Translating the Metastasis Paradigm from Scientific Theory to Clinical Oncology

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Abstract
Cancer metastasis contributes to significant morbidity and mortality. Patients with metastatic cancer, often considered incurable, are provided with either supportive care or aggressive management without curative intent. Despite decades of research toward unraveling cancer progression mechanisms, the current body of knowledge has not translated into effective antimetastasis therapies, but recent findings challenge the classic notion that metastases develop during the late stages of carcinogenesis. Here, we evaluate the scientific evidence in the context of the multistage metastasis model. The resolution of current controversies has implications on both the prognostic value of molecular technology and the future of targeted therapies for the clinical benefit of metastasis patients.

Metastasis and the Cancer Patient
Cancer is a heterogeneous disease, and patients can experience a variety of outcomes following diagnosis, including metastasis of the primary cancer to a distant site. Metastatic cancer is particularly challenging for the medical field and is largely responsible for complications and mortality associated with most epithelial malignancies such as lung, colon, breast, prostate, liver, stomach, and pancreatic cancers. Furthermore, metastasis is typically difficult to cure by conventional surgery, radiation therapy, and chemotherapy and confers poor prognosis for the affected patient. As a result, 90% of human cancer deaths are attributed to local invasion and distant metastases (1).

The consideration of metastasis is clinically relevant in both the evaluation and the treatment of the cancer patient. At the time of diagnosis, evaluating the patient for the risk of developing clinical metastasis is important for prognosis and for determining the potential benefit of systemic therapy. Patients at high risk for metastasis would ideally be treated with therapeutic agents that disrupt the spread of disease or hinder growth at the distant site.

Early Theories of Cancer Metastasis
The development of clinically useful therapies against metastasis must begin with a detailed understanding of the origins and mechanisms of cancer spread, concepts that have been studied for more than 100 years (Fig. 1). Significant insight was first attained through Stephen Paget’s (2) observations that cancer spread seemed to be dependent on the cross-talk between cancer cells (seeds) and the specific metastatic organ site (soil). Ewing (3) challenged this notion by proposing an anatomic mechanism for cancer spread, whereby dissemination occurred through mechanical factors associated with the structure of the vascular system. These notions, which persisted for half a century, were followed in 1975 by Bross’ (4) metastatic cascade theory, which showed that the spread of metastasis was not random but rather occurred in steps that required one or more disseminating sites.

Controversy over the Metastasis Paradigm
In recent years, scientific research has blossomed and is in a prime position to address both clinical prognosis and the treatment of metastatic cancer. The excitement in the field is fueled by the likelihood that fundamental biological differences exist between metastatic and nonmetastatic cancers. The identification of these differences is of clinical importance because they may be used to discriminate patient subpopulations and represent a starting point for developing targeted therapies against the metastatic process.

Despite the scientific progress in cancer biology, therapies that specifically disrupt molecular pathways associated with metastasis have not been clinically successful, and this failure of clinical translation warrants a reevaluation of our understanding of metastasis. In this regard, two opposing viewpoints have been debated in recent years. Whereas some have shown that molecular alterations of the primary cancer dictate the ability to metastasize, others contend that metastasis develops from a minority cell population following seeding and selection at a distant site. These viewpoints are contradictory but not mutually exclusive.
It has generally been accepted that success in forming a primary tumor depends on a series of genetic and epigenetic changes in premalignant cells that allow them to escape from normal cell growth signaling controls, to resist proapoptotic stimuli, and to induce angiogenesis (1). As a metastatic lesion is an extension of a primary tumor, the traditional view on metastatic progression has leaned heavily toward the clonal selection hypothesis. However, the traditional view has been challenged as being conceptually inconsistent because the acquisition of a metastasis phenotype may not be equated with an increase in proliferative capacity at the primary site (5). Under such conditions, a cell that is “metastasis enabled” would remain extremely rare in the primary tumor mass, and thus the probability of selecting and expanding a metastatic lesion would likely be minimal. One question in particular is at the heart of this issue: is metastatic capacity embedded within the genetic identity of the primary tumor? This notion has led to the dual proclivity model, in which conditions other than clonal selection lead to metastasis (5). The answer has direct implications on the use of molecular technology for patient evaluation and treatment.

The Classic Metastasis Model: Clonal Expansion and Selection

Cancer develops from normal human tissue via a series of genetic and epigenetic events (1). In their landmark studies, Vogelstein et al. (6) showed that sequential mutations involving the loss of tumor suppressor genes and the gain of an oncogene are responsible for cancer development in the majority of patients with colorectal adenocarcinoma. These observations supported the model of carcinogenesis as a clonal expansion of mutant cells that acquire proliferative advantage and invasive properties.

Metastasis has been thought to be a logical extension of this model, in which at a late stage of disease, invasive cells acquire the capacity to systemically disseminate and grow at a distant site. Because metastatic properties may not be selective for clonal proliferation, the cells responsible for metastasis may be rare within the primary tumor. This viewpoint is supported by the clinical observation that metastatic lesions are rare events despite the continual dissemination of significant numbers of tumor cells into the circulation (7–9). To experimentally support this model, Fidler and Kripke (10) developed subclones from melanoma cells in a murine model and first showed metastatic heterogeneity, including the existence of a distinct subpopulation of highly metastatic cancer cells.

Since those initial experiments, the murine selection model has been used in conjunction with comparative genomics to identify the genetic mechanisms related to metastasis. Specifically, cancer cell lines were implanted into host mice and were allowed to grow and metastasize. The metastatic cells were then isolated and expanded in vitro. Repeated selection by this method resulted in cell lines with enhanced metastatic potential upon further implantation compared with the parental cell line. Clark et al. (11) used this model to describe distinct gene expression patterns in high versus low metastatic melanoma cell lines, including the metastasis-related

Fig. 1. The evolution of metastasis theories. A timeline is presented outlining the major conceptual advances in our understanding of the origins and mechanisms of metastasis, from Paget’s seminal “seed versus soil” hypothesis to more recent theories about the role of stem cells and early developmental transitions.
enancement of RhoC. These studies suggest that the capacity to metastasize is an intrinsic property of a subpopulation of malignant cells and that metastatic heterogeneity is a defining characteristic of the primary tumor.

Other studies have shown that cancer cells isolated from metastases in a murine xenograft model not only have enhanced metastatic potential but also retain specificity for the distant organ of metastasis. Massagué et al. (12) selected for a subpopulation of MDA-MB-231 breast cancer cells with tropism for bone metastasis, which showed differential expression of a distinct set of genes with multiple functions. A unique gene expression pattern was also observed in a MDA-MB-231 subpopulation with tropism for lung metastasis (13). Interestingly, a subset of the differentially expressed genes from the in vivo selection for metastasis could also predict clinical lung metastasis based on gene expression profiles from primary cancers, suggesting that some of the genes involved in the selective and site-specific nature of metastasis may also be reflected in the genetics of primary cancer.

### Metastasis as a Property of the Primary Cancer

A pairwise comparison between primary and metastatic cancer tissues is possible with the availability of high-resolution and high-throughput technology for gene expression profiling. Such experiments have challenged the clonal selection model of metastasis. Gene expression profiling analysis has shown that paired primary tumors and metastases are similar, whereas a significant difference is observed when primary tumors with or without metastases are compared. Consistently, multiple reports have used gene expression profiles of primary tumor samples to predict metastasis and poor clinical outcome (14–16). These studies take a bioinformatics approach and offer little with regard to the exact biological mechanisms underlying the metastatic process. Nonetheless, the success of the molecular profile of the bulk tumor in predicting metastasis defies the theory that a rare variant within the tumor population is chiefly responsible for the spread of disease.

Direct comparisons of genetic profiles have been done between primary tumors of the breast and liver and their matched metastases. When unsupervised clustering is done, samples from the same patient almost always clustered together (16–18). Moreover, identical expression patterns are observed between primary liver cancer (16) and their extrahepatic metastases. That a metastasis is more similar to its paired primary tumor compared with other metastases suggests that there may not be an integral set of changes that are selected for during the metastatic process. Rather, the genetics of the primary cancer may determine the capacity of the tumor to metastasize. In addition, epigenetic mechanisms such as methylation status or microRNA activities may affect the ability to metastasize. Consistently, a recent study suggests that the genetic machinery that causes metastasis is hardwired into the primary tumor because metastatic foci harbor few genetic alterations compared with their corresponding primary cancer (19). Furthermore, clinical observations reveal that ~5% to 10% of patients with metastasis have a cancer of unknown primary site (20), and recent experimental studies indicate that early disseminated cancer cells may account for metachronous metastases (21), suggesting that systemic dissemination may be an early event in cancer development. These studies suggest that metastatic capacity is embedded in the majority of cells within the primary tumor and may be determined at an early stage of carcinogenesis.

### Limitations and Reconciliations

To establish the current metastasis models, researchers have used experimental mouse systems or have established genetic profiles based on patient samples. These approaches have led to contradictory conclusions about the nature of metastatic cancer. Unfortunately, both methods are imperfect and may not be able to capture the true biology of metastatic disease.

In the mouse models, repeated cycles of in vitro culture and in vivo selection result in highly metastatic variants. However, the evidence is based on cell lines adapted to culture conditions, and the repeated selection process does not recapitulate the nature of human disease. These experimental types could certainly be confounded by artifacts in cell lines occurring through extended passaging and ensuing genetic instability. In addition, the host environment of xenograft transplantation models does not necessarily recapitulate the human scenario, and thus the extension of these findings to the human condition should not be taken as an exact correlate. Meanwhile, in the gene profiling studies comparing primary human samples, the similarities observed between the primary cancer and its corresponding metastasis could likely reflect two intertwined scenarios. The first involves alterations of a few important “true” metastasis genes that are necessary to promote this phenotype but do not alter global gene expression; thus, they may not be readily identifiable through microarray technology. Although these few changes may be absolutely necessary to generate this phenotype, the actual magnitude of the change or type of change (expression alteration, for example) may not be large or deemed significant by the chosen cutoff variables of the study and, thus, may not be extracted as a significant alteration in high-throughput global analyses such as microarrays. In other words, those genes with the largest and most significant changes are those that are usually chosen for follow-up in array-based studies, whereas those with minimal changes are usually not pursued. But perhaps, these smaller changes in genes are where the true alteration necessary for metastasis lies. Thus, we may have left the true metastasis genes by the wayside in pursuit of other genomic changes that alter phenotypes such as proliferation, etc., which affect not only the process of metastasis but also the growth of the primary tumor. The second involves the role of tumor cell heterogeneity and tumor-associated stroma cells present in the bulk of tumor specimens. The alterations in these tumor-surrounding tissues, affecting a vast number of genes, may overshadow that of true metastasis-promoting events. Significant alterations of gene expression profiles in noncancerous tissues from patients with metastatic cancer have been observed (22, 23). Such alterations may be due to tumor-infiltrating immune cells from the microenvironment, which may contribute to metastasis either positively or negatively. This occurs due to tumor heterogeneity and the diversity of inflammatory cells that are located in the stroma or infiltrate the tumor. Examples of inflammatory cell infiltrates include tumor-associated macrophages, considered

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to be associated with angiogenesis and poor outcome, and dendritic cell infiltration, typically associated with good outcome due to their induction of T-cell responses and presentation of tumor antigens (24, 25). In addition, myeloid-derived suppressor cells inhibit immune responses and facilitate tumor growth and metastasis, whereas T-cell infiltration is generally associated with a good immune response (26). Certain populations of T cells, however, such as the T-regulatory cells or those alternatively activated by Th2 cytokines are associated with metastasis and poor outcome (27). Therefore, the accurate assessment of immune cell distribution, phenotypes, and the status of inflammation are critical factors for the assessment of metastasis proclivity, and thus the inflammatory status may therefore contribute to metastatic tendency. In addition, the alteration of the tumor microenvironment may reflect host genetics, imparting a high risk for metastasis as a result of inherited factors (28). It is possible that a few tumor-associated metastasis genes are necessary but not sufficient to form a metastatic lesion unless they are supported by stroma-associated events, an underlying concept of Paget's metastasis model. Thus, both the methods (i.e., array technology) and the role of the microenvironment/inflammatory status may significantly and dually affect the ability to identify true metastasis genes.

Based on the evidence described above, it is likely that both the intrinsic properties of the primary tumor and selective mechanisms are involved in metastasis. Within the framework of the metastatic cascade, we speculate that the early steps of metastasis may be governed by the intrinsic properties of the primary tumor, which are defined by the cell of origin and the genetic and epigenetic events of tumorigenesis. These properties are likely to determine the capacity of the primary cells to exit the primary site and survive systemic dissemination. Such characteristics would be reflected in the genetic signature of the primary cancer and would allow for the prediction of metastasis by molecular methods. Consistently, Gupta et al. (29) recently identified four genes that can promote both primary tumor growth and metastatic outgrowth. Moreover, the intrinsic properties of the tissue of origin may play a role in the organ specificity of metastasis as shown by Paget's classic observation that certain types of cancer tend to metastasize to specific organs (2).

However, the intrinsic properties of the primary cancer do not confer the complete set of genetic changes for disseminated cells to complete the metastatic process. This view is supported by the observation that relatively few metastatic lesions develop from up to $10^7$ circulating tumor cells within a cancer patient (7–9). In this case, distant metastases may represent stochastic or selective events. The enhancement of metastatic potential following serial transplantation in an animal model suggests that metastatic cells undergo phenotypic changes that lead to their growth advantage at distant sites. This advantage may be a result of selection or cellular adaptation for proliferation in a foreign microenvironment. Alternatively, the inefficiency of tumor cells to metastasize may be due to the presence of cancer stem cells because these cells generally represent a minor population of tumor cells and share many common properties of metastasis (30). Another viewpoint comes from the studies of the critical role of the epithelial-mesenchymal transition in metastatic progression. It has been shown that most carcinoma cells access and exploit components of the epithelial-mesenchymal transition program to acquire malignant cell traits (31, 32). It is possible that cancer cells may act autonomously by creating an epithelial-mesenchymal transition state or recruiting stromal cells to induce the epithelial-mesenchymal transition program, although these two scenarios seem difficult to distinguish. Taken together, the evidence suggests that the metastatic capacity of any cancer cell is most likely a function of both the intrinsic properties of the bulk tumor and the ability of individual cells to withstand selection pressures at a distant site.

### Implications for Prognosis and Targeted Therapy

Understanding the mechanisms behind metastasis has clear implications for the future of clinical oncology. Early reports
have already established gene expression profiles that are able to predict metastasis and outcome, including metastasis profiles identified in our laboratory based on the expression of mRNA and microRNA patterns in primary hepatocellular carcinoma (HCC) specimens and in their surrounding noncancerous tissues (16, 33, 34). These models may be useful for prognostic and identifying patients who require systemic therapy, but further validation is necessary to establish their value in comparison with existing clinical variables.

Additionally, understanding the sequence of molecular events at various stages of metastasis may aid the identification of therapeutic targets. In particular, a patient who develops metastasis likely has disease that has undergone distant colonization at the time of diagnosis; therefore, only the mechanisms governing growth at the distant site are of clinical significance to his or her treatment. This reinforces the need to develop therapeutic targets with specificity for the metastasis itself. There have been many attempts to develop HCC therapeutic agents; however, many are geared toward processes shared between tumorigenesis and growth rather than a particular metastasis-specific event (Table 1). A lesson may be taken from the failed phase III trials of inhibitors of matrix metalloproteinases. Although matrix metalloproteinases have been shown to have multiple roles in the metastatic process, their most important functions may be in facilitating the escape of the cancer from the primary site. In addition, a growth factor family targeted in HCC is epidermal growth factor receptor or human epidermal growth factor. These receptors are involved in a signaling pathway that eventually leads to the activation of a tyrosine kinase intracellular domain, which serves as a docking location for intracellular signaling molecules that bind to phosphotyrosine and lead to the modulation of cell proliferation, invasion, apoptosis, angiogenesis, and metastasis. Small-molecule compounds such as erlotinib and lapatinib (35, 36), which inhibit tyrosine phosphorylation, have been reported in clinical trials for HCC. Although these compounds were well tolerated, their antitumor effect was only modest. Cetuximab is a chimaeric monoclonal antibody directed against the epidermal growth factor receptor and blocks the binding of this receptor to its ligands. Although cetuximab leads to growth inhibition, its antitumor activity has been found to be quite low (37). These drugs, however, are mainly targeting tumor growth and not the process of metastasis.

In contrast, the anti–vascular endothelial growth factor antibody bevacizumab targets angiogenesis in the growth of metastatic cancer following colonization and is currently used as a first-line agent against metastatic colorectal cancer (38). The use of this drug in an adjuvant setting and in combination with other therapeutics is also warranted. Other vascular endothelial growth factor–related agents include sorafenib (an oral multikinase inhibitor that blocks tumor cell proliferation and angiogenesis mainly by targeting Raf/mitogen-activated protein kinase kinase), sunitinib, and TSU-68 (oral antiangiogenesis compounds). Sorafenib has recently been described to improve survival in a recent randomized control trial in advanced HCC; however, the survival benefit is only by an extension of 3 months, and thus other drugs that provide longer prognosis are needed (39). Sunitinib has also shown promise in phase II trials of advanced HCC and warrants a large-scale randomized trial (40). Results from clinical trials using individual or combined therapeutic agents suggest that synergistic effects may be achieved when drug regimens are combined. In fact, combinations of epidermal growth factor receptor and vascular endothelial growth factor inhibitors such as erlotinib and bevacizumbab show promising results (41). Thus, drugs targeting more specific alterations associated with metastasis, such as angiogenesis, are more likely to be beneficial in blocking this phenotype.

The Wnt/β-catenin signaling pathway is another important target for the development of novel HCC therapeutics. A large percentage of HCCs show activation of this pathway, which leads to nuclear translocation of β-catenin and up-regulation of downstream transcription factors. Various strategies are being developed and tested in preclinical studies to target this signaling pathway. These include blocking the interaction between β-catenin and TCF and monoclonal antibodies against Wnt-1 and Wnt-2. In our laboratory, we have found that a HCC subtype correlating with stem cell–like features and poor prognosis displays a molecular profile consistent with activation of the Wnt-β-catenin pathway (42–44). This suggests that certain patients with a stem cell–like HCC subtype and activation of Wnt-β-catenin signaling may specifically benefit from Wnt-β-catenin inhibitor regimens. This would allow for more beneficial stratification of patients for the most appropriate treatment regimens, a notion that requires further studies. Although Wnt pathway antagonists have not been efficacious in HCC, the importance of this signaling pathway in HCC warrants future investigation and development of therapies geared toward the modulation of Wnt signaling.

Summary

The identification of clinically useful drug targets is possible with current technology and careful study design. The challenge is to identify molecular changes that distinguish the metastasis from the matched primary tumor. Current reports comparing samples from patients lack statistical power due to the difficulty in obtaining metastatic tissue for analysis. Moreover, to be truly comparative and to ascertain metastasis changes that are not confounded by tumor/sample heterogeneity, experiments comparing primary tumors and metastases must be conducted in a paired/matched fashion. However, the evidence from profiling studies thus far has shed light on the metastatic process and suggests that metastasis may occur through the alteration of rare metastasis-promoting genes and by changes in the nature of the environment for it to thrive. We suggest that molecular profiling of the bulk tissue, which can dually ascertain tumor- and microenvironment-associated changes, may delineate the factors that distinguish a primary tumor mass from its metastasis. We suspect that such analysis will reveal the molecular mechanisms of metastasis and essential genetic changes that allow for survival and drive proliferation within a foreign microenvironment. If these pathways can be targeted with specificity, there will be rekindled hope of finding a cure for metastatic cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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