Review

Molecular biology of breast cancer metastasis
'Has it spread?': disarming one of the most terrifying questions

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Introduction

There is good news to report. I list below a number of significant advances in breast cancer metastasis research. Each of these shifts has the potential to influence translational research (ie drug development, preclinical studies and clinical trials). I offer my thanks to the authors who contributed the accompanying reviews.

The barn door has not been left open! Metastatic colonization as a translational target

The number of clinicians whose eyes glaze over as metastasis researchers dutifully recite the many steps of the metastatic process, and go on to examine tumor cell invasion in minute detail, signals either that we give boring lectures or that we have evoked the 'so what?' response. Those who would favor the latter response might state that, even for the greater than 90% of patients without detectable distant metastases at surgery, it remains possible that tumor cells have already invaded out of the primary tumor and are sitting contentedly in distant sites undetected. Only 'growth' and angiogenesis remain. Why study metastasis when it may be virtually complete by the time the patient walks into the clinic? Has the barn door been left open? Should we all drop our experiments and switch to antiangiogenesis projects?

Two reviews in this series have addressed this critical question, and arrived at similar answers. Investigators from Dr Ann Chambers' laboratory have watched it all happen. She and others have tagged tumor cells and watched them metastasize to the livers and lungs of experimental animals using *in vivo* videomicroscopy [1]. She reports that the clinicians are partially correct; both metastatically competent and poorly metastatic cell lines arrive at the metastatic site and extravasate at high frequencies. What separates the cells with high from those with low metastatic potential is their ability to colonize in that distant site. Differences in metastatic colonization potential are observable at the micrometastatic stages, before angiogenesis is a rate-limiting step. In addition to Dr Chambers' *in vivo* work, other researchers using nonbreast cancer cell lines have recently identified different points of metastatic blockade in distant organs, such as metastatic colonization attached to lung endothelium [2]. Both findings, however, point to colonization at the secondary site as the metastasis-limiting point. Thus, intravital videomicroscopy efforts have confirmed metastatic colonization as a critical limiting step, and currently suggest heterogeneity in its mechanism.

These studies have several interesting implications. What is the significance of the detection of isolated cancer cells in metastatic sites? How is metastatic colonization of a distant site different from that of primary tumor growth, and does it represent an understudied but valuable translational target? What are we learning when we test lead agents in xenograft model systems, and measure changes in primary tumor size?

Dr Danny Welch and colleagues, in their review [3], report a similar conclusion from a completely different line of investigation. The investigators study metastasis-suppressor genes, which suppress metastasis but not primary tumor growth, upon injection of transfected cells into experimental animals. There is a growing and vibrant literature on these genes, which is reviewed. Intriguingly, the mechanism of action of many of the metastasis-suppressor genes is unknown (ie not among the traditional adhesion, protease and motility factors studied in invasion). Although this has represented a 'kiss of death' to editors and study sections, emerging evidence from multiple metastasis-sup-

ECM = extracellular matrix.

pressor genes suggests that it may represent a strength, because it supports the hypothesis that these genes 'work' by a different mechanism. Studies in breast and prostate carcinoma model systems indicate that tumor cells from metastasis suppressed *kai1* or *kiss* transfectants, as well as their and metastatically competent control transfectants, arrived in the distant organ at comparable frequencies and with equivalent viabilities. What distinguished metastatic competence was the ability to complete nonangiogenesis-dependent colonization in the distant site.

That two different fields of inquiry have developed similar conclusions is noteworthy. I propose that the metastasis field focus attention on metastatic colonization as a distinct, translationally important facet of breast cancer progression. Although angiogenesis at the distant site is undoubtedly clinically important, the work summarized above demonstrates that nonangiogenic processes inherent in metastatic colonization also exist and are amenable to translational development.

What is metastatic colonization? How is it different from primary tumor growth? The literature implies that metastatic colonization is more than proliferation, and includes facets such as dormancy, apoptosis, novel sets of cell–cell and cell–tissue interactions, altered responsiveness to paracrine factors, etc. The discovery of *mkk4* as a prostate cancer metastasis-suppressor gene suggests that the stress kinase signal transduction pathway may be germane to metastatic colonization [4], and we await confirmation of this trend in breast cancer metastasis. Perhaps a distinguishing factor in metastatic colonization is the adaptive response to the stress of being on foreign 'soil', and that specific signal transduction pathways will be involved. In *nm23*-transfected breast carcinoma cells, the ubiquitous soft agar anchorage-independent growth assay was used as an imperfect measure of metastatic colonization [5]. Metastasis-suppressed *nm23* transfectants colonized poorly in soft agar, and remained unresponsive to the addition of transforming growth factor-β, whereas metastatically competent control transfectants were more proficient at colonization and were stimulated by transforming growth factor-β. This switch in responsiveness to a widely available cytokine may be part of the stress response to foreign 'soil'. The progress of this new field of inquiry will undoubtedly depend on the development of *in vitro* model systems that accurately replicate the *in vivo* process, as well as continued development of *in vivo* model systems to enable the visualization, quantitation, and characterization of micrometastatic cells.

Finally, we need to acknowledge that most of our breast cancer metastasis model systems rely on metastases to the draining lymph nodes and lungs. An emerging literature is reviewed elsewhere on metastasis studies to the bone [6,7], and attempts have been made to optimize models for breast cancer metastasis to the brain [8,9] and other sites [10]. It is conceivable that we may obtain different answers to our metastatic colonization questions as these relevant models evolve.

Are there nonendothelial targets for angiogenesis in breast cancer?

Recently the laboratories of Drs Mary Hendrix and Robert Folberg have put forth a hypothesis in ocular melanoma, which proposes that aggressive tumor cells can form extracellular matrix (ECM) channels that contain blood and may augment endothelial-derived angiogenesis [11–13]. This hypothesis has generated discussion and controversy, but areas of concordance appear in the field. Does it apply to breast cancer?

A brief digression into uveal melanoma is required. The team of Drs Hendrix and Folberg used uveal melanomas, which lack lymphatics and therefore present a simpler system for study. A periodic acid–Schiff immunohistochemical stain identified nonendothelial channels that appeared delimited by ECM and ultimately melanoma cells, and which contained erythrocytes. It remains controversial whether traditional endothelial-derived capillaries also service these tumor cells, with opinions based on the stain used, the observer, and the intratumoral location of the observation. The looping patterns have also been proposed to represent ECM-surrounding nests of tumor cells [11,13]. The overall significance of these looping structures is unknown, but of high interest. Do they represent 1%, 10% or 100% of bloodflow? Do they generally conduct blood, or is this the result of hemorrhage in surgery [14]? Affiliated *in vitro* experiments suggest additional possibilities; culture of aggressive, but not nonaggressive melanoma cell lines in ECM resulted in patterned, ECM-channel network formation. Dr Mina Bissell [15] noted that the time course of channel formation, and its localization inside the ECM, are distinct from the rapidly formed networks that many of us observe when cells are plated onto matrigel. Array analysis of the channel-forming melanoma cells revealed that they expressed two sets of genes of interest: genes of the 'interconverted' phenotypes, suggesting a dual differentiation or redifferentiation of the melanomas expressing both epithelial and mesenchymal phenotypes; and genes associated with traditional angiogenesis. The latter observation also prompts us to ask what exactly we are detecting when we stain for factor VIII, or other 'endothelial' proteins.

The accompanying review by Dr Mary Hendrix *et al* [16] now expands this hypothesis to breast cancer using the *in vitro* culture system. We anxiously await *in vivo* evidence for or against this hypothesis in breast cancer, because it will impact on our thinking on clinical antiangiogenic strategies. Will endothelial based therapies 'work' if alternate vascular systems exist? Do these new vascular systems

represent additional therapeutic targets? These questions will undoubtedly be addressed using tagged cell lineages in animal models, and we invite involved researchers to consider the breast cancer models available.

The heterogeneity of breast cancer: inflammatory breast cancer

So much of our cancer research work is delineated by the cell lines and mouse systems available, and metastasis research is no exception. It is with great pleasure that we read a review from Dr Sofia Merajver and colleagues [17] on recent progress in inflammatory breast cancer. This cancer type occurs rarely, but is exceptionally aggressive. It is defined both clinically and pathologically, with tumor cell infiltration of the dermal lymphatics leading to inflamed appearing skin as a hallmark. Cell lines from inflammatory breast tumors were established by Dr Steve Ethier and colleagues [18], and a xenograft mouse model system was reported by Alpaugh *et al* [19]. Using the cell lines, Dr Merajver reports that relatively unstudied molecular alterations occur in inflammatory breast cancer, and her focus is on RhoC GTPase in signal transduction and an insulin-like growth factor-binding protein. These findings advance the field toward the day when a diagnosis of inflammatory breast cancer signals the patient's entry into molecularly defined clinical trials of treatments that are specific for this disease.

The impressive results reviewed herein in inflammatory breast cancer highlight the importance of investigation into the many subtypes of aggressive breast cancer. A corollary of this observation is our need for funding of model system and cell line development to facilitate these critical studies.

The number crunch: will mathematics contribute meaningfully to metastasis research?

This is where the molecular biologists' eyes glaze over. Is metastasis too complex, heterogeneous, and unstable to model mathematically? Similar arguments were forwarded to predict that molecular genetic alterations would not be instructive to metastasis research years ago.

Dr Susan Clare and colleagues [20] have reviewed the recent progress in the field of mathematical modeling with respect to breast cancer natural history, its metastatic initiation, the effect of surgery, tumor dormancy, and the effectiveness of chemotherapy. Quantitative predictive approaches should be familiar to the cell biologist who tests the metastatic potential of cell lines in mice and the pharmacologist who performs *in vivo* testing of lead agents, as well as the clinician who is concerned with the dosing and timing of drugs. All three types of research, and not just the latter, need to be evaluated and understood mathematically. At a minimum we should be able to judge which preclinical models best fit the human situation. Optimally, we continue to refine our modeling so that reasonable predictions can improve the development of *in vivo* experiments and clinical trials.

Conclusion

The tumor metastasis field holds great promise for translating our developing understanding of the molecular and signal transduction pathways underlying aggressive cell behavior to the clinic. The obstacles to such advances, in my opinion, include: (a) the availability of model systems that reproduce the heterogeneity of human disease; (b) a hesitancy to test a pathway in a human tumor cohort study, to see if a trend observed in a cell line actually correlates with aggressive clinical course; and (c) the need for better interactions between bench researchers and industry, where many lead agents are under development. The breast cancer field has been invigorated by the participation of cancer survivors/advocates, and their message is germane: it is not enough to discover an interesting pathway. How will it lead to the treatment of metastatic disease?

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