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Mechanisms of Disease: epithelial-mesenchymal transition and back again: does cellular plasticity fuel neoplastic progression?

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SUMMARY
Epithelial-mesenchymal transition (EMT) is a conversion that facilitates organ morphogenesis and tissue remodeling in physiological processes such as embryonic development and wound healing. A similar phenotypic conversion is also detected in fibrotic diseases and neoplasia, which is associated with disease progression. EMT in cancer epithelial cells often seems to be an incomplete and bi-directional process. In this Review, we discuss the phenomenon of EMT as it pertains to tumor development, focusing on exceptions to the commonly held rule that EMT promotes invasion and metastasis. We also highlight the role of the RAS-controlled signaling mediators, ERK1, ERK2 and PI3-kinase, as microenvironmental responsive regulators of EMT.

INTRODUCTION
Simple epithelia are composed of cohesive sheets of cells connected by tight junctions and polarized in an apical-basal orientation relative to an underlying basement membrane (BM; Figure 1). The surrounding mesenchymal cells are embedded within the interstitial extracellular matrix (ECM); lacking intercellular junctions, they manifest primarily anterior-posterior polarity.¹,² These structural differences are reflected in the characteristic genes each cell type expresses: epithelial cells express distinct junctional proteins like E-cadherin, and epithelial-specific cytoskeletal proteins like cytokeratins, while mesenchymal cells express N-cadherin and mesenchymal-specific vimentin³ (Table 1).
Desmoplasia, the appearance of fibrous, mesenchymal-like tissue in the peritumor stroma, is associated with poor clinical outcome. Recent gene-profiling experiments suggest that the presence of a mesenchymal gene signature in tumors is predictive of poor clinical outcome in colorectal, breast and ovarian cancers. The principal cell types that contribute to the desmoplastic stromal reaction and to the mesenchymal gene signature are fibroblasts, which reside in the stroma and produce interstitial ECM molecules, and myofibroblasts, which produce growth factors, cytokines, and ECM, and which also act to contract the ECM. Myofibroblasts have long been thought to derive from fibroblasts, but recent studies show that a substantial proportion of these cells are derived from EMT associated with tumor progression, tissue fibrosis and other pathologies.

EMT involves fundamental changes in gene expression that disrupt epithelial polarity and that establish a mesenchymal phenotype with concomitant alterations in cytoskeletal organization, cell adhesion, and ECM production (Figure 2). This process of phenotype conversion is well-conserved throughout the vertebrata, having emerged more than 500 million years ago. More recent observations have led to suggestions that EMT contributes to the phenotypic conversions observed in tissue fibrosis, chronic inflammation, similar to conversions that occur in rheumatic diseases and cancer progression. Several recent reviews have summarized key signaling pathways involved in EMT and have probed the link between the tumor microenvironment, fibrosis, EMT, and cancer progression. In this Review, we expand upon these ideas by analyzing the mechanistic processes involved in this EMT conversion as part of the broader function of epithelial plasticity in tumor progression. We focus on the role of RAS signaling in epithelial tissue plasticity because EMT in cancer is a dynamic and often incomplete process regulated by the microenvironment and RAS and its effector pathways, most notably ERK1, ERK2 and PI3-kinase/Akt. Surprisingly, these pathways are responsive to the microenvironment even when RAS is mutated into an activated form.
EMT IN CULTURE IS PART OF AN INTRINSIC EPITHELIAL TUMOR CELL PLASTICITY

When Boyer and colleagues studied cultured cells they first described EMT as a morphological change from epithelial-like tumor cell sheets to scattered, fibroblast-like cells capable of invading the basement membrane (Figure 3A). EMT has more recently been shown to occur during normal mammary gland morphogenesis and seems to be required for formation of ducts. Since the initial observations, EMT in cultured cancer cells has been characterized on the molecular level; altered expression profiles, subcellular localizations, and activity levels are now commonly used to identify EMT in culture (Table 1). EMT in culture can be either stable, i.e., the mesenchymal phenotype is sustained after the stimulus provoking the conversion is removed, or reversible, i.e., the cells revert, or undergo a mesenchymal-epithelial transition (MET), when the stimulus is removed. Experiments that quantitatively define the transient and incomplete phenotypic changes often observed in cultured tumor cells provide insight into the dynamic role EMT may play in neoplastic processes.

Insight into the complexity of EMT has been provided by studies that have used gene transcription and proteomic microarrays to assess EMT and MET. Additional data suggest that the gene signatures for EMT and more generally, tumor epithelial cell plasticity are controlled by tissue and microenvironmental factors. A comparison of transcriptional analyses of TGF-β1-induced EMT in EpH4 mouse mammary epithelial cells (transient EMT associated with a scattering phenotype) and EMT induced in EpH4 derivatives such as c-Fos-ER-EpH4 (stable EMT without induction of malignancy) and RAS-EpH4 (stable EMT with induction of malignancy) revealed a common EMT gene signature distinct from that associated with scattering, metastasis or oncogene expression (Figure 4A). Furthermore, a number of the genes in this signature have been linked to poor outcome in breast cancer. Intriguingly, some changes in gene-expression profiles also overlap with those documented to occur during EMT conversion of the medial edge epithelial seam in the embryonic palate.

Figure 4 B,C shows the extent of this overlap and compares expression of genes that are upregulated by at least two-fold during EMT of EpH4 mammary cells with those altered during EMT in the embryonic palate. There are similar but not identical
alterations in the expression of gene sets in EpH4 mammary cells and embryonic palate. There is overlap in genes that are down-regulated during EMT of EpH4 cells compared to the gene profile altered during palatogenesis: 70% of genes down regulated in EpH4 cell EMT-specific are also altered by more than two-fold during palatogenesis (Figure 4D). The similarity between these two EMT signatures might result from the important role of TGF-β1 in driving mesenchymal conversion of both EpH4 cells and the embryonic palate medial ridge epithelium. Even though an EMT involves acquisition of at least some mesenchymal properties, the EMT-specific gene signature of EpH4 cells shares surprisingly few gene expression changes in common with stromal signatures. Such gene changes include the fibroblast serum response that encompasses genes that are commonly upregulated in fibroblasts from different tissues following serum stimulation in culture, and which predicts both poor outcome in breast and other cancers and enhances the prognostic value of an “invasiveness” gene signature that predicts poor outcome in breast cancer. A limited overlap in altered gene expression is observed between the EMT-specific EpH4 cell gene signature and tumor stromal signatures such as head and neck squamous cell carcinoma (HNSCC) and prostate cancer (Figure 5). Furthermore, the EpH4 mammary cell EMT gene signature bears little resemblance to an EMT signature of HNSCC (Figure 5). Although these comparisons are limited, gene-signature analysis indicates that EMT is a process that is distinct from metastasis or tumorigenesis per se and could be tissue and microenvironment-specific, but has some resemblance to the embryonic process, at least when driven by a common factor such as TGF-β1.

These studies illustrate that EMT in cancer is a complex process that seems to be a subset of an extensive conversion program. The dynamic nature of tumor phenotype inter-conversion is more difficult to capture in vivo and has rarely been documented. A conversion of breast tumor ductal epithelial cells into myoepithelial cells and myofibroblasts, however, is suggested by both the residual expression of epithelial keratin markers in myoepithelial and myofibroblast cells and the simultaneous expression of myoepithelial (e.g. K14, K17 and vimentin) and myofibroblast (e.g. vimentin and alpha smooth muscle actin) markers. Retention of some epithelial and myoepithelial markers in “transdifferentiated” myofibroblasts, as well as evidence of non-random X-chromosome inactivation patterns also demonstrate epithelial plasticity towards the
fibroblast phenotype. These results suggest that adult epithelial cells have a capacity to acquire aspects of a mesenchymal phenotype and vice versa in culture and in breast cancer. The apparent rarity of an EMT in human tumor samples likely reflects its transient nature and its possible function as a brief pro-invasion conversion program that is required for colonizing distant tissues.

**CLINICAL SIGNIFICANCE OF EMT**

Although EMT has been clearly documented in cultured human cancer cell lines and in some human tumors, its prevalence in aggressive tumors and its role in clinical progression are still controversial.\(^{33,39}\) A clear demonstration of EMT in most human neoplastic disease has been compromised by the cellular heterogeneity of most human tumors and by the lack of clear mesenchymal and epithelial tumor in solid tumor biopsies. Evidence that EMT might be highly localized and transient or limited to specific steps in metastatic colonization\(^{32,40}\) further complicates clinical analysis of this process. Uncertainty regarding a clinical role for EMT in tumor progression is fueled by the rarity of morphological changes observed in primary tumors by pathologists. Nonetheless, a number of studies showing that expression of EMT-related genes (Table 1)\(^{1,41}\) are associated with the metastatic/invasive phenotype. Furthermore, a recent study that compared the gene signature of metaplastic breast cancer with breast ductal carcinoma, showed unique downregulation of epithelial genes and upregulation of mesenchymal genes in metaplastic breast carcinoma.\(^{42}\) EMT might therefore be a feature of breast carcinoma subtypes. These studies justify further assessment of EMT as an essential component of malignancy.

Nevertheless, increased expression of EMT markers has been detected at the invasive fronts of aggressive tumors.\(^{1}\) Data illustrate an association between known regulators of EMT (e.g. Snail, Twist, Slug) and aggressive tumor behavior in animal models and poor clinical outcome in cancer patients, suggesting a role for EMT in tumor progression.\(^{1,23,43}\) The pleiotropic nature of EMT regulators such as Snail, make it difficult to determine the extent to which they are causative of EMT in human cancer.\(^{44}\) One approach used to detect diagnostic, tissue-specific EMT markers is to identify gene expression alterations associated with conversion in human tumors, animal cancer
models, or cultured cells and then assess whether or not these gene signatures are correlated with clinical outcome. Such transcriptional profiling experiments resulted in the identification of a ‘wound responsive’ gene signature that predicts poor outcome in breast cancer patients and that increases the predictive value of other gene signatures for poor outcome in this disease. Similarly, identification of key transcriptional alterations associated with the response of human breast epithelial cells to organotypic three-dimensional culture conditions was also predictive of outcome in breast cancer patients.

While the prognostic value of an EMT to breast cancer progression per se has to our knowledge not been reported, a number of genes in the EpH4 mammary cell EMT/metastatic gene signatures and mammary tumor cell signature are associated with poor prognosis in breast cancer. Comparison of the EMT-specific EpH4 mammary cell gene signature has limited overlap with two metastasis/invasion gene signatures that predict poor clinical outcome in breast cancer (Figure 5). More in-depth studies of this type could help to clarify the clinical significance of an EMT to tumor progression.

POSSIBLE FUNCTIONS OF EMT DURING TUMOR PROGRESSION

Numerous studies have shown that blocking the expression or impairing the function of EMT-regulating factors blocks migration and invasion in cultured epithelial cells. Invasion and metastasis of epithelial tumors, however, can occur in the absence of any detectible EMT, and even in the presence of EMT, invasion and metastasis may not occur. For example, metastasis of some bladder cancer cell lines was associated with conversion to an epithelial phenotype (MET) rather than with retention of the mesenchymal phenotype, while desmoid tumors are mesenchymal and locally invasive but do not metastasize. EMT can be functionally uncoupled from the processes of invasion and metastasis. Conditional expression of TGF-β1 in mouse keratinocytes in the presence of a functional TGF-β1 receptor promoted EMT and metastasis in chemically-induced papilloma; however, expression of a dominant-negative TGF-β1 receptor blocked the induction of EMT but did not influence the ability of TGF-β1 to promote metastasis. Such observations indicate that it might be an oversimplification that EMT as responsible only for increased migratory and invasive capacities.
A broader perspective of the role of EMT in cancer can be gleaned from the study of EMT in normal development. Growing evidence suggests that EMT is integral to normal tissue repair and renewal processes and may contribute to fibrosis when these processes are sustained or otherwise aberrant. As predicted by analysis of EMT gene signatures, EMT has been documented in keratinocyte migration at wound sites and in response to UV irradiation. EMT also occurs transiently at the tips of growing mouse mammary gland branches as they invade the fat pad during branching morphogenesis, a process that resembles tumor invasion into adjacent tissues. In such instances, EMT-related processes coordinate epithelial cell movement rather than dissemination.

Transient EMT in cancer can provide fibroblast-like properties to tumor cells even in the absence of complete morphological alteration. Several reports suggest that conversion of non-small-cell lung carcinoma (NSCLC) to a mesenchymal phenotype affects their sensitivity to mitogens and to anti-proliferative drugs. For example, EMT in NSCLC, which was detected by a mesenchymal gene signature, predicted loss of response to epidermal growth factor receptor (EGFR) activation and insensitivity to the EGFR inhibitor erlotinib. Experimental model studies have shown that acquisition of resistance to drugs such as tamoxifen is associated with and may even promote EMT. These studies demonstrate that EMT can be associated with changes in responsiveness to mitogens and anti-hormone therapy. It is not clear whether these effects are a direct or indirect consequence of a phenotypic conversion. EMT could directly affect responses to mitogens and hormones as a result of altered expression of specific growth factor receptors, such as EGFR. Mesenchymal cells also differ from epithelial cells in their expression of transporters, and this could affect sensitivity to specific drugs.

EMT may also perform key immune modulatory functions during tumor progression. For instance, fibroblasts possess distinct immunomodulatory activities; they can permit leukocyte infiltration and retention within tissues at wound sites, by presenting antigens to the immune system, and by modifying T-cell responses. Thus, fibroblasts could act to mask tumor antigens and to protect tumors from immune surveillance. Fibroblasts produce and respond to a different set of cytokines and growth factors than epithelial cells and are more responsive to the mitogenic and motogenic
effects of platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) than epithelial cells. These growth factors are abundant in the microenvironment of tissues undergoing extensive remodeling as well as in tumors, and transiently or reversibly, EMT might facilitate growth of epithelial tumor cells.\footnote{62} Transient EMT allows epithelial cells to temporarily evade the effects of growth-inhibitory factors. Using a tissue micropatterning approach, Nelson and coauthors showed that a transient EMT occurred in regions of lowest concentration of the branching inhibitor TGF-β1.\footnote{27} Transient EMT may provide the ductal cells with a temporary release from the inhibitory growth effects of TGF-β1, and allow response to other mitogens in the microenvironment.\footnote{63} In summary, the likely transient nature of EMT and paucity of mesenchymal markers for EMT have limited an assessment of the extent this conversion contributes to tumor progression. Microdissection techniques that enable sampling of the tumor edge as well as improved imaging resolution \textit{in vivo} will also contribute in clarifying the role of EMT in tumor metastasis.

**MOLECULAR PATHWAYS THAT REGULATE EMT**

Identifying the molecular pathways that regulate EMT in cancer cells has been the subject of intense investigation.\cite{1,19,64–67} While the processes involved in EMT have distinct characteristics in different tissues, RAS-regulated ERK1/ERK2 and PI3-kinase signaling pathways are increasingly recognized as key mediators of tumor cell plasticity. Gene signatures of de-regulated RAS pathways are common in human tumors and these as well as other oncogenic pathway signatures have permitted risk stratification in many types of human cancers.\footnote{68,69}

RAS proteins act as switches controlling many downstream signaling pathways and are triggered by microenvironmental factors such as growth factors and ECM molecules.\footnote{70–72} Increased expression and/or mutation of RAS is a common early event in human tumors.\footnote{66,71} In breast cancer, mutations that result in increased expression of RAS are more common than mutations that result in constitutive activation,\footnote{73} and increased activity of the RAS-regulated downstream mediators, PI3-kinase and ERK1/ERK2, is a poor prognostic indicator.\footnote{74–79} RAS-regulated pathways can induce autonomous, stable EMT in mammary epithelial cells. For example, exposing EpH4 cells with activating
RAS mutations to TGF-β1 stimulates autocrine production of mesenchymal factors such as PDGF-A, PDGF-B, and the PDGF receptors alpha and beta, which maintain EMT even when exogenous TGF-β1 is withheld. In the absence of constitutively activated RAS signaling pathways in parental EpH4 mammary cells, TGF-β1 induces an incomplete and transient mesenchymal conversion that is reversible when TGF-β1 is removed.\textsuperscript{80,81}

ERK1/ERK2 and PI3-kinase-regulated pathways play central roles in tumor cell EMT. PI3-kinase stimulates proliferation, blocks apoptosis, and promotes cadherin isotype switching upon exposure to interstitial collagens;\textsuperscript{65,82} ERK1/ERK2 disassembles adherens junctions and induces expression of mesenchymal ECM components such as tenascin-C as well as matrix metalloproteinases (MMPs).\textsuperscript{83,84} Both pathways regulate the transcription factors Slug and Snail, which in turn promote EMT by suppressing expression of E-cadherin, genes encoding epithelial tight junction components, and epithelial-specific cytokeratins; loss of E-cadherin induced by extracellular MMPs can induce EMT as well.\textsuperscript{85–87} Activation of ERK1/ERK2 and PI3-kinase pathways regulate tumor suppressive effects of environmental factors such as TGF-β1, promoting growth and stabilization of EMT,\textsuperscript{88} an effect that is achieved by linking TGF-β1 receptor activity to PI3-kinase and ERK1/ERK2 signaling pathways.\textsuperscript{28,49,80} RAS, PI3-kinase, and ERK1/ERK2 mediators are controlled through alteration of integrin-responsive signaling pathways: β1-integrins modulate the activity of growth factor receptors such as EGFR and PDGFR, which activate ERK1/ERK2 and PI3-kinase.\textsuperscript{89,90} Additionally, the nuclear localization of activated ERK1/ERK2, which is required for its effects on gene transcription, is regulated by hyaluronan.\textsuperscript{91} Thus, ECM molecules in combination with growth factors present in the tumor microenvironment control the localization and activation status of these RAS-effectors thereby determining the precise effect of these pathways on tumor cell behavior and differentiation/plasticity (Figure 6).

CONCLUSIONS
A more-complete definition of how EMT contributes to cancer progression requires analysis of EMT during normal tissue renewal and development of mechanistic assays for \textit{in situ} detection of EMT as well as continued identification of effectors of EMT that are
prognostic for tumor outcome. Ras-regulated ERK1/ERK2 and PI3 kinase signaling pathways are modulated by elements of the tumor microenvironment suggesting functions beyond their well-studied roles in motility and proliferation. Another active field involves identification of EMT regulatory pathways in the context of epithelial plasticity to identify potential targets for therapy. In parallel, technical advances in accurate sampling and visualization of individual cells will enable isolation and analysis of the key regulators of tumor EMT.

Figure legends

**Figure 1** Common morphological characteristics of epithelial and mesenchymal cells. Epithelial morphology is characterized by an apical-basal polarity, contact with a basal basement membrane and formation of extensive cell-cell contacts including tight junctions. An anterior-posterior polarity is lost if any cell-cell junctions and residency within a more unstructured interstitial matrix characterize mesenchymal morphology.

**Figure 2** EMT of mammary epithelial cells. Treatment of mouse mammary epithelial cells with MMP-3 stimulates breakdown of epithelial structure and acquisition of a mesenchymal morphology. Red, f-actin; blue, DAPI.

**Figure 3** Dynamic role of EMT in mammary gland neoplastic processes. EMT during mammary tumor progression is postulated to facilitate tumor cell invasion and colonization of distant tissues. EMT permits efficient penetration of vessels and escape into distant tissues such as the lung or bone. A mesenchymal phenotype might be retained or may revert to an epithelial phenotype (MET) depending upon the tissue microenvironment. For example, some microenvironments such as those provided by bones can offer selective growth for a mesenchymal phenotype while others (e.g. lung) may favor growth of an epithelial phenotype. Abbreviations: EMT, epithelial–mesenchymal transition; MET, mesenchymal–epithelial transition
Figure 4 Overlap between the EMT gene signature of EpH4 mammary cells and embryonic palate overlap. (A) A Venn diagram illustrates the overlap between the Eph4 metastasis and EMT gene signatures for both upregulated and downregulated expression of genes. These results show that EMT can be distinguished from metastasis as a molecular process. (B) A Venn diagram shows the number of upregulated EMT-specific genes in Eph4 cells that are also altered in embryonic palate undergoing EMT, and are up and down-regulated by at least two fold. Both EMT processes are in response to TGFβ1. Approximately 50% of EMT-specific genes upregulated in EpH4 cells are also upregulated during EMT associated with embryonic palate morphogenesis. (C) The table identifies the genes that are commonly altered during EpH4 mammary cell and embryonic palate EMT. Of the 21 upregulated EpH4 mammary cell EMT genes, 10 are increased (+) in embryonic palate undergoing EMT, 8 are not altered (-) and 3 are down regulated (arrow). (D) The table shows the number of down-regulated EMT-specific genes of EpH4 mammary cells that are commonly altered in embryonic palate undergoing EMT.

Figure 5 Comparison of EpH4 mammary cell EMT gene signature with cancer-related gene signatures. Both upregulated and down regulated EMT-specific genes from EpH4 mammary cells were compared with gene-expression profile changes during EMT of head and neck squamous cell carcinoma (HNSCC), stromal gene signatures that have prognostic value in breast and other cancers, and metastasis invasion gene signatures that predict poor outcome in breast cancer. Limited overlap is seen between the EpH4 EMT-specific gene signature and these cancer-related gene signatures.

Figure 6. Microenvironmental and spatial regulation of signaling pathways controlling EMT. The RAS-ERK1/ERK2 pathway is an example a signaling module that is responsive to microenvironment cues and requires specific subcellular localization in determining the consequences to gene expression and tumor phenotype. A simplified version of this complex process is illustrated in the diagram. Extracellular matrix components interact with integrin receptors at the cell surface (step 1) and the affinity of this interaction is modified by growth factor-regulated signaling in a process known as
outside-in signaling (step 2). Conversely, the affinity of the integrin:extracellular matrix interaction affects growth factor regulated signaling in a process known as outside-in signaling (step 3). The collective interactions between integrins and growth factor receptors promote the localization and activation of lipid-modified RAS (2) at the inner cell membrane leaflet (step 4). Activated RAS then selectively activates kinases such as ERK1/ERK2 (3) but also other pathways such as the PI3 kinase/AKT pathway (step 5). RAS also blocks the tumor suppressing activity of TGF-β1 (step 6) by linking this pathway to ERK1/ERK2 signaling pathways (step 5). This linkage promotes the pro-invasion properties of TGF-B1. Activated ERK1/ERK2 must translocate to the nucleus or cell adhesion sites known as focal contacts to have access to target proteins that regulate EMT/motility/invasion. ERK1 and ERK2 regulate gene expression (e.g. MMP-9) further modifies the tumor cell microenvironment thereby affecting integrin/growth factor receptor signaling pathway activation status. This downstream consequence of RAS/ERK1/2 activation and the reversal of Steps 1–5 have profound effects on tumor phenotype even when other pathways are mutated.

References


Figure 1. Common morphological characteristics of epithelial and mesenchymal cells

- **Epithelial morphology**
  - Apical domain
  - Basolateral domain
  - Apical-basal polarity
  - Tight junctions
  - Basement membrane

- **Mesenchymal morphology**
  - Interstitial matrix
  - Anterior-posterior polarity
  - No intercellular junctions
Figure 2. EMT of mammary epithelial cells.
Figure 3. Dynamic Role of EMT in mammary gland neoplastic processes
From Jechlinger et al., 2003

Data in B,C are from Jenchlinger et al., 2003 and LaGamba et al., 2005
Figure 4 cont  D.

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Figure 5. Comparison of EpH4 mammary cell EMT gene signature with cancer-related gene signatures

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Figure 6. Microenvironmental and spatial regulation of signaling pathways controlling EMT