# Adaptation versus Selection: The Origins of Metastatic Behavior

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## Introduction

During the formation of a primary tumor, premalignant cells acquire a series of genetic and epigenetic changes that enable them to proliferate in the absence of growth factors, to resist proapoptotic stimuli, and to stimulate angiogenesis (1). Each of these acquired traits confers a distinct selective advantage on those cells that develop it. In contrast, it is less clear how the initial steps of the invasion-metastasis cascade can be acquired purely as a result of selection in the primary tumor. These steps include the loss of cell-to-cell adhesion, invasion into the local microenvironment, intravasation into the blood and lymphatic vasculature, and extravasation into the parenchyma of distant tissues. Accordingly, in the absence of such local growth advantage, cells acquiring these malignant traits will not undergo clonal expansion within the primary tumor, and the alleles favoring invasion and metastasis will not be represented in large numbers of neoplastic cells within this tumor. Hence, as argued below, in most human tumors, acquisition of these malignant traits is unlikely to arise as a consequence of selection occurring in the context of the primary tumor.

Most, and possibly all, carcinoma cells that disseminate from primary tumors are poorly suited to survive and proliferate in the foreign tissue environments in which they happen to have landed. This suggests that Darwinian selection is indeed important during the last step of the invasion-metastasis cascade, termed colonization, which depends on the ability of the disseminated cancer cells forming a micrometastasis to adapt to the foreign tissue microenvironments at the site of final dissemination. It is clear that the vast majority of micrometastases fail to devise the means to survive and thrive in such novel microenvironments and eventually disappear. Here, enormous selective pressure operates to favor the outgrowth of the cells in a rare micrometastasis that can indeed solve the problem of adaptation. Still, this colonization step does not illuminate the processes occurring during the preceding steps of the invasion-metastasis cascade—the topics of this article.

As we argue here, many of the cellular phenotypes associated with the earlier steps of this cascade, such as motility and invasiveness, do not arise as purely cell-autonomous processes. Instead, recruited stromal cells seem to play key roles in the acquisition of these traits by carcinoma cells, and adaptation of cancer cells to these signals, rather than selection, seems to be key to initiating these phenotypes (2).

## The Epithelial-Mesenchymal Transition

Our mechanistic insights into how adaptation may operate have been greatly increased by the discovery that within normal and neoplastic cells, a normally latent transcriptional circuitry resides that can confer on cancer cells many of the traits needed to complete the initial steps of the invasion-metastasis cascade. Developmental genetics has revealed that this circuitry is controlled by a set of master regulators that induce the transdifferentiation program termed the epithelial-mesenchymal transition (EMT), through which epithelial cells lose their epithelial traits and acquire instead many of the attributes of mesenchymal cells, including a loss of association with epithelial cell sheets and acquisition of cell motility, invasiveness, and resistance to apoptosis (3, 4).

Normally, EMT programs are used to effect a number of critical morphogenetic steps during development, such as the formation of mesoderm and the migration of neural crest cells. Reflecting this complexity, this transdifferentiation program can be choreographed by a number of distinct, pleiotropically acting transcription factors, such as Snail, Twist, Slug, and Goosecoid (5–8). When active, each of these factors can initiate the complex transcriptional program that imparts to cancer cells many of the traits associated with high-grade malignancy. The involvement of these transcription factors in cancer pathogenesis exemplifies a situation in which cancer cells opportunistically co-opt cell biological programs that are normally operative during early embryogenesis (and perhaps during certain types of wound healing). Because expression of these transcription factors by embryonic cells is provoked by contextual signals that these cells receive from their surroundings, it is likely that such signals act similarly during cancer pathogenesis to trigger expression of these EMT-inducing transcription factors in cancer cells.

Although hardly proven, it is plausible at present that most human carcinoma cells access and exploit components of the EMT program to acquire malignant cell traits. Indeed, it is even possible that nonepithelial cancer cells also use this program when they progress to highly malignant growth states.

At the biochemical level, the EMT program involves the downregulation of epithelial protein expression, notably E-cadherin and cytokeratins, and the induction of mesenchymal protein expression, including vimentin, N-cadherin, fibronectin, platelet-derived growth factor receptor, and matrix metalloproteinases. These changes are accompanied by the aforementioned cell biological changes, such as acquisition of motility and invasiveness (reviewed in ref. 5). When active in cancer cells, this program should enable them to complete the initial steps of the invasion-metastasis cascade, specifically local invasion, intravasation, survival while in the circulation, and extravasation. Although a transition from a completely epithelial to a completely mesenchymal state may occur in some human tumors, it seems likely that, more often than not, cancer cells advance partway into the EMT program, shedding some but not all epithelial markers and acquiring concomitant expression of some but not all mesenchymal markers. Importantly, the EMT is reversible, and carcinoma cells that have acquired a mesenchymal phenotype can revert to an epithelial state via a mesenchymal-epithelial transition (9).

The question of whether invasive and metastatic behavior is acquired as a consequence of selection or adaptation can best be

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addressed by studying the state of the EMT program in a variety of tumors, posing the question of whether the EMT program is turned on reversibly in tumor cells or is constitutively activated in such cells. As argued here, if the EMT program is reversibly activated in carcinoma cells, then the case for adaptation is strongly supported; conversely, if the EMT program is activated irreversibly (constitutively), then the case for selection is favored.

## Regulation of the EMT Program

We actually know rather little about the signals and circumstances that are responsible for activating the EMT program in cancer cells. The seminal work of the Beug group showed that this program can be activated in *ras*-transformed mouse mammary carcinoma cells simply by the application of transforming growth factor-β to cells *in vitro* (10). This discovery represented the first demonstration that the EMT program in cancer cells can be triggered through signals that they receive from their microenvironment. Hence, cancer cells bearing multiple mutated genomes need not sustain additional mutations to activate this program, doing so instead in response to contextual signals that they receive from nearby cells, ostensibly those forming the tumor-associated stroma.

This model has been sustained by many other subsequent reports, indicating that a variety of extracellular signals can contribute to triggering the EMT in the context of neoplasia; included here are the signals conveyed by hepatocyte growth factor (11), tumor necrosis factor-α (12), Notch (13), Hedgehog (14), Wnts (15), and platelet-derived growth factor (16). These reports and others suggest that an EMT is usually triggered by a convergence of several of these signals. (Because the EMT is used to effect morphogenetic steps in a variety of tissue sites during embryogenesis, it seems unlikely that a common, invariant set of heterotypic signals is responsible for inducing this program in all types of tumor cells.).

Implicit in these models is an important assumption—specifically that such EMT-inducing signals do not originate in the cancer cells themselves, and therefore do not form a self-initiating autocrine signaling loop. Instead, they seem to originate in other cell types, for example those forming the stroma. As such, the signals responsible for activation of the EMT would seem to exemplify paracrine signaling, more specifically, heterotypic signals flowing from stroma to epithelium within a carcinoma.

The notion that microenvironmental signals are responsible for activating the EMT holds a number of important further implications. Because the putative inductive signals are likely to act only over short distances, as is the case with most paracrine factors, it seems likely that only a subset of cells within an epithelial cell island will experience these signals and undergo an EMT. In fact, in the context of colon cancer pathogenesis, the EMT has been associated specifically with the cells at the invasive front at the epithelial-stromal interface and with individual carcinoma cells that have left the invasive front and invaded singly into the nearby stroma (17). Moreover, in the authors' laboratory, when studying the behavior of human tumor xenografts, the EMT can be clearly observed to affect a cell layer one to two cells deep that is directly apposed to the stroma; cells lying more internally in epithelial cells islands maintain their characteristic epithelial morphology and continue to express epithelial markers.[1]

[1] K.A. Hartwell and T.A. Ince, unpublished observations.

The notion that contextual signals trigger the EMT suggests its transience and reversibility: When cancer cells within a primary tumor disseminate to distant sites within the body, they will no longer encounter the mix of contextual signals that they experienced in the primary tumor, specifically the signals released by the activated stroma within the primary tumor. In the absence of an ongoing flux of these inductive signals, the disseminated cancer cells may then revert, via a mesenchymal-epithelial transition, to the epithelial phenotype of their ancestors within the core of the primary tumor (5).

Yet a third idea is implied in this model: Although hardly addressed experimentally, it seems likely that certain tumor cells are responsive to the paracrine, EMT-inducing signals whereas others are not. Such responsiveness may be dictated by the differentiation program expressed by the cancer cells, and may thus be reflective of their normal cell of origin. Even more important, however, may be the spectrum of genetic and epigenetic lesions that the cancer cells have accumulated during the course of multistep tumor pathogenesis. Included here are both mutations as well as DNA methylation events and resulting silencing of gene promoters. One set of these somatically acquired changes may render a cancer cell responsive to these EMT-inducing signals, whereas another set may not confer such responsiveness, leaving the cancer cells refractory to induction of an EMT. Moreover, it seems likely that fully normal epithelial cells will not be responsive to the EMT-inducing heterotypic signals that succeed in eliciting an EMT from derived carcinoma cells.

To summarize, successful induction of an EMT would seem to be dictated by a confluence of at least three factors: (*a*) the differentiation program inherited by tumor cells from their normal cell of origin; (*b*) the set of heritable, somatically acquired changes accumulated during the course of tumor development; and (*c*) the mix of heterotypic signals that islands of carcinoma cells receive from the nearby activated stroma.

## Implications of Induced EMT on the Adaptation versus Selection Debate

It remains to be seen how often the EMT program is activated reversibly in commonly occurring human tumors. Progress in addressing this issue has been held back by several obstacles. First, antibodies that can be used to detect EMT-inducing transcription factors have been lacking. Second, in the event that epithelial cancer cells undergo a full EMT, it is difficult to resolve them from the mesenchymal cell types that exist naturally within the tumor-associated stroma. Third, and perhaps most importantly, as depicted above, the EMT may only involve a small subset of carcinoma cells—the minority that reside at the interface between epithelium and stroma within a carcinoma. Consequently, analytic procedures that are used to assess the importance of EMTs may often fail to do so, simply because they analyze entire carcinoma cell populations within a tumor, rather than the small minority of carcinoma cells at the invasive edge.

Validation of the frequent and critical role of the EMT in carcinoma pathogenesis holds important implications for the adaptation versus selection debate. We imagine that during the early stages of multistep tumor progression, preneoplastic cells successively acquire a range of alleles that confer on them increasing proliferative and survival advantage. As mentioned, at this stage, the role of selection is clear and unambiguous. Importantly, these cells are undergoing such Darwinian evolution

in a tissue microenvironment that still contains relatively normal stroma. Hence, these cells are not evolving in a context defined by EMT-inducing heterotypic signals.

All this changes as the primary tumor reaches its final preinvasive state. The tumor cells may have acquired an array of alleles that render them responsive to EMT-inducing heterotypic signals. Equally important, at a certain point during progression, the surrounding stroma becomes "activated," as indicated by the accumulation of myofibroblasts and a variety of inflammatory cells; for the first time, the stroma can release EMT-inducing signals, and only now does the induction of an EMT in the carcinoma cells become possible.

This scenario represents a clear example of the acquisition of highly malignant cell biological traits through an adaptive mechanism. Thus, as preneoplastic cells proceed through the multistep evolution that leads them to the final preinvasive stage, these cells cannot anticipate future encounters by their descendants with EMT-inducing signals that will eventually be released by the nearby stroma. Moreover, because these preinvasive cells do not yet display the phenotypes of an EMT, such phenotypes cannot affect their reproductive fitness during these earlier, preinvasive steps of tumor progression. Finally, because the EMT is often exhibited by hundreds of cells at the outer edges of an epithelial cell island, it is highly unlikely that these cells are the results of a stably heritable trait (which is required for selection to operate), rather than a localized adaptation to a stromal signaling environment.

## Gene Expression Arrays Provide Further Evidence for Adaptation

Use of gene expression arrays to provide prognostic information about primary human breast cancers sheds further light on the adaptation versus selection debate. It is clear that in a subset of human breast cancers, the spectrum of expressed genes provides a clear indication of a greatly increased risk of eventual metastatic relapse (18). Importantly, because the pattern of expressed genes is readily detectable, it seems highly likely that this set of genes is expressed by the dominant cell clone in each primary tumor. (Conversely, it is experimentally unlikely that this pattern reflects the gene expression pattern of a small minority subclone of neoplastic cells residing in the primary tumor.) Hence, for whatever reason, the clonal expansion of cells expressing this set of genes was favored during multistep tumorigenesis.

In these primary tumors, histopathologic analyses will quite often fail to reveal any of the cellular and tissue phenotypes that are associated with aggressive invasion and metastasis. (Indeed, success in detecting invasiveness and metastatic ability at the histologic level would obviate the use of gene expression arrays as a prognostic tool.) Accordingly, some tumors have a low probability of metastasizing, whereas other histopathologically identical tumors have a high tendency to do so.

Here, one is confronted with cell clones that have undergone clonal expansion in the absence of any demonstrable phenotypic advantage conferred by high-grade invasiveness and metastasis. Hence, to the extent that they eventually become invasive and metastatic, these are unselected phenotypes that have been acquired adventitiously—unintended side products of tumor progression. Indeed, it seems to us increasingly likely that the metastatic powers of such breast cancer cells are strongly predetermined by the differentiation programs of their respective normal cells of origin as well as by mutations that are sustained in the early steps of tumor progression, long before invasive and metastatic traits could possibly be operative. These early influences will, by necessity, become widely represented in the descendant cell clones arising years later, when invasion and metastasis do indeed become phenotypically apparent.

## When Might the Invasiveness and Metastatic Ability Be a Selected Trait?

The scenario depicted above argues strongly for adaptation as the prime driving force that underlies the acquisition of highly malignant traits by neoplastic cells. (Yet other terms, such as "induction" or "education," may ultimately prove to be more useful than "adaptation.") However, adaptation cannot hold true for all types of human cancer, because of a highly interesting subset of these tumors that enter into a state in which the EMT is expressed constitutively (i.e., essentially irreversibly). We refer here to situations in which genetic or epigenetic lesions within the cancer cell genome create a cell autonomous, stably expressed EMT state.

The most apparent of these states derives from numerous types of human carcinoma cells in which the expression of E-cadherin is compromised, either by reading-frame mutations or by methylation of the *CDH1* gene promoter (19). Although we have depicted E-cadherin as one of the downstream effectors of epithelial phenotype and a target of repression by EMT-inducing transcription factors (see above), in fact, E-cadherin, on its own, may function as a pleiotropically acting regulator to elicit most, if not all, of an EMT. More specifically, work from our own laboratory and earlier work of others indicates that when E-cadherin is lost, many of the changes associated with an EMT are triggered (20). In the event that E-cadherin loss is essentially permanent, transdifferentiation to a mesenchymal (or quasi-mesenchymal) state by carcinoma cells may also be.

This alternative way of inducing an EMT clearly depends on a cell-autonomous change, that is, a change acquired by the carcinoma cells themselves. In this respect, it starkly contrasts with the depiction of EMTs that are induced by heterotypic signals originating in the tumor-associated stroma. Because this change is cell autonomous, it qualifies as a possible object of Darwinian selection. Stated differently, if E-cadherin loss is documented in many if not all neoplastic cells within a carcinoma, this loss must have conferred a selectively advantageous, cell biological phenotype, enabling the clonal expansion of the E-cadherin–negative cells.

Of course, the downstream consequences of acquiring an EMT may extend beyond the immediate acquisition of local invasiveness within a primary tumor. It seems highly likely, at this juncture, that cells that are invasive are well equipped to execute multiple additional steps of the invasion-metastasis cascade, including intravasation, translocation through vasculature, and extravasation.

Granting this, we still cannot perceive how metastatic dissemination can be a selectively advantageous trait during the course of primary tumor development. This forces us to confront the remaining possibility: that localized invasiveness (rather than metastatic dissemination) is indeed advantageous to certain primary tumor cells. Precisely why it might be so is hardly dictated by existing observations. However, we can speculate on at least one: Invasion into the stroma affords carcinoma cells greatly

improved access to many of the mitogenic and trophic factors that are usually released by stromal cells and directed through intact basement membranes to the overlying epithelium.

Localized invasiveness may also be advantageous by enabling cancer cells to escape from the hypoxic cores of primary tumors. Recent evidence suggests that hypoxia itself may be an inducer of the EMT (21). It is possible to envision that once the EMT program is turned on, it not only allows cells to escape hypoxia but confers upon them the ability to resist additional apoptotic stimuli, thereby giving them a clear selective advantage. In fact, it has become increasingly evident that embedded in the EMT program induced by master regulators such as Snail and Twist is the ability to resist apoptosis (22–24).

Given the paucity of existing data, the adaptation versus selection debate is far from resolution. We straddle this issue, believing that both sides may be correct: Adaptation is likely to be favored in the great majority of tumors; in these, the EMT is induced reversibly (i.e., facultatively); selection may be favored in the minority of tumors in which the EMT is fixed by alterations in the *E-cadherin* gene. Unaddressed by the present arguments is the possibility that the EMT may be fixed and constitutive in a small minority of tumors by a constellation of alleles (not including E-cadherin) that have been acquired during tumor progression, having been selected initially for their growth-promoting and survival functions. Much remains to be learned.

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# Response

The Counterpoint by Weinberg et al. (1) provides an excellent overview of epithelial-mesenchymal transition (EMT) as a potential mechanism in the metastatic process. Despite the controversy identified by these two reviews (1, 2), there are several points of consensus; first is the assumption that metastasis is a complex process and the implicit agreement that control of this process is critical to clinical outcomes. Within the process of metastasis, as acknowledged in the article by Weinberg et al., there is interplay between tumor cells and infiltrating and pericapsular nonparenchymal cells. Although these are identified by the Counterpoint as epithelial cancer cells that have undergone EMT, it is also recognized in their review that such an origin is difficult, potentially impossible, to differentiate from mesenchymal cells within the tumor-associated stroma. Nonetheless, it is argued that secondary to EMT, epithelial cells lose their epithelial traits and acquire mesenchymal attributes including an increased malignant phenotype. From a histiopathologic viewpoint, and supported by morphology and cellular isolation of nonparachemical cells (3), the presence of tumor-infiltrating mesenchymal cells is associated with infiltration by host inflammatory and vascular cells.

EMT has been a prior theme in the "Point-Counterpoint Reviews" published in *Cancer Research* i.e., those of David Tarin (4) and Erik Thompson and Donald Newgreen (5). In the present Point-Counterpoint Reviews, there are again opposing viewpoints based on pathology (4) and cell biology (3). In our Point (4), we have extended the clonal selection hypothesis to incorporate the timeframe over which this process occurs. We posit, from this perspective, that many competing hypotheses coalesce into the

clonal selection hypothesis; i.e., that the process of tumor cell dissemination, circulation, extravagation, and growth at a secondary site results in the survival of only a minority of tumor cells with the formation of a metastatic foci. One potentially important point of disagreement between the two articles is the concept that clonal selection during metastasis selects for the survival of tumor cells capable of completing the metastatic process and not necessarily for tumor cells that have improved tumor growth. Although this may seem as a semantic issue, it is a critical to understanding the clonal selection hypothesis of metastasis.

The role of EMT in tumor progression and metastasis provides an intriguing hypothesis from which to study the process of metastasis. However, as identified in the counterpoint by Weinberg et al., it is only weakly supported by data from *in vivo* studies (1). Indeed, the cellular heterogeneity shown within tumors, including infiltration by nonparachymical (3) and immature myeloid cells (6), provides an alternative origin for the morphologic, cellular, and molecular processes identified as EMT within metastasis. Further, as discussed by Weinberg et al. (1), some tumor cell characteristics believed to be important to the metastatic process may also facilitate primary tumor growth, providing a second point of consensus with the clonal selection hypothesis. Although, unlikely correct for all metastatic phenotypes, invasive cancer cells are histopathologically apparent as cellular foci adjacent to, yet slightly discontinuous, to the primary tumor. Similarly, some metastatic cells can be identified as paravascular cuffs after arrest, extravasation, and growth as micrometastatic foci. Thus, the histopathologic study of primary tumors, margins, draining lymph nodes, and metastatic foci can provide prognostic as well as mechanistic insight into the process of metastasis. Indeed, biopsies, most notably of the marrow, have been shown to have prognostic significance (7).

The opportunity to engage in a discussion of the role clonal selection has in the process of metastasis is appreciated. It is our fervent hope that these polemic articles will stimulate thought, discussion, research, and most importantly data as regards this topic. However, we would like to stress the need for any discussion of metastasis to incorporate data from experimental animal models with confirmation from human tissue and processes.

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