Breast Cancer Metastasis: Challenges and Opportunities

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Introduction

Despite exciting progress in the understanding of breast cancer development and progression, and in the development of novel therapeutic strategies, breast cancer remains the second leading cause of cancer-related death in women, with a yearly toll of more than 40,000 deaths in the United States alone. Breast cancer–related deaths are mainly due to the "incurable" nature of metastatic breast cancer (MBC) at the current time. It is estimated that ~6% of patients have metastatic disease at the time of diagnosis and 20% to 50% patients first diagnosed with primary breast cancer will eventually develop metastatic disease. Even with the remarkable advances in research and clinical management, the current treatment strategies for breast cancer metastasis still largely rely on the use of systemic cytotoxic agents, which frequently deteriorate the patient's life quality due to severe side effects and, in many cases, have limited long-term success. The prognosis for MBC patients is poor, with an estimated 5-year survival of only 26%. Therefore, MBC remains the most challenging task facing both cancer researcher and oncologist. To tackle this challenge, scientists and physicians of the Breast Cancer Research Program at the M.D. Anderson Cancer Center held a symposium to (a) provide a better understanding of breast cancer metastasis at the molecular and cellular level; (b) introduce cutting-edge technologies in metastatic breast cancer detection, including clinicopathologic detection, circulating tumor cells (CTC) detection, and advanced imaging; and (c) solicit innovative ideas in basic, translational research and clinical patient management. The symposium led to a positive consensus notion that we will be able to prevent, and to a lesser degree, treat metastasis and ultimately save most patients from metastatic deaths in the foreseeable future.

The Challenges of Breast Cancer Brain Metastasis

Understanding the molecular and biological basis of metastasis is essential for paving the way to conquering it. Dr. Patricia Steeg (National Cancer Institute, Bethesda, MD) set the stage on the challenges of understanding metastasis in her keynote speech: *A Molecular Portrait of Brain Metastasis of Breast Cancer*. Brain metastasis represents a devastating consequence of breast cancer, with a particularly dismal prognosis of ~80% mortality within

1 year of diagnosis. The classic tools in treating brain metastasis, including neurosurgery, stereotactic radiosurgery, or whole brain radiation, are partially effective in treating large macrometastasis; however, these tools have limited effects on controlling small micrometastasis, many of which will grow and eventually become deadly to patients. Therefore, novel molecular therapies to eliminate or stabilize micrometastasis are urgently needed. However, the development of effective chemotherapy in treating brain metastasis has been especially challenging due to the limited permeability of the blood-brain barrier, which is considered a "sanctuary site" for tumor cells. To study brain metastasis, a "brain-seeking" MDA-MB-231 human breast cancer cell subline (named 231-BR) was developed that forms brain metastasis after 21 to 28 days following left cardiac ventricle injection (1). The brain metastasis of this 231-BR xenograft model shared properties with clinical human brain metastases of breast cancer, including a brain inflammatory response with extensive reactive gliosis surrounding the metastases, high proliferation, and minimal apoptosis. Several chemotherapy agents were tested for blood-brain barrier penetration using this mouse model, with doxorubicin showing the most efficient penetration to the brain metastasis. This pioneering preclinical study is followed by systematic investigation of the blood-brain barrier penetration of chemotherapeutic agents to select the ones that are capable of achieving functional dose distribution at the sites of brain metastases.

Brain metastases are prevalent in triple negative and HER2-overexpressing (HER2+) subpopulations, and the incidence of brain metastases approaches one-third of all metastatic HER2+ patients. HER2+ status was found to specifically increase brain colonization 2.5-fold to 3-fold, although not affecting tumor cell arrival or intravasation into the brain. Excitingly, a study of ^{14}C-lapatinib distribution in a 231-BR xenograft model showed a significant increase of its distribution to the metastasis over normal brain. Indeed, lapatinib inhibited the formation of clinical metastases in HER2/EGFR-expressing 231-BR cells by 54%, suggesting activity in the adjuvant setting (2). Current work is examining rational combinations to elicit synergistic effects with radiation and other targeted agents.

Animal Models and Molecular Basis of Metastasis

Metastasis is the most difficult cancer phenotype to simulate. Dr. Janet Price (The University of Texas, M.D. Anderson Cancer Center, Houston, TX) summarized the current animal models of cancer metastasis for both analysis of molecular mechanisms of metastasis and testing of antimetastatic therapies. The majority of the models are rodents, predominantly mice, including xenograft mouse models and genetically engineered mouse models. A variety of transplantable mouse and established human breast cancer cell lines also have tumorigenic and metastatic properties

in appropriate host animals. Although a number of mouse models of breast cancer develop metastases to the lungs and lymph nodes, limited models showed metastasis to the bones, brain and liver, the common sites of metastatic disease in humans. To circumvent this, different injection techniques have been developed to target breast cancer cells to these organs in order to investigate the properties of these cells in the colonization of the specific organ, such as the 231-BR model (1, 3). Additionally, the development of noninvasive imaging technologies for rodents continues to facilitate the monitoring of the growth of metastases and responses to therapy.

The molecular defects driving each step of metastasis have been categorized as metastasis initiation genes, metastasis progression genes, and metastasis virulence genes (4). Dr. Dihua Yu (The University of Texas, M. D. Anderson Cancer Center, Houston, TX) summarized data demonstrating that *erbB2* amplification can modulate almost every step in the metastatic cascade and is involved in metastasis to the lungs, bones, and brain. Several of the HER2+ downstream signals (Src, Stat3, Akt, mTOR, VEGF, etc.) may serve as therapeutic targets for the intervention of breast cancer metastasis.

Detection of MBC

Detection of MBC by currently available imaging modalities depends on accurate targeting of the lesion and adequate tissue size for a pathologic diagnosis. Dr. Savitri Krishnamurthy (The University of Texas, M. D. Anderson Cancer Center, Houston, TX) introduced the newly developed technologies of detecting small-sized metastasis in lymph nodes that are below the detection limit of commonly used imaging techniques. Two recently developed automated platforms for molecular testing of sentinel lymph nodes can generate results comparable to histopathologic examination. The Gene Search BLN assay (Veridex, LLC, Warren, NJ) uses reverse transcription-PCR to detect transcripts of CK19 and mammaglobin as biomarkers for detecting metastasis in the lymph nodes. Another platform (RD-100i analyzer; Sysmex, Japan) uses one-step nucleic acid amplification for CK19 to detect metastasis. These comprehensive examinations of the entire lymphoid tissue using a combination of the traditional histopathologic methods and molecular testing enable the detection of small-sized metastasis which would otherwise remain buried in the paraffin block, never to be detected during routine examination. The limitations of this method at the current stage include possible false-positive results due to contamination by epithelial cells during the surgical procedure, and inconclusive results if the molecular markers are not expressed due to the heterogeneous nature of tumors. Therefore, the extent of improvement in staging and prognostic information that can be obtained in patients using such comprehensive examinations of lymph nodes remains to be established in the near future.

Detection of CTCs in MBC

The detection of tumor cells that have disseminated from the primary breast cancer to peripheral blood (CTCs) and bone marrow (DTCs, as disseminated tumor cells) has substantially facilitated our understanding of their prognostic value, and may serve as early markers to evaluate the effects of therapy. Due to the ever-growing importance of detecting CTCs in MBC, Drs. Savitri Krishnamurthy, James Reuben, and Massimo Cristofanilli (all from The University of Texas, M. D. Anderson Cancer Center, Houston, TX) discussed the most recent progress in the detection of CTCs and its implications in both research and clinical patient care. Because CTCs occur at very low frequencies of 1 in 10^6 to 1 in 10^7 nucleated hematopoietic cells, several enrichment methods have been developed to increase the detection sensitivity, including density gradient separation, immunomagnetic separation, and filtration (5). In particular, immunomagnetic-based technologies allowed for CTC detection in the peripheral blood of patients with MBC by using epithelial markers for enrichment and characterization. The clinical relevance of detecting CTCs in different patient populations is rapidly evolving. For example, detection of CK19 mRNA-positive CTCs was shown to indicate poor prognosis, particularly in estrogen receptor (ER)–negative, triple-negative, and HER2-positive groups, and their presence before the initiation of adjuvant chemotherapy indicates poor prognosis (6). Furthermore, the prognostic value of CTCs was independent of the biological characteristics of the primary or recurrent disease, and superior to standard imaging traditionally used to measure tumor load and assess therapeutic response (7). Although the detection of CTCs and DTCs is emerging as an important predictor of progression and treatment response, as well as early and advanced breast cancer prognosis, there is an urgent need to conduct large multi-institutional trials using standardized well-validated techniques that can lead to their possible utilization as prognostic and predictive markers in routine clinical practice. A very interesting phenomenon observed in CTC studies is that HER2+ CTCs can be found in patients with HER2-negative primary tumors. The demonstration of discordant HER2 status raises questions of selecting appropriate treatment strategies for specific patients, and also raises the possibility of the involvement of "cancer stem cells" in breast cancer development and progression.

"Breast Cancer Stem Cell" in Metastasis and the Involvement of Epithelial-Mesenchymal Transition

The "cancer stem cell" hypothesis has attracted enormous attention because of its perceived potential of improving cancer prevention, diagnosis, and treatment. In contrast to the classic "stochastic" model of carcinogenesis, in which any cell in an organ can initiate tumor formation by certain combinations of mutations, the cancer stem cell hypothesis supports the origin of tumor from stem and/or progenitor cells through the dysregulation of the normally tightly regulated process of self-renewal (8). Dr. Gabriela Dontu (University of Michigan, Ann Arbor, MI) elegantly identified aldehyde dehydrogenase 1 (ALDH1) as a novel cancer stem cell marker. Her *in vitro* studies and *in vivo* mouse xenograft models revealed a cellular hierarchy in the normal mammary gland in which ER-negative, ALDH-positive stem cells generate ER-positive progenitor cells. This hierarchy of differentiation is maintained in a subset of ER-positive human breast carcinomas. Importantly, this subset of ER-positive cancers, derived from and driven by an ER-negative stem cell, has a significantly worse clinical outcome than other ER-positive breast cancers. These studies indicate that different subtypes of ER-positive breast cancer may have different cellular origins. Therefore, it is crucial to identify these subtypes at the molecular level in order to develop more tailored treatments. Dr. Sendurai Mani (The University of Texas, M. D. Anderson Cancer Center, Houston, TX) reported the unexpected convergence of two lines of studies: cancer stem cell and epithelial-mesenchymal transition (EMT). He reported that EMT in mammary epithelial cells generated cells sharing properties of self-renewing stem cells, including the CD44-high/CD24-low configuration, mammosphere formation, and the ability to give rise to populations containing a mixture of luminal cells, myoepithelial cells, and a small subpopulation of bipotential cells. Consistently, both normal and neoplastic human breast stem cell-like cells

expressed EMT-associated markers. This discovery of EMT generating cells with stem cell properties highlights the importance of EMT in breast cancer metastasis by facilitating the execution of the invasion-metastasis cascade, from dissemination from the primary site to colonization of secondary organs. Overall, these provided compelling evidence that breast cancer stem cells may play a pivotal role in initiating the primary tumor as well as in promoting metastasis.

Advanced Imaging for Breast Cancer

Cutting-edge imaging technology features a combination of multidimensional (e.g., three-dimensional and above) and multimodality (i.e., a combination of various modalities) imaging. Dr. Luc Bidaut (The University of Texas, M. D. Anderson Cancer Center, Houston, TX) compared the detection of metastasis using current major cancer imaging modalities, including computed tomography (CT), magnetic resonance imaging, positron emission tomography (PET), single photon emission computed tomography (SPECT), and the smaller physical footprint but more ubiquitous ultrasound. For breast cancer metastases, whole body imaging is key in surveying and potentially targeting all possible sites as well as primary lesions. Currently, only PET intrinsically provides such a capability in three dimensions (Supplemental Fig. S1), but its clinical limitation to a single tracer (e.g., fluorodeoxyglucose) limits its effectiveness in pinpointing disease type and staging more specifically. SPECT suffers from more physical limitations than PET but its reliance on many more available tracers makes it a viable contender, and even more so in its SPECT/CT manifestation. Other modalities, e.g., magnetic resonance imaging, are making strides toward whole body imaging as well and could soon become a good alternative or at least a complement to PET/CT for identifying and characterizing metastases. Advanced imaging has become a key component in the management of MBC. By combining ever improving biological knowledge with sophisticated instruments and advanced computer imaging techniques, breast cancer metastasis will likely be better detected and targeted, and potential therapies will be more objectively evaluated, all of which should lead to an improved outcome for patients.

State-of-the-Art Clinical Management of Breast Cancer Metastasis

Dr. Vicente Valero (The University of Texas, M. D. Anderson Cancer Center, Houston, TX) presented an overview of the clinical aspect of MBC and emphasized "*Current Therapeutic Approaches and New Developments in the Treatment.*" Currently, MBC is generally incurable and only 1% to 3% of patients with chemotherapy-naïve MBC may remain long-term disease–free after systemic therapy. Current therapeutic goals for patients with MBC are prolongation of survival, palliation of symptoms, and maintenance of quality of life. Prolongation of survival has recently improved from 12 to nearly 36 months due to more effective therapies including new agents like docetaxel, doxorubicin, and paclitaxel; docetaxel and capecitabine; gemcitabine plus paclitaxel; trastuzumab and chemotherapy; and aromatase inhibitors, etc. It is believed that with the accelerated acquisition of knowledge in both basic and clinical breast cancer research, we have a reason to be optimistic that MBC will be a curable disease in the future (Supplemental Fig. S2).

This retreat has served as a platform for both physicians and scientists to communicate and share insights from different perspectives on MBC. Excitingly, the extensive interactions between physicians and scientists during this retreat had initiated the planning of a novel clinical trial for breast cancer brain metastasis involving scientists investigating breast cancer brain metastasis, neuropathologists, breast medical oncologists, and neuro-oncology surgeons. The extensive discussions and communications between clinicians and scientists also closely connected research and patient management for breast cancer metastasis. The retreat ended with a consensus that the united force of experts of different disciplines is crucial to win the battle against MBC. In summary, inhibiting metastasis and ultimately saving patients from metastatic deaths can be a dream coming true, when research scientists and physicians closely communicate, team up, and persistently work together with the same vision, as we showcased during this retreat.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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