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Breast Cancer Progression: Controversies and Consensus in the Molecular Mechanisms of Metastasis and EMT

Pamela Cowin and
Departments of Cell Biology and Dermatology, New York University School of Medicine, New York, NY 10016, USA, e-mail: cowinp01@med.nyu.edu

Danny R. Welch
Departments of Pathology, Cell Biology, Pharmacology and Toxicology, Comprehensive Cancer Center, National Foundation for Cancer Research-Center for Metastasis Research, University of Alabama at Birmingham, Birmingham, AL, 35294, USA

Breast cancer is the second most frequent form of female cancer and a major source of cancer death worldwide. Although breast cancer has long been part of the human condition, appearing in the writings of ancient Egyptians, modern breast cancer shows a disturbing statistical increase from 1 per 20 women in the 1960s to one in eight today [1]. Although early detection by mammography and adjuvant therapies has improved survival, the absence of good prognostic criteria continues to result in overtreatment of patients with benign disease and failure to identify and eliminate the source of metastatic breast cancer.

This issue is concerned with the myriad stages of tumor progression that lead to the dissemination of breast cancer cells from the primary tumor and propagation at distant sites. The first stage in this progression involves loss of local constraints, both physical and regulatory, emanating from neighboring normal cells and surrounding stroma. The second step termed intravasation, involves entering a circulatory system (blood and/or lymphatic), the third dissemination and survival within hostile ectopic environments, and the fourth organotropic colonization of compatible sites.

Considerable debate has arisen between elements in the scientific and clinical community concerning the proposed mechanisms of metastatic spread [2,3]. The controversy centers on whether metastasis involves inappropriate transient re-enactment of developmental migratory processes; Darwinian selection within a population of genomically unstable, rapidly evolving cancer cells for attributes that facilitate growth in and exploitation of new environs; or a combination of both.

Seeing parallels between the metastatic process and the long-distance migration of cells during development, cell and developmental biologists have invoked epithelial–mesenchymal transition (EMT) as the mechanistic basis for breast cancer metastasis [4-6]. In its strictest sense, EMT and its converse MET, have been used to describe conversions in cell shape and migratory behavior that accompany cell fate changes during embryonic development. A classical example of EMT is the emergence from the coherent neural plate of migratory neural crest cells, which travel long distances and adopt many different mesenchymal cell fates. These events involve the loss of “epithelial” characteristics such as cell–cell adhesion mechanisms and junctions and switching on of vimentin expression as the major intermediate filament protein. Further analyses have revealed the essential role of a genetic program tightly

Correspondence to: Pamela Cowin.

choreographed by negative regulators of E-cadherin transcription such as TGF-beta, Snail, Slug, Goose-coid and Twist. Embodied within this strict definition of EMT are two concepts: (1) that developmental EMT involves an ordered series of transcriptionally regulated events and (2) that EMT involves switch in cell fate.

However, few cell biologists studying cultured cell models cleave to the strict and narrow view of EMT expounded above. Rather they have used the term more liberally to define a recognizable change in cellular phenotype, generally involving loss of cell junctions and gain of a migratory behavior. Indeed, it is common practice to use EMT as shorthand for changes in cell shape from a coherent “epithelial” monolayer to a “mesenchymal” or migratory fibroblastic phenotype. In this more inclusive definition, EMT is viewed as a complex, co-ordinated process that produces rapid morphological and behavioral phenotypic change [3]. In this “big tent” view, the terms “epithelial” and “mesenchymal” refer to two extremes in a spectrum of phenotypic states within a lineage rather than to lineage conversion. In this usage “epithelial” refers to social cells that tend to stay put, express cadherins and cytokeratins and display apico–basal–lateral polarity whereas mesenchymal refers to individualistic cells with fibroblastic morphology and behavior that are frequently bipolar, lack cell–cell contacts, have transcytoplasmic actin and express vimentin [3,7]. The expanded view of EMT also encompasses reversible change. Importantly, cell culture studies have conclusively demonstrated that these complex programs of co-ordinated molecular and phenotypic changes can be reproducibly instigated by experimental agents that antagonize cell–cell adhesion or by the introduction into cells of transcriptional repressors of cadherins such as snail, slug and Twist [8-11].

Pathologists have questioned the relevance of EMT for cancer and raised two main issues with its widespread assumed role in cancer progression [2]. First, they contend that transmutation, or cell fate switch from one lineage to another, does not occur in tumor samples. Second, they argue that the chaotic histopathological features seen in breast tumors do not fit with the concept of a synchronized series of co-ordinated events that occur during developmental EMT. They conclude that although EMT has an attractive and compelling logic, a large measure of wishful thinking by scientists lacking experience in diagnostic pathology propels extrapolation of its relevance for metastasis. They further argue that where similarities exist between metastatic events and EMT they have arisen randomly through genomic instability. Though harsh, there is a valid kernel of truth to the fact that few scientists working in the field of breast cancer are well-informed about breast tumor pathology. However, while pathologists strive to derive prognostic significance from observing tumors, biologists seek to explain dynamic processes of tumor etiology and progression. Cell and developmental biologists have countered that the failure to see molecular evidence of EMT in histological analyses of clinical specimens may relate to the fact that such changes are transient and fleeting and occur in a minor population at the margins of primary tumors and that full and detailed molecular analyses are not routinely performed [3].

Thus, while both parties to this debate agree that metastasis involves adhesion change, directed migration, dissemination, and preferred destinations for outgrowth, they disagree on definitions and on the pathological relevance of EMT. Amid this debate it is perhaps important to ask if there is any evidence of functional convergence in the mechanisms underlying EMT and metastasis, or approaching the question another way, have studies on EMT resulted in findings of clinical significance? Here breast pathologists must concede that the study of changes loosely described as EMT that result from addition of adhesion-inhibitory cadherin antibodies to epithelial cells paved the way to the significant discovery that loss of heterozygosity in the 16q21.1 locus containing the E-cadherin gene, CDH1, is a critical event in 85% of lobular breast cancer [12-14]. Lobular breast cancer accounts for 5–10% of all breast cancer, and loss of E-cadherin expression is now widely utilized in its diagnosis. Thus, the concept of EMT has

borne fruit in the clinical arena by fueling discovery of the most important breast tumor suppressor protein after p53.

That said, individual cell migration as postulated by EMT, may not be the only way that cells can metastasize around the body. Indeed there are many examples of developmental pilgrimages where cells travel together. As such emigrations may equally inform the clinical setting, we begin this issue with a lucid review by Montell and colleagues highlighting the powerful use of genetic screens to study cell migration in *Drosophila*. Denise Montell's work identifying genes involved in border cell migration is familiar to audiences working on ovarian cancer and has profound implications for the study of breast cancer. These studies provided genetic evidence linking pubertal hormonal transactivation through the Drosophila homolog of AIB1 "Amplified in Breast Cancer" gene to cadherin modulation and cell migration. Further underscoring the import and relevance of their approach for our understanding breast cancer is the identification of C/EBP, STATs, and EGFR in the control of directed migration. This work was one of the first studies to suggest the counterintuitive view that cadherin *modulation* rather than cadherin loss of expression is critical to cell migration.

The connections between estrogen and cadherins have been further strengthened by recent studies on members of the metastasis tumor antigen family, reviewed by Rakesh Kumar and colleagues. MTAs 1–3 are integral components of the chromatin remodeling machinery and transcriptional co-regulators of the steroid hormone response. MTA-3, for example, is itself transcriptionally regulated by estrogen and functions to co-repress Snail, which in turn inhibits E-cadherin transcription. By this mechanism estrogen positively regulates E-cadherin expression. These studies provide a clear understanding of how estrogen regulates tissue morphology during normal development and illuminate why hormone-negative and hormone-unresponsive breast cancer have poor outcome. Though the role of E-cadherin loss in lobular breast cancer is clear, the involvement of cadherin loss in ductal breast cancer is less well-understood [15]. Indeed, following from seminal observations of Walsh and Doherty on the effects of N-cadherin on FGFR signaling during neurite outgrowth, a series of studies have suggested that the migratory behavior of breast cell lines correlates more tightly with N-cadherin up-regulation than with E-cadherin down-regulation [16-20]. Rachel Hazan presents an overview of her recent work on this topic, which has provided a mechanistic understanding of the proinvasive effects of N-cadherin promotion of FGFR function and, moreover, provided important validation of the role of N-cadherin EMT-like changes in human clinical samples.

Moving on from mechanisms initiating tumor cell dissemination to the forces driving cell migration, William Muller and colleagues review the role of integrins in locomotion. In addition to the well-known functions of integrins in cell-matrix attachment this article reviews recent studies on their role in modulating the effects of growth factors, extracellular proteases and chemotactic molecules. Integrin activation of small GTP-binding proteins is tightly coupled to actin cytoskeletal rearrangements that generate traction and alter cell shape during cell migration. The ability of integrins to augment matrix and cell junction degradation via promoting the expression and clustering of MMP and uPA proteases along invadopodia is critically linked to their adhesive function in steering cells through collagenous and endothelial barriers. Co-operation between integrins and growth factor receptors contributes to chemotactic guidance. Integrin knock-out mice also show that integrins are essential for the emergence of metastatic tumor cells from a dormancy into an actively proliferating state and for some aspects of directed migration.

The theme of chemotactic guidance is expanded upon in the following article by Jeff Segall and colleagues. As is the case with primitive organisms, such as slime molds, breast cells sense and move towards gradients of chemokines and growth factors. These are released by stromal cells and macrophages, and stimulate G-protein-coupled receptors and receptor tyrosine

kinases, which promote directed cell migration by stimulating highly localized dynamic changes in the actin cytoskeleton leading to focal cell protrusions. This process is critical to migration towards and intravasation into blood vessels. This article also highlights the role played by stromal paracrine factors that stimulate directed cell migration and the cytoskeletal changes induced within breast tumor cells.

The manner in which tumor cells co-opt normal stromal cells to support their growth is the topic of the following article co-written by Sarah Hatsell and Andra Frost. Recent work from Sarah Hatsell has demonstrated the importance of repressing Hedgehog signaling for the normal development of the embryonic mammary gland whereas Andra Frost has shown that Hedgehog activation occurs in breast carcinoma [21,22]. This article reviews recent data that show that failure to repress Hedgehog activation in carcinoma cells and stromal misactivation of Hedgehog signaling critically influence tumor progression in both human and mouse models of cancer.

A fascinating aspect of cancer metastasis, also related to the specificity of tumor-stromal or seed and soil attributes, concerns the distinct patterns of metastatic colonization. As reviewed by Yibin Kang and colleagues, considerable strides in the imaging of metastatic cells and functional genomics have set the stage for a fuller understanding of the concept of organotropism. Selection of human breast cell lines that show specific organotropic patterns of dissemination to bone, lung, brain and liver has paved the way for refined functional genomic analysis [23]. Gene signatures derived by this approach have been able to accurately predict from primary tumor samples patients at high and low risk for lung metastasis, suggesting that propensity to metastasize is, to some extent, ‘hardwired’. Derivation of distinct profiles for lung and bone metastases has set the stage for a greater understanding of the biological mechanisms underlying organotropism of metastasis (i.e., the ‘Seed and Soil Hypothesis’ of metastatic colonization) and the advent of tailored therapeutic intervention.

The last two articles in this issue deal with experimental approaches that are critical for the identification, development and testing of biological therapeutics. Kedar Vaidya and Dan Welch review the literature on the functional identification and biological characterization of metastasis suppressors, a complex group of proteins that act at multiple stages of the metastatic cascade to specifically prevent metastasis without blocking primary tumor growth. These factors have diverse cellular functions and act at distinct stages in the metastatic process. Understanding their function is necessary to provide a rational basis for the development of drugs that can mimic their effects. And, while this issue began with *Drosophila* models, mouse models have been pivotal to the development of our understanding of the etiology of breast cancer. In the last article, Jos Jonkers and colleagues review the contributions and limitations of transgenic, knock-out, allograft, xeno-graft and humanized mouse models. Examples of recent developments in the creation of more sophisticated conditional and regulatable models of human breast cancer are described in the context of their potential use in understanding disease progression and providing physiologically relevant test systems for assaying therapeutics.

In summary, we solicited and arranged articles that touch on aspects of tumor progression beginning with events that facilitate initial dissemination, forces that propel and direct cell migration and intravasation, factors that promote specific patterns of colonization and experimental approaches to inhibit and model the process more effectively. In shaping this issue, we attempted to provide the reader with a flavor of the exciting new studies and approaches that are being taken in order to understand individual steps in the complex process of tumor progression. Our goal is to present data so that healthy deliberations on the merits of the data supporting and/or refuting particular stances can be done. The relevance of EMT to metastasis and whether metastasis is an adaptive or selective phenotype will continue, and

dialog between the various camps will foster experimentation that will resolve the differences. Another positive aspect of these debates is that they highlight the need to develop a common language from bench to bedside if we are to solve breast cancer. We appreciate the efforts of our colleagues who, recognizing this chasm, have written balanced papers that mete out understanding of both the biology and pathology of the breast and feel that this journal also serves a special role in fostering this synthesis.

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