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Authors

Derynck, Rik
Weinberg, Robert A

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EMT and Cancer: More Than Meets the Eye

Rik Derynck^{1,*}, Robert A. Weinberg^{2,*}

¹Department of Cell and Tissue Biology, and Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California at San Francisco, San Francisco, CA 94143, USA

²Whitehead Institute for Biomedical Research, MIT Ludwig Center for Molecular Oncology, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142, USA

Abstract

Epithelial cells acquire mesenchymal characteristics during development, wound healing and inflammation, and in cancer and fibrosis. With increasing appreciation of different roles of epithelial-mesenchymal transition (EMT), we address the question of how to define and recognize EMT processes and discuss their properties in cancer progression.

Epithelial cells can repress their epithelial morphology, elongate, and acquire motile and invasive properties during the course of development, wound healing, and propagation in cell culture. This reversible transition was named “epithelial-to-mesenchymal transition” (EMT) because of the repression of existing epithelial characteristics and the induced expression of certain genes that are commonly expressed in mesenchymal cells. The term “transdifferentiation” was initially not embraced to describe this cell-biological program, since it went against the belief at the time that cells cannot readily transition from one differentiated phenotype to another, let alone that this could be reversible—another property of the EMT. Furthermore, the observation that a growth factor, such as fibroblast growth factor or TGF- β , could induce in cultured cells the phenotypic shift that is now routinely termed an EMT was initially met with disbelief, since prevailing opinion held that growth factors should only control proliferation and were unable to change cell differentiation states. During the past three decades, we have seen an increasing acceptance that epithelial and endothelial cells can indeed undergo EMT (or EndMT in the case of endothelial cells) and that they do so often in response to contextual signals, such as those conveyed by various cytokines and even components of the extracellular matrix (ECM) (Lamouille et al., 2014; Nieto et al., 2016; Dongre and Weinberg, 2019).

In the context of normal organismic physiology, EMT is now seen as a normal transdifferentiation process that gives rise to new cell and tissue types during the course of development. It is also seen as a transient and often reversible process during wound healing

*Correspondence: rik.derynck@ucsf.edu (R.D.), weinberg@wi.mit.edu (R.A.W.).

DECLARATION OF INTERESTS

R.D. is a founder of Pliant Therapeutics, Inc. and a consultant to Genentech, Inc. and NGM Biopharmaceuticals, Inc. R.A.W. is a founder of Theracrine, Inc. and Verastem, Inc., serves as chair of the scientific advisory board of Verastem, Inc., and is a member of the scientific advisory board of Syros Pharmaceuticals, Inc.

and at sites of inflammation, where EMT plays a central role in restoring epithelial and endothelial integrity. During carcinoma pathogenesis, EMT contributes to cancer progression by imparting the mesenchymal phenotypes associated with the cells of highly aggressive tumors. Finally, EMT and EndMT are increasingly thought to play a role in fibrosis and may be required for the fibrotic response (Lamouille et al., 2014; Nieto et al., 2016; Dongre and Weinberg, 2019). There are now almost 15,000 reports describing the roles of EMT programs in these various processes. With the rapid expansion of this research field, questions often arise on how to best define EMT and to recognize cells that are undergoing EMT or have transitioned through an EMT program, and whether a unifying model for EMT applies. Furthermore, with EMT involved in cancer progression and fibrosis, pathologists and researchers wonder how to histologically recognize EMT in complex tissues. Here, we briefly elaborate on how EMT can be defined and recognized and the roles that EMT plays in cancer pathogenesis.

Defining and Recognizing EMT

EMT programs manifest in diverse ways, which follows in no small part from their involvement in a diverse array of normal and pathological processes, tissue types, differentiated cell types, and the mix of signals that the epithelial cells receive from the stromal microenvironment. This diversity is compounded by the often-incomplete suppression of preexisting epithelial characteristics and incomplete acquisition of mesenchymal ones. Indeed, the display of mixed epithelial and mesenchymal traits by individual cells appears to be the norm rather than exception. Stated differently, completion of an EMT program with generation of fully mesenchymal cells may be rare in normal and neoplastic human tissues.

Despite differences in molecular manifestations, certain commonalities are shared by the EMT programs operating in various epithelial cells (Table 1, EMT-associated changes in normal and neoplastic cells). EMTs often enable increased cell migration through extracellular matrices, designated as invasion, which stands in striking contrast to the controlled and seemingly stable interactions of epithelial and endothelial cells with one another and with underlying basement membranes in intact tissues. To undertake directed cell migration, such as that displayed by high-grade carcinoma cells, epithelial cells must redirect their apical-basal cell polarity toward a front-rear polarity. Since apical-basal polarity is coupled to epithelial or endothelial junction integrity, redirecting cell polarity is coupled to the deconstruction of epithelial or endothelial lateral cell-cell junctions. These changes are accompanied by cytoskeletal changes, with a reorientation of the cortical organization of the actin cytoskeleton toward bundles of stress fibers that assemble near the ventral surface of cultured cells. Finally, the ability of cells to invade through extracellular matrix requires the activation of proteases at the invasive fronts of individual cells or cohorts of these cells (Lamouille et al., 2014; Nieto et al., 2016; Dongre and Weinberg, 2019).

Specific changes in gene expression that define all EMT programs operating both *in vitro* and *in vivo* have been proposed (Table 1, EMT-associated changes in normal and neoplastic cells). However, realization of this goal has been elusive, given the diversity of EMT programs and the often partial completion of these programs. For this reason, no simple,

shared set of EMT-defining gene expression changes has been produced. Moreover, we note that it remains unclear whether the various EMT-induced cell states can be arrayed along a linear spectrum of phenotypes that begins with a fully epithelial cell state at one end and ends with a fully mesenchymal state at the other. Instead, diverse partial EMT phenotypes may need to be portrayed in a two-dimensional landscape of phenotypic states (Nieto et al., 2016).

Many if not all EMT programs have in common the expression of highly conserved EMT-inducing transcription factors (EMT-TFs)—including Snail, Slug (Snail2), Zeb1 and Zeb2, and Twist—that operate in various combinations with one another and serve as master regulators of these programs (Nieto et al., 2016; Stemmler et al., 2019). However, the specific combination of expressed EMT-TFs activated in a particular EMT program depends on the cellular context (e.g., normal versus neoplastic), the differentiation lineage of the cell-of-origin, and the extent to which an EMT is completed (Stemmler et al., 2019; Aiello et al., 2018; Pastushenko and Blanpain, 2019). The confounding diversity of EMT programs makes it difficult to pinpoint further changes in gene expression that are diagnostic of all EMT programs. Repression of epithelial junction protein expression is often observed, but is not required for initiation of EMT, since the expression of these proteins is often retained even though they may no longer participate directly in junction formation. Increased expression of certain mesenchymal proteins, including mesenchymal adhesion proteins, is often seen but depends on the extent to which cells have activated major portions of the EMT program (Table 1, EMT-associated changes in normal and neoplastic cells). Partial, heterogeneously expressed EMT-associated phenotypes are apparent in the carcinoma cells participating in “collective cell migration,” which involves the migration of cohesive, largely epithelial cell cohorts, similar to that observed in developmental contexts and epithelial wound closure. The “leader” cells at the invasive edges of these cohorts often if not invariably express mixed epithelial and mesenchymal traits, whereas the closely associated “follower” cells retain epithelial characteristics and associate with one another through epithelial junctions (Campbell and Casanova, 2016).

While generally seen as reversible, it is important to point out that EMT programs operating developmentally often yield cells that stably retain the newly acquired mesenchymal phenotypes. Neuro-ectodermal, endothelial, and epithelial cells that have undergone EMT during development often do not revert to their original phenotypes and instead stably retain acquired, mesenchymal traits. Prolonged exposure of cultured epithelial and carcinoma cells to TGF- β also yields cells that reside stably in a mesenchymal phenotype, even after TGF- β is removed (Katsuno et al., 2019). Hence, reversibility of EMT occurring via the process of mesenchymal-epithelial transition (MET) is not intrinsic to all EMT programs.

EMT in Carcinoma Cells: More Than Epithelial-Mesenchymal Transition

EMT processes affect both the carcinoma cells and, indirectly, the stromal cells within a tumor (Shibue and Weinberg, 2017; Dongre and Weinberg, 2019). Carcinoma cells exhibiting a partial EMT, as evidenced by increased expression of EMT-associated proteins such as EMT-TFs, are frequently seen. However, as stated earlier, full loss of the preexisting epithelial cell phenotype and entrance into an entirely mesenchymal state are likely to be

rare. During cancer progression, the proportion of carcinoma cells with partial EMT phenotypes increases, yielding subpopulations of cells with diverse patterns of intermediate EMT-associated gene expression and differences in invasive properties and preferred localization within the tumor (Aiello et al., 2018; Pastushenko and Blanpain, 2019). The secretion of TGF- β by carcinoma cells that have activated EMT programs operates in an autocrine fashion to ensure maintenance of these programs in the carcinoma cells. At the same time, this TGF- β may impinge in a paracrine manner on the fibroblast-like cells in the nearby stroma, sometimes termed carcinoma-associated fibroblasts (CAFs), to induce the formation of myofibroblasts. The extent to which EMT programs operating in carcinoma cells generate fully mesenchymal cells that participate in the formation of the tumor-associated stroma remains a topic of debate.

As described above, EMT programs enable directional cell migration and invasion of carcinoma cells through the basement membrane and extracellular matrix. Eventually, a portion of the invading cells may intravasate and disseminate hematogenously to distant tissues. Cancer cell dissemination does not, on its own, imply that these cells migrate and initiate invasion as individual cells. Indeed, invasive multi-cell cohorts are prevalent, and invasive behavior often associates with collective cell migration. Furthermore, cancer cells captured in circulation are seen as single cells or clusters of cells that express both epithelial and mesenchymal characteristics. In addition, it is now increasingly accepted that partial EMTs yielding cells with mixed epithelial and mesenchymal characteristics are essential for carcinoma cell invasion and dissemination. Such mixed traits may also be critical to the subsequent success of disseminated cells to found metastatic colonies.

In a variety of carcinomas, activation of EMT programs increases the number of carcinoma cells exhibiting stem cell properties (Shibue and Weinberg, 2017). Stemness in carcinoma cells is defined operationally by the ability of the cells to initiate tumor formation following experimental implantation in suitable mouse hosts, but is often more conveniently inferred by the ability of cells to generate multicellular spheres from single cells in three-dimensional matrix cultures. By definition, the cancer stem cells (CSCs) generated by partial EMTs can self-renew or revert to a more epithelial phenotype through MET. At the same time, the more epithelial cells in a tumor may spawn new CSCs through the activation of EMT programs. Taken together, these behaviors suggest dynamic interconversion between phenotypic states, likely influenced by signals from the tumor microenvironment and between the minority subpopulations of more mesenchymal CSCs and the majority subpopulations of more differentiated carcinoma cells (Shibue and Weinberg, 2017; Dongre and Weinberg, 2019). Of note, in a number of normal bilayered epithelia, the Slug EMT-TF is expressed in basal cells and associates with the normal stem cell phenotype (Guo et al., 2012). This suggests that the alliance between EMT and stem cell programs may operate in at least a subset of normal epithelial cells, and that this association, when operating in carcinoma cells, echoes aspects of normal epithelial cell physiology.

Precisely how, at the cellular and molecular levels, stem cell properties are conferred by EMT programs is currently unclear. Acquisition of stemness may be an intrinsic component of EMT programs. If so, EMT programs in normal and neoplastic epithelial tissues would be required for and enable the acquisition of stemness. A major gap in existing observations is

the precise localization of CSCs along the spectrum of epithelial-mesenchymal phenotypic states. Moreover, the regulatory coupling between residence in the CSC state and the EMT program is poorly understood. As one example of this complexity, carcinoma cells arising after prolonged TGF- β exposure show a far higher proportion of CSCs than do cells observed after short-term TGF- β exposure, even though the two cell populations exhibit similar EMT characteristics (Katsuno et al., 2019).

At present, it is plausible that the acquired abilities of all types of carcinoma cells to invade and disseminate depend on the activation, at least transiently, of EMT-associated traits, and conversely that blockage of EMT prevents these processes from occurring. Nonetheless, this notion has not been universally accepted, and definitive proof of it will depend on effectively blocking EMT programs in diverse types of primary carcinoma cells and observing the loss or retention of the ability of such cells to invade and disseminate.

As of late, it has become apparent that EMT programs confer additional properties on carcinoma cells beyond those enumerated above (Table 1, Physiological changes associated with EMT in carcinoma cells). Notably, EMT enables carcinoma cells to exert local immunosuppressive powers, thereby compromising immunosurveillance and immune attack on tumors (Terry et al., 2017; Dongre and Weinberg, 2019). As an important example, carcinoma cells expressing EMT programs show increased release of chemokines and cytokines, including, as mentioned above, TGF- β 1. TGF- β 1, for its part, promotes formation of immunosuppressive regulatory T (Treg) cells and inhibits the cytotoxic activities of CD8⁺ cytotoxic T cells, antigen presentation by dendritic cells, and the cytolytic activities of natural killer (NK) cells (Sanjabi et al., 2017). EMT in carcinomas is also accompanied by increased expression of PDL1, the ligand of the PD1 checkpoint receptor displayed on cytotoxic T cells (Dongre et al., 2017), further contributing to suppression of anti-tumor immune attacks launched by cytotoxic T cells. These and other observations predict that EMT should enable cancer cells to escape from antitumor immunity and decrease their susceptibility to checkpoint blockade-based immunotherapy.

As has been apparent for almost two decades, expression of EMT programs correlates with increased resistance of carcinoma cells to cytotoxic anticancer drugs (Shibue and Weinberg, 2017; Steinbichler et al., 2018). Thus, among carcinomas, those with higher proportions of mesenchymal cells are found to be less sensitive to cytotoxic therapies. Among experiments that correlate EMT with acquired resistance to chemotherapy are those that associate a strongly elevated resistance to killing by anti-cancer chemotherapeutics with the strongly increased stem cell properties following long-term TGF- β treatment (Katsuno et al., 2019). The increased cancer drug resistance that is associated with EMT may relate to increased efflux activity of multi-drug resistance (MDR) export pumps, which may result from increased expression of *MDR* genes. Additionally, EMT and the CD44^{high}CD24^{low} CSC cell-surface antigen phenotype have been correlated with a decreased ability of carcinoma cells to repair double-stranded DNA breaks (Pal et al., 2017). The resulting accumulation of genomic changes may then link EMT to cell-heritable genotypic diversification that can, in turn, yield variants that are selected for during subsequent cancer progression. Finally, EMT has been linked to protection against cellular transition to the senescent state (Smit and Peeper, 2010), although the underlying mechanisms need to be thoroughly defined.

To summarize, EMT programs, initially identified based on changes in cell morphology and behavior, encompass far more than simple shifts in epithelial versus mesenchymal cell characteristics that are apparent under the microscope. When assessing the overall contributions of EMT to discrete steps of multi-stage cancer progression, the aggregate of EMT-associated changes, including stem cell properties, changed genomic stability, increased invasiveness and disseminating ability, avoidance of senescence and apoptosis, sensitivity to cytotoxic therapies, and localized effects on the tumor-associated microenvironment, need to be taken into account (Table 1, Physiological changes associated with EMT in carcinoma cells). Indeed, this list makes it apparent that most of the distinctive phenotypes that associate specifically with high-grade carcinoma cells are orchestrated partially or entirely by EMT programs. While EMT also confers stem cell properties on normal epithelial cells, it remains to be seen whether the other EMT-associated changes observed in carcinoma cells represent manifestations of this program operating in a similar fashion in normal, non-neoplastic epithelial cells. The diversity of partial EMT phenotypes and the varied developmental, physiological, and pathological contexts in which EMT programs operate illustrate that EMT does not result from a simple linear succession of transdifferentiation events. As is apparent, extensive further studies are required to determine precisely how the distinct cell phenotypes associated with partial EMT programs contribute to cancer progression, fibrosis, and wound repair.

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Table 1.

Changes Associated with Epithelial-to-Mesenchymal Transitions

EMT-Associated Changes in Normal and Neoplastic Cells	
Cell-biological changes	
Deconstruction of epithelial cell-cell junctions	
Transition from apical-basal to front-rear polarity	
Cytoskeletal rearrangements, e.g., actin reorganization	
Motility	
Invasion	
Cell-associated proteolytic activity	
Reprogramming of gene expression	
Changes in gene expression often used as EMT indicators	
Activated expression of EMT transcription factors: Snail, Slug/Snai2, ZEB1, ZEB2, Twist	
Decreased expression of epithelial adhesion proteins: E-cadherin (or VE-cadherin in EndMT), ZO1, desmoplakin	
Activated expression of mesenchymal adhesion proteins: N-cadherin, N-CAM	
Increased vimentin expression	
Increased fibronectin expression	
Physiological Changes Associated with EMT in Carcinoma Cells	
Cancer stem cell characteristics	
Cancer cell motility, invasion and dissemination	
Increased cancer drug resistance	
Localized immunosuppression	
Changes in genomic stability	
Protection against senescence	