adjuvant-like therapy of micrometastatic breast cancer or melanoma where we recently reported of accelerated metastatic growth – in contrast to suppression of established (primary) tumours. From our results we concluded that postoperative adjuvant treatment of early stage cancers with antiangiogenic drugs may actually be less effective compared to established metastatic disease. The recent disappointing results of the first adjuvant trial of antiangiogenic therapy suggest this hypothesis may have merit. However, they also suggest a number of strategies to improve the impact of antiangiogenic drugs for both early and late stage disease; one such approach is the combination with metronomic chemotherapy. We have been evaluating this treatment combination for advanced metastatic disease using a series of new xenograft models developed for this purpose, the results of which will be discussed.

DR. JANUSZ RAK received his MD degree in 1980, and PhD degree in tumor biology from the Ludwik Hirszfeld Institute in Poland (1986). He was a postdoctoral Fullbright-Hays Scholar (1988-1990) at the Michigan Cancer Foundation in Detroit, MI, and later Postdoctoral Fellow (1990-1993), Research Associate and Scientist at the Sunnybrook Health Sciences Centre in Toronto, ON (1993-1999). In 2000 he became Assistant Professor at McMaster University and Henderson Research Centre in Hamilton, ON (2000-2006), and is presently Associate Professor of Pediatrics and Jack Cole Chair in Pediatric Hematology/Oncology, at the Montreal Children's Hospital Research Institute (MCHRI) and McGill University Health Centre (MUHC). Dr. Rak's 120 published research papers pertain to the role of oncogenes in tumour angiogenesis, oncogene-bearing microvesicles, tissue factor, glioma, vascular aging, pathobiology of pediatric malignancies and related subjects. His work is supported by Canadian Cancer Society, Cancer Research Society, Canadian Institutes of Health Research, Cole Foundation and MUHCRI.

Impact of vascular ageing on angiogenesis and antiangiogenesis

Janusz Rak

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The onset of tumour angiogenesis is a function of the interplay between cancer cells and their host microenvironment. In this regard, oncogenic mutations trigger and exacerbate proangiogenic changes in tumour initiating cancer cells, including their expression of endothelial growth factors (e.g. VEGF), procoagulant receptors (e.g. tissue factor) and the release of angiogenesis-stimulating microvesicles (oncosomes). Recent studies suggest that vascular responses to these various signals are not only defined by their own nature, but also depend on host-related processes of vascular aging and age-dependent vascular comorbidities, such as atherosclerosis. The latter condition is highly prevalent in the human population, but is absent in mice. Thus, the same type of tumour initiating cancer cells that form aggressive tumours in young (pediatric-type) mice, exhibit much slower growth, reduced ability to recruit microvasculature and to elicit endothelial cell proliferation in aged tumour recipients, and especially in agend ApoE-/-, with full-blown atherosclerosis. Moreover, the molecular properties of tumour associated endothelial cells (TEM1 expression), sprouting of endothelial cells ex vivo, and recruitment of endothelial progenitor-like, VEGFR-2(+)/CD45(-) cells into the circulation were all reduced in the latter (adult-type) hosts. Importantly, such vascular ageing/disease impacted tumour metastasis and the disease responsiveness to antiangiogenic therapies, including metronomic protocols of cyclophosphamide and sutent. Age-specific vascular features were also observed in human renal cell carcinoma. Thus, we propose that age-dependent vascular properties may necessitate new strategies to develop and test more age-appropriate antiangiogenic therapies.

JOSE G. TEODORO, PhD, is a Virologist and Cancer Biologist at the Goodman Cancer Center and an Assistant Professor in the Department of Biochemistry at McGill University. His research focuses on identifying tumor suppressor mechanisms that negatively regulate angiogenesis. He also studies viral mechanisms of cell death and how such pathways can be harnessed to specifically destroy cancer cells. Research in Dr Teodoro's laboratory is supported by operating grants from the Canadian Institute of Health Research (CIHR) and the Natural Science and Engineering Research Council (NSERC). Dr Teodoro received his PhD from McGill University and performed post-doctoral studies at the University of Massachusetts Medical School.

Regulation of Collagen-derived Antiangiogenic Factors by p53

Sarah Assadian, Wissal El-Assaad, and Jose G. Teodoro

Rosalind and Morris Goodman Cancer Centre and
Department of Biochemistry, McGill University, Montreal, Quebec, Canada

The p53 tumour suppressor is well recognized as a key factor protecting humans from cancer. There are several known mechanisms by which p53 limits the formation of tumours including the induction of apoptosis and cell cycle arrest. In addition, p53 is believed to exert non-cell autonomous effects such as inhibition of metastasis and angiogenesis. Increasing evidence suggests that loss of p53 activity contributes to aggressiveness of tumours by increasing their ability to vascularize. Angiogenesis is the physiological process by which new blood capillaries are formed and is an absolutely necessary step in tumour growth in order to allow delivery of oxygen and nutrients. Inhibiting tumour angiogenesis is a promising intervention point for new cancer therapies and identifying novel mechanisms that inhibit angiogenesis is crucial to this endeavor. Angiogenesis is controlled by a balance of pro- and antiangiogenic factors that regulate the growth of endothelial cells, the cellular precursors of the vasculature. A large number of antiangiogenic factors have been identified that are proteolytic cleavage products of extracellular matrix components. In particular, several types of collagen proteins have proteolytic fragments that act as antiangiogenic factors. Our studies have demonstrated that tumour cells expressing p53 secrete a potent cocktail of such collagen-derived antiangiogenic factors (CDAFs). Collagen biosynthesis is dependent upon prolyl hydroxylase enzymes for stability. We have shown that p53 transcriptionally activates the α (II) collagen prolyl-4-hydroxylase gene resulting in the extracellular release of CDAFs from collagen type IV and XVIII. In addition we have shown that p53 is able to directly stimulate expression of antiangiogenic collagen genes including *COL4A1*. Our studies suggest that p53 initiates a powerful transcriptional program to limit tumor angiogenesis by increasing production of CDAFs.

DR. WILLIAM C. SESSA has been a faculty member at the Yale University School of Medicine since 1993, and a Professor of Pharmacology since 1999. Sessa holds a Ph.D. from New York Medical College and a B.S. from the Philadelphia College of Pharmacy and Sciences. He was a post-doctoral fellow at both the William Harvey Research Institute of St. Bartholomew's Hospital Medical College in London and the University of Virginia School of Medicine's Health Sciences Center. Dr. Sessa's research interests are in endothelial function and angiogenesis and its role in the progression of diseases such as atherosclerosis, diabetes and cancer. His laboratory studies the etiologic factors and genes that regulate the transition from normal to dysregulated endothelium at the molecular, cellular and functional levels. Dr. Sessa has received several awards for his research accomplishments, including the American Heart Association Established Investigator Award, the Philadelphia College of Pharmacy and Sciences Young Alumnus Award, John J. Abel Award in Pharmacology, Robert Berne Award in Cardiovascular Biology and a MERIT Award from the NIH. He presently serves on several editorial boards included JCI, Cell Metabolism, Circulation Research and is an Associate Editor for ATVB.

Role of miRNAs in tumor angiogenesis

William C. Sessa

Boyer Center for Molecular Medicine, Vascular Biology and Therapeutics Program,
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miRNAs have been shown to be regulators of gene expression participating in the control of a wide range of multiple physiological pathways. Using siRNA or endothelial specific knockout of the terminal endonuclease responsible for the generation of mature miRNAs, Dicer, leads to defective angiogenesis in vitro and in vivo. A reduction of endothelial Dicer, reduces post-natal angiogenic response to a variety of stimuli, including exogenous VEGF, tumors, limb ischemia and wound healing. Furthermore, VEGF regulated the expression of several miRNAs, including the upregulation of components of the c-Myc oncogenic cluster miR-17-92. Transfection of endothelial cells with components of the miR-17-92 cluster, induced by VEGF treatment, rescued the induced expression of thrombospondin-1 (Tsp1) and the defect in endothelial cell proliferation and morphogenesis initiated by the loss of Dicer. Thus, endothelial miRNAs regulate post-natal angiogenesis and VEGF induces the expression of miRNAs implicated in the regulation of an integrated angiogenic response. Recent insights into control of pericyte/smooth muscle recruitment to sites of angiogenesis via miRNAs will be discussed.

JEAN-PHILIPPE GRATTON, Ph.D. is, since 2002, the Director of the Endothelial Cell Biology research unit at the Institut de recherches cliniques de Montréal (IRCM) and Associate Research Professor in the Department of Medicine of Université de Montréal. He earned his Ph.D. at Université de Sherbrooke and was a post-doctoral fellow in the Department of Pharmacology at Yale University School of Medicine. He holds a Canada Research Chair in Endothelial Cell Signalling and Angiogenesis (Tier 2) and was the recipient of the Wilbert J. Keon New Investigator Award from the Institute of Circulatory and Respiratory Health of the CIHR. His research interests include the regulation of nitric oxide production from endothelial cells and studying the contribution of vascular permeability to tumour angiogenesis. He is currently Associate Director of Academic Affairs at IRCM.

Regulation of VEGF- stimulated NO release from endothelial cells: Insights into vascular permeability

Jean-Philippe Gratton

Laboratory of Endothelial Cell Biology, Institut de recherches cliniques de Montréal (IRCM), Montreal, Quebec, Canada

Increased vascular permeability is an initial and essential step of angiogenesis. The passage of plasma macromolecules across the endothelium is tightly controlled and inhibition of the endothelial nitric oxide synthase (eNOS) results in decreased vascular leakage and angiogenesis. Vascular endothelial growth factor (VEGF) is a potent angiogenic cytokine that also increases vascular permeability. Nitric oxide (NO) release from endothelial cells, following activation of eNOS, contributes to proangiogenic and permeability effects of VEGF. Angiopoietin-1 (Ang-1) shares many of the proangiogenic properties of VEGF on endothelial cells. However, in contrast to VEGF, Ang-1 protects blood vessels from increased permeability which contributes to their stabilization and maturation. Since eNOS-derived NO is central to increased permeability induced by VEGF, we investigated if Ang-1 interferes with VEGF signaling to eNOS. We demonstrate that Ang-1 stimulation of endothelial cells inhibits VEGF-induced NO release and transendothelial permeability. In contrast to VEGF, Ang-1 causes a marked increase in phosphorylation of eNOS on the inhibitory threonine 497 residue. We also demonstrate that inhibition of atypical PKCζ prevents the phosphorylation of eNOS on Thr497 and abrogates the capacity of Ang-1 to inhibit VEGF-induced NO release and endothelial permeability. Thus, inhibition of VEGF-stimulated vascular permeability by Ang-1 is dependent on inhibition of NO production via phosphorylation of eNOS on Thr497 by PKCζ. The mechanisms by which NO increases endothelial permeability remain obscure, we are interested in the effects of NO on the adherens junction protein complex, responsible for inter-endothelial cell contacts. We provide evidences that NO can directly S-nitrosylate β-catenin which results in the disassembly of the adherens junction complex and results in increased endothelial permeability. Using mass spectrometry, we identified Cys residues on β-catenin that are targets of NO. These data indicate that S-nitrosylation of β-catenin following eNOS activation participates in VEGF-dependent increase in endothelial permeability.

DR. NICOLE BEAUCHEMIN is a professor at McGill University in the Depts. of Biochemistry, Medicine and Oncology. Her laboratory is situated in the Metabolism and Cancer unit within the Goodman Cancer Centre. Dr. Beauchemin is an expert of the Carcinoembryonic Antigen gene family. She has a long-standing expertise in the development of mouse models for one member of the family, namely CEACAM1. Her team has indeed shown that CEACAM1 is down-regulated in microadenomas and functions as a tumor suppressor in the context on colon cancer. The availability of the *Ceacam1*-null mouse, which is currently used by 18 laboratories world-wide, has highlighted that this protein regulates proliferation and apoptosis in this tissue and in addition, has shown its interaction with β-catenin and as a modifier of the Wnt signaling pathway. In the last 5 years, Dr. Beauchemin has also studied the functions of CEACAM1 in endothelial cells and shown that CEACAM1 regulates neo-vascularization. Her studies are supported by the CIHR, the NCIC and the CRS.

CEACAM1 deficiency induces endothelial vascular dysfunction.

Anne-Laure Nouvion[1], Sarah LeBlanc[1], Malika Oubaha[2], Jean-Philippe Gratton[2], Suleyman Ergun[3], Valérie Breton[1], and Claire Turbide[1], Nicole Beauchemin[1].

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CEACAM1 exhibits angiogenic properties and functions as a VEGF effector. We have shown that CEACAM1 plays a major role in vascular integrity and formation of the vascular network *in vivo*, particularly in normal neo-vascularization. Accordingly, CEACAM1 is expressed in newly-formed vessels during physiological angiogenesis such as in wound healing and endometrial proliferation. In addition, downregulation of epithelial CEACAM1 in prostate intraepithelial neoplasia (PIN), bladder and kidney cancers is accompanied by its upregulation in the adjacent vasculature that correlates with vascular destabilization and increased vascularization of tumors. To identify the mechanisms regulating CEACAM1's endothelial activities, we have investigated whether CEACAM1 plays a role in modulating endothelial signaling pathways. *Ceacam1*-deficient lung primary ECs (MLECs) exhibit increased proliferation and decreased VEGF-induced migration with no changes in p-Erk1/2 and p-p38 MAPK levels. Absence of CEACAM1 leads to defective acute VEGF-dependent permeability in Miles assays. VEGF-induced signaling in *Ceacam1*-null MLECs highlighted a defective VEGF-elicited eNOS signaling pathway with truncated NO production, deficient Akt activation and sustained eNOS activation. In contrast, CEACAM1 deficiency increases tumour hyperpermeability with concomitant augmented tumour growth. These results suggest that cross-talk between VEGFR2 and CEACAM1 regulate vessel permeability and that inhibiting CEACAM1 expression in tumour ECs may lead to increased chemotherapy drug efficacy in tumours.

DR. ALEXANDRA EICHTEN conducted her graduate work on regulation of p53 by the human papillomavirus type 16 E7 oncoprotein in Dr. Karl Münger's laboratory at Harvard Medical School. Based on this work received her PhD in June 2002 from Hannover University, Germany. After a brief postdoctoral period at Harvard Medical School, she joined the laboratory of Dr. Lisa M. Coussens at UCSF Comprehensive Cancer Center as a postdoctoral fellow in April 2003, where she worked on microenvironmental regulation of tumor progression and angiogenesis using a transgenic skin carcinogenesis mouse model. Currently, Dr. Eichten is a scientist at Regeneron Pharmaceuticals in the Oncology & Angiogenesis group focusing on mechanisms of VEGF inhibition and tumor resistance to anti-angiogenic therapies.

Rapid changes in tumor vessels following treatment with Aflibercept (VEGF Trap)

Alexandra Eichten, Alexander Adler, Chiayi Kao, Yi Wei,
Wen Fury, Calvin Lin, Gavin Thurston,
Regeneron Pharmaceuticals, Tarrytown, New York, USA

Blockade of VEGF has been shown to be effective at inhibiting tumor growth in various preclinical models and in diverse human cancers. Treatment of tumors with VEGF blockers results in a rapid loss of tumor vessels and changes in the phenotype of the tumor vessels, including decrease sprouting and branching. In this study, we sought to characterize in more detail the rapid changes in vascular gene expression and tumor perfusion following VEGF blockade. SCID mice bearing established tumors of different origins were treated with a single subcutaneous dose of aflibercept (VEGF Trap, 25 mg/kg), a potent inhibitor of all isoforms of VEGF-A and placental growth factor (PlGF) that is currently in Phase III clinical trials. To assess gene expression changes, at 8, 24 or 72 hr after treatment, tumors were excised and split in half: one half was processed for gene expression analysis, and the other half was stained by immunocytochemistry for blood vessels (PECAM/CD31) and measured morphometrically for tumor vessel density. We found that expression levels of a number of vascular genes, including ICAM2, eNOS, and VE-cadherin, were decreased by VEGF Trap at 24 and 72 hr, but not at 8 hr. The time course and magnitude of the reduced expression of these genes correlated with the decrease in vessel density in the different tumor types, thus these genes may serve as markers of tumor vessel density. In concordance with the decrease in tumor vessel density, treatment with VEGF Trap also resulted in decreased functional tumor perfusion, as assessed using contrast-enhanced micro-ultrasound 2-dimensional imaging. Expression levels of several other vascular genes, including Angiopoietin-2 and Nidogen2, were already decreased at 8 hr after treatment of multiple tumor types with VEGF Trap, and remained decreased at 24 and 72 hr. Expression of these latter genes appears to be dependent upon continued VEGF signaling, suggesting that these represent direct VEGF target genes. These results show that at least 2 phases of vascular gene expression changes can be discerned in tumors following VEGF blockade: the initial phase involves VEGF target genes and the latter phase involves constitutive endothelial cells genes. The most rapidly changed genes may provide biomarkers of VEGF-blockade, whereas the slower changing genes may be useful as indicators of overall tumor vascularity.

Accelerated metastasis after short-term treatment with a potent inhibitor of tumour angiogenesis

JOHN M.L. EBOS[1, 4], Christina R. Lee[1], William Cruz-Munoz[1], Georg A.Bjarnason[2], James G. Christensen[3], and Robert S. Kerbel[1, 4]

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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.

This work was supported by grants from the Ontario Institute for Cancer Research (OICR) and the NCIC (all to R.S.K.). 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.

Gene expression profiling in breast cancer microvasculature identifies subtypes linked to vessel maturity and disease outcome

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[6]Current address: Institut de recherches cliniques de Montréal, Quebec, Canada

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. 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. 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. Several studies have helped characterize the gene expression of tumor endothelial cells in different cancers and identify tumor-specific endothelial markers. 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 work was supported through US Department of Defense Breast Cancer Predoctoral Traineeship Award (BC050298)

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).

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Dr. Mark W. Kieran received his PhD in 1983 from the University of Alberta, Edmonton, Canada, and his MD in 1986 from the University of Calgary. He completed postgraduate training in molecular biology at the Pasteur Institute in Paris. After a pediatric residency in Montreal, he received postdoctoral education at Children's Hospital Boston. In 1999, he became Director of Pediatric Neuro-Oncology at Dana-Farber Cancer Institute. He also received the Nick Palmer Award and Lecture in London, UK in 2002. Dr. Kieran also worked on angiogenesis in the laboratory of the late Dr. Judah Folkman and is renowned for his research efforts. His laboratory selects promising agents and evaluates them in human tumors which have been orthotopically implanted into mice. This process quickly moves potential therapies into the clinic. At present, more than five such projects are ongoing in conjunction with different groups at Boston's DFCI, BWH, BIDMC, and CHB.

Anti-Angiogenic (Metronomic) Chemotherapy: From Bench to Bedside

Mark W. Kieran
Dana-Farber Cancer Institute and Children's Hospital Boston,
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Chemotherapy was developed to damage rapidly dividing tumor cells, a hallmark of cancer. Optimal tumor cell damage is achieved by increasing chemotherapy to the maximally tolerated dose (MTD), defined as the dose beyond which unacceptable toxicity would occur. The toxicity of this approach causes significant organ damage and requires prolonged interruption of therapy to provide sufficient recovery (usually three to four weeks) before the next dose can be given. In spite of this aggressive approach, most patients diagnosed with cancer will not be long term survivors, largely the result of selection of resistant clones within the tumor mass. In order to survive and grow, tumors, like all living things, require oxygen, nutrients and removal of waste products. This is achieved by tumor mediated induction of angiogenesis. This complex process involves a large number of components including the proliferation of endothelial cells and pericytes which provide the building blocks for neovascularization. In a landmark study, Browder and Folkman proposed a simple but novel idea. Could chemotherapy be used to kill endothelial proliferation and thus inhibit angiogenesis? The discovery that low, repetitive doses of chemotherapy (hence the name metronomic chemotherapy) could effectively target neovascularization without causing the toxicity that traditional-dosed chemotherapy does has opened up an array of new drugs (or old drugs given in new schedules and doses) that are commercially available and easily translated into the clinic. Because endothelial cells are biologically normal, classical drug resistance is much less likely to occur. This presentation will discuss the approaches used to identify agents that could be considered anti-angiogenic (metronomic) chemotherapy, development of combinations of these agents with other inhibitors, and human clinical trial data demonstrating proof of principle.

DR. JULIA GLADE BENDER is the Director of the Pediatric Cancer Foundation Developmental Therapeutics Program at Columbia University, one of 21 pediatric phase I research programs supported by the National Cancer Institute (NCI) and the Children's Oncology Group (COG). Dr. Glade Bender serves as the institutional principal investigator for all phase I trials sponsored by the COG, New Approaches to Neuroblastoma Therapy (NANT) and Therapeutic Advances in Childhood Leukemia (TACL) consortia. Dr. Glade Bender chaired the first pediatric trial of bevacizumab in children with relapsed and refractory solid tumors, and co-chairs a randomized trial of chemotherapy with bevacizumab for patients with relapsed Ewing sarcoma. She has helped to develop pediatric trials of sorafenib, sunitinib, and VEGF-Trap and will lead the first study of pazopanib in children. Within COG she sits on the Steering Committees for Developmental Therapeutics and Bioethics, and Disease Committees for bone sarcoma and neuroblastoma.

Clinical Development of Anti-Angiogenic Therapy for Pediatric Solid Tumors

Julia Glade Bender

Department of Oncology, Columbia University Medical Center, New York, New York, USA

Anti-angiogenic therapy using vascular endothelial growth factor (VEGF) blocking agents has been validated as clinically efficacious for adults with metastatic cancer. The prototypic neutralizing agent, bevacizumab, received its first FDA approval in early 2004. Despite robust preclinical data supporting the potential for tumor growth inhibition in xenograft models of the common malignancies of childhood and adolescence including neuroblastoma, Wilms tumor, rhadomyosarcoma, osteosarcoma and Ewing sarcoma, pediatric development of this class of agents for solid tumors has been slow. A Phase I study of bevacizumab in children with clinically refractory solid tumors was conducted through the Children's Oncology Group (COG). Minimal adverse effects were seen, with no dose-limiting toxicities observed and a maximum tolerated dose was not reached. Analysis of blood pressures demonstrated statistically significant increases in systolic and diastolic pressures, although not meeting clinical criteria for hypertension. Limited pharmacokinetic data supported dosing schedules for children similar to those used in adults. Encouraging disease stabilization was observed in subset of patients with bone and soft-tissue sarcomas. Analysis of apoptotic and viable circulating endothelial cells was feasible in children, and the results promising for mechanistic validation and potential biomarker application. Pediatric phase 1 studies of sunitinib, sorafenib, VEGF Trap (Aflibercept) and pazopanib are currently ongoing. Short term concerns regarding cardiac ventricular dysfunction, intratumoral hemorrhage and epiphyseal dysplasia following VEGF-blockade in children have emerged and long-term toxicity data is still required. Evidence supporting the need for combination trials using bevacizumab and limited patient numbers initially presented unique challenges for the design of pediatric phase 2 efficacy trials, but these are now underway for bone and soft tissue sarcomas.

DR. SYLVAIN BARUCHEL is a Professor of Pediatrics University of Toronto, Member of Institute of Medical Science University of Toronto and Director, New Agent and Innovative Therapy Program (NAIT) and Biooncopharmacology, Laboratory, Co-Head of Comprehensive Sick Kids Cancer Center experimental therapeutics program The NAIT program is a translational and clinical research program focusing on Pediatric Cancer Developmental Therapeutics and is one of only 21 centers in North America, funded by the NCI and the only Canadian center to belong to the TACL (Therapeutic Advance Childhood Leukemia) and NANT (New Advance Neuroblastoma Therapy) consortia. The NAIT program is the Reference Laboratory for the Children's Oncology Group angiogenesis clinical trials.

Biomarkers of angiogenesis in pediatric oncology clinical trials: Are they useful to establish an optimum biological dose of new anti-angiogenic agents?

Sylvain Baruchel

Hospital for Sick Children, Department of Haematology and Oncology,
University of Toronto, Toronto, Ontario, Canada

Tumor angiogenesis is a critical event in cancer growth and progression. A significant number of pediatric tumors rely on active angiogenesis to grow and metastasize. Tumors rely on their vasculature to provide them with oxygen and nutrients however; this vasculature also delivers cytotoxic drugs. It has been observed that tumor vasculature, though extensive, is abnormal and ineffective due to the large number of leaky, dilated and tortuous vessels that have a random pattern of interconnection. Recent evidence suggests that chemotherapy drugs when administered in a low dose metronomic fashion and in combination with drugs that specifically target tumor angiogenesis such as bevacizumab sunitinib, sorafenib can normalize tumor vasculature. Dose dense chemotherapy in addition to antiangiogenic therapy, may result in effective drug delivery to the tumor and increased cytotoxic efficacy. clinical response evaluation classically using the RECIST criteria may not be appropriate to assess the anti-angiogenic activity of anti-angiogenic and metronomic chemotherapy In light of this, it is crucial that surrogate markers be developed to monitor biological response and therapeutic activity of these regimen. Ideal markers are those which are minimally invasive and reproducible validated in a multicenter modality such that they can be used at multiple time points during the course of therapy. We will review the potential biological markers used to define the antiangiogenic therapy in preclinical models and in pediatric clinical trials using metronomic chemotherapy, and direct / indirect ant-angiogenic agents. These include circulating levels of various plasma proteins, viable circulating endothelial cells and their progenitors, the expression of endothelial-specific genes and a new diagnostic imaging technique, dynamic contrast magnetic resonance imaging that allows tumor blood vessels to be visualized.

DR. DANIEL SINNETT earned his Ph.D. in Biochemistry from the University of Montreal in 1991 for his research on the genetic basis of several X-linked diseases as well as on phenomena such as retroposition that influence complex genome structure and evolution. Then he went to the Children's Hospital, Harvard Medical School (Boston) for his postdoctoral training in Dr Marc Lalande's group studying the genetic basis of Angelman Syndrome, an imprinting disorder. In 1994, Dr Sinnett established his molecular oncogenetics laboratory at the Sainte-Justine University Health Center one the largest pediatric hospital in Canada. Dr Sinnett's group has made significant contributions in the understanding of genetic determinants of childhood leukemia both at the gene and at the genomic levels. Dr Sinnett is also involved in a Genome-Quebec/Canada funded initiative to study genetic variation that affects gene regulation in the context of complex disease aetiology. The significance of Dr Sinnett's research has been acknowledged by becoming the first holder of a Research Chair in Pediatric Oncogenomics and by receiving a FRSQ national scientist scholar award. Dr Sinnett is currently Professor in the Departments of Paediatrics and Biochemistry in the University of Montreal as well as a member of the Robert Cedergren Centre in Bioinformatics and Genomics.

Regulatory genomics and susceptibility to childhood leukemia

Daniel Sinnett
Mother and Child University Hospital Centre, St-Justine Hospital, Montreal, Quebec, Canada

Acute lymphoblastic leukemia (ALL), the most frequent cancer affecting children, is a complex genetic disease where the effect of a series of "low penetrance" genes is modulated by external factors, thus modifying the individual's risk of cancer. Genetic variation plays a significant role in determining individual's childhood leukemia susceptibility. Indeed, we provided evidence that childhood ALL might originate through the collective contribution of genes controlling the efficiency of carcinogen metabolism, the capacity of maintaining DNA integrity and the response to oxidative stress. This illustrates the importance of thorough genetic studies to define the genetic determinants in this disease. Until recently, most association studies targeted variants in coding regions. Sequence variation in gene regulatory sequences has gained much importance in the study of complex disorders due to the quantitative effect on gene expression. We have performed a comprehensive study of functional regulatory variation in genes involved in highly controlled cellular processes known to contribute to cancer susceptibility, such as the cell cycle. We focused on cis-acting regulatory polymorphisms (rSNPs) that could disrupt transcription factor (TF) binding and lead to abnormal expression and variation in gene dosage and therefore influence the risk of disease. The proximal promoter region of 197 candidate genes has been screened and a total of 1838 rSNPs were identified. Progressively, population genetic studies are carried out to evaluate promoter genetic diversity worldwide and the functional impact of these rSNP/haplotypes is assessed combining *in silico* TF binding site prediction analysis and *in vitro* functional assays. Thus far, the influence of promoter variation on transcriptional activity has been tested for 17 cell cycle genes and 6 DNA repair genes and DNA-protein binding disruption has been validated for several of these rSNPs. Population- and family-based genetic association studies are performed in parallel to evaluate the impact of functional rSNPs in the risk of childhood ALL. Our findings demonstrate how the expected variability in expression levels due to regulatory polymorphisms in key metabolic genes can influence the risk of childhood leukemia and contribute to carcinogenesis, and reveal the functional relevance of the study of regulatory genetics in cancer research.

DR. VIVEK MITTAL is an Associate Professor of Cardiothoracic Surgery and Director of Lehman Brothers Lung Cancer Center at Cornell University Medical Center, New York. His laboratory is actively engaged in research to understand the cellular and molecular mechanisms of tumor metastasis. Dr. Mittal and colleagues have published seminal papers describing the role of bone marrow-derived microenvironments in angiogenesis-mediated progression of primary tumors, initiation of metastasis and development of micrometastasis to macrometastases. Dr. Mittal held appointments in the faculties of Cold Spring Harbor Laboratory and State University of New York, Stony Brook. He lectures widely to diverse audiences throughout the world and has been published in leading journals including Science, Nature, Cancer Cell, PNAS, and other leading peer-reviewed journals and text books. Dr. Mittal completed his postdoctoral research at Cold Spring Harbor Laboratory, New York and remained there as a faculty member until 2008.

Cellular and molecular mechanisms of metastasis

Vivek Mittal

Cornell University Medical Center, New York, New York, USA

Tumor-derived endocrine signals systemically induce a permanent switch in the inflammatory bone marrow (BM) cells that allow the activated BM cells to function as instigators (pro-angiogenic/pro-tumorigenic) of tumor growth and metastasis. We have identified subsets of BM cells including vascular and myeloid progenitors that regulate key tumorigenic processes including angiogenesis, metastatic initiation and progression. Notably, specific ablation of these defined populations markedly impaired macrometastasis formation associated with severe angiogenesis inhibition. Overall, these studies provide key insights into the cellular and molecular mechanisms of metastasis and have implications for the design of anticancer therapies.

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DR. MARK BASIK, born in Montréal, Québec, is a surgical oncologist practicing at the Jewish General Hospital, and an assistant professor of surgery and oncology at McGill University. His research interests include the genomics of breast and colon cancer as well as the discovery and validation of novel biomarkers in breast and colon cancer, while his clinical practice is limited to the care of breast cancer patients. He is the medical director of the inter-disciplinary breast cancer team at the Segal Cancer Center and is a member of the Breast Site Executive Committee of the NCI-C's Clinical Trial Group.

The role of SDF-1 and CXCR4 in metastasis in breast cancer

Mark Basik

Departments of Surgery and Oncology, McGill University, Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, Montreal, Quebec, Canada

The process of metastasis involves several steps starting from tissue invasion, intravasation, circulation, extravasation and finally, growth at the metastatic site. Recent work found that chemokines, such as stromal cell-derived factor (SDF)-1, overexpressed in organs, such as lung, liver, and bone, that are frequent targets of metastasis, may serve to home in cancer cells that express their receptors, like CXCR4. We further explored the role of the SDF-1/CXCR4 ligand/receptor axis in breast cancer. Our initial study reported the frequent over-expression of the CXCR4 receptor in breast cancer in association with hormone-independent and HER2 positive breast cancer. We then found that a low level of plasma SDF-1 is a strong independent prognostic marker, suggesting that the concentration gradient between low plasma SDF-1 and high tissue SDF-1 may be critical in driving the extravasation of cancer cells from the circulation to the target organ. We further determined that the levels of plasma SDF-1 were tumor-independent, identifying the first host-derived blood marker predictive of distant metastasis. In the same cohort of breast cancer patients, patients with tumors that highly expressed the activated form of the receptor, phosphorylated-CXCR4, and with low plasma SDF-1 levels, had a much poorer prognosis than those patients with either risk factor alone. These results highlight the importance of the dysfunctional relationship between the tumor and the host in the metastatic process. Finally, the therapeutic potential of targeting CXCR4 with a small molecule inhibitor was investigated in a transgenic mouse model. In combination with an anti-angiogenic agent, targeting CXCR4 resulted in a 40% decrease in primary tumor volume and 75% reduction in distant metastasis. Together, these results underline the potential role both for plasma SDF-1 as a prognostic tool that may assist in the selection of adjuvant therapy, and for tumor CXCR4, as a promising druggable target.

DR. ROSANDRA KAPLAN is Assistant Professor of Pediatrics at Weill Cornell Medical College and Memorial Sloan-Kettering Cancer Center and the Charles Lillian and Betty Neuwirth Scholar in Pediatric Oncology. She has received the ASCO Young Investigator Award and the Doris Duke Clinical Scientist Grant Award. She graduated from Dartmouth Medical School with high honors and completed her clinical training at Harvard Children's Hospital with her subspecialty training at Memorial Sloan-Kettering Cancer Center and postdoctoral research with David Lyden, MD, PhD. Dr Kaplan's work identified a novel role for bone marrow-derived progenitor cells in metastatic initiation. She is investigating the systemic impact a primary tumor has on its host and how effective exploitation of stem cell properties by cancers may characterize their ability to metastasize. The goal of this work is to develop novel strategies for preventing cancer progression by better identifying critical mediators of the metastatic process in patients with cancer.

Bone marrow-derived hematopoietic progenitor cells as mediators of metastasis

Rosandra N. Kaplan, Daniel Rutigliano, Selena Granitto, Lauren Rotman,
Daniel Rafii, Elan Bomsztyk, Kendra Kadas, Emma Sidebotham,
Elisa Port, Linda Vahdat, Allyson Ocean, David Lyden.
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The role of host cells in tumor progression and metastasis is now well recognized. We show that bone marrow-derived hematopoietic progenitor cells (HPCs) help to initiate the metastatic cascade by creating a supportive microenvironment in distant tissue sites. In addition to detection of these cells in pre-metastatic and metastatic tissues, we can now monitor HPCs in the circulation in mouse models as well as for patients in the clinical setting. Patients with advanced carcinoma show elevated levels of circulating HPCs by flow cytometry compared to low levels in healthy controls. We identify a defined circulating cell population that correlates with the presence of tissue-specific HPCs at the pre-metastatic niche. These circulating cells express CD34 and VEGFR1 as well as cKit, CD133, and CXCR4, with a subset expressing CD11b. Moreover, the degree of elevation of these cells correlates with clinical stage with significant increase in mobilized HPCs in patients with metastatic disease as compared to localized disease at presentation and in ongoing studies is being correlated with metastatic progression. We also show that patients with high circulating HPCs have greater colony forming assay capacity than healthy controls, suggesting these cells functionally maintain their progenitor status. Beyond the HPC elevation observed in newly diagnosed patients, these cells appear to be mobilized in the setting of tumor surgical resection and may explain the finding shown previously of enhanced metastasis observed after surgical removal of the primary tumor in mouse models. This process can potentially be inhibited and thereby derail the early systemic changes occurring even in those patients with so-called localized cancers. Targeting these cells at different clinical time points may significantly impact the outcome of metastatic spread, and monitoring patients for HPC mobilization may help define a population of cancer patients at higher risk for metastatic disease, enabling more tailored therapies.

DR. FRANÇOIS BÉNARD received his MD (1991) and earned his Fellowship in Nuclear Medicine (1995) from the Université de Sherbrooke. In 1998 he did a Research Fellowship in Positron Emission Tomography at the University of Pennsylvania. Bénard was a Professor in the Department of Nuclear Medicine and Radiobiology at the Université de Sherbrooke, the Chief of the Sherbrooke Molecular Imaging Center and a Clinician-Scientist at the Clinical Research Center of the Centre hospitalier universitaire de Sherbrooke till 2008. He is now the Scientific Director of the Centre of Excellence for Functional Imaging of Cancer at the BC Cancer Agency and a senior scientist at the BC Cancer Research Center. Dr Bénard holds the Leadership Chair in Functional Cancer Imaging and is Professor in the Department of Radiology at the University of British Columbia. His research interests are in positron emission tomography (PET), nuclear medicine, and cancer imaging. François Bénard was the recipient of a Clinician-Scientist training Award from the Medical Research Council of Canada (MRC). Since the beginning of his academic career, he has received uninterrupted research scholarships from the Canadian Institutes of Health Research (Clinician-Scientist award), the Quebec Health Research Funds (Junior II and Senior scholarships), and the Michael Smith Foundation for Health Research (Senior Scholar). His research work has been supported by the Canadian Institutes of Health Research, the Canadian Breast Cancer Research Alliance and more recently the National Institutes of Health.

PET/CT imaging to monitor and characterize metastases

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Cancers exhibit distinctly different biological behaviour from surrounding tissues which can be used for targeted imaging. Many years ago, Warburg documented increased anaerobic metabolism and glucose consumption in cancer cells, which forms the basis of using radiolabelled glucose analogues for cancer imaging with positron emission tomography (PET), using 18F-Fluorodeoxyglucose (FDG). Most of the common cancers show enhanced utilization of glucose and overexpression of glucose transporters, which has led to the growing use of PET imaging to detect metastases and assess cancer response to treatment. FDG uptake has been linked with cell density and influenced by hypoxia and cell division rate, and is probably one of the best non-invasive method to assess the tumour microenvironment. In several malignancies, primary tumour FDG uptake has been shown to correlate with prognosis as well as with regional and distant metastases. In addition, PET/CT imaging with FDG currently constitutes the most sensitive imaging method to detect metastases for many cancers, including lung, colorectal, breast, melanoma, head and neck cancers and lymphoma. Some cancers, such as prostate cancers, lobular breast carcinomas, renal cell carcinomas and hepatocellular carcinomas have highly variable FDG uptake and PET imaging is generally less sensitive in these cases. In renal

cell carcinomas for example, primary tumours are often FDG-negative while metastases are often FDG-positive. The significance of FDG uptake in primary tumours and metastases remain to be fully explored. Cancers also overexpress a variety of hormonal, growth factor and peptide receptors which have variable biological significance. In breast cancer, estrogen receptor imaging can be used to predict the success of hormone therapy at relapse: recent data shows that receptor expression heterogeneity can occur across metastatic sites. PET imaging agents have also been developed to image integrin receptors associated with angiogenesis (notably $\alpha_v\beta_3$) as well as other receptors associated with angiogenesis or tumour growth (VEGFR, EGFR and HER2/neu). Several other peptide receptors and proteins overexpressed in cancer cells can be targeted for imaging with PET. While primary tumours can be sequenced and analyzed for DNA, RNA or protein expression, metastases frequently remain inaccessible due to limited possibilities for resection or biopsy. PET imaging provides a unique method to explore the growth and biology of metastases *in vivo.*

PROF. MICHAL NEEMAN's research focuses on angiogenesis, the process by which new blood vessels grow. Angiogenesis is an essential component of reproductive and developmental processes, and also plays a key role in many pathological conditions, including cancer. Prof. Neeman combines magnetic resonance and optical imaging with molecular analyses to elucidate the mechanisms that regulate angiogenesis. Michal Neeman received her PhD from the Weizmann Institute after completing undergraduate studies at the Hebrew University in Chemistry and Biology, and postdoctoral training at The Los Alamos National Laboratory. Since 1991 she is a member of the Faculty of Biology at The Weizmann Institute of Science.

Imaging the recruitment of tumor stroma fibroblasts

Michal Neeman

Department of Biological Regulation, Weizmann Institute of Science, Rehovot, Israel

Angiogenesis, the growth of blood vessels, is induced by tumors through expression of proangiogenic factors and attenuation of endogenous inhibitors of angiogenesis. Plasticity of the vasculature is frequently initiated by vascular endothelial growth factor (VEGF), leading to endothelial cell fenestration and enhanced vascular permeability that is easily visualized by MRI using contrast media. Perivascular mural cells, such as pericytes and vascular smooth muscle cells, which control vaso-reactivity and blood flow, dissociate from the vessels to allow proliferation and migration of new vascular sprouts. Such changes in vasoreactivity can be detected by MRI using BOLD contrast. The combination of both tools was applied to demonstrate the rapid angiogenic response induced by tumors, and the inherent instability of immature vessels, lacking the perivascular mural cells. In tumors, the lack of bona fide pericytes can be compensated by tumor associated fibroblasts and myofibroblasts. These cells can be labeled ex vivo with contrast media to allow non-invasive imaging of their recruitment to the tumor vasculature. Recent experiments suggested a possible role for p53 expression by tumor fibroblasts in regulation of tumor progression.

DR. PETER SIEGEL received his Ph.D. from McMaster University (1999), where he utilized transgenic mouse models to study mechanisms of ErbB-2 receptor activation during mammary tumorigenesis. As a post-doctoral fellow at the Memorial Sloan-Kettering Cancer Center in New York (1999-2003), he investigated the collaboration between the ErbB-2 and TGF-β signaling pathways in promoting breast cancer metastasis and helped define organ-specific gene expression profiles of human breast cancer cells that were metastatic to bone and lung. In 2004, Dr. Siegel joined the Department of Medicine at McGill University and is a full member of the Goodman Cancer Centre. His research focuses on the interaction between the TGF-β and ErbB-2 signaling pathways in breast cancer as well as defining determinants of organ-specificity in breast cancer metastasis. Dr. Siegel received the Harold E. Johns Award from the National Cancer Institute of Cancer (2004) and is currently a research scientist of the Canadian Cancer Society.

Claudin-2 expression is associated with breast cancer liver metastasis

Peter Siegel
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Breast cancer displays a propensity to metastasize to distinct organs and tissues; with the liver representing the third most frequent site of distant metastasis, following the bone and lung. Breast cancer patients with hepatic metastases display a poor prognosis, with median survival times between 14-16 months and 5 year survival rates that reach only 5.5-8.5%. Despite its clinical importance, little is known about the molecular mechanisms governing breast cancer metastasis to the liver. To identify genes that are associated with the liver metastatic phenotype, we have performed *in vivo* selection to isolate 4T1 breast cancer cells that are capable of aggressively growing in the liver. Through gene expression-profiling of weak and aggressively liver metastatic breast cancer populations, we demonstrate that numerous tight-junctional proteins, including Claudins-3, -4, -5 and -7, are lost in highly liver aggressive cell populations. In contrast, Claudin-2 overexpression is associated with the liver-aggressive phenotype in our *in vivo* selected breast cancer cells. Furthermore, Claudin-2 overexpression in liver-weak breast cancer cells is sufficient to promote their ability to colonize and grow in the liver. Finally, we demonstrate that Claudin-2 is expressed in liver metastases from patients with breast cancer. To understand how Claudin-2 may function to promote breast cancer metastasis to the liver, we have examined a potential role for Claudin-2 in mediating tumor cell/extracellular matrix (ECM) adhesion. Liver-aggressive breast cancer cells display enhanced adhesion to Fibronectin and Collagen IV, but not Collagen I or Laminin when compared to the weakly aggressive counterparts. Interestingly, stable knock-down of Claudin-2 resulted in impaired adhesion of the liver aggressive cells to Fibronectin and Collagen IV. Together, these results implicate Claudin-2 as a novel and important determinant of breast cancer liver metastasis which may function to enhance breast cancer adhesion to ECM components found in the liver.

DR. PNINA Brodt is a Professor in the Departments of Surgery, Medicine and Oncology at McGill University and a McGill University Health Center investigator with previous visiting appointments at Harvard, the NIH Bethesda and Hadassah Hospital, Jerusalem. During her tenure at McGill, she was a Medical Research Council of Canada Scholar and a Fonds de la Recherche en Santé du Québec (FRSQ) Chercheur Boursier senior. Dr Brodt's laboratory is studying molecular aspects of metastasis with a focus on tumor-host interactions during the early stages of liver metastasis. Her laboratory was the first to identify hepatic IGF-I as a major regulator of liver metastasis and to describe the pro-metastatic, host inflammatory response triggered by tumor cells entering the liver. Recently, her group has engineered a novel IGF-decoy for the treatment of liver metastases and published extensively in this area. She edited several invited books and book chapters in the field of metastasis. Dr Brodt has served on Metastasis grant panels both in Canada and the US and as a reviewer in this field for international granting agencies. Her research has been supported by funding agencies in Canada and abroad as well as by industry and her work has been presented in numerous national and international conferences.

Role of ECM-derived survival signals in site-specific liver metastasis

Pnina Brodt

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The liver is a major site of metastasis for some of the most prevalent human malignancies, particularly carcinomas of the upper and lower GI tract. At present, surgical resection is the only curative option for liver metastases but its success rate is partial, producing a 25-30%, 5 year disease-free survival rate for malignancies such as colorectal carcinoma. In order to improve these statistics, the biology of liver metastasis needs to be better understood. Our laboratory has been studying the molecular factors regulating the process of liver colonization using murine and human, lung and colon carcinoma models. These studies identified the IGF-I receptor as a major promoter of the liver-metastasizing potential. Using gene chip microarray analysis, we subsequently identified IGF-IR-regulated genes that were differentially expressed in highly metastatic cells and their functional relevance to liver metastasis was explored. The talk will focus on the role of the extracellular matrix (ECM) and in particular, type IV collagen in the process of liver colonization. Data will be presented, based on gain and loss of function strategies, that identify type IV collagen as a conveyer of survival signals to the tumor cells in the liver and thereby an essential component of the liver colonization process. Data based on analysis of surgical specimens that support the relevance of these findings to the clinical course of the disease will also be presented. Finally, recent data, based on the utilization of a novel IGF-Trap for inhibition of liver metastasis will be presented and their translational implications for therapeutic targeting of metastatic disease will be discussed. These studies were supported by Canadian Institute for Health Research grants MOP- 81201 (PB) and MOP- 77677 and a Terry Fox Research Institute grant.

Dr. Kent Hunter received a B.S. in biochemistry at the Pennsylvania State University in 1985 and earned his Ph.D. in Biology from the Massachusetts Institute of Technology in 1991. After completing his doctoral work, Dr. Hunter continued his training as a Postdoctoral Fellow in the laboratory of Dr. David Housman at MIT. In 1996 Dr. Hunter joined the faculty of Fox Chase Cancer Center as an associate member. While at the Fox Chase Cancer Center, Dr. Hunter first demonstrated the importance of inherited factors in metastatic mammary tumor progression. In 1999, Dr. Hunter became an Investigator in the Laboratory of Population Genetics (LPG) at the NCI, where he identified the first metastasis efficiency modifier gene, *Sipa1*. In 2007 Dr. Hunter was promoted to Senior Investigator and became the head of the Metastasis Susceptibility Section of the Laboratory of Cancer Biology and Genetics (LCBG).

The genetics of inherited breast cancer metastasis susceptibility

Kent Hunter

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Metastatic disease is the primary cause of breast cancer mortality. Despite decades of research however, the etiology of the terminal stages of cancer progression is poorly understood. Recent evidence has suggested that many factors other than somatic mutation play an important role in the development of metastatic disease. One of these previously unrealized factors is inherited metastatic susceptibility. Using systems biology approach based on a highly metastatic mouse mammary tumor model we demonstrated that the genetic background upon which the tumor arose has a significant impact on the metastatic efficiency. Subsequent work identified *Sipa1* as the first candidate metastasis efficiency locus. Further efforts have identified a number of additional metastasis efficiency genes that interact either physically or transcriptionally with *Sipa1*. The polymorphic metastasis efficiency genes have been found not only to impact the physiologic manifestation of metastasis, but also to induce prognostic gene expression signatures that can discriminate human breast cancer patient outcome. Finally, association studies have demonstrated that these polymorphisms are significantly associated with distant metastasis-free survival in human populations as well as mouse inbred strains. These data suggest not only that metastasis susceptibility is segregating throughout the human population, but also that genetic testing might be possible as a clinical tool to discriminate between patients of low or high risk of metastatic disease to better personalize their therapeutic interventions.

DR. PHIL GOLD is the Douglas G. Cameron Professor of Medicine, and Professor of Physiology and Oncology, at McGill University. He has served as Chairman of the Department of Medicine at McGill and Physician-in-Chief at the Montreal General Hospital. He is presently the Executive Director of the Clinical Research Centre of the McGill University Health Centre. Dr. Gold's early research led to the discovery and definition of the Carcinoembryonic Antigen (CEA), the blood test most frequently used in the diagnosis and management of patients with cancer. For this work, other studies, and his outstanding contributions as a medical educator, he has gained national and international recognition. He has been elected to numerous prestigious organizations and has been the recipient of such outstanding awards as the Gairdner Foundation Annual International Award, the Isaak Walton Killam Award in Medicine of the Canada Council, and the National Cancer Institute of Canada R.M. Taylor Medal. He has been elected to membership in the Royal Society of Canada, the Association of American Physicians, and Mastership in the American College of Physicians. He has been honored by his country, his province his city, and his university by appointment as a Companion of the Order of Canada, an Officer of l'Ordre National du Québec, a member of the Academy of Great Montrealers; and a the recipient of the Gold Medal of the McGill University Graduate Society, respectively. He has been the Sir Arthur Sims Traveling Professor to the British Commonwealth.

PHIL GOLD STUDENT AWARDS

As part of the meeting and a start of the tradition, Dr. Phil Gold will deliver a short speech and present prizes to research trainees for the science presented at the Montreal International Symposium on Angiogenesis and Metastasis. In recognition of the great discovery and development of a cancer diagnostic tool used commonly today, those awards will carry the designation "Dr. Phil Gold Student Awards". In its inaugural year, three awards for best abstract and three awards for best poster presentation will be awarded during the closing ceremony on June 12, 2009.