The basics of epithelial-mesenchymal transition

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The origins of the mesenchymal cells participating in tissue repair and pathological processes, notably tissue fibrosis, tumor invasiveness, and metastasis, are poorly understood. However, emerging evidence suggests that epithelial-mesenchymal transitions (EMTs) represent one important source of these cells. As we discuss here, processes similar to the EMTs associated with embryo implantation, embryogenesis, and organ development are appropriated and subverted by chronically inflamed tissues and neoplasias. The identification of the signaling pathways that lead to activation of EMT programs during these disease processes is providing new insights into the plasticity of cellular phenotypes and possible therapeutic interventions.

What is epithelial-mesenchymal transition?

An epithelial-mesenchymal transition (EMT) is a biologic process that allows a polarized epithelial cell, which normally interacts with basement membrane via its basal surface, to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype, which includes enhanced migratory capacity, invasiveness, elevated resistance to apoptosis, and greatly increased production of ECM components (1). The completion of an EMT is signaled by the degradation of underlying basement membrane and the formation of a mesenchymal cell that can migrate away from the epithelial layer in which it originated.

A number of distinct molecular processes are engaged in order to initiate an EMT and enable it to reach completion. These include activation of transcription factors, expression of specific cell-surface proteins, reorganization and expression of cytoskeletal proteins, production of ECM-degrading enzymes, and changes in the expression of specific microRNAs. In many cases, the involved factors are also used as biomarkers to demonstrate the passage of a cell through an EMT (Figure 1).

The pioneering work of Elizabeth Hay first described an "epithelial-mesenchymal transformation" using a model of chick primitive streak formation (2). In the intervening time, the term "transformation" has been replaced with "transition," reflecting in part the reversibility of the process and the fact that it is distinct from neoplastic transformation (1). The phenotypic plasticity afforded by an EMT is revealed by the occurrence of the reverse process — a mesenchymal-epithelial transition (MET), which involves the conversion of mesenchymal cells to epithelial derivatives. Relatively little is known about this process; the best-studied example is the MET associated with kidney formation, which is driven by genes such as paired box 2 (*Pax2*), bone morphogenetic protein 7 (*Bmp7*), and Wilms tumor 1 (*Wt1*) (3–5).

Why does EMT occur?

The concept of cell division as a way to generate more cells and expand tissue size emerged about 150 years ago (6). Central to this concept was an understanding that all cells in a body derive from other cells and the resulting deduction that ultimately all are derived from a single cell, the fertilized egg. An additional level of complexity came from the realization that cells can assume various phenotypic states during development, that is, they can undergo the process of differentiation. As was learned more recently, during specific steps of embryogenesis and organ development, the cells within certain epithelia appear to be plastic and thus able to move back and forth between epithelial and mesenchymal states via the processes of EMT and MET (7). Upon completion of the development of epithelial tissues, the epithelial cells typically exert tissue-specific function, while the mesenchymal cells in such tissue play a supporting role. Implied in this notion was the further idea that a state of terminal differentiation is necessary to carry out such specialized functions and that cells are maintained in a permanent state of differentiation once development is complete.

This concept has been challenged by numerous observations that the cells within a terminally differentiated epithelium can indeed change their phenotype through activation of an EMT program, which enables transdifferentiation, resulting in the conversion of epithelial cells to mesenchymal derivatives during development and adulthood. These programs can also be activated in association with tissue repair and pathological stresses, including those creating various types of inflammation and high-grade carcinomas. Accordingly, EMTs now constitute recognized mechanisms for dispersing cells in embryos, forming mesenchymal cells in injured tissues, and initiating the invasive and metastatic behavior of epithelial cancers.

Classification of EMT into three different subtypes

EMTs are encountered in three distinct biological settings that carry very different functional consequences (Figure 2). While the specific signals that delineate the EMTs in the three discrete settings are not yet clear, it is now well accepted that functional distinctions are apparent. A proposal to classify EMTs into three different biological subtypes based on the biological context in which they occur was discussed at a 2007 meeting on EMT in Poland and a subsequent meeting in March 2008 at Cold Spring Harbor Laboratories. The EMTs that are associated with implantation, embryo formation, and organ development are organized to generate diverse cell types that share common mesenchymal phenotypes. This class of EMTs, which we and others (8) propose to term

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Nonstandard abbreviations used: BMP, bone morphogenetic protein; EMT, epithelial-mesenchymal transition; EndMT, endothelial-mesenchymal transition; FSP1, fibroblast-specific protein 1; LEF, lymphoid enhancer binding factor; MET, mesenchymal-epithelial transition.

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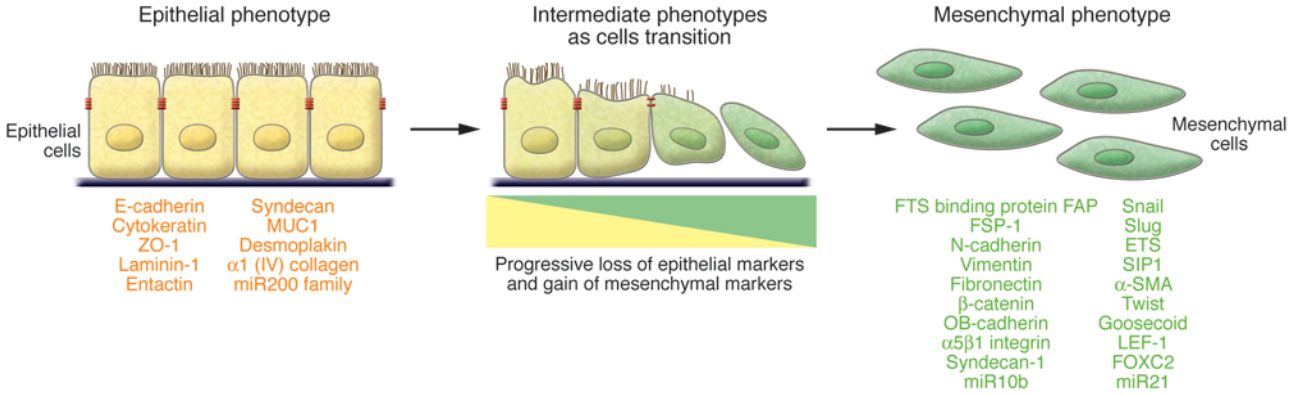

Figure 1
EMT. An EMT involves a functional transition of polarized epithelial cells into mobile and ECM component–secreting mesenchymal cells. The epithelial and mesenchymal cell markers commonly used by EMT researchers are listed. Colocalization of these two sets of distinct markers defines an intermediate phenotype of EMT, indicating cells that have passed only partly through an EMT. Detection of cells expressing both sets of markers makes it impossible to identify all mesenchymal cells that originate from the epithelia via EMT, as many mesenchymal cells likely shed all epithelial markers once a transition is completed. For this reason, most studies in mice use irreversible epithelial cell–lineage tagging to address the full range of EMT-induced changes. ZO-1, zona occludens 1; MUC1, mucin 1, cell surface associated; miR200, microRNA 200; SIP1, survival of motor neuron protein interacting protein 1; FOXC2, forkhead box C2.

"type 1," neither causes fibrosis nor induces an invasive phenotype resulting in systemic spread via the circulation. Among other outcomes, these type 1 EMTs can generate mesenchymal cells (primary mesenchyme) that have the potential to subsequently undergo a MET to generate secondary epithelia.

The EMTs associated with wound healing, tissue regeneration, and organ fibrosis are of a second type. In these type 2 EMTs, the program begins as part of a repair-associated event that normally generates fibroblasts and other related cells in order to reconstruct tissues following trauma and inflammatory injury. However, in contrast to type 1 EMTs, these type 2 EMTs are associated with inflammation and cease once inflammation is attenuated, as is seen during wound healing and tissue regeneration. In the setting of organ fibrosis, type 2 EMTs can continue to respond to ongoing inflammation, leading eventually to organ destruction. Tissue fibrosis is in essence an unabated form of wound healing due to persistent inflammation.

Type 3 EMTs occur in neoplastic cells that have previously undergone genetic and epigenetic changes, specifically in genes that favor clonal outgrowth and the development of localized tumors. These changes, notably affecting oncogenes and tumor suppressor genes, conspire with the EMT regulatory circuitry to produce outcomes far different from those observed in the other two types of EMT. Carcinoma cells undergoing a type 3 EMT may invade and metastasize and thereby generate the final, life-threatening manifestations of cancer progression. Importantly, cancer cells may pass through EMTs to differing extents, with some cells retaining many epithelial traits while acquiring some mesenchymal ones and other cells shedding all vestiges of their epithelial origin and becoming fully mesenchymal. It is still unclear what specific signals induce type 3 EMTs in carcinoma cells. For example, such signals may originate in the tumor stroma that is associated with many primary carcinomas.

While these three classes of EMTs represent distinct biological processes, a common set of genetic and biochemical elements appears to underlie and thus enable these outwardly diverse phenotypic programs. As more experiments are performed in the future, we will learn more regarding the similarities and differences among the three classes of EMTs.

Type 1 EMT: EMT during implantation, embryogenesis, and organ development

Following the earliest stages of embryogenesis, the implantation of the embryo and the initiation of placenta formation are both associated with an EMT that involves the parietal endoderm (9). In particular, the trophoectoderm cells, which are precursors of the cytotrophoblast, undergo an EMT in order to facilitate invasion of the endometrium and the subsequent proper anchoring of the placenta, enabling its function in nutrient and gas exchange (10, 11). As described briefly below, this is only the first of many type 1 EMTs that accompany and underlie embryonic morphogenesis; more detail regarding some of the EMTs that occur during development are discussed elsewhere (12).

A fertilized egg undergoes gastrulation, generating three germ layers. Initially, a primitive streak is generated in the epiblast layer (13). The epithelial cells in this tissue express E-cadherin and exhibit apical-basal polarity. Formation of the primitive streak is considered to be the first sign of gastrulation, which leads in turn to the formation of the three germ layers that generate all tissue types of the body. The primitive streak is formed from a furrowed invagination in the midline of the epiblast layer that forms initially at the lower extremity of the embryo and, later on, extends in the direction of the future head. The epithelial-like cells of the epiblast undergo programmed changes dictated by specific expression of proteins associated with cell migration and differentiation (14).

Once formed, the primitive streak, acting via invagination or ingression (depending on the species studied), generates the mesendoderm, which subsequently separates to form the mesoderm and the endoderm via an EMT (also known as epiblast-mesoderm transition) (2) by replacing the hypoblast cells, which presumably either undergo apoptosis or contribute to the mesoderm layer via an EMT. The embryonic mesoderm that forms between the epiblast and hypoblast eventually gives rise to primary mesenchyme associated with the axial, paraxial, intermediate, and lateral

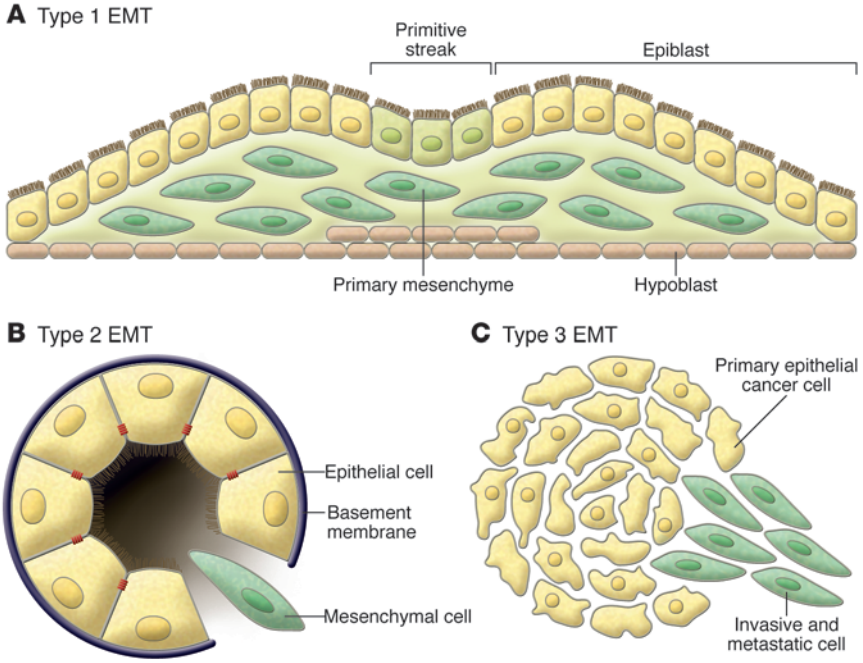

Figure 2
Different types of EMT. (**A**) Type 1 EMT is associated with implantation and embryonic gastrulation and gives rise to the mesoderm and endoderm and to mobile neural crest cells. The primitive epithelium, specifically the epiblast, gives rise to primary mesenchyme via an EMT. This primary mesenchyme can be re-induced to form secondary epithelia by a MET. It is speculated that such secondary epithelia may further differentiate to form other types of epithelial tissues and undergo subsequent EMT to generate the cells of connective tissue, including astrocytes, adipocytes, chondrocytes, osteoblasts, and muscle cells. (**B**) EMTs are re-engaged in the context of inflammation and fibrosis and represent the type 2 EMTs. Unlike the type 1 EMT, the type 2 EMT is expressed over extended periods of time and can eventually destroy an affected organ if the primary inflammatory insult is not removed or attenuated. (**C**) Finally, the secondary epithelia associated with many organs can transform into cancer cells that later undergo the EMTs that enable invasion and metastasis, thereby representing type 3 EMTs.

plate mesodermal layers (15). Cells of the primary mesenchyme exhibit enhanced migratory properties when compared with those of the epiblast and the hypoblast (15).

At the biochemical level, the EMT associated with gastrulation is dependent on and orchestrated by canonical Wnt signaling, and embryos deficient in Wnt3 cannot undergo the EMT associated with gastrulation (16, 17). The subsequent formation of the primitive streak is associated with expression of Wnt8c, and ectopic expression of Wnt8c in embryos leads to multiple primitive streaks (18, 19). TGF-β superfamily proteins, notably Nodal and Vg1, mediate the action of Wnts, and their deficiencies can lead to mesodermal defects due to the absence of functional EMTs (20–24). Wnts also cooperate with FGF receptors to help regulate an EMT associated with gastrulation (25–28). The Snail, Eomes, and Mesps transcription factors orchestrate the EMT associated with gastrulation (29–31). For example, Snail represses E-cadherin and induces EMT mediated by cell adhesion molecules, such as occludins and claudins, and by polarity genes, such as Discs large (*Dlg*) and Crumbs homolog 3 (*Crb3*) (32–34).

During embryonic development, an EMT involving the epithelial cells of the neuroectoderm gives rise to migratory neural crest cells (35). Initially, the premigratory neural crest cells express genes such as *Sox*, *Snail*, *Slug*, and forkhead box D3 (*FoxD3*), and these cells subsequently undergo an EMT (36, 37). As a consequence, they then dissociate from the neural folds, become motile, and disperse to the different parts of the embryo, where they undergo further differentiation into, among other cell types, the melanocytes that provide pigment to the skin.

This EMT occurring in the neural crest is triggered by signaling pathways similar to those orchestrating the EMT associated with gastrulation. Thus, signaling pathways mediated by Wnts, FGFs, BMPs, c-Myb, and msh homeobox 1 (Msx-1) conspire to induce EMT (38–40). Among them, BMP most prominently induces the migratory property of neural crest cells. Noggin, an inhibitor of BMP, is essential in negatively regulating this activity, and its expression places a hold on EMT (41, 42). Additionally, E-cadherin and N-cadherin, two cell adhesion molecules, need to be repressed in order for neural crest EMT to occur (43). By some estimates, EMT programs are deployed during several subsequent phases of embryogenesis, indicating the continued involvement of these programs later in development, such as the endothelial-mesenchymal transition (EndMT) that takes place during heart valve formation.

Type 2 EMT: EMT associated with tissue regeneration and organ fibrosis

Organ fibrosis, which occurs in a number of epithelial tissues, is mediated by inflammatory cells and fibroblasts that release a variety of inflammatory signals as well as components of a complex ECM that includes collagens, laminins, elastin, and tenacins (Figure 3). More specifically, such EMTs are found to be associated with fibrosis occurring in kidney, liver, lung, and intestine (44–47). Some of the earliest proof of this came from the study of transgenic mice bearing germ-line reporter genes whose expression was driven by epithelial cell–specific promoters. The behavior of these reporters provided direct evidence for epithelial cells serving, via EMTs, as important precursors of the fibroblasts that arise during the course of organ fibrosis (48–50).

Fibroblast-specific protein 1 (FSP1; also known as S100A4 and MTS-1), an S100 class of cytoskeletal protein, α-SMA, and collagen I have provided reliable markers to characterize the mesenchymal products generated by the EMTs that occur during the development of fibrosis in various organs (48, 49, 51). These markers, along with discoidin domain receptor tyrosine kinase 2 (DDR2), vimentin, and desmin, have been used to identify epithelial cells of the kidney, liver, lung, and intestine that are in the midst of undergoing an EMT associated with chronic inflammation. Such cells continued to exhibit epithelial-specific morphology and molecular markers, such as cytokeratin and E-cadherin, but showed concomitant expression of the FSP1 mesenchymal marker and α-SMA. Such cells are likely to represent the intermediate stages

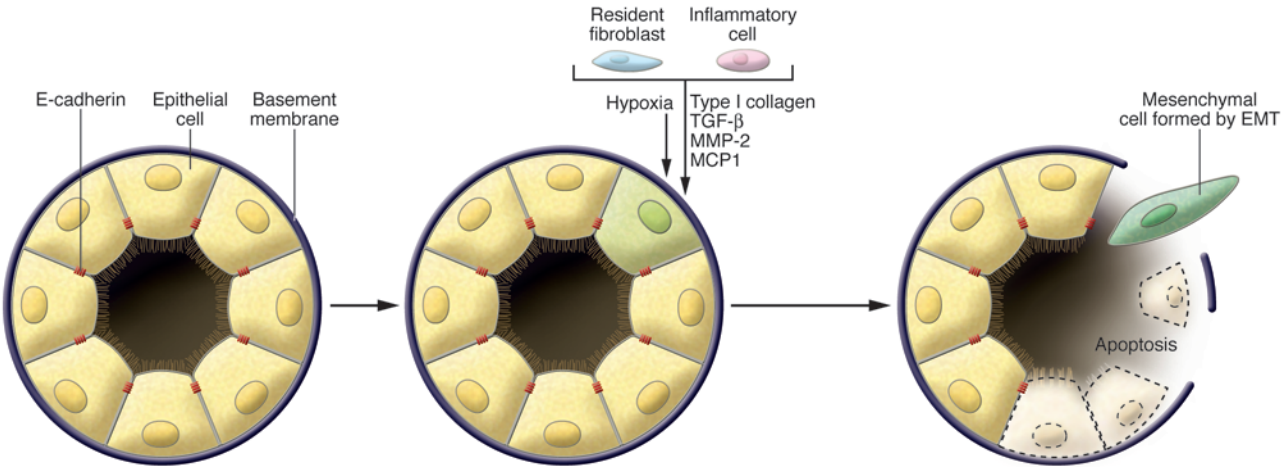

Figure 3
EMT and fibrosis. The EMTs associated with fibrosis are associated with inflammation and the generation of numerous types of molecules by inflammatory cells and resident activated fibroblasts (myofibroblasts). These molecules cause disruption of the epithelial layers via degradation of the basement membrane. The epithelial cells lose polarity and either undergo apoptosis (the majority of cells) or EMT (the minority of cells). MCP1, monocyte chemoattractant protein 1.

of EMT, when epithelial markers continue to be expressed but new mesenchymal markers have already been acquired. The behavior of these cells provided one of the first indications that epithelial cells under inflammatory stresses can advance to various extents through an EMT, creating the notion of "partial EMTs" (Figure 1). Eventually these cells leave the epithelial layer, negotiate their way through the underlying basement membrane, and accumulate in the interstitium of the tissue (52), where they ultimately shed all of their epithelial markers and gain a fully fibroblastic phenotype.

Recent experiments in mice have demonstrated that endothelial cells associated with the microvasculature can also contribute to the formation of mesenchymal cells during the course of fibrosis, doing so via an analogous process known as EndMT (44). This pathologic process echoes a similar normal process occurring during development (45). Thus, during embryogenesis, an EndMT occurs during the organization of the endocardial cushion and the heart valves. In cardiac fibrosis associated with post-ischemic injury of the heart, EndMT, which involves both the endocardium (the inner endothelial layer of the heart) and the microvascular endothelium of the heart, has been demonstrated to play a key role in contributing to the emergence of newly formed fibroblasts (45) (Figure 4). In tissue culture models of EndMT, TGF-β1 induces EndMT of capillary endothelial cells and the loss of endothelial markers, such CD31 and integrin αVβ3, as well as the acquisition of fibroblast- and myofibroblast-specific markers such as FSP1, α-SMA, DDR2, collagen I, and vimentin. While not yet documented, it is plausible that many of the molecular regulators of EMTs also play critical roles in orchestrating EndMTs.

In one analysis (1), lineage-tagging experiments and bone marrow transplant studies demonstrated that during the course of kidney fibrosis in mice, about 12% of fibroblasts are derived from the bone marrow and about 30% are derived via EMT from the tubular epithelial cells of the kidney. In addition, it has been shown more recently that in kidney fibrosis about 35% of fibroblasts are derived via EndMT from the endothelial cells normally residing within the kidney. The remaining portions are speculated to arise via activation of resident fibroblasts or other mesenchymal cells, such as perivascular smooth muscle cells/pericytes and fibrocytes (53) in the circulation (Figure 4). It is likely that these proportions may vary dramatically, depending on the stage of fibrosis, the organ, and the particular experimental model being studied.

This complex pathologic process may reflect the developmental origins of the kidney. Thus, the entire epithelium of the kidney, including the tubular epithelial cells, is derived from the intermediate mesoderm during the development of the urogenital system. More specifically, a mesenchyme condenses over the ureteric bud (derived from the endoderm) to give rise to the glomerular and tubular epithelial structures of the kidney (54), doing so via a MET. This hints at a peculiar aspect of renal biology: by retaining some imprint of their mesenchymal origins, kidney epithelial cells may be particularly prone to return to this state, via the EMTs that occur in response to inflammatory stress and lead to pathologic fibrosis (55, 56).

Inflammatory injury to the mouse kidney can result in the recruitment of a diverse array of cells that can trigger an EMT through their release of growth factors, such as TGF-β, PDGF, EGF, and FGF-2 (57). Most prominent among these cells are macrophages and activated resident fibroblasts that accumulate at the site of injury and release these growth factors. In addition, these cells release chemokines and MMPs, notably MMP-2, MMP-3, and MMP-9. Epithelial cells come under the influence of these signaling molecules and, acting together with the inflammatory cells, induce basement membrane damage and focal degradation of type IV collagen and laminin (57). Delaminated epithelial cells may then migrate toward the interstitial area (the space between epithelial layers) under the influence of gradients of growth factors and other chemoattractants (57). This initial recruitment of epithelial cells into an EMT can be inhibited by blocking the expression of MMP-9 through the disruption of tissue plasminogen activator (tPA) (58). Other studies have also demonstrated that HGF can decrease levels of TGF-β, restore TGF-β–mediated loss of E-cadherin, and potentially decrease amounts of active MMP-9 (59). β1 integrin and integrin-linked kinase (ILK) are also identified as important mediators of the TGF-β–induced EMT associated with tubular epithelial cells

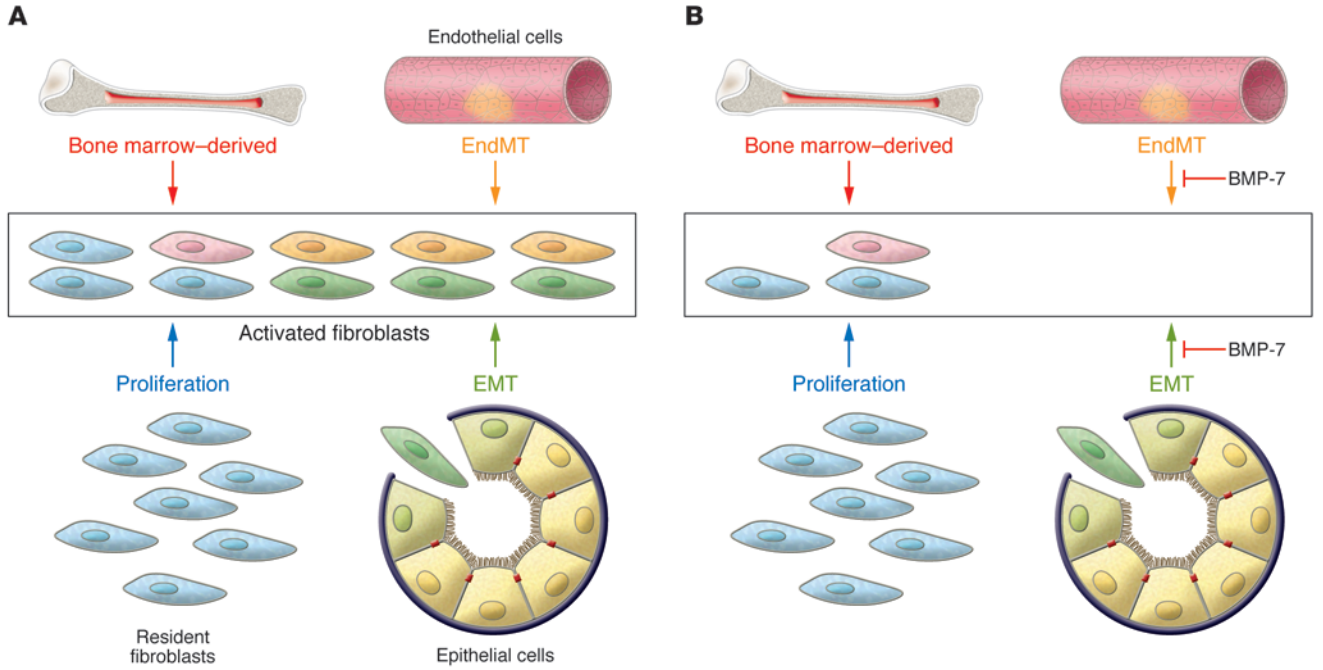

Figure 4
Origin of fibroblasts during fibrosis and its reversal by BMP-7. (**A**) Different sources of fibroblasts in organ fibrosis. Four possible mechanisms are depicted. One study suggests that about 12% of fibroblasts are from bone marrow, about 30% can arise via local EMT involving tubular epithelial cells under inflammatory stress, and about 35% are from EndMT (1). The remaining percentage likely emerge via proliferation of the resident fibroblasts and other still unidentified sources. (**B**) Systemic treatment of mice with renal fibrosis with recombinant human BMP-7 reverses renal disease due to severe attenuation of the formation of EMT- and EndMT-derived fibroblasts.

(60). TGF-β induces EMT via both a Smad2/3-dependent pathway and a MAPK-dependent pathway. Recent experiments have also demonstrated a key role for the E-cadherin/β-catenin signaling axis for EMT involving epithelial cells (61, 62).

The significance of TGF-β–induced EMT for progression of organ fibrosis has been demonstrated in studies using BMP-7, an antagonist of TGF-β signaling, in mouse models of kidney, liver, billiard tract, lung, and intestinal fibrosis (51, 63). BMP-7 functions as an endogenous inhibitor of TGF-β–induced EMT (51, 63) (Figure 4). Among other effects, it reverses the TGF-β–induced loss of the key epithelial protein, E-cadherin (63). Restoration of E-cadherin levels by BMP-7 is mediated via its cognate receptors, activin-like kinase–2/3/6 (ALK-2/3/6), and the Smad4/5 downstream transcription factors (63). Systemic administration of recombinant BMP-7 to mice with severe fibrosis resulted in reversal of EMT and repair of damaged epithelial structures, with repopulation of healthy epithelial cells, all presumably mediated via a MET (63). This reversal was also associated with restoration of organ function, a substantial decrease in $FSP1^+$ and $\alpha\text{-SMA}^+$ interstitial fibroblasts, and the de novo activation of BMP-7 signaling (63).

Studies using fibrosis tissue from humans have also demonstrated EMT (50). In a study of 133 patients with kidney fibrosis, an EMT was demonstrated in a substantial number of the samples, as evaluated using double labeling of the tubular epithelial cells with cytokeratin, vimentin, α-SMA, or zona occludens 1 (ZO-1). Similarly, in patients with Crohn disease, an EMT was demonstrated in areas of fibrosis in the colon (64). Such studies should provide the necessary confidence to develop novel therapeutic interventions in order to suppress EMTs and potentially reverse organ fibrosis.

Type 3 EMT: EMT associated with cancer progression and metastasis

Excessive epithelial cell proliferation and angiogenesis are hallmarks of the initiation and early growth of primary epithelial cancers (65). The subsequent acquisition of invasiveness, initially manifest by invasion through the basement membrane, is thought to herald the onset of the last stages of the multi-step process that leads eventually to metastatic dissemination, with life-threatening consequences. The genetic controls and biochemical mechanisms underlying the acquisition of the invasive phenotype and the subsequent systemic spread of the cancer cell have been areas of intensive research. In many of these studies, activation of an EMT program has been proposed as the critical mechanism for the acquisition of malignant phenotypes by epithelial cancer cells (66).

Many mouse studies and cell culture experiments have demonstrated that carcinoma cells can acquire a mesenchymal phenotype and express mesenchymal markers such as α-SMA, FSP1, vimentin, and desmin (67). These cells typically are seen at the invasive front of primary tumors and are considered to be the cells that eventually enter into subsequent steps of the invasion-metastasis cascade, i.e., intravasation, transport through the circulation, extravasation, formation of micrometastases, and ultimately colonization (the growth of small colonies into macroscopic metastases) (66, 68, 69).

An apparent paradox comes from the observation that the EMT-derived migratory cancer cells typically establish secondary colonies at distant sites that resemble, at the histopathological level, the primary tumor from which they arose; accordingly, they no longer exhibit the mesenchymal phenotypes ascribed to metastasizing carcinoma cells. Reconciling this behavior with

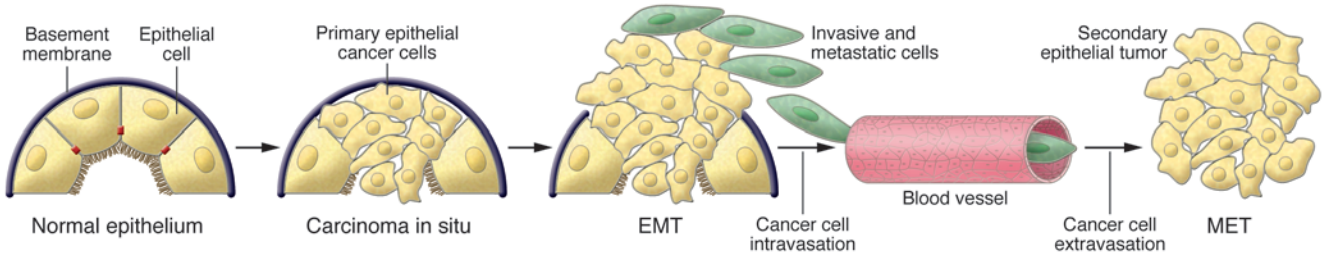

Figure 5
Contribution of EMT to cancer progression. Progression from normal epithelium to invasive carcinoma goes through several stages. The invasive carcinoma stage involves epithelial cells losing their polarity and detaching from the basement membrane. The composition of the basement membrane also changes, altering cell-ECM interactions and signaling networks. The next step involves EMT and an angiogenic switch, facilitating the malignant phase of tumor growth. Progression from this stage to metastatic cancer also involves EMTs, enabling cancer cells to enter the circulation and exit the blood stream at a remote site, where they may form micro- and macro-metastases, which may involve METs and thus a reversion to an epithelial phenotype.

the proposed role of EMT as a facilitator of metastatic dissemination requires the additional notion that metastasizing cancer cells must shed their mesenchymal phenotype via a MET during the course of secondary tumor formation (70). The tendency of disseminated cancer cells to undergo MET likely reflects the local microenvironments that they encounter after extravasation into the parenchyma of a distant organ, quite possibly the absence of the heterotypic signals they experienced in the primary tumor that were responsible for inducing the EMT in the first place (66, 71, 72). These considerations indicate that induction of an EMT is likely to be a centrally important mechanism for the progression of carcinomas to a metastatic stage and implicates MET during the subsequent colonization process (Figure 5). However, many steps of this mechanistic model still require direct experimental validation. Moreover, it remains unclear at present whether these phenomena and molecular mechanisms relate to and explain the metastatic dissemination of non-epithelial cancer cells.

The full spectrum of signaling agents that contribute to EMTs of carcinoma cells remains unclear. One suggestion is that the genetic and epigenetic alterations undergone by cancer cells during the course of primary tumor formation render them especially responsive to EMT-inducing heterotypic signals originating in the tumor-associated stroma. Oncogenes induce senescence, and recent studies suggest that cancer cell EMTs may also play a role in preventing senescence induced by oncogenes, thereby facilitating subsequent aggressive dissemination (73–75). In the case of many carcinomas, EMT-inducing signals emanating from the tumor-associated stroma, notably HGF, EGF, PDGF, and TGF-β, appear to be responsible for the induction or functional activation in cancer cells of a series of EMT-inducing transcription factors, notably Snail, Slug, zinc finger E-box binding homeobox 1 (ZEB1), Twist, Goosecoid, and FOXC2 (66, 71, 76–79). Once expressed and activated, each of these transcription factors can act pleiotropically to choreograph the complex EMT program, more often than not with the help of other members of this cohort of transcription factors. The actual implementation by these cells of their EMT program depends on a series of intracellular signaling networks involving, among other signal-transducing proteins, ERK, MAPK, PI3K, Akt, Smads, RhoB, β-catenin, lymphoid enhancer binding factor (LEF), Ras, and c-Fos as well as cell surface proteins such as β4 integrins, α5β1 integrin, and αVβ6 integrin (80). Activation of EMT programs is also facilitated by the disruption of cell-cell adherens junctions and the cell-ECM adhesions mediated by integrins (67, 75, 81–86).

TGF-β is an important suppressor of epithelial cell proliferation and thus primary tumorigenesis. However, it is now clear that in certain contexts it can also serve as a positive regulator of tumor progression and metastasis (87–89). Thus, in vitro studies have demonstrated that TGF-β can induce an EMT in certain types of cancer cells (90). Two possible signaling pathways have been identified as mediators of TGF-β–induced EMT. The first of these involves Smad proteins, which mediate TGF-β action to induce EMTs via the ALK-5 receptor (51, 91–95). Smad-mediated signaling induced by TGF-β facilitates motility. Inhibitory Smads modulate differential effects of relevant transcription factors and cytoplasmic kinases and induce the autocrine production of TGF-β, which can further reinforce and amplify the EMT program (91, 92, 96, 97). Signaling pathways that mediate the action of β-catenin and LEF also cooperate with Smads (61, 98) in inducing an EMT (61, 99, 100). In this regard, the involvement of LEF and β-catenin in PDGF-induced EMT was recently described (98). These studies collectively demonstrate that the TGF-β/Smad/LEF/PDGF axis is an important inducer of an EMT phenotype in cancer.

Evidence for the involvement of a second TGF-β–induced pathway in EMT is also compelling. More specifically, some data indicate that p38 MAPK and RhoA mediate an autocrine TGF-β–induced EMT in NMuMG mouse mammary epithelial cells (96, 101). This process also requires integrin β1–mediated signaling and the activation of latent TGF-β by αVβ6 integrin (96, 101). Fibulin-5, an ECM molecule, augments TGF-β–induced EMT in a MAPK-dependent mechanism (102). TGF-β can induce an EMT in Ras-transformed hepatocytes, mammary epithelial cells (via MAPK), and MDCK cells; at the same time, Ras-activated PI3K inhibits TGF-β–induced apoptosis to facilitate this transition (103–106). Evidence for these connections comes from observations that ERK/MAPK and PI3K/Akt pathways mediate Ras mutant–induced EMT, and that such an EMT is reversed by either wild-type Ras or MAPK kinase 1 (MEK1) inhibitors (106). In this regard, Raf also mediates TGF-β–induced EMT and promotes invasiveness of cancer cells. In mouse models of skin carcinoma and human colon cancer, the absence of TGF-β receptor expression actually confers better prognosis (107, 108). The connection between inflammation and EMT was demonstrated when COX-2 was shown to inactivate Smad signaling and enhance EMT stimulated by TGF-β through

a PGE_2-dependent mechanism (109). Changes in the expression of certain cell polarity proteins may also play an important role in TGF-β–induced EMT, since evidence of a role for partitioning-defective protein 6 (Par6) in this process is emerging (66, 110).

The connection between loss of E-cadherin expression by cancer cells and passage through an EMT has been established by many studies (111, 112). For example, induction of the *c-Fos* oncogene in normal mouse mammary epithelial cell lines induces an EMT and is associated with a decrease in E-cadherin expression (99). Moreover, epithelial cell adhesion complexes reorganize and cell proliferation is suppressed when the full-length or the cytoplasmic portion of E-cadherin (containing the β-catenin binding site) is ectopically expressed in cells that have passed through an EMT, causing such cells to lose their mesenchymal phenotype (99, 113). Sequestration of β-catenin in the cytoplasm is important for the preservation of epithelial features of cancer cells, and acquisition of the mesenchymal phenotype correlates with the movement of β-catenin to the nucleus, where it becomes part of Tcf/LEF complexes (100, 114). Such β-catenin accumulation in the nucleus, which is often associated with loss of E-cadherin expression, correlates with susceptibility to enter into an EMT and acquisition of an invasive phenotype (61, 66). Thus, cells that lose cell surface E-cadherin become more responsive to induction of an EMT by various growth factors (61).

Some studies have demonstrated that the epigenetic control of E-cadherin and β-catenin/LEF activity is important in establishing the metastatic potential of cancer cells (115–118). Cell lines that lack E-cadherin show increased tumorigenicity and metastasis when transferred into immunodeficient mice (118). E-cadherin expression levels vary dramatically in different human tumors, and an inverse relationship between levels of E-cadherin and patient survival has been documented (117). In this regard, mutations in the E-cadherin gene have been identified in cancer cells, making them more susceptible to EMT and metastasis (115, 116). A more thorough analysis of such mutations and their correlation to metastatic progression is still required.

The central role played by E-cadherin loss in the EMT program is further illustrated by the actions of several EMT-inducing transcription factors that facilitate acquisition of a mesenchymal phenotype, such as Snail and Slug, as well as those encoding two key zinc finger–containing basic helix-loop-helix transcription factors, survival of motor neuron protein interacting protein 1 (SIP1) and E12 (also known as E47-E2A). These transcription factors are induced by TGF-β exposure and, once expressed, repress E-cadherin expression (78). Snail also facilitates an invasive phenotype in mice (31). Loss of E-cadherin promotes Wnt signaling and is associated with high levels of Snail in the nucleus (119). SIP1 represses E-cadherin expression and binds, along with Snail, to the E-cadherin promoter in an overlapping fashion (120, 121). The expression of *Snail* and E-cadherin correlates inversely with the prognosis of patients suffering from breast cancer or oral squamous cell carcinoma (119, 122). The use of gene expression analyses to compare expression of genes in metastatic and non-metastatic mouse breast cancer cell lines has led to the identification of *Twist* and *Goosecoid* as important genes that facilitate EMT and induce metastasis (85, 123). Some have reported that matrix-degrading enzymes such as MMP-3 facilitate EMT by inducing genomic instability via Rac1b and ROS (124).

Noncoding microRNAs are also components of the cellular signaling circuitry that regulates the EMT program. For example, microRNA 200 (miR200) and miR205 inhibit the repressors of E-cadherin expression, ZEB1 and ZEB2, and thereby help in maintaining the epithelial cell phenotype (125–129). In breast carcinoma, a loss of miR200 correlates with increased expression of vimentin and a decrease in the levels of E-cadherin in cancer cells (125–127, 129). Acting in the opposite direction, miR21 is upregulated in many cancers and facilitates TGF-β–induced EMT (130). Interestingly, $CD44^{hi}CD20^{lo}$ cells purified from normal and malignant breast cancer tissue exhibit features of an EMT, and human cancer cells induced to undergo EMT exhibit stem cell–like properties and increased metastatic potential (84). Therefore, EMT may play a role in the generation of high-grade invasive cells with stem cell–like features, and the latter phenotype, which includes self-renewal potential, may facilitate the formation of secondary tumors by disseminating cancer cells, a notion that still requires direct demonstration.

Perspective

It is now clear that EMTs occur in three distinct biological settings. While the outcome is the generation of motile cells of mesenchymal phenotype, the mechanisms of EMT induction and progression vary dramatically from one setting to another. The EMTs associated with implantation, embryogenesis, and organ development (type 1 EMTs) are driven by the evolutionary need to remodel and diversify tissue in order to enable proper morphogenesis and generate a functional organism. Such EMTs occur transiently and are not associated with inflammation, fibrosis, and systemic dissemination. Type 2 EMTs are associated with tissue regeneration and fibrosis, and they depend on inflammation-inducing injuries for their initiation and continued occurrence. Accordingly, these EMTs continue to occur until the provoking injuries or infections are removed and, in the case of injuries, the tissue is repaired. Type 2 EMTs generate activated mesenchymal cells, notably myofibroblasts that produce excessive amounts of collagen-rich ECM. Type 3 EMTs occur in the context of tumor growth and cancer progression, when cancer cells at the invasive front of the tumors convert to a mesenchymal phenotype. The induction of type 3 EMTs is facilitated by the genomic alterations acquired by cancer cells and these EMTs generate cells with invasive properties that enable them to move into the blood stream and spread systemically to other organs. Future research will surely focus on uncovering the molecular similarities and differences among the EMT programs that occur in the three distinct settings.

EMT research in the next few years promises to be exciting, as new mouse models and molecular probes are identified to address important, still-unanswered questions. For example, what are the identities of the EMT-inducing microenvironmental signals? What is the nature of the changes in cells that render them responsive to such signals? And what signaling machinery within epithelial cells orchestrates the various EMT programs?

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