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MEETING REPORT

Invasion and metastasis—recent advances and future challenges

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Introduction

Most cancer deaths are due to metastasis—the spread of cancer from its site of origin to distant, vital organs—and the physiological damage caused by tumor growth in those organs. While the broad outlines of the process of metastatic spread are known, much of the details of the process remain poorly understood. To continue to improve cancer survival rates, we must face and tackle the problems inherent to metastatic disease. Cancers that are detected early, before they are believed to have spread to other organs, are generally treated with more success than cancers that are metastatic at diagnosis. However, even cancers that are detected early will recur in some patients, but our ability to predict which individuals will have recurrences is limited. Thus, adjuvant therapy is often given to patients with early-stage disease who are believed as a group to be at risk for recurrence, leading to overtreatment of some patients to benefit a subset of them and possibly failing to treat other patients who will eventually develop recurrent disease. Some recurrences can occur years or even decades after apparently successful primary treatment, and research on tumor dormancy is providing insights into these delayed recurrences. Progress has been made in the basic biology of tumor invasion and metastasis, and in understanding some of the complexities of cancer cell interactions with host cells in their microenvironment. Great advances have been made for many cancers, in terms of molecular markers/subtypes that are associated with favorable versus poor outcome, as well as prediction of response to a growing list of molecularly targeted agents. However, we also recognize that tumors are not static entities, but instead evolve and change over time, and information from a primary tumor specimen may poorly characterize individual metastases that occur years later. Bioinformatic analyses of tumors and their metastases as well as detection and characterization of disseminating tumor cells in blood or bone marrow, over time, are providing a wealth of data to be interpreted. New models are being developed to address problems in metastasis. The challenge is to learn how to harness this growing body of information to help patients with cancer. Can we prevent metastasis? Can we delay appearance of metastases following primary treatment, either through information inherent to the primary tumor, or through life style or anti-metastatic chemoprevention strategies? Can we learn how to better treat metastases once they have developed?

In 1983, 10-year-old William (Billy) Guy Forbeck was diagnosed with neuroblastoma. Unfortunately, he succumbed to the disease at age 11. In 1985, Billy's parents, George and Jennifer Forbeck, established the William Guy Forbeck Research Foundation (WGFRF) in Billy's memory. The WGFRF website provides more information: www.wgfrf.org. The mission of the Foundation is "to promote advances in the field of oncology, particularly pediatric oncology, by shortening the cancer research timetable." The Annual Forbeck Forum is a cornerstone of the Foundation and was conceived as a small and intimate "think tank" for open

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discussion, sharing and collaboration for cancer research. Each year, the WGFRF Scientific Advisory Board selects a focused topic for the upcoming year. Each participant may show only five slides and discuss only unpublished data. As a result, the majority of the Forum is comprised of informal, open discussions. The Foundation also supports young investigators, elected as Forbeck Scholars for a 4-year period, to enable them to attend the Forum and other targeted, mentoring meetings.

The 2014 Forum on Invasion and Metastasis was chaired by Dr. Ann Chambers of the University of Western Ontario, London, Ontario, Canada, and Dr. Zena Werb from the University of California, San Francisco. The goal was to bring together leaders from multiple disciplines to help understand current progress and discuss ways forward to translate this information to the clinic to prevent deaths from metastasis. Below, we summarize the presentations of invited participants at the meeting.

Tumor progression and molecular genetics of metastatic disease

Zena Werb, University of California, San Francisco Despite major advances in understanding the molecular and genetic basis of cancer, disease progression to metastasis remains the cause of >90 % of cancer-related mortality. Dr. Werb posited that understanding metastasis initiation is critical for the development of new therapeutic strategies to specifically treat metastatic disease. Prevailing theories hypothesize that metastases are seeded by rare tumor cells with unique properties, which may function like stem cells in their ability to initiate and propagate new tumors in metastatic sites through self-renewal and differentiation [1]. This hypothesis is supported by studies in human colon and pancreatic cancer, which demonstrate that metastases arise from cancer stem cells (CSCs). Recent studies have indicated that the microenvironment of metastases differs from that of tumors and is likely to regulate CSC dormancy or growth to macrometastases [2, 3]. However, the identity of metastasis-initiating cells in human breast cancer remains elusive and, specifically, whether metastases are hierarchically organized is unclear. Dr. Werb showed at the single-cell level that early-stage human disseminated tumor cells (DTCs) possess a distinct stem cell-like gene expression signature. To identify and isolate DTCs from patient-derived xenograft (PDX) models of human breast cancer, her group developed a highly sensitive FACS-based assay, which allowed them to compare gene signatures in DTCs at different stages of metastasis. She found that "early" DTCs comprised a distinct population from "late" DTCs and primary tumor cells due to their increased expression of stem cell, EMT, pro-survival, and dormancy-associated genes. These findings support a hierarchical model for metastatic cell initiation and progression, and open up new targets for the management of metastatic disease.

Yibin Kang, Princeton University Dr. Kang discussed the origin and evolution of metastatic traits in breast cancer. How and when cancer cells acquire metastatic traits is a topic of intense investigation and debate in the field. It has become clear that the development of metastatic capability in cancer cells is a continuous process that is shaped by the tissue of origin of the primary tumor, early oncogenic events, as well as the stresses tumor cells endure when they encounter different microenvironments and therapeutic treatments [4]. Many genes, such as Metadherin, play multiple functions during primary tumorigenesis and metastatic progression, and may represent ideal targets for therapeutic intervention [5]. Dr. Kang discussed recent findings regarding our understanding of the origin and evolution of metastasis traits, with emphasis on the connection of metastasis genes to early events of tumor initiation, and speculated on the potential for developing therapeutic strategies against metastatic cancer.

Daniel Haber, Massachusetts General Hospital Cancer Center, Harvard Medical School Dr. Haber has used circulating tumor cell (CTC) isolation technologies to study the process of blood-borne metastasis. Using a series of microfluidic devices built by his MGH bioengineering collaborator, Dr. Mehmet Toner, Dr. Haber's lab has focused on breast cancer metastasis, both in a mouse model and in blood specimens from women with breast cancer [6]. They showed that clusters of CTCs in the blood are rare compared with single CTCs, but they are more highly prone to generate metastases. These CTC clusters are derived from oligoclonal fragments of primary tumors and are held together by the cell junction protein Plakoglobin. Single cell RNA sequencing of CTC clusters versus single CTCs from the blood of women with metastatic breast cancer showed a 200 fold increase in plakoglobin expression, and its knockdown in mouse models suppressed the generation of CTC clusters and lung metastases without affecting the size of the primary tumor. Dr. Haber finished with a discussion of hypothetical ways in which CTC clusters may navigate through capillary beds. Reconstitution of capillary-sized channels showed that clustered cancer cells can pass through them under physiological pressures as they realign into single rows, only to regroup as clusters when they emerge on the other side.

Christine Iacobuzio-Donahue, Memorial Sloan Kettering Cancer Center Dr. Iacobuzio-Donahue discussed the observations of different patterns of metastatic failure, oligometastatic and widely metastatic, in patients diagnosed with pancreatic cancer and the genetic features that underlie each [7, 8]. She presented new data on the role of TGFβ signaling in these two phenotypes. For example, loss of

expression of TGFβ1 and TGFβR2 in human tissues was more frequent in oligometastatic pancreatic cancers, and conditional loss of one *TGFBR2* allele in the KPC mouse model (Ptf1aCre/+; LSL-KRASG12D/+; LSL-Trp53R172H/+; Tgfbr2flox/+) led to an oligometastatic phenotype. Moreover, she demonstrated that loss of TGFβ signaling reduced distant metastasis in experimental metastasis models that included a reduction in extravasation from the vasculature in the liver. She also presented data building upon her work of the phylogenetic relationships of coexistent primary and metastatic tumors in patients at autopsy based on whole genome and targeted whole exome sequencing [9], with the interpretation that metastases are derived from more than one subclone in the primary tumor. Finally, she presented data on the mutational spectra and epigenetic alterations that occur in pancreatic cancers and how these alterations have spatially distinct patterns.

Issues and progress in pediatric brain tumors

Julie Park, Children's Hospital and Medical Center, Seattle Neuroblastoma is a heterogeneous cancer arising from primordial neural crest cells that give rise to sympathetic neural ganglia and adrenal medulla. It has a widely divergent clinical spectrum ranging from spontaneous tumor regression to widely metastatic, aggressive disease (high-risk neuroblastoma) [10]. Approximately 50 % of high-risk neuroblastoma disease will not be cured by dose intensive multimodal therapy. In contrast to adult carcinomas, there is a striking lack of recurrent neuroblastoma somatic mutations. Alternatively, preclinical studies have identified altered molecular pathway signaling for cellular differentiation, metastasis, angiogenesis, and inflammation that are associated with aggressive tumor behavior. Multigene expression profiles have identified cohorts of tumors with aggressive behavior including a 14-gene classifier that includes genes important in inflammation and immune responses. Moreover, recent clinical trials using immunotherapy have demonstrated encouraging anti-tumor activity. Unfortunately, despite the addition of antibody-directed immunotherapy in the clinical setting of non-detectable disease, greater than 30 % of patients will experience tumor recurrence. Novel immunotherapeutic approaches that better harness the anti-tumor activity of cellular therapy approaches are underway. However, results from both pre-clinical and clinical trials highlight the need for a combination of both immunological approaches with those that target the microenvironment to better treat the most aggressive forms of the neuroblastoma.

Nada Jabado, McGill University, Montreal, Canada Dr. Jabado's group was one of two to first identify a histone mutation in human disease. High-frequency recurrent somatic mutations at specific residues in histone 3 (H3) variants occur in a particularly lethal form of brain tumor, high-grade astrocytomas affecting children and young adults [11]. H3 mutants, or oncohistones as we label them, were later identified by another group in two bone cancers affecting children and young adults. There is limited knowledge on how oncohistones act in tumor formation and affect the tumor epigenome, micro-environment, and potential invasion and metastatic spread, and this gap impedes the design of effective therapies [12]. These aspects as well as the cross-talk between tumor and microenvironment which may be regulated by epigenetic alterations that favor implementation and growth of tumor cells at distant sites were discussed at the meeting and are the subject of our ongoing investigations [13].

Tumor cell and host/microenvironmental interactions

Sara Courtneidge, Oregon Health Sciences University, Portland Dr. Courtneidge discussed recent research from her laboratory on membrane protrusions known as invadopodia, which are associated with invasive behavior of cancer cells. By studying the obligate invadopodia scaffold protein Tks5, her laboratory has been able to determine what role these structures play in cancer progression. She reported that expression of high levels of Tks5 mRNA correlates with a worse outcome for breast cancer patients, particularly those with early stage disease. In keeping with this, reduction in Tks5 expression not only reduces the invasiveness of breast cancer cells, but also inhibits their growth in three-dimensional tissue culture systems, and in xenograft assays. This growth inhibitory phenotype is accompanied by specific changes in gene expression. Her current research seeks to exploit these gene expression changes to define the mechanisms by which invadopodia control growth, to develop a signature that would define which tumors elaborate invadopodia in vivo, and to use genetically engineered models of cancer to study the role of Tks5 in more detail.

Gregg Semenza, Johns Hopkins University School of Medicine, Baltimore Triple-negative breast cancers (TNBCs) are defined by the lack of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression, and are treated with cytotoxic chemotherapy such as paclitaxel or gemcitabine, with a durable response rate of less than 20 %. TNBCs are enriched for the basal subtype gene expression profile and the presence of breast cancer stem cells, which are endowed with self-renewing and tumor-initiating properties and resistance to chemotherapy. Dr. Semenza showed that hypoxia-inducible factors (HIFs) and their target gene products are highly active in TNBCs [14]. He demonstrated that HIF expression and transcriptional activity are induced by treatment of MDA-MB-231, SUM-149, and SUM-159 TNBC cells, as well as

ER^+/PR^+ MCF-7 cells, with paclitaxel or gemcitabine. Chemotherapy-induced HIF activity enriched the breast cancer stem cell population through interleukin-6 and interleukin-8 signaling and increased expression of multidrug resistance 1 protein. Coadministration of HIF inhibitors overcame the resistance of breast cancer stem cells to paclitaxel or gemcitabine, both in vitro and in vivo, leading to tumor eradication. Increased expression of HIF-1α or HIF target genes in breast cancer biopsies was associated with decreased overall survival, particularly in patients with basal subtype tumors and those treated with chemotherapy alone. Based on these results, clinical trials are warranted to test whether treatment of TNBC patients with a combination of cytotoxic chemotherapy and HIF inhibitors will improve patient survival.

Erik Sahai, London Research Institute, London, UK Dr. Sahai's presentation focused on insights from imaging invasion and metastasis. The problem of different modes of cell migration and invasion was introduced. Depending on how cells move they have different responses to potential "anti-invasive" drugs. This problem is further exacerbated by the ability of cancer cells to switch between different modes of migration. To understand these complex issues, the Sahai group has been collaborating with modelers to develop computational models of cell migration [15]. These models can then be used to explore the plasticity of cancer migration strategy and have helped to uncover a key role for the STRIPAK complex in cancer invasion and metastasis [16]. Dr. Sahai then discussed how tumor invasion is influenced by stromal cells, in particular stromal fibroblasts. These cells play a key role in remodeling the extracellular matrix in tumors and thereby guiding patterns of migration. The possibility of targeting them therapeutically was discussed. Further, their role in modulating the response of melanoma cells to targeted therapy was presented. This work further emphasizes how it is crucial to consider the effects of kinase targeted therapies the tumor stroma, and not just the cancer cells. Given the importance of the tumor stroma in determining the response to therapies, Dr. Sahai proposed that varying stromal environments at metastatic locations might explain the differential responses of metastases in the same patient to systemic therapies.

Therapeutic strategies to combat metastasis—prevent, delay, or treat?

Klaus Pantel, University Medical Center Hamburg-Eppendorf, Hamburg, Germany Improved early detection and adjuvant therapy have facilitated progress in diagnosis and therapy for patients with solid tumors; however, the prognosis of cancer patients is still limited by the occurrence of distant metastases. In patients with completely resectable primary tumors, this relapse is largely due to clinically occult micrometastasis present in secondary organs at primary diagnosis but not detectable even with high resolution imaging procedures. Dr. Pantel has found that sensitive and specific immunocytochemical and molecular assays enable the detection and characterization of disseminated tumor cells (DTC) at the single cell level in bone marrow (BM) as a common homing site of carcinoma-derived DTC. Because of the high variability of results in DTC detection, there is an urgent need for standardized methods. While the prognostic impact of DTC in BM has clearly been shown for primary breast cancer patients [17], less is known about the clinical relevance of DTC in patients with other carcinomas. Current findings suggest that DTC are capable to survive chemotherapy and persist in a dormant nonproliferating state over many years. To what extent these DTC have stem cell properties is subject of ongoing investigations. Since BM sampling is invasive, detection of circulating tumor cells (CTCs) in the peripheral blood of cancer patients has received great attention. CTCs are usually detected by immunostaining or RT-PCR assays, and more recently by the EPISPOT assay which measures the number of cells releasing/secreting tumor-associated marker proteins [18]. Interestingly, detection of cell-free nucleic acids released by tumor cells such as tumor-associated DNA or microRNAs into the blood might become an indirect way to detect micrometastatic disease. At present, most CTC assays rely on epithelial markers and miss CTCs undergoing an epithelial-mesenchymal transition (EMT). New markers, such as the actin bundling protein plastin-3, that are not downregulated during EMT and not expressed in normal blood cells might overcome this important limitation and, therefore, increase the sensitivity of CTC assays [18]. Recently, in vivo capture of CTCs with an antibody-coated wire placed into the peripheral arm vein has become feasible and allows the capture of CTCs from 1.5 l of blood within 30 min. CTC enumeration and characterization with certified systems provides reliable information on prognosis and may serve as liquid biopsy. Moreover, monitoring of CTCs before, during and after systemic therapy (e.g., chemotherapy, hormonal therapy, antibody therapy) might provide unique information for the future clinical management of the individual cancer patient and might serve as surrogate marker for response to therapy. Besides CTCs, the analysis of circulating tumor DNA and circulating cell-free microRNAs may provide complementary information as "liquid biopsy" [19]. This information can be used as companion diagnostics to improve the stratification of patients and to obtain insights into therapy-induced selection of cancer cells.

Ann Chambers, London Health Sciences Center, London, Canada Dr. Chambers discussed three questions. First, are dormant tumor cells a therapeutic target in cancer? The answer is a tentative "Yes," based on experimental studies and results from the MA.14 clinical trial [20, 21], which showed benefit for very long-term anti-hormonal therapy in women with

hormone-responsive breast cancer. Breast and prostate cancer, and likely other tumor types, appear to be chronic, relapsing diseases. Even in cancers diagnosed early, recurrences can happen, years or decades after apparently successful primary treatment. We cannot predict well which individual patients will recur and which will not, nor do we know whether this information lies within the primary tumor or is affected by posttreatment lifestyle or other influences. Second, do we know how to target dormant tumor cells therapeutically? This answer is a clear "No" at our current stage of understanding of the biology of dormancy. While we are learning much about molecular aspects of dormancy and recurrence (e.g., [22]), translating this information to individual patients is not yet feasible. Third, would we know which patients to treat? Again, the answer is, at present, "No." We simply do not know the extent of micrometastatic burden in patients for whom there is no current evidence of disease. We are not able to tell which patients are cured and which harbor undetected "minimal residual disease." We need improved methods to assess for micrometastatic burden in patients who are at risk for later recurrences. Only when we understand which patients harbor undetected, dormant disease will we be in position to consider individualized treatment to prevent recurrence of dormant disease or attack and destroy dormant cells. Until then, we will continue to overtreat some patients in a group at risk for recurrence, only some of whom would otherwise develop recurrence, as well as undertreat some patients in groups of good prognosis who nonetheless develop recurrence.

Patricia Steeg, National Cancer Institute, Bethesda The translation of metastasis experiments to the clinic remains problematic. Dr. Steeg discussed the fact that standard phase I–III trials in the metastatic setting quantify the shrinkage of metastatic lesions, not the prevention of their occurrence. Two new trial designs were discussed, primary metastasis prevention and secondary metastasis prevention [23, 24]. Primary prevention may enroll patients at very high risk of metastases, for instance those who underwent neoadjuvant therapy and did not obtain a pathological complete response, those with multiple positive lymph nodes, or those with chest wall recurrences. Secondary metastasis prevention trials could enroll patients with limited, treated metastatic disease at high risk of relapse; the endpoint would be time until the development of a new metastasis.

New investigator presentations

The Forbeck Forum invited several young investigators to participate in the meeting, to meet experts in the field, and to present their own work.

Karla Williams, London Health Sciences Center, London, Canada Dr. Williams discussed cellular remodeling of the extracellular matrix (ECM), facilitating tumor cell invasion, as a key intrinsic quality in metastatic cells. Invasive tumor cells form specialized structures called invadopodia which remodel the ECM through the precise trafficking and localization of proteins involved in actin dynamics and ECM degradation. Past research into invadopodia dynamics identified key trafficking events regulated by SNAREs (soluble *N*-ethylmaleimide-sensitive factor activating protein receptors), which function to localize vesicles to invadopodia transporting proteins such as membrane-type 1 MMP (MT1-MMP) and epidermal growth factor receptor (EGFR) [25, 26]. Her current research investigating the in vivo function of invadopodia and their role in the metastatic cascade has demonstrated that inhibitors of invadopodia formation, such as PAK1, impair tumor cell extravasation. The importance of proper invadopodium regulation was discussed since dysregulation enhancing ECM degradation did not correlate with increased tumor cell invasion or extravasation. While proper regulation of invadopodia was considered an important avenue of research, there was also a consensus that this regulated process was strongly similar to existing cellular processes such as podosome formation in immune cells. The co-opting of such existing cellular mechanisms by tumor cells to mediate metastasis remains an important consideration in ongoing research. The dialogue highlighted the potential of invadopodia research as there is still much to learn about how these structures function in vivo, their role in the metastatic process, and if they can be successfully targeted to impair metastasis.

Rosandra Kaplan, National Cancer Institute, Bethesda (Forbeck Scholar) Dr. Kaplan presented her research on the pre-metastatic niche and its role in metastasis. In order to understand the process of metastasis and the role of niche biology in metastatic progression, the Kaplan lab uses both orthotopic mouse models and blood samples from patients with localized and metastatic disease to investigate early systemic changes in response to cancer that promote metastatic progression. Dr. Kaplan's lab has identified the early events leading to formation of the pre-metastatic niche, which is a metastasis-promoting microenvironment composed of bone marrow-derived cells and stromal cells that enhance disseminated tumor cell survival and proliferation [27]. Her team has demonstrated that the bone marrow microenvironment is altered during tumor progression with expansion of hematopoietic stem and progenitor cells that proliferate and are mobilized into the circulation. The hematopoietic stem/progenitor cells in distant tissue sites differentiate into myeloid-derived suppressor cells that contribute to immune suppressive microenvironment [28, 29]. This hematopoietic stem cell niche expansion can be marked by increased circulating hematopoietic

stem/progenitor cells in patients and may serve as a biomarker to predict which patients are at highest risk for metastatic progression. In addition to the hematopoietic component of the pre-metastatic niche, the Kaplan lab has also demonstrated stromal cell activation contributing to the niche environment. Understanding metastatic niches in regulating metastatic cell fate can lead to approaches to target these unique microenvironments and prevent metastasis.

Mario Shields, Cold Spring Harbor Laboratory (Forbeck Scholar) A hallmark of pancreatic cancer is the fibrotic stroma, consisting of an extensive deposition of fibrillar type I collagen and infiltration of myeloid cells [30]. To understand the role of the stroma in pancreatic cancer cell invasion and metastasis, Dr. Shields developed a live animal imaging platform to examine the dynamics of the interaction between the tumor microenvironment and cancer cells. Using a novel genetically engineered mouse model of pancreatic cancer, in which the KrasG12D oncogene is co-expressed with tetracycline-regulated shRNA against PTEN, he showed that cancer cell invasion occurs in early stage tumors. Restoration of PTEN expression resulted in tumor regression with a marked increase in cell death and recruitment of stromal cells. To examine the contribution of myeloid cells or type I collagen to cancer cell invasion, he imaged pancreatic tumors in which cancer cells were orthotopically implanted in transgenic mice expressing a fluorescent reporter specific to myeloid cells or GFP-labeled type I collagen. Dr. Shields showed that cancer cells invaded independently of myeloid cells, but were more migratory in the vicinity of linear collagen. Further, perturbing type I collagen architecture, by increasing collagen thickness and alignment promoted the spread of cancer cells to the liver. His future studies will focus on delineating the signaling mechanisms that dictate collagen-mediated invasion, with the aim of limiting metastatic spread.

Louise van der Weyden, Wellcome Sanger Institute, Cambridge, UK (Forbeck Scholar) Dr. van der Weyden used Dr. Stephen Paget's "seed and soil" hypothesis of metastasis as the foundation of her studies on understanding melanoma metastasis. The incidence of melanoma is less than 2 % of all skin cancers, yet it is responsible for 75 % of skin cancer-related deaths—this is due to its inherent ability to metastasize early on. To understand the nature of the "seed," whole-genome DNA and RNA sequencing of mouse melanoma cell lines with differing metastatic capacities is being performed, to look for genes whose loss or gain of expression, or mutation, correlates with enhanced metastatic ability. Similarly, series of canine oral melanomas are being exome sequenced. To ensure relevance of the findings, cross-species comparison will be used to identify which differentially expressed or mutated genes in the animal model datasets are also found in human datasets, and more importantly, that correlate with survival. To understand the "soil" Dr. van der Weyden is using a mouse melanoma cell line to perform an "experimental metastasis assay" on mutant mouse lines coming through the Mouse Genetics Program at the Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/mouseportal/). This provides a unique opportunity to interrogate the genome in an unbiased manner and identify host genes that are able to regulate the ability of melanoma cells to successfully metastasize to the lung. From the mutant lines screened to date, they have found that metastatic efficiency can be modulated by immune system, and inflammatory and stress mediators [31]. Understanding genes that are altered in metastasis or host genes that can regulate metastasis will hopefully pave the way for identifying potential new drug targets.

Chad Pecot University of North Carolina Lineberger Comprehensive Cancer Center, Chapel Hill (Forbeck Scholar) Dr. Pecot presented his work studying lymphatic metastasis in lung cancer. On average, lung cancer patients die within a year of presentation due to development of distant metastasis. Even if caught in the early stages, patients often die following surgical resection due to rapid recurrence, suggesting primary tumors are very "fit" for dissemination. Intriguingly, patients with micrometastases in surgically resected lymph nodes are known to have a significantly higher chance of distant relapse. This suggests that the lymphatic route of spread may not be a "dead end," and in fact may represent a parallel process to hematogenous metastases with unique mechanisms. To address this question, Dr. Pecot presented data from lung cancer subclones, developed using in vivo selective pressure, which have markedly enhanced capacity to metastasize to lymph nodes. With this approach, Dr. Pecot's team found these subclones have dramatic changes in their microRNA profiles. Future directions will be to characterize the molecular pathways regulated by select microRNAs that may have important roles in lymphatic metastases.

Conclusions from the meeting

We recognize that improved cancer survival rates will require improved understanding of the metastatic process and a stronger research focus on these issues.

We are beginning to define new mechanisms that contribute to tumor invasion and metastasis. We are recognizing that cancer is a heterogeneous and dynamic disease that can evolve in patients, and new therapies need to address this concept.

Progress has been made in advancing our understanding of metastatic disease but much more is needed. We appreciate the willingness of patients to participate in clinical trials and in tissue and clinical data banking as a partnership to further our understanding and lead to improved treatments.

There is slow progress in developing new approaches to prevent, treat, or delay metastatic disease. However, we are learning that cancer can affect the whole body, and that the body in some cases can limit metastatic spread. We need to understand how this occurs, in order to harness it therapeutically.

There is a need for comprehensive basic research to learn more about metastasis and there is a great need for translational research to improve responses to targeted agents. The first of the new ideas are beginning to be translated to the clinic, but it is early days.

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Each participant was asked to supply up to three references to their own work, as background.

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