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Review

Epithelial-Mesenchymal Transition: At the Crossroads of Development and Tumor Metastasis

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The epithelial-mesenchymal transition is a highly conserved cellular program that allows polarized, immotile epithelial cells to convert to motile mesenchymal cells. This important process was initially recognized during several critical stages of embryonic development and has more recently been implicated in promoting carcinoma invasion and metastasis. In this review, we summarize and compare major signaling pathways that regulate the epithelial-mesenchymal transitions during both development and tumor metastasis. Studies in both fields are critical for our molecular understanding of cell migration and morphogenesis.

An Overview of Epithelial-Mesenchymal Transition

The development of metazoan organ systems starts with a single layer of epithelial cells, which constitutes the primary building block for constructing organismic complexity. Epithelial cells form a sheet or layers of cells that are tightly connected laterally by specialized junction structures, including adherens junctions, desmosomes, tight junctions, and gap junctions. Among these, adherens junctions play a particularly important role in assembling and constructing lateral cell-cell adhesions in epithelial cell sheets (Yap et al., 1997). Epithelial cells establish an aligned apical-basal polarity through their association with a lamina layer at their basal surface, often called the basement membrane. Under normal conditions, this anchoring to the basement membrane ensures that epithelial cells can only migrate laterally along the basal surface, thereby maintaining their positioning within the epithelium and precluding their entrance into the underlying extracellular matrix (ECM).

During early embryogenesis of most metazoans, mesenchymal cells arise from the primitive epithelium. In contrast to epithelial cells, mesenchymal cells exhibit a front-back end polarity and rarely establish direct contacts with neighboring mesenchymal cells (Hay, 1995). Unlike epithelial cells, mesenchymal cells can invade as individual cells through ECM constructed by epithelial sheets and by mesenchymal cells themselves.

While epithelial and mesenchymal cell types have long been recognized in early embryos, the conversion of epithelial cells into mesenchymal cells was only defined as a distinct cellular program in 1980s. In a series of elegant experiments, Greenburg and Hay showed that when epithelial cells from embryonic and adult anterior lens were cultured in 3D collagen gels, these cells elongated, detached from the explants, and migrated as individual cells (Greenburg and Hay, 1982, 1986, 1988). Based on the mesenchymal morphology and the pseudopodia and filopodia structures of these migrating cells, they concluded that differentiated epithelial cells could be transformed into mesenchymal cells through a cellular program they named Epithelial-Mesenchymal Transition (EMT).

Subsequent cell-biological and molecular studies of EMT resulted in this program being loosely defined by three major changes in cellular phenotype (Boyer and Thiery, 1993; Hay, 1995): (1) morphological changes from a cobblestone-like monolayer of epithelial cells with an apical-basal polarity to dispersed, spindle-shaped mesenchymal cells with migratory protrusions; (2) changes of differentiation markers from cell-cell junction proteins and cytokeratin intermediate filaments to vimentin filaments and fibronectin (in addition, certain integrins [Zuk and Hay, 1994] and splicing variants of FGFR2 [Savagner et al., 1994] switch from epithelial to mesenchymal subtypes); and (3) the functional changes associated with the conversion of stationary cells to motile cells that can invade through ECM. Not all three changes are invariably observed during an EMT; however, acquisition of the ability to migrate and invade ECM as single cells is considered a functional hallmark of the EMT program.

The EMT program is activated at multiple steps of embryonic development to enable the conversion of various types of epithelial cells into mesenchymal cells. Passage through an EMT, however, does not necessarily represent an irreversible commitment to switch differentiation lineages. Thus, the reverse program, termed the Mesenchymal-Epithelial Transition (MET), also occurs both during embryonic development and during several pathological processes (Boyer and Thiery, 1993; Davies, 1996). The reversibility of this process underscores the enormous plasticity of certain embryonic and adult cells that participate in processes of disease pathogenesis.

EMT in Development

During development, the EMT program has been observed to underlie a variety of tissue remodeling events, including mesoderm formation, neural crest development, heart valve

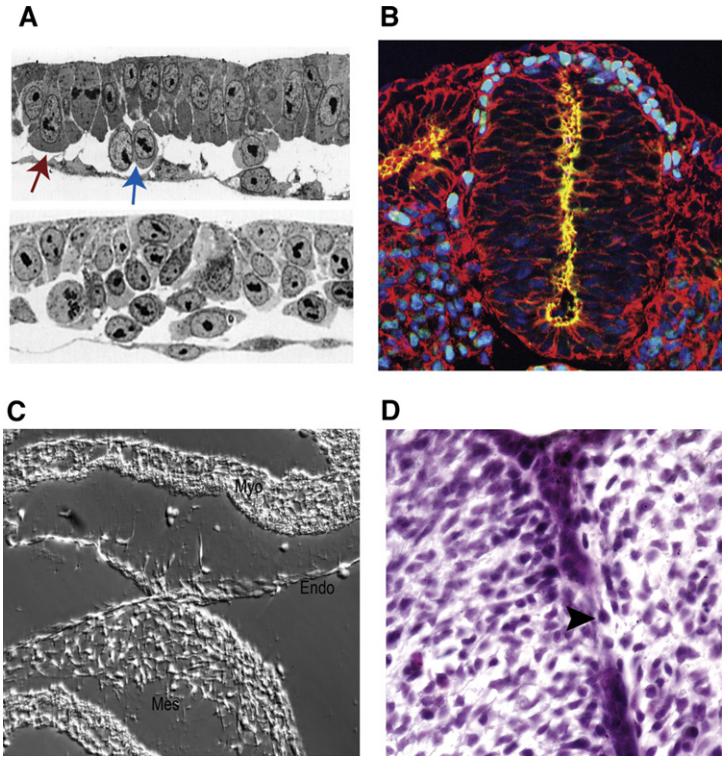

Figure 1. Images of EMT Processes during Embryogenesis

(A) Primitive streak formation. The top panel shows the posterior third of a midsagittal 1 μm plastic section from early streak stage rabbit embryo at 6.6 days post coitum (d.p.c.) with three definitive mesoderm cells (blue arrow) between epiblast and hypoblast and a bottle-shaped epiblast cell (red arrow), which is about to ingress to become a definitive mesoderm cell (Viebahn, 1995). The lower panel shows the center of a transverse 1 μm plastic section through the center of the primitive streak of a full primitive streak stage rabbit embryo at 6.7 d.p.c. with multiple bottle-shaped epiblast cells near the primitive pit (center of the picture) and mesoderm cells filling the space between epiblast and hypoblast (Viebahn et al., 1995). Image is courtesy of Christoph Viehahn (University of Göttingen, Göttingen, Germany) and S. Karger AG, Basel.

(B) Neural crest formation. Immunofluorescence image showing that neural crest cells (light blue) emerge from the neural epithelium (yellow). Neural crest cells are stained with SOX9 antibody (light blue); neural epithelia cells are stained with N-cadherin (yellow). Phalloidin (red) is used to label all cells, and DAPI (deep blue) is used to label cell nuclei. Image is courtesy of James Briscoe (National Institute for Medical Research, London, UK).

(C) Cardiac valve formation. Light microscopy on a section of the atrium and ventricular flow of an E9.5 mouse embryo heart. Note that mesenchymmal cells (Mes) are emerging from the cardial endothial cell layer (Endo). Image is courtesy of Raymond B. Runyan (University of Arizona, Tucson, Arizona, USA).

(D) Secondary palate formation. A hematoxylin- and eosin-stained section of rodent palate during palatogenesis in vitro is shown. The palatal seam begins to disintegrate and epithelial cells break away from the intact seam to become mesenchymal within 24 hr (arrowhead). Image is courtesy of Ali Nawshad (University of Nebraska Medical Center, Lincoln, Nebraska, USA).

development, secondary palate formation, and male Müllerian duct regression. In this section, we discuss in more detail four examples of the best-studied EMT events that occur during embryogenesis (Figure 1). Mesoderm formation and neural crest development represent the key EMT programs that occur during early embryonic development, and the resulting mesenchymal and neural crest cells maintain oligopotentiality, enabling them to further differentiate into various cell types. In contrast, heart valve development and secondary palate formation occur in relatively well-differentiated epithelial cells that are destined to become defined mesenchymal cell types. The latter two processes, which occur in well-differentiated epithelia, raise the possibility that EMTs may also be induced under certain physiological or pathological conditions in adult tissues, including tumor invasion and metastasis processes that will be discussed below.

Mesoderm Formation

The earliest example of an EMT program participating in embryogenesis is the formation of mesoderm from the primitive ectoderm, a process that is initiated during gastrulation. Most of our molecular understanding of the EMT program during gastrulation is derived from model organisms, including *Drosophila* and amphibian and avian embryos (Kimelman, 2006). More recent studies indicate that the same basic principles apply to mammalian embryos (Viebahn, 1995).

The induction of mesoderm begins in a specific area of the primitive ectoderm, termed the ventral furrow in *Drosophila*, the blastopore lip in *Xenopus*, and the primitive streak in birds and mammals. The first event in mesoderm formation is the invagination of the epithelial cells. This step is characterized by drastic morphological changes in a small population of epithelial cells, which include the narrowing of their apical compartments, the redistribution of organelles to the apical location, and the bulging of the basal compartments. When the epithelial cells are ready to ingress, the basement membrane is breached locally. At this point, the ingressing epithelial cells lose their tight cell-cell adhesions and remain attached to neighboring cells only by sparsely distributed focal contacts. Subsequently, these cells undergo mesenchymal differentiation and migrate along the narrow extracellular space underneath the ectoderm (Viebahn, 1995). The newly gained ability for such ectoderm-derived cells to migrate along and through ECM marks the completion of the EMT program during gastrulation.

Neural Crest Formation

Another dramatic example of EMT participating in embryogenesis involves the generation of the neural crest, a defining tissue of vertebrates. The neural crest is composed of a population of precursor cells that are equipped with the ability to migrate over extraordinarily long distances in the embryo. Based on certain similarities, formation of the neural crest has been portrayed as a second gastrulation event in vertebrates (Duband et al., 1995).

The neural crest develops at the boundary between the neural plate and the epidermal ectoderm. The emergence of neural crest cells begins with the presence of a distinct population of cells with rounded and pleiomorphic shapes, which contrast with those of the polarized neural tube cells that form nearby. Like the EMT program during gastrulation, the presumptive neural crest cells proceed to lose N-cadherin-mediated cell-cell adhesion while becoming excluded from the neural epithelium (Tucker et al., 1988). Detailed immunolabeling and electron microscopic studies revealed that disruption of the basal lamina occurs immediately before or at the onset of neural crest cell migration in the cranial regions of avian and mouse embryos

(Duband and Thiery, 1987; Nichols, 1981). These observations indicate that neural crest cells actively invade through the basal lamina, just as in the case of gastrulation. However, in the trunk region of both avian and mouse embryos, a basal lamina is not present at the neural fold before the onset of neural crest migration, obviating a rate-limiting step in the release of neural crest cells (Martins-Green and Erickson, 1987). These findings suggest that the cranial and trunk neural crest cells might employ distinct types of subcellular machinery to initiate EMT, invade the ECM, and migrate.

As presumptive neural crest cells are released from the neural epithelium, they concomitantly upregulate genes required for mesenchymal phenotype and migratory ability. One critical component of neural crest migration is ECM that is laid down along the migratory path, helping to ensure that these cells reach appropriate destinations. Studies of this process have revealed that high levels of fibronectin and hyaluronan appear in the presumptive neural crest area before the onset of migration (Poelmann et al., 1990), which raises the intriguing possibility that expression of these molecules is critical to the subsequent migratory ability of these cells.

Cardiac Valve Formation

The endocardial cushion is the precursor structure to the heart valve in vertebrate embryos and begins to form soon after the primitive linear heart tube begins to loop. Initially, the myocardial cells secrete a large amount of ECM, which displaces the endocardium away from the myocardium, thereby creating endocardial cushions. After cushion formation, mesenchymal cells fill the cushion space (Markwald et al., 1977). Video microscopy of isolated atrioventricular (AV) canals in culture has demonstrated that these mesenchymal cells derive from the endocardial cell layer by undergoing an EMT (Bolender and Markwald, 1979). Through use of an elegant AV canals explant culture system in a 3D, type I collagen gel, detailed molecular studies have shown that signals secreted from the AV myocardium are essential for induction of an EMT in the AV endocardial cells (Mjaatvedt and Markwald, 1989; Rezaee et al., 1993). After receiving EMT-inducing stimuli, AV endocardial cells exhibit decreased expression of N-CAM, lose cell-cell adhesion, and invade the newly deposited endocardial cushion, thereby establishing the presumptive cardiac septa and valves (Person et al., 2005).

The ability to model this EMT event in the explant culture has greatly facilitated molecular characterization of individual signals involved in this program. Several inducing signals, transcription factors, and ECM components have been tested for their involvement in cardiac valve formation. Below we discuss several EMT-inducing signals that are involved in EMT that occurs during cardiac valve formation.

Secondary Palate Formation

Another relatively well-studied EMT event involving differentiated epithelial cells involves formation of the secondary oral palate. Dissecting the palatal tissue and introducing it into organ cultures has made it possible to trace the entire program of palate remolding by electron microscopy and immunohistochemistry. Development of the secondary palate requires fusion of the palatal shelves at the midline. As the shelves approach one another from opposite sides of the developing oral cavity, epithelial cells covering the tip of each shelf intercalate and form the medial epithelial seam. Soon after fusion, these medial epithelial cells undergo an EMT and are integrated into the mesenchymal compartment of the palate, thereby completing the program of palatogenesis (Fitchett and Hay, 1989). Interestingly, blocking fusion of the palatal shelves has been shown to prevent basal epithelial cells from undergoing an EMT, indicating that signals from the epithelial midline seam are critical to the triggering of the EMT process (Griffith and Hay, 1992).

MET in Development

During embryogenesis, the EMT program does not always represent an irreversible process that permanently commits cells to one or another fate. Instead, in several cases, the converted mesenchymal cells can revert to an epithelial cell state by passing through an MET. The best-studied MET event during embryogenesis is the formation of the nephron epithelium in the developing kidney. During this process, nephric mesenchymal cells aggregate around individual branches of the ureteral bud, begin to express laminin, polarize, and eventually develop cell-cell adhesions and differentiate into epithelial cells that form kidney tubules (Davies, 1996). The ability of a mesenchymal cell to revert to an epithelial identity demonstrates, once again, substantial cell plasticity and additionally suggests that interconversion between epithelial and mesenchymal cell states may also occur under certain pathological conditions.

Molecular Regulation of EMT

Both developmental studies in various organisms and tissue culture studies have yielded a number of distinct signaling pathways that regulate EMT. Here we focus our discussion on the extracellular signals responsible for inducing EMTs and the key transcriptional factors that respond these signals; such transcription factors function as master regulators of the EMT programs in vertebrates (Table 1).

TGF-β Signaling

Members of the transforming growth factor-β (TGF-β) superfamily have been implicated as major induction signals of EMT during almost all of the morphogenetic events discussed above. Extensive studies in various developmental EMT systems provide convincing evidence that TGF-β signaling is a primary inducer of EMT, although the precise signaling pathways activated by individual family members may differ during various EMT events.

In both *Xenopus* and zebrafish embryos, mesoderm induction is initiated mainly by members of the Nodal subfamily of TGF-β (Kimelman, 2006). Several elegant studies in *Xenopus* using activin as a Nodal pathway activator or using different doses of Nodal inhibitors indicate that activation of Nodal signaling is essential for mesoderm induction in both the dorsal and ventral regions (McDowell and Gurdon, 1999). Another Nodal family member, termed Vg1 in *Xenopus* and chicken and GDF3 in mice, is also known to play a critical role in mesoderm induction. Overexpression of chick Vg1 on its own is sufficient to induce the formation of a secondary primitive steak (Shah et al., 1997). In mice, *Gdf3* null mutants frequently exhibit defects in mesoderm formation (Chen et al., 2006).

Induction of neural crest requires the activation of BMP, another TGF-β superfamily member. In *Xenopus*, experiments examining differential activation of the BMP signal indicate that BMPs set up a competency zone between neural plate and ectoderm from which the neural crest can be induced (Raible,

Table 1. Examples of EMTs during Vertebrate Embryonic Development and the Corresponding Signaling Pathways

Developmental Process	Transition	The TGF-ß Pathway	The Wnt Pathway	The Notch Pathway	Tyrosine Kinase Receptors	Transcription Factors
Mesoderm formation	primitive ectoderm to mesoderm	Nodal	canonical Wnts	N.D.	FGFR	Snail, Slug, Goosecoid
Neural crest formation	neural epithelium to neural crest cells	BMP	canonical Wnts, noncanonical Wnt11/Frizzled7	regulate BMP signaling	FGFR, PDGFR	Snail, Slug, Twist1, SIP1
Cardiac valve formation	endocardial cells to cardiac septa and valves	TGF-β 1, 2, 3, BMP	canonical Wnts	Jagged 1/ Notch1	ErbB3	Snail, Slug
Secondary palate formation	palatal shelf epithelial cells to palatal mesenchymal cells	TGF-β3	N.D.	N.D.	EGFR, PDGFR	N.D.

N.D., not determined.

2006). In chickens and mice, BMPs have also been shown to be required for the induction and/or migration of neural crest cells (Correia et al., 2007).

TGF-β signals also play critical roles in the activation of EMT during cardiac valve formation and secondary palate fusion. In a series of elegant antibody-blocking experiments using chicken AV explants, TGF-β2 was shown to be essential for the initiation of the EMT program and the separation of endothelial cells; in contrast, TGF-β3 was specifically required for the migration of the separated endothelial cells into ECM (Boyer et al., 1999). In mice, experiments using individual *TGF-β1* or *TGF-β2* knockout mice and heart explant culture also revealed the involvement of TGF-β1 and TGF-β2 in the EMT process occurring during cardiac morphogenesis (Mercado-Pimentel and Runyan, 2007). Interestingly, the *TGF-β3* knockout mice present a cleft palate phenotype, and the role of this TGF-β variant in initiating EMT to enable palatal fusion has been further confirmed using a TGF-β3-blocking antibody in isolated mouse palatal shelf explants (Ahmed et al., 2007).

One of the key questions about the role of TGF-β signaling in EMT is how various TGF-β signals are translated into the transcriptional signaling networks and the cellular responses required for EMT and cell migration. In cultured mammary epithelial cells, TGF-β receptors are localized at tight junctions and directly interact with two important regulators of epithelial cell polarity and tight-junction assembly, Par6 (Ozdamar et al., 2005) and Occludin (Barrios-Rodiles et al., 2005). Phosphorylation of Par6 by TGF-β type II receptor leads to a loss of tight junctions and apical-basal polarity (Ozdamar et al., 2005). Most developmental studies also suggest that the TGF-β pathway collaborates with Wnt, Notch, and receptor tyrosine kinase signals to generate the specificities required for EMT in various morphogenetic steps. In cultured MDCK epithelial cells, activation of the TGF-β/Smad pathway has been shown to coordinate with Ras activation to promote a full EMT phenotype (Grunert et al., 2003). Future studies are needed to reveal how these complex networks interact to coordinate and specify individual steps of the EMT program.

The Wnt Signal

The canonical Wnt pathway is implicated in the initiation and maintenance of mesoderm formation. In *Xenopus* and zebrafish, Wnt8 is required for the formation of dorsal mesoderm (Kelly et al., 1995; Smith and Harland, 1991; Sokol et al., 1991). In mice, *Wnt3* null mutants show defects in the formation of the primitive streak (Liu et al., 1999).

Activation of neural crest cell delamination and migration involves both canonical and noncanonical Wnt signaling. In *Xenopus*, gain-of-function experimental approaches demonstrate that activation of Wnt signaling induces ectopic neural crest, while depletion of β-catenin confirms that the canonical Wnt pathway is required for initial neural crest induction (Wu et al., 2005). Furthermore, activation of Wnt11/Frizzled7-mediated noncanonical Wnt signaling also plays an essential and specific role in neural crest migration in *Xenopus* (De Calisto et al., 2005). In avian embryos, Wnt is necessary and sufficient to induce neural crest cells (Garcia-Castro et al., 2002).

The role of Wnt signaling in heart valve induction is also well documented. β-catenin transcription activity has been observed in the newly formed mesenchyme of cardiac cushions during EMT in both zebrafish (Gitler et al., 2003; Hurlstone et al., 2003) and mouse embryos (Liebner et al., 2004). In mice, inactivation of *β-catenin* in endothelial cells inhibits EMT and cardiac cushion formation both in vivo and in tissue explants (Liebner et al., 2004).

The Notch Pathway

Notch signaling has also been implicated in modulating the EMT program during embryogenesis. For example, in frog and chick, Notch signaling regulates cranial neural crest cells indirectly through its effect on expression of BMP family members (Cornell and Eisen, 2005). Another study identified a role for Notch in promoting EMT during cardiac valve formation and in cultured epithelial cells from mammary gland, kidney tubules, and epidermis (Timmerman et al., 2004). In mice and zebrafish, embryos lacking components of the Notch pathway maintain endocardial adhesion complexes and fail to undergo endocardial EMT. Conversely, overexpression of activated Notch1 induces EMT in immortalized endothelial cells (Timmerman et al., 2004). Furthermore, in epithelial cells from the mammary gland, kidney tubules, and epidermis, the Notch ligand Jagged 1 and the Notch target Hey1 are induced by TGF-β at the onset of EMT in a Smad3-dependent fashion (Zavadil et al., 2004). Unlike the TGF-β and Wnt pathways, activation of the Notch signaling pathway is not conserved among all the EMT processes that occur during the course of embryogenesis. It is therefore reasonable to predict

that Notch signaling is not sufficient and needs to be coordinated with additional signaling inputs in order to promote an EMT.

Signals from Tyrosine Kinase Receptors

In addition to TGF-β, several other tyrosine kinase receptors, including Met, FGF, IGF, EGF family members, and more recently PDGF, also play critical roles in regulating EMT-like morphogenetic events that occur during development. For example, FGFR1 orchestrates the EMT and morphogenesis of mesoderm at the primitive streak by controlling E-cadherin expression in mice (Ciruna and Rossant, 2001). In mice, P38 MAP kinase downregulates E-cadherin independent of the FGF signaling at the primitive streak (Zohn et al., 2006). Furthermore, activation of ErbB2-ErbB3 is required for the EMT program during mouse cardiac valve formation (Camenisch et al., 2002). In mice, ablation of the *HGF* or *Met* genes results in the complete absence of all muscle groups that are derived from long-range migrating progenitor cells. This defect is due to an absence of delamination by the migratory progenitors (Dietrich et al., 1999). The similarity between muscle progenitor cell release and the EMT program suggests a role for HGF signaling in regulating an EMT-like morphological change occurring during this critical developmental step as well.

A partial EMT has been implicated in the branching morphogenesis that occurs during the formation of several organs, including the trachea, the kidneys, and the mammary glands. During *Drosophila* tracheal development, FGF signaling is essential in guiding the migration of the tracheal cells during branch budding (Ghabrial et al., 2003), and this function of FGF is also conserved in mice. In the mouse mammary gland, overexpression of HGF causes hyperplastic branching morphogenesis, while inhibition of HGF/Met signaling blocks the budding of side branches (Rosario and Birchmeier, 2003). Therefore, it is likely that receptor tyrosine kinase pathways contribute to this EMT-like program during these branching morphogenesis events.

Receptor tyrosine kinase pathways are also clearly involved in EMT in certain cell types in culture. The HGF/Met pathway promotes the partial EMT (''scattering'') phenotype of MDCK epithelial cells in monolayer culture. Extensive biochemical analyses have shown that multiple downstream signaling pathways, including Ras, MAP kinase, PI3 kinase, and Rac/Cdc42, are activated to coordinate the change in cell adhesion and motility that underlies this scattering (Birchmeier et al., 1997). In a mouse carcinoma cell line, NBT-II, the scattering phenotype induced by FGF1, EGF, or HGF, is due to the delocalization of E-cadherin from adherens junctions, suggesting that receptor tyrosine kinases can induce E-cadherin downregulation during an EMT (Boyer et al., 1997). Recently, a PDGF/PDGF receptor autocrine loop was induced during TGF-β-induced EMT, which was essential for acquisition of a complete EMT phenotype (Jechlinger et al., 2003).

Transcriptional Regulation

During the execution of the EMT program, many genes involved in cell adhesion, mesenchymal differentiation, cell migration, and invasion are transcriptionally altered. The best-studied transcriptional modulation during EMT is that involving the *E-cadherin* gene promoter. Indeed, functional loss of E-cadherin in an epithelial cell has been considered a hallmark of EMT. Detailed analyses of the human *E-cadherin* promoter have identified E-box elements that are responsible for its transcriptional repression in non-E-cadherin-expressing mesenchymal cells (Giroldi et al., 1997; Hennig et al., 1995). In 2000, the zinc-finger transcription factor Snail was found to directly bind to the E-boxes of the *E-cadherin* promoter and to repress transcription of this gene (Batlle et al., 2000; Cano et al., 2000). Since this important discovery, several additional zinc-finger transcription factors have been found to be capable of repressing *E-cadherin* transcription, thereby causing the dissolution of cell-cell adhesion that occurs during EMT; these include Slug, a close relative of Snail (Hajra et al., 2002), and two members of the ZEB family of transcription factors, ZEB1 (δEF1) (Eger et al., 2005) and ZEB2 (SIP1) (Comijn et al., 2001). More recent studies have found that ZEB1, Snail, and Slug are capable of repressing the transcription of several polarity factors, including *Crumbs3* and *Lgl2* (Aigner et al., 2007; Spaderna et al., 2008), indicating their roles in suppressing critical components of epithelial cell traits.

These transcription factors are involved in various EMT processes occurring during embryogenesis. During fly mesoderm formation, for example, both Snail and Slug are expressed to promote the dissociation of cell adhesion, thereby allowing the migration and differentiation of epithelial cells. This particular function of Snail in gastrulation is also apparent in mice, as Snail knockout mice display a severe defect in mesoderm formation (Carver et al., 2001). In *Xenopus* and chicken embryos, Snail and Slug are induced in the premigratory neural crest precursor cells and play essential roles in the subsequent delamination and migration of neural crest cells (Nieto, 2002). Similarly, *SIP1* knockout mice display a delamination arrest of cranial neural crest cells, suggesting SIP1's involvement in regulating neural crest development (Van de Putte et al., 2003).

In addition to these zinc-finger transcription factors that have a high affinity for the E-box elements of the *E-cadherin* promoter, transcription factors belonging to other families also regulate EMT in culture and during development. E47, a widely expressed bHLH transcription factor, has been shown to repress *E-cadherin* transcription in MDCK cells directly by binding to E-boxes in the *E-cadherin* promoter (Perez-Moreno et al., 2001), albeit with lower affinity. In a search for genes involved in mouse mammary tumor metastasis, the bHLH factor Twist1 has been found to be capable of inducing EMT in human mammary epithelial cells (Yang et al., 2004). One unique aspect of Twist1 is that it does not seem to directly repress the transcription of *E-cadherin* (E. Ruiz, E. Danis, and J.Y., unpublished data). Twist1 was originally identified as being required for mesoderm induction in *Drosophila* (Thisse et al., 1987; Leptin and Grunewald, 1990; Leptin, 1991). In vertebrates, Twist1 is predominantly expressed in neural crest cells (Gitelman, 1997). *Twist1* mutation in mice causes failure in cranial neural tube closure, indicating its role in proper migration and differentiation of neural crest and head mesenchymal cells (Chen and Behringer, 1995; Soo et al., 2002). Like Twist1, two additional embryonic transcription factors, FOXC2 (Mani et al., 2007) and Goosecoid (Hartwell et al., 2006), have also been demonstrated to induce EMTs in certain epithelial cells, while they also seem to lack the ability to directly bind to the *E-cadherin* promoter.

All of the aforementioned factors are capable of repressing *E-cadherin* directly or indirectly when overexpressed in cultured epithelial cells. We still do not understand, however, how these genes are involved in the mesenchymal differentiation, cell

motility, and invasion occurring during an EMT. The Snail and ZEB transcription factors are generally considered to be transcription repressors based on their domain structures. Extensive studies suggest that they play major roles in repressing the transcription of epithelial genes, such as *E-cadherin*, during embryogenesis and in tumor cells. The bHLH transcription factors homodimerize or heterodimerize to function as both activators and repressors in a context-dependent manner. In *Twist1* null mice, a promigratory cadherin, Cadherin 11, is downregulated (Soo et al., 2002), suggesting a more specific role of Twist in activating the migratory machinery.

Gene expression profiling analyses have been carried out in order to understand how these various transcription factors orchestrate this complex program. Identification of a group of common genes controlled by several of the EMT-inducing transcription factors suggests that a gene signature of the EMT program might be shared, at least in certain cell types (Moreno-Bueno et al., 2006). Precisely how these groups of genes orchestrate the entire cellular program of cell motility and invasion is an area of active research. It is also worth noting that most of the EMT signaling pathways have been examined under overexpression conditions in cultured epithelial cells. During development, several of these transcription factors are often activated simultaneously, such as Snail/Slug and Twist1 in neural crest cells. Additional studies are needed to recapitulate the endogenous activation of these transcription factors to fully understand their biological contributions to the EMT program.

Since the functional loss of E-cadherin is a common feature of EMT, one additional attractive candidate transcriptional pathway regulating EMT is the β-catenin-mediated transcription program. The disappearance of E-cadherin from adherens junctions results in the release of its partner, β-catenin, into the cytosol, which then has the potential to enter the nucleus where it can activate LEF/TCF-mediated transcription. Although the contribution of the adherens-junction-associated β-catenin pool to transcription is still a matter of debate, several studies suggest that β-catenin-mediated transcription can induce the expression of Slug (Conacci-Sorrell et al., 2003) and Twist1 (Onder et al., 2008), thereby contributing to the EMT program. Recently, two studies found that Ajuba LIM proteins, also components of adherens junctions, could travel to the nucleus to serve as functional corepressors of the Snail family to repress *E-cadherin* (Ayyanathan et al., 2007; Langer et al., 2008). Together, these studies highlight the dynamic communication between cell adhesion and its nuclear regulators during the EMT program.

EMT in Carcinoma Progression and Metastasis

The great majority of human solid tumors are carcinomas that originate from various epithelial cell types throughout the body. As mentioned earlier, in order for carcinoma cells to break away from neighboring cells to invade adjacent cell layers, these tumor cells must lose cell-cell adhesion and acquire motility. The resulting cellular motility shares many similarities with the extensive cell migration and tissue rearrangements that occur during the various developmental events discussed above. Indeed, this highly conserved EMT program has been implicated in giving rise to the dissemination of single carcinoma cells from primary epithelial tumors (Thiery, 2002).

E-cadherin *Mutations and Posttranscriptional Control*

Since it functions as a key gatekeeper of the epithelial state, the partial loss of E-cadherin has been associated with carcinoma progression and poor prognosis in various human and mouse tumors (Vincent-Salomon and Thiery, 2003). In both tumor cell cultures and in vivo mouse tumor models, forced expression of E-cadherin in certain invasive carcinoma cells can inhibit their ability to invade and metastasize; conversely, blocking E-cadherin function in noninvasive tumor cells activates their invasiveness and metastatic powers (Frixen et al., 1991; Perl et al., 1998). In several types of human carcinomas, E-cadherin expression is lost at an early stage of tumor development, causing the resulting tumors to exhibit an essentially permanent mesenchymal phenotype, i.e., the phenotype of cells that have passed through an irreversible EMT. The best demonstration of this process is in familial gastric cancers, in which *E-cadherin* germline mutations play a causal role. Depending on the position of the nonsense or frameshift mutation involved, the E-cadherin protein can either be absent or truncated, and thus incapable of mediating cell-cell adhesion (Guilford et al., 1998).

E-cadherin-mediated adherens junction formation is also negatively regulated by the actions of several tyrosine kinases in tumor cells. Accumulating evidence suggests that receptor and nonreceptor tyrosine kinases, such as Met and Src, can phosphorylate both β-catenin and the short cytoplasmic tail of E-cadherin that are associated with adherens junctions. Phosphorylation of E-cadherin promotes its binding to a Hakai E3-ligase. Ubiquitinated β-catenin is degraded in the proteasome, while ubiquitinated E-cadherin-Hakai complexes are internalized to endosomes and thereafter recycled back to the cell surface or degraded in lysosomes. Therefore, activation of tyrosine kinases in carcinoma cells could lead to rapid disruption of cell adhesion and a scattering phenotype (Fujita et al., 2002; Pece and Gutkind, 2002).

Transcriptional Regulation of EMT

In the majority of human carcinoma cell populations, the loss of E-cadherin appears to be heterogeneous, with foci of E-cadherin-positive carcinoma cells scattered among E-cadherin-negative areas. Moreover, E-cadherin is often detected in distant metastases, which may derive from cells that have previously passed through an EMT (Bukholm et al., 1998, 2000). This suggests that after their initial dissemination, some metastatic cells may revert to an epithelial state, and it indicates that other, nongenetic mechanisms controlling transcription of the *E-cadherin* gene or posttranslational modification of the E-cadherin protein may be responsible for the lack of expression of this protein in some tumor cells.

Indeed, many EMT-inducing transcription factors, including Snail, Slug, δEF1, SIP1, Twist1, FOXC2, and Goosecoid, have been associated with tumor invasion and metastasis. Expression of almost all of these has been detected in certain invasive human and mouse tumor cell lines. Expression of Twist1 has been shown to be essential for a mouse breast carcinoma cell line to metastasize from the mammary gland to the lung (Yang et al., 2004). And overexpression of FOXC2 or Goosecoid increases the ability of weakly metastatic human carcinoma cells to disseminate (Hartwell et al., 2006; Mani et al., 2007). The involvement of several EMT-inducing transcription factors has also been reported in human carcinomas. For example, when gene expression profiling was performed for "poor prognosis" signatures, overexpression

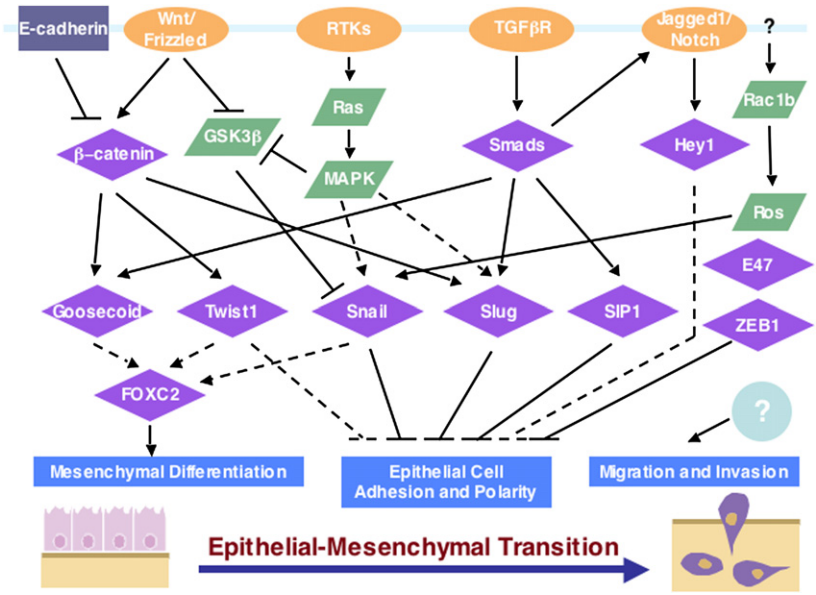

Figure 2. The Molecular Network of Known Signaling Pathways and Transcription Factors that Regulate the Epithelial-Mesenchymal Transition Program in Carcinoma Cells

In cancer cells, the TGF-β signaling pathway induces multiple EMT-inducing transcription factors, including Slug, SIP1, and Goosecoid, via activation of Smads. The Wnt pathway and loss of E-cadherin from adherens junctions activate β-catenin, which in turn induces several EMT-inducing transcription factors as well, such as Slug, Twist1, and Goosecoid. Multiple tyrosine kinase receptor (TKR) pathways, including FGFR, EGFR, PDGFR, and HGFR, can induce the expression of Snail and Slug through the Ras-MAPK pathway. The Wnt and tyrosine kinase receptor pathways also modulate Snail nuclear transport and degradation through GSK3β. The Notch pathway is induced by and required for TGF-β-induced EMT. Activated ROS, through Rac1b, is capable of promoting an EMT in tumor cells, though the inducing signal is unknown. Among all the EMT-inducing transcription factors, Snail, Slug, SIP1, and E47 directly suppress *E-cadherin* transcription, while Twist1, Goosecoid, and FOXC2 seem to function directly. FOXC2 is induced in tumor cells expressing Twist1, Snail, and Goosecoid and mediates mesenchymal differentiation. Although almost all the aforementioned pathways are associated with cell migration and invasion, the specific inducers of migration and invasion during EMT are not well understood. Solid lines indicate direct transcriptional or posttranscriptional regulations. Dashed lines indicate indirect regulation.

of Twist1 was associated with distant metastasis and poor survival in N-*Myc*-amplified neuroblastomas (Valsesia-Wittmann et al., 2004) and in melanomas (Hoek et al., 2004). A recent review has presented an excellent survey on the published reports of their expression in human cancers (Peinado et al., 2007).

Inducing Signals of EMT

Signals responsible for inducing EMTs during embryogenesis have also been implicated in tumor invasion and metastasis. Once again, the best-studied pathway is that involving TGF-β signaling. In various human cancer cell lines and in mouse tumor models, activation of the TGF-β pathway at the later stages of tumor progression has been shown to promote EMT in carcinoma cells, which allows these cells to invade the ECM in culture and to spread to distant organs in mice (Janda et al., 2002; Oft et al., 1998). In various tumor cell lines, TGF-β signaling induces Snail, Slug, and SIP1, which may then proceed to repress the expression of E-cadherin, thereby causing a loss of cell-cell adhesion (Comijn et al., 2001; Peinado et al., 2003). Glycogen synthase kinase 3β (GSK3β) can phosphorylate Snail, which promotes its nuclear export and ubiquitin-mediated degradation. Hence, inactivation of GSK3β via the Wnt pathway or receptor tyrosine kinases may promote Snail stability and nuclear import, thus activating its ability to promote EMT (Bachelder et al., 2005; Zhou et al., 2004). More recently, two studies have reported that hypoxia can induce EMT in carcinoma cells, doing so via activation of either Snail (Lester et al., 2007) or Twist1 (Yang et al., 2008). Together, these data suggest that in response to various inductive signals, EMT-inducing transcription factors may serve as major signaling mediators of the EMT program to promote metastasis. In Figure 2, we summarize the major players that have been reported to play a direct role in regulating the EMT program in carcinoma cells.

The Controversy of EMT in Metastasis

One major difficulty in demonstrating the role of EMT in metastasis in human cancers is identifying carcinoma cells that have passed through an EMT in primary human tumor samples. Most of the EMT markers currently used are expressed in either epithelial or mesenchymal cells, and therefore are not specific for identifying tumor cells at the EMT stage that are scattered among the many stromal cells present within a primary tumor. A few studies have shed some light on the existence of such EMT carcinoma cells in primary human cancers. The EMT-inducing transcription factor Twist1 is overexpressed in two human cancer types, lobular breast carcinoma and diffused-type gastric cancer. Over 90% of these tumors do not express E-cadherin and exhibit a permanent EMT phenotype (Rosivatz et al., 2002; Yang et al., 2004). In colorectal cancers that contain *APC* or *β-catenin* mutations, β-catenin expression is predominantly observed in tumor cells localized at the invasion front and scattered in the adjacent stromal compartment. More interestingly, tumor cells with nuclear β-catenin accumulation appear to have undergone an EMT, as demonstrated by the progressive loss of E-cadherin expression and acquisition of mesenchymal markers such as fibronectin (Fodde and Brabletz, 2007).

Despite these various observations, the involvement of EMT in human cancer metastasis is still highly debated, due in part to clinical observations showing that the majority of human breast carcinoma metastases express E-cadherin and maintain their epithelial morphology, suggesting that they have disseminated without switching to a mesenchymal phenotype (Tarin et al., 2005; Thompson et al., 2005). Several studies in mouse tumor models have also suggested that tumor invasion and metastasis can be achieved without an obvious EMT phenotype. In a TGF-β1-induced mouse skin tumor model, blocking the EMT program

with a dominant-negative TGF-β type 1 receptor did not inhibit the occurrence of distant metastases (Han et al., 2005). Collective cell invasion (Wicki et al., 2006) and fibroblast-led collective invasion (Gaggioli et al., 2007) have also been responsible for carcinoma cell local invasion into the extracellular space. Indeed, cell migration during several developmental events does not require a complete EMT phenotype. For example, in the *Drosophila* egg chamber, border cells delaminate from the follicular epithelium and migrate posteriorly with polar cells as a compact cluster with well-defined cell contacts (Prasad and Montell, 2007). However, partial EMT and collective cell migration are not sufficient to explain how individual tumor cells can intravasate into the blood circulation and travel to distant organs.

Some of this controversy, we believe, is due to the depiction of EMT as a permanent, irreversible process occurring during the course of tumor metastasis. Indeed, a reversible EMT model has been proposed to describe the transient activation of the EMT program that carcinoma cells undergo during tumor metastasis (Thiery, 2002). In this model, carcinoma cells activate the EMT program to achieve local invasion and dissemination to distant organs. Once they have reached those organs, these mesenchymal cells may revert via an MET to an epithelial identity and thereby regain proliferative ability and the ability to form epithelial growths in distant organ sites. Moreover, cancer cells may, more often than not, pass through a partial EMT program rather than a complete one; such cells may concomitantly express epithelial and mesenchymal markers. Better EMT mouse tumor models will clearly be needed to test this hypothesis rigorously.

An alternative model suggests that carcinoma cells may gain motility through activation of an Epithelial-Amoeboid Transition. During this process, upon loss of cell adhesion, carcinoma cells switch to a spherical morphology, which allows them to squeeze through the ECM without extracellular proteolysis (Wolf et al., 2003). Recently, this amoeboid movement has been observed in migrating mesoderm cells in the gastrula-stage zebrafish embryo (Weiser et al., 2007). It is likely that carcinoma cells utilize both EMT and Epithelial-Amoeboid Transition programs to achieve tumor metastasis: breaching the dense basement membrane requires the activation of EMT and extracellular proteolysis, while an Epithelial-Amoeboid Transition might enable cells to pass through thinner ECM.

The above possibilities might also explain why genes involved in the EMT program have not been frequently detected as being part of the poor prognosis or distant metastasis gene expression signatures. In many gene expression profiling studies using human tumor samples, detecting gene expression patterns from small populations of tumor cells that are undergoing an EMT program is experimentally unlikely in a heterogeneous tumor sample. Another class of studies that addresses these questions uses mouse or human cancer cell lines to select highly metastatic variants in mice and compare gene expression signatures between the highly metastatic and parental cell lines. Unfortunately, almost all such studies use tumor cell lines that already have a mesenchymal phenotype and directly introduce such tumor cells into the circulation, thereby bypassing most of the steps of the invasion-metastasis cascade that may depend on an EMT program.

Future Perspectives

Currently, the EMT program is only loosely defined by certain cell morphological changes, changes of differentiation markers from epithelial to mesenchymal patterns, and the functional changes required for cells to migrate and invade through ECM. As such, a clear molecular definition of the EMT program during both development and tumor metastasis is still elusive. Since EMT-associated changes occur in coordination with other cellular programs, such as cell survival and proliferation, it is often not clear whether a specific molecule or pathway under investigation is specific to the EMT program or is operating in parallel with another program or programs. Loss of E-cadherin (N-cadherin in the neural crest) may be the only consistently reported molecular change occurring during the various EMTs involved in both development and tumor metastasis. Since loss of E-cadherin alone in normal epithelial cells results in cell death rather than EMT, it appears that the core EMT molecular program includes more than E-cadherin repression. Indeed, several studies showed that the same factors that induce EMT, such as Snail/Slug (Nieto, 2002) and many receptor tyrosine kinases, could also help cells to survive in the absence of cell adhesion. Identification of the molecular signature of the EMT may be aided by the recent genomic analyses of the EMT program occurring in embryogenesis and tumor progression. Such a specific molecular signature of the EMT program would create the framework for elucidation of the complete signaling network that regulates the EMT program.

By undergoing an EMT, cells not only change their cellular characteristics to acquire motility and invasiveness, but also develop novel interactions with the extracellular environment. Most studies have focused on the afferent signaling pathways received by or activated within the cells undergoing an EMT. Importantly, such cells also release various signals to modify the microenvironment and interact with other cell types during the EMT program. For example, during mesoderm formation, primitive mesoderm cells deposit hyaluronan (Sanders, 1979) and syndecan (Sutherland et al., 1991) to prepare the microenvironment for migration. Understanding the changes associated with the microenvironment surrounding cells undergoing an EMT will be critical in dissecting how tumor cells interact with various tissues during the metastatic journey, which begins in a primary tumor and ends at distant sites of dissemination.

During embryogenesis, activation of the EMT program in vivo requires the coordination of multiple signaling inputs in a context-dependent manner. For example, TGF-β superfamily members are prominent inducers of EMT at many sites within the developing embryo. However, distinct members of this superfamily are involved in particular EMTs, and they interact with different signaling pathways in order to achieve these EMTs. The developmental stages of cells and their respective tissue contexts are largely responsible for these differences, which have been demonstrated using model organisms such as *Drosophila, Xenopus*, and zebrafish. Unfortunately, it is still extremely challenging to localize these complex cellular programs in the far more complex mouse embryo. Additional new 3D or organ culture systems will be required to identify the critical pathways and their interactions in the physiological context.

Studying EMT under physiologic conditions is also critical for elucidating its role in tumor metastasis. In the past, the majority

of studies on the role of EMT in cancer pathogenesis were performed in cultured tumor cell lines. Given the various tissue-specific interactions occurring during tumor metastasis, including those that depend on the tissue microenvironment, the molecular steps that create EMT must be dissected in vivo or in organotypic 3D cultures. Furthermore, the transient nature of the EMT program in metastasis requires us to build better genetic mouse models and more sensitive reporters of the EMT program to monitor its activation in real time in vivo. Together, studies in both development and tumor metastasis will reveal the complex molecular networks that orchestrate the EMT program and hold the promise of suggesting new types of cancer therapeutics.

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