Leading Edge
Review

Cell

Epithelial-Mesenchymal Transitions in Development and Disease

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The epithelial to mesenchymal transition (EMT) plays crucial roles in the formation of the body plan and in the differentiation of multiple tissues and organs. EMT also contributes to tissue repair, but it can adversely cause organ fibrosis and promote carcinoma progression through a variety of mechanisms. EMT endows cells with migratory and invasive properties, induces stem cell properties, prevents apoptosis and senescence, and contributes to immunosuppression. Thus, the mesenchymal state is associated with the capacity of cells to migrate to distant organs and maintain stemness, allowing their subsequent differentiation into multiple cell types during development and the initiation of metastasis.

Most adult tissues and organs arise from a series of conversions of epithelial cells to mesenchymal cells, through the epithelial to mesenchymal transition (EMT) and the reverse process (mesenchymal to epithelial transition [MET]). Epithelial cells establish close contacts with their neighbors and an apicobasal axis of polarity through the sequential arrangement of adherens junctions, desmosomes, and tight junctions. The epithelial cell layer maintains global communication through gap junctional complexes, and it remains separated from adjacent tissues by a basal lamina. Epithelia have the capacity to function as barriers or in absorption. Conversely, mesenchymal or stromal cells are loosely organized in a three-dimensional extracellular matrix and comprise connective tissues adjacent to epithelia. The conversion of epithelial cells to mesenchymal cells is fundamental for embryonic development and involves profound phenotypic changes that include the loss of cell-cell adhesion, the loss of cell polarity, and the acquisition of migratory and invasive properties.

This review presents the events in development that involve EMT and discusses its relevance in tissue homeostasis, tissue repair, fibrosis, and carcinoma progression. We also examine the impact of EMT on drug resistance and explore recent findings that reinforce the concept of EMT as a major driver of morphogenesis and tumor progression.

Development: Primary, Secondary, and Tertiary EMT

The transition of epithelial to mesenchymal cells is not irreversible, as several rounds of EMT and MET are necessary for the final differentiation of specialized cell types and the acquisition of the complex three-dimensional structure of internal organs. Accordingly, these sequential rounds are referred to as primary, secondary, and tertiary EMT (Figure 1). Examples of primary EMT include those evident during mammalian implantation, during gastrulation in various metazoans, and in the neural crest of all vertebrates.

Gastrulation

Although the morphogenetic movements associated with gastrulation vary among metazoans, it is the universal process by which the body plan is established. The necessary changes in cell shape are followed by internalization of the mesendoderm, convergence to the midline, and extension along the anteroposterior axis. A crucial structure in all organisms is the region where cells involute or ingress (the ventral furrow in *Drosophila*, the blastopore in *Xenopus*, and the primitive streak in the chick and mouse). In vertebrates, this region contains an organizing center known as the Spemann organizer in *Xenopus*, the shield in fish, and the node in birds and mammals. To understand how gastrulation proceeds, it is necessary to consider the successive inductive processes that occur before or at the time when the organizer forms and to identify the molecular elements involved. Interestingly, some of the most important elements are conserved throughout evolution.

Gastrulation in Invertebrates

The sea urchin is amenable to detailed fate mapping and molecular embryology approaches, leading to the generation of an extensive gene regulatory network at gastrulation (Oliveri et al., 2008) (Figure 2A). Key in this network are the transcription factors Snail and Twist, which are evolutionary conserved repressors of E-cadherin and inducers of EMT (Peinado et al., 2007). In the sea urchin, Snail inhibits *E-cadherin* transcription and promotes cadherin endocytosis as well as the delamination of primary mesenchyme cells (PMCs) by EMT (Wu et al., 2007), whereas its inhibition blocks PMC ingression. In turn, inhibition of Twist function delays the ingression of PMCs (Wu et al., 2008).

As in sea urchin, Twist and Snail are crucial factors in fly gastrulation (Figure 2B). Apical constriction is necessary for ventral furrow formation and cell invagination, and this process requires Twist and its target, T48. These proteins are recruited

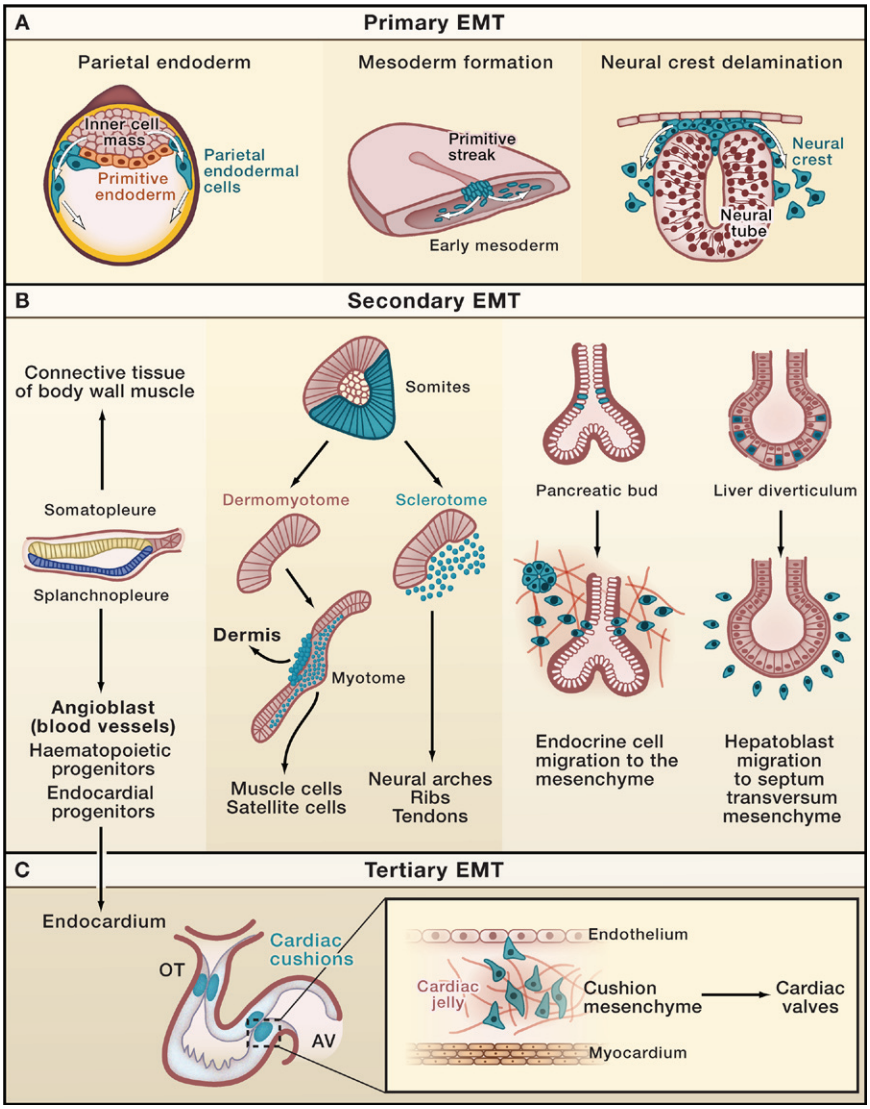

Figure 1. Successive EMT during Embryonic Development

(A) Primary EMT occurs early during embryonic development, even before implantation such as during the formation of the parietal endoderm in mice. The first EMT after implantation is that undergone by the mesendodermal progenitors during gastrulation, whereas the delamination of neural crest cells from the dorsal neural tube is a later event.

(B) Early mesodermal cells are subdivided into axial, paraxial, intermediate, and lateral plate mesodermal cells that will condense into transient epithelial structures: the notochord, the somites, and the somatopleure and splanchnopleure, respectively. These transient structures will undergo secondary EMT, leading to the generation of mesenchymal cells that differentiate into specific cell types. Endodermal tissues, including the pancreas bud and the liver diverticulum, exhibit morphological changes reminiscent of a secondary EMT to induce the dissociation of endocrine cells and hepatoblasts from their respective epithelial primordia.

(C) An example of tertiary EMT arises during the formation of the cushion mesenchyme in the heart from the atrioventricular canal (AV) or the outflow tract (OT). The cushion mesenchyme is the precursor of the cardiac valves.

to the adherens junctions, producing rapid changes in cell shape in conjunction with RhoGEF2, a Rho GTP-exchange factor and cytoskeletal regulator that concentrates at the site of apical constriction (Kolsch et al., 2007). Snail is also required for ventral furrow formation, the cells of which express string, a cdc25 homolog essential for entry into mitosis. Snail-dependent string inhibition generates the mitotic block necessary for gastrulation to occur (Grosshans and Wieschaus, 2000). Simultaneously, Snail represses *E-cadherin* transcription (Oda et al., 1998) and generates the pulses of myosin contraction required for apical constriction while Twist maintains the constricted state between pulses (Martin et al., 2009). In vertebrates, T48 is not conserved, and Twist is not crucial for gastrulation, suggesting that Snail may fulfill all of these functions.

Gastrulation in Vertebrates

In *Xenopus*, the Spemann organizer is induced by the Nieuwkoop center, a group of dorsal blastula cells characterized by the nuclear accumulation of β-catenin. Wnt signaling initiates the process, and Goosecoid is induced in the Spemann organizer by its target, Siamois, and by several transforming growth factor β (TGFβ) superfamily members, including Nodal (Gilbert, 2006). In amniotes, activation of Wnt signaling confers competence to the posterior part of the embryo in the formation of the primitive streak (Figure 2C). Subsequently, members of the TGFβ superfamily, including Nodal and Vg1, induce gastrulation. Nodal signaling, together with fibroblast growth factor (FGF), controls the specification of the mesendoderm in all vertebrates (Figure 2C). Thus, in preparation for EMT, numerous signaling pathways help establish an organizing center that in turn controls morphogenetic movements and specification (Heisenberg and Solnica-Krezel, 2008).

There are two main *Snail* genes in vertebrates, *Snail1* and *Snail2* (called *SNAI1* and *SNAI2* in humans). They are induced by TGFβ superfamily members, and FGF signaling is necessary to maintain their expression and for gastrulation to proceed (Barrallo-Gimeno and Nieto, 2005; Ciruna and Rossant, 2001). *Snail*-deficient embryos fail to gastrulate, and "mesodermal" cells are unable to downregulate E-cadherin accumulate at the streak (Carver et al., 2001; Nieto et al., 1994). Snail proteins are not essential for mesodermal fate specification as a "mesodermal" population expressing the appropriate markers still forms in *Snail* mutant mice, although cells fail to migrate because it cannot undergo EMT (Carver et al., 2001). Furthermore, diploblasts (animals derived from only two germ layers) that do not form mesoderm also express *snail* in the regions of involution or ingression during endoderm formation. Hence, Snail activity is not associated with

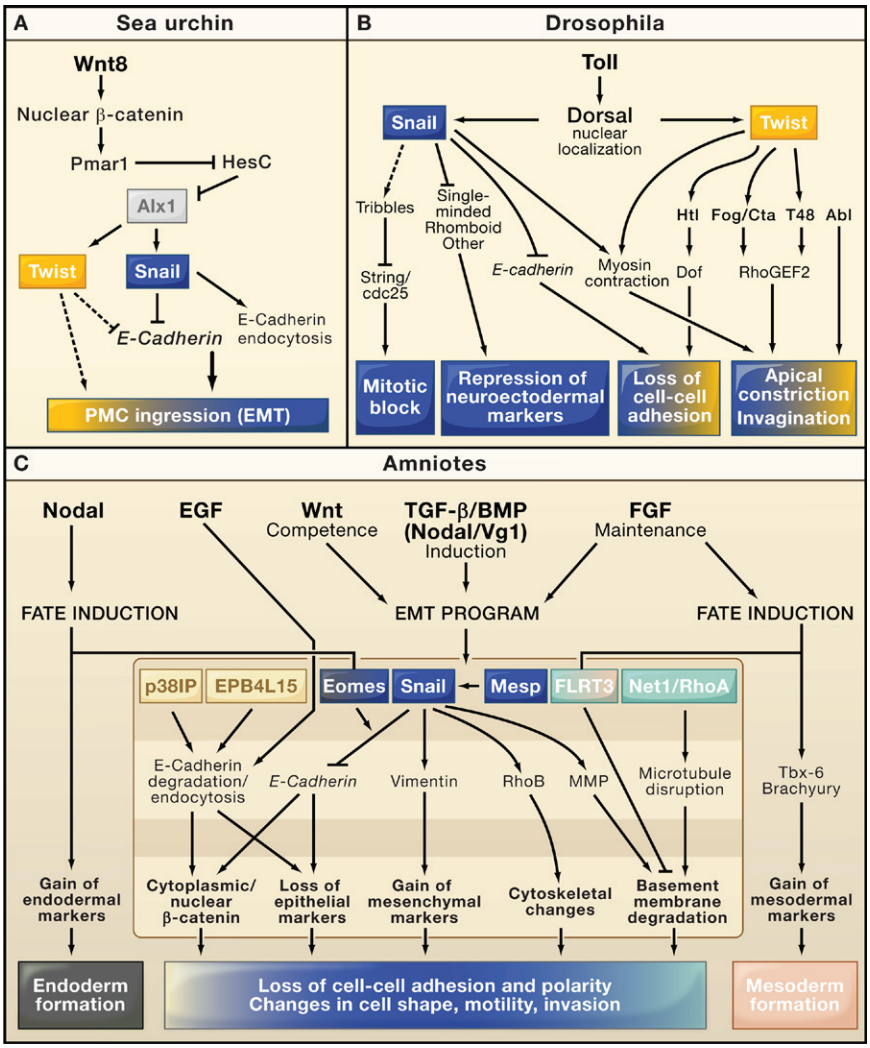

Figure 2. Genetic Pathways Governing Gastrulation

(A) The gene regulatory network governing EMT during gastrulation in the sea urchin embryo. A specification step involving Wnt8 signaling leads to HesC repression, switching on the EMT regulatory program, and inducing the ingression of the primary mesenchymal cells (PMCs). Alx1, arista-less-like 1.

(B) Mesoderm invagination in *Drosophila*. Twist and Snail pathways cooperate to modulate cell adhesion and cytoskeletal changes to undergo gastrulation movements and mesoderm spreading. The arrows indicate the flow of the pathway, not direct transcriptional regulation. Abl, Abelson kinase; Htl, Heartless (*Drosophila* FGF receptor); Dof, downstream of FGFR; Fog/Cta, folded in gastrulation/concertina.

(C) Genetic pathways controlling gastrulation in amniotes. Convergence of signaling pathways at the posterior part of the embryo leads to primitive streak formation and initiation of the EMT as well as the mesodermal fate program. Snail genes are key regulators of the EMT program during gastrulation in amniotes as they control cell-cell adhesion, cell shape, and motility. Additional mechanisms such as endocytosis, lysosomal targeting, and degradation of the E-cadherin protein together with the control of basement membrane integrity explain the rapid and drastic changes occurring in ingressing cells during gastrulation. The induction of endodermal and mesodermal fates is mainly governed by the FGF and Nodal pathways through specific regulators and the contribution of some of the genes involved in the EMT program. EPB4L5, FERM and actin-binding domains-containing band 4.1 superfamily member; FLRT3, Fibronectin-leucine-rich-transmembrane protein-3; Net-1, neuroepithelial transforming factor 1; MMP, metalloproteinases; p38IK, p38 interacting kinase.

the mesodermal lineage but rather with changes in cell shape, cell adhesion, and cell movements, consistent with the notion that cell fate specification and morphogenetic movements are independent processes even though they occur simultaneously.

Given that gastrulation is a very rapid process, the regulation of *E-cadherin* transcription alone is insufficient. E-cadherin is also controlled at the protein level by the P38 interacting protein (IP), p38-MAP kinase, and the FERM protein (EPB4.1L5) (Figure 2C) (Zohn et al., 2006; Hirano et al., 2008; Lee et al., 2007).

Other transcription factors such as Eomesodermin (Eomes) and Mesp1 and 2 are important for EMT during mouse gastrulation (Figure 2C). Eomes is a T-box transcription factor expressed in the posterior epiblast prior to streak formation, in the streak and in nascent mesendoderm at gastrulation. In turn, the basic helix-loop-helix transcription factors Mesp1 and 2 are also expressed in the posterior epiblast of the mouse embryo. Mesodermal delamination from the streak is blocked in mice lacking Eomes in the epiblast and in double *Mesp1/Mesp2* mutants (Kitajima et al., 2000; Arnold et al., 2008). This is consistent with the ability of Mesp proteins to induce Snail and EMT in differentiated embryonic stem cells (Lindsley et al., 2008).

Cells need to break the basal membrane to successfully delaminate from the primitive streak. A pathway mediated by the RhoGEF protein Net1 induces RhoA downregulation in the primitive streak and disrupts the interaction between epiblast cells and the underlying the basal membrane (Figure 2C) (Nakaya et al., 2008). Snail factors contribute to basal membrane degradation by activating metalloproteases (Jorda et al., 2005) and by repressing some components such as Laminin5 and its receptors (Haraguchi et al., 2008). Importantly, the integrity of the basal membrane must be maintained in areas outside of the primitive streak and the transmembrane protein FLRT3 seems to offer protection against its disruption in addition to regulating cell fate (Figure 2C) (Egea et al., 2008).

Unlike in the sea urchin or in *Drosophila*, the gene regulatory networks operating at gastrulation in vertebrates are far from complete. Future studies of EMT would benefit from the application of genome-wide approaches and in vivo cell imaging.

Indeed, attempts to define the transcriptome in the gastrulating mouse embryo have already provided interesting information (Mitiku and Baker, 2007).

The Neural Crest

After gastrulation in vertebrates, the epidermal and neural territories are progressively defined along the rostrocaudal axis, and the neural crest forms at the boundary between these two territories. Neural crest cells undergo EMT within the dorsal neural epithelium, and individual cells migrate before giving rise to different derivatives, including craniofacial structures, most of the peripheral nervous system, some endocrine cells, and melanocytes.

Our understanding of how the neural crest territory is specified has increased substantially over the last 10 years, although the gene regulatory network operating during neural crest development is less complete than at gastrulation (Sauka-Spengler and Bronner-Fraser, 2008). A complex set of inductive events initiated before and during gastrulation define a distinct territory at the junction between neural and nonneural ectoderm. The neural crest territory is delimited by FGF, Wnt, Notch, and retinoic acid signaling pathways in cooperation with opposing gradients of bone morphogenetic protein 4 (BMP4) and its antagonists Noggin, Follistatin, and Chordin. Although canonical Wnt signaling is important for both the induction and stabilization of neural crest precursors and for their delamination, noncanonical Wnt signaling is important for neural crest migration (De Calisto et al., 2005; Carmona-Fontaine et al., 2008). Thus, most of the signaling pathways involved in defining the neural crest territory are common to those used during gastrulation.

The presumptive neural crest is modified by the neural plate border specifiers Msx1, Msx2, Dlx3, Dlx5, Pax3, Pax7, and Zic1, which induce genes that trigger different programs in the neural crest such as Snail and Sox factors, FoxD3, AP2, c-Myc, and Id. Although organized in a hierarchy, these factors can also regulate expression of each other (Sauka-Spengler and Bronner-Fraser, 2008).

Regulation of Cell Adhesion and Movement

Cadherin-mediated adhesion also plays a major role in neural crest cell EMT, where delamination from the neural fold involves the downregulation of N-cadherin and Cadherin 6 as well as the de novo expression of type II cadherins, such as Cadherin 7 and 11 (Nakagawa and Takeichi, 1995; Vallin et al., 1998). The less adhesive type II cadherins allow crest cells to migrate away from the neural tube (Chu et al., 2006). In the chick embryo, the onset of delamination involves N-cadherin protein cleavage by the ADAM 10 protease. The membrane bound fragment generated is then further digested by γ-secretase. It then translocates to the nucleus together with β-catenin where it activates cyclin D1 and promotes exit from the G1 phase, a prerequisite for the emigration of these cells (Shoval et al., 2007). Interestingly, Snail factors prevent entry into the S phase of the cycle by repressing *cyclin D* transcription (Vega et al., 2004), probably synchronizing the premigratory crest population so that all the cells can simultaneously enter into the S phase upon N-cadherin cleavage and cyclin D activation. At least in the chick embryo, Cadherin 6B (Cad6B) is transiently expressed at the premigratory phase, and its downregulation leads to premature neural crest cell migration, whereas its overexpression induces accumulation of crest cells at the dorsal border of neural tube (Coles et al., 2007). The precise timing of Cad6B downregulation is directly controlled by Snail2 (Taneyhill et al., 2007).

In addition to cadherins, small GTPases also play an important role in neural crest EMT as they do during gastrulation. RhoV downregulation affects Sox9, Snail2, and Twist expression in *Xenopus* embryos (Guemar et al., 2007). Rac1 can induce Snail2 expression in the neural crest in *Xenopus*, and its dominant negative or activated forms markedly affect crest cell delamination. RhoA has the opposite effect, as its activated form abrogates crest cell delamination (Broders-Bondon et al., 2007) and RhoB lies downstream of Snail2 and Sox5 in the chick neural crest (del Barrio and Nieto, 2002; Perez-Alcala et al., 2004). RADIL, a downstream effector of Rap GTPase that links the plasma membrane with the actin cytoskeleton, controls neural crest cell adhesion and migration in zebrafish (Smolen et al., 2007), whereas RhoA and Rac1 control both FoxD3 and Snail1 expression. Thus, the small Rho-GTPases play a major role in establishing a transcription factor autoregulatory network at the time of neural crest specification and EMT.

As in gastrulation, in vivo cell imaging analysis of chick neural crest has provided interesting information about the individual cell movements and the contacts that crest cells maintain or create while migrating (Teddy and Kulesa, 2004). Elegant analyses at the single-cell resolution in *Xenopus* embryos have shown that contact inhibition of locomotion mediated by Wnt noncanonical signaling is a crucial mechanism for the directional movements of neural crest cells toward their target tissue (Carmona-Fontaine et al., 2008).

In summary, analogous signaling pathways operate during EMT in gastrulation and neural crest formation. It is worth noting here that while defects in individual genes lead to very strong EMT phenotypes at gastrulation, there is a high degree of cooperation and plasticity during neural crest development. The existence of regulatory loops among the different EMT inducers in the neural tube explains why the absence of one player may be compensated for by the others. For instance, although Snail is crucial for gastrulation in all metazoans analyzed (Carver et al., 2001; Nieto et al., 1994, Wu et al., 2007), mice mutant for *Snail1* and *Snail2* still generate neural crest even though they develop multiple craniofacial defects (Murray and Gridley, 2006). This plasticity and cooperation endows the system with robustness, perhaps reflecting the importance of the neural crest as an evolutionary novelty fundamental for the development of the vertebrate head.

Secondary EMT: Somites, Palate, Pancreas, Liver, and Reproductive Tracts

The primary EMTs are followed by differentiation events that generate different cell types. Indeed, the migratory neural crest cells follow stereotyped pathways and then differentiate into neurons, cartilage, or bone cells, and mesodermal cells subdivide into axial, paraxial, intermediate, and lateral mesoderm after gastrulation. These populations condense into transient epithelial structures through a MET process, thereby forming

the notochord, the somites, the precursors of the urogenital system and the somatopleure and splanchnopleure, respectively (Figure 1B). Except for the notochord and in response to signals from their microenvironment, these secondary epithelia undergo a secondary EMT to generate mesenchymal cells with a more restricted differentiation potential.

The repression of Snail factors controls the timing of presomitic axial mesoderm MET, which leads to somite epithelialization (Dale et al., 2006; Morales et al., 2007). This epithelialization is strictly controlled by Rac1 and Cdc42 (with high levels of Cdc42 promoting a mesenchymal phenotype), whereas Paraxis drives epithelialization by regulating the levels of activated Rac1 (Nakaya et al., 2004). The somites later undergo various secondary EMT processes (Figure 1B). For example, the dorsal part of the somite converts into dermal mesenchyme, and myoblasts that delaminate from the myotome contribute as progenitors of muscle and satellite cells (Gros et al., 2005). A distinctive EMT event occurs in myotomes at the axial limb bud level, which become populated by actively migrating myoblasts that detach from the tip of the myotome (Buckingham et al., 2003). Hepatocyte growth factor/scatter factor (HGF/SF) activation of the PI3K and Src pathways control this process through its receptor c-Met (Maina et al., 2001). In turn, the ventral part of the somites transforms into sclerotomal mesenchyme cells that will later form the vertebrae. Inducers produced by the notochord and the ventral neural tube control this EMT. Noggin, which antagonizes BMP signaling, and sonic hedgehog (SHH) are required for the induction of Pax1, Pax9, and Nkx3.1, the earliest markers of sclerotomal cells (Monsoro-Burq, 2005). Yet it remains unclear how this process is controlled given that EMT still occurs in the ventral compartment in *Pax1/9* or *Nkx3*.1 mutants.

The lateral plate mesoderm condenses into two epithelia separated by a cavity, the coelom. Cells from the ventral epithelia, the splanchnopleure, undergo EMT and generate endocardial progenitors, angioblasts, and hematopoietic stem cells. Cells from the dorsal epithelia, the somatopleure, mostly conserve their epithelial morphology, although some cells undergo a further EMT to form the connective tissue of body wall muscle.

Endodermal derivatives also appear to use EMT during liver development (Tanimizu and Miyajima, 2007), although this is not well documented at the molecular level (Figure 1B). Pancreatic endocrine cells specified in the bud also delaminate and migrate through the surrounding mesenchyme before they undergo MET to form the langerhans islets (Johansson and Grapin-Botton, 2002).

EMT processes are also important during normal development of secondary palate and reproductive tracts. After the fusion of the epithelia of the two palatal halves, it is still not clear whether these cells undergo EMT, die, or migrate to the oral epithelium. Each of these processes probably contribute, with some cells undergoing EMT and rapidly dispersing within the adjacent mesenchyme and others undergoing apoptosis (Martínez-Álvarez et al., 2004; Ahmed et al., 2007; Dudas et al., 2007). In male reproductive tracts, the Mullerian duct regresses after the EMT induced by the Mullerian-inhibiting substance (Zhan et al., 2006). Furthermore, testicular cords form upon the migration of mesonephric endothelial cells most likely after undergoing EMT or, more precisely, endothelial to mesenchyme transition (EndMT) (Combes et al., 2009).

From Primary to Tertiary EMT: Heart Development

The heart forms through three successive cycles of EMT and MET. Although cardiac mesodermal cells are specified during the EMT at gastrulation (Figures 1B and 1C), cardiac progenitors in the splanchnopleure quickly become organized into a two-layered epithelium via MET. A secondary EMT occurs when the two cardiogenic areas fold around the primitive foregut. Mesenchymal cells arising from this delamination give rise to the endothelial cell lining of the heart through another MET, forming an endocardial tube surrounded by the myocardial epithelium. Subsequently, these two concentric tubes develop into the four compartments of the heart primordium. Endothelial cells from the atrioventricular canal undergo a tertiary EMT (here also called EndMT due to the nature of the tissue of origin), invade the cardiac jelly, and form the endocardial cushion, the cells that later assemble into the atrioventricular valvulo-septal complex (Nakajima et al., 2000).

During cardiac valve formation in the chick, TGFβ2 activates TGFβRIII to initiate EMT, and TGFβ3-activating TGFβRII promotes invasion into the cardiac cushion (Mercado-Pimentel and Runyan, 2007). Notch regulates TGFβ2 production through translocation of its cytoplasmic domain to the nucleus, where it coactivates the Su(H)/RBPjk/CBf1 transcription factor. Target genes include *Snail1* and the three members of the bHLH *Hey* family of transcription factors. Snail1 represses *VE-cadherin* transcription and promotes EMT (Timmerman et al., 2004), and inactivation of *Hey2* causes major congenital heart defects and inactivation of *Hey1* and *HeyL*, suggesting that these two factors cooperate in EMT and that their combined inactivation induces ventricular septal and atrioventricular pulmonary valve defects (Fischer et al., 2007). By contrast, heterozygous mutations in the Gata4 transcription factor lead to severe cardiac defects in humans, as it lies upstream of MAPK in the ErbB3 signaling pathway required for the EMT of endocardial cells in the atrioventricular canal (Rivera-Feliciano et al., 2006). Thus, the EMT that forms the valves is controlled by three distinct pathways, triggered by TGFβR, Notch, and ErbB.

EMT as a Physiological Response to Injury

Processes similar to EMT also occur as a physiological response to injury. During wound healing, keratinocytes at the border of the wound recapitulate part of the EMT process. They appear to acquire an intermediate phenotype known as the "metastable" state, which allows them to move while maintaining loose contacts rather than migrating as individual cells. Snail2 expression in keratinocytes at the migratory front influences this state, as its inactivation or overexpression compromises or accelerates wound healing, respectively (Arnoux et al., 2008). In addition, in each menstrual cycle the ovarian surface epithelium undergoes an EMT-like process during postovulatory wound healing. This EMT is induced by epidermal growth factor (EGF) and involves the activation of metalloproteases and of ILK and ERK kinases (Ahmed et al., 2006).

During zebrafish cardiac regeneration, reactivation of developmental programs stimulates a rapid expansion of the entire epicardial cell layer, whereas the myocardium is regenerated by

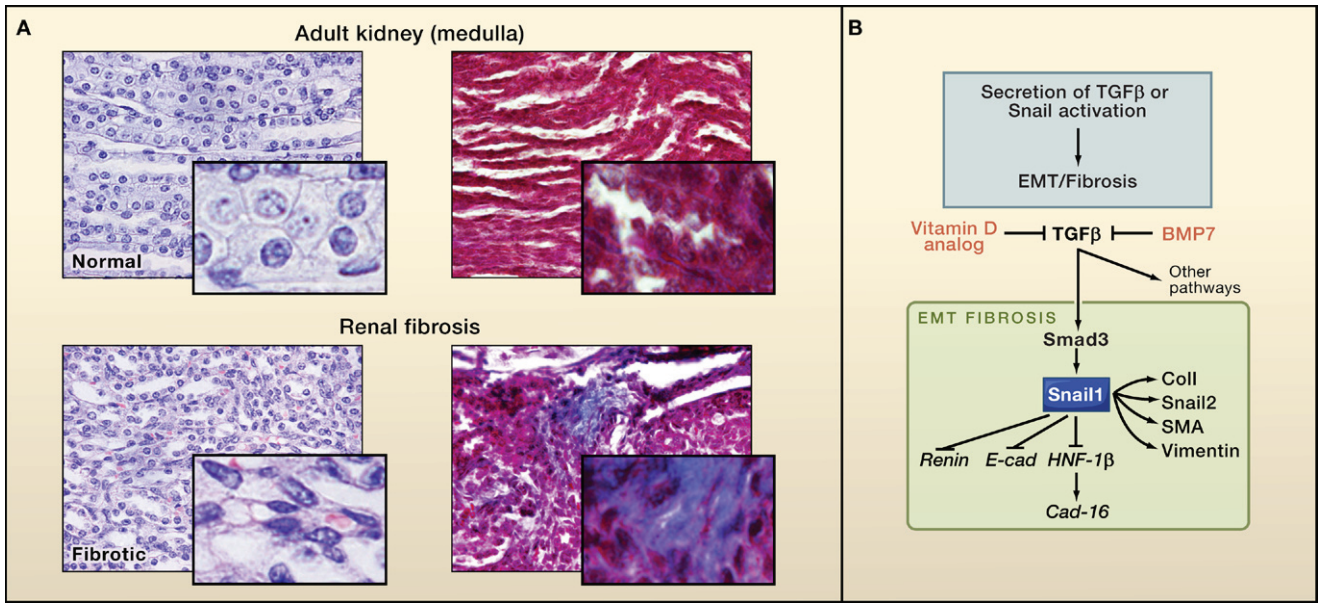

Figure 3. EMT and Renal Fibrosis

(A) Aberrant Snail1 activation in the adult kidney is sufficient to induce renal fibrosis and renal failure in transgenic mice. The morphology of the tubules is disrupted and there is aberrant accumulation of Collagen fibers in the extracellular matrix. (Images from Boutet et al., 2006. Reprinted by permission from Macmillan Publishers Ltd.: EMBO J. *25*, 5603–5613, copyright 2009.)

(B) TGFβ signaling regulates several pathways including that leading to Snail1 activation, which triggers EMT and the conversion of epithelial cells into myofibroblasts expressing and secreting collagen I. Inhibition of TGFβ signaling by BMP7 or the vitamin D analog paricalcitol are promising strategies to attenuate renal fibrosis. The pathways activated by TGFβ signaling are shown in bold.

progenitor cells. A subpopulation of the *tbx18*-positive activated epicardial-derived cells must undergo an Fgf17b/Fgfr2, Fgfr4-dependent EMT, required to invade the regenerating myocardium and facilitate coronary neovascularization (Lepilina et al., 2006). In this sense, the organism may reactivate EMT-like programs as a strategy to recover tissue homeostasis.

Pathological EMT

Organ Fibrosis

EMT not only occurs during embryonic development or as a physiological response to injury, but is also an important element in cancer progression and other pathologies that involve organ degeneration, such as fibrosis. At the cellular level, pathological EMTs are very similar to physiological EMTs in that they are governed by similar signaling pathways, regulators, and effector molecules.

In fibrotic tissues, myofibroblasts accumulate and secrete an excessive amount of collagen that is deposited as fibers, thereby compromising organ function and leading to its failure. Fibrosis had been thought to originate through the pathological activation of interstitial fibroblasts that convert to myofibroblasts to form the fibrotic collagen network. However, elegant cell tracing studies have shown that a significant portion of these myofibroblasts arise from the conversion of epithelial cells through an EMT process (Iwano et al., 2002). Initially demonstrated in differentiated cells of renal tubules and ducts, it is now clear that lens epithelium, endothelium, hepatocytes, and cardiomyocytes can all undergo EMT and contribute significantly to tissue fibrosis. Indeed, lineage-tracing in transgenic mice also indicates that hepatocytes undergo EMT during CCL_4-induced liver fibrosis (Zeisberg et al., 2007b), as do the alveolar epithelial cells during the pulmonary fibrosis induced by TGFβ (Kim et al., 2006). Interestingly, hepatocytes derived from cirrhotic livers also display characteristics of EMT, which has implications for the progression to hepatocellular carcinoma (Nitta et al., 2008).

EMT involving transformation of endothelial cells into mesenchymal cells is evident during cardiac and renal fibrosis (EndMT) (Zeisberg et al., 2007a, 2008). Mesothelial cells are also converted to mesenchyme in patients that receive ambulatory peritoneal dialysis and that develop peritoneal fibrosis (Yanez-Mo et al., 2003), a process that involves the MAPK pathway and Snail1 activation (Strippoli et al., 2008). Furthermore, the EMT undergone by lens epithelial cells contributes to capsular opacification after cataract surgery. Prevention of this EMT process is achieved by transient adenoviral gene transfer of the TGFβ signaling inhibitor Smad7 (Saika et al., 2004). TGFβ also participates in the renal fibrosis induced after unilateral ureteral obstruction, and high levels of TGFβ have been found in fibrotic tissues from patients. Accordingly, mouse models lacking Smad3, a signaling molecule downstream of TGFβ receptors, are protected against renal fibrosis (Sato et al., 2003), indicating that inhibition of TGFβ signaling is a promising strategy to treat the disease. Accordingly, systemic injection of the endogenous TGFβ antagonist BMP7 can revert renal fibrosis in mice (Zeisberg et al., 2003), and paricalcitol, a synthetic vitamin D analog that suppresses the expression of TGFβ and of its type I receptor, also attenuates ureteral obstruction-induced renal fibrosis (Figure 3) (Tan et al., 2006). TGFβ is the main inducer of Snail1 in different contexts, and,

interestingly, activation of Snail1 alone in the adult kidney is sufficient to induce renal fibrosis and renal failure (Boutet et al., 2006). Furthermore, high levels of Snail1 have been detected in fibrotic kidneys from patients subjected to nephrectomy. Given that the high levels of TGFβ observed during fibrosis may be part of the physiological response to an insult or pathological state, and given that Snail appears to transduce the deleterious effects of TGFβ, inhibition of Snail may perhaps be a more specific alternative to treat kidney disease that would preserve the beneficial effects of TGF-β secretion.

Cancer Progression

Although EMT processes are documented in many in vitro cancer cell models, the significance of EMT during cancer progression and even its relevance in human cancer tissues has remained a matter of debate until very recently. This resistance was mainly due to the lack of convincing evidence of EMT in clinical samples. Yet, EMT may be a focal event that is easily overlooked. Interestingly, systemic spread has been detected from early lesions in HER-2 transgenic mice and in human ductal carcinoma suggesting that metastasis is not necessarily a late event in tumor progression (Hüsemann et al., 2008). More importantly, individual mesenchymal cells derived from epithelial tumor cells after EMT are very difficult to distinguish from stromal cells or other tumor-associated fibroblasts. The description of cords or small aggregates of tumor cells extending or detaching from the tumor mass into the adjacent stroma have recently provided morphological evidence of EMT at invasive fronts of human tumors (Prall, 2007). Similarly, in colon carcinoma, EMT occurs at the invasive front and produces single migratory cells that lose E-cadherin expression. This is concomitant with deregulation of the Wnt pathway and a selective loss of the basement membrane (Brabletz et al., 2001). This phenomenon is recapitulated by other solid tumors, as the invasive fronts in papillary thyroid carcinoma or in some breast carcinoma reveal an EMT expression profile, and those in cervical carcinoma show increased vimentin and loss of E-cadherin (see the Supplemental Data available online for additional discussion and references for different types of carcinoma). The invasive front of carcinoma is the immediate interface for tumor and stromal signals. EMT at this interface reflects the intricate counterbalance between internal growth pressure exerted by the expanding main tumor nest and the free edge of the tumor periphery. However, EMT-independent machinery such as podoplanin-mediated actin remodeling governs collective movements at the tumor invasive front in the majority of squamous cell carcinoma (Wicki et al., 2006). This suggests that EMT-dependent and -independent invasion can occur synchronously at the invasive front. Direct in vivo imaging has also yielded evidence of EMT in cancer progression (Wyckoff et al., 2007).

Many important EMT drivers such as SNAIL1 and SNAIL2 have been shown to correlate significantly with disease relapse and survival in patients with breast, colorectal, and ovarian carcinoma, which indicates that EMT leads to poor clinical outcomes. Many studies have demonstrated that EMT profiles are associated with certain clinicopathological parameters such as histological grades and tumor subtypes as in basal-like and metaplastic breast carcinoma, usually belonging to the group

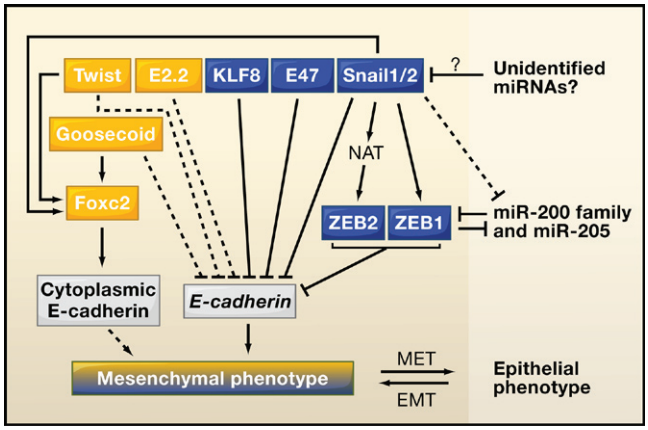

Figure 4. *E-cadherin* Transcription in Normal and Cancer Cells
SNAIL, ZEB, E47, and KLF8 factors directly repress *E-cadherin* transcription whereas Twist, Goosecoid, E2.2, and Foxc2 are indirect *E-cadherin* repressors. Snail1 activates the expression of the ZEB genes by different mechanisms, including the induction of a natural antisense transcript for ZEB2 (NAT). The miR-200 family and in some cases also miR-205, represses the transcription of ZEB genes preventing EMT. A loop of miRNAs and ZEB factors cross-regulation plus the cooperation of several EMT inducers reinforces the control of the EMT process. Preliminary data indicate that Snail1 may also repress the expression of the miR-200 family. Whether miRNAs can also control Snail expression awaits further investigation. EMT, epithelial to mesenchymal transition; MET, mesenchymal to epithelial transition.

with worse outcomes. In addition, suppression of EMT can increase the sensitivity to EGF receptor-targeted treatments in cell line models (hepatoma and pancreatic carcinoma), as well as in lung cancer patients. Thus, the identification of EMT features in tumor samples might provide a tool to better stratify patients and predict outcomes.

EMT Inducers

The loss of E-cadherin expression is considered a crucial step in the progression of papilloma to invasive carcinoma (Perl et al., 1998), and it is also a fundamental event in EMT. Much effort has been devoted to understanding how E-cadherin is regulated during cancer progression. We can now classify E-cadherin repressors into two groups depending on their effects on the *E-cadherin* promoter. Snail, Zeb, E47, and KLF8 factors bind to and repress the activity of the E-cadherin promoter (Peinado et al., 2007; Wang et al., 2007b) (see Table S1 for references), whereas factors such as Twist, Goosecoid, E2.2, and FoxC2 repress *E-cadherin* transcription indirectly (Figure 4) (Yang and Weinberg, 2008; Sobrado et al., 2009).

Snail factors bind to E-box consensus sequences in the *E-cadherin* promoter with the help of local modifications of chromatin structure after the recruitment of SIN3A, histone deacetylases HDAC1 and HDAC2, and components of the Polycomb 2 complex (Cano et al., 2000; Batlle et al., 2000; Fraga et al., 2004; Herranz et al., 2008). In addition to being tightly regulated at the transcriptional level, Snail factors undergo posttranslational modifications that control their nuclear localization or degradation. These modifications include phosphorylation by PAK and GSK3β, dephosphorylation by the small C-terminal domain phosphatase (SCP), and lysine oxidation by

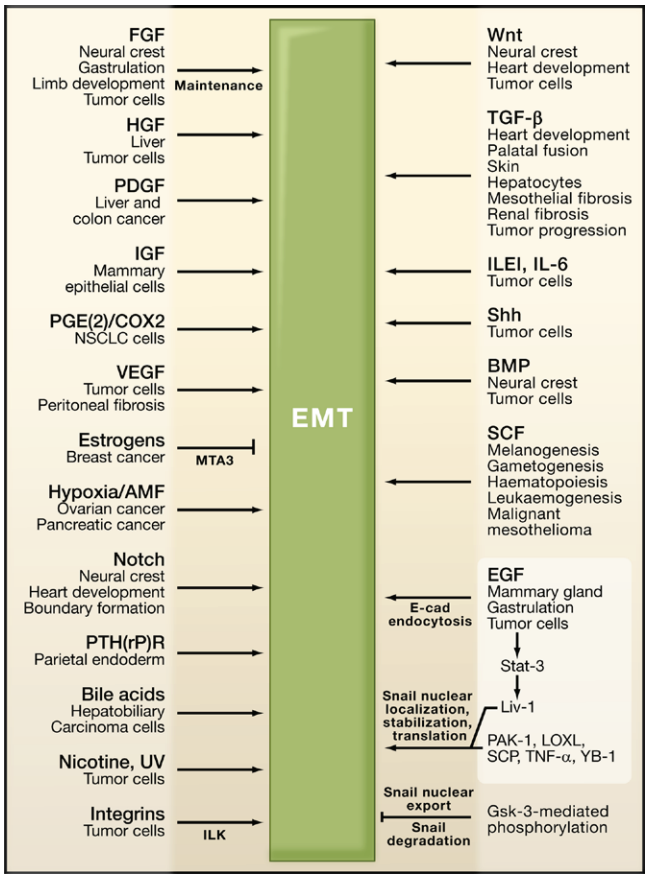

Figure 5. EMT Signaling Pathways

A plethora of signaling pathways and agents induce EMT in numerous cellular contexts, both during embryonic development and in human pathologies. The figure shows the target tissues and the biological process below the corresponding signal that promotes EMT. Although the expression of several transcription factors is an essential aspect of the EMT program, some of these transcription factors are also regulated at the posttranslational level through their subcellular localization and the regulation of their degradation by the proteasome. AMF, autocrine motility factor; E-cad, E-cadherin; EGF, epidermal growth factor; FGF, fibroblast growth factor; BMP, bone morphogenetic protein; IGF, insulin-like growth factor; ILEI, interleukin-related protein; ILK, integrin-linked kinase; IL-6, interleukin-6; LOXL, lysyl oxidase-like proteins; MTA3, metastasis-associated protein 3; PDGF, platelet derived growth factor; TGFβ, transforming growth factor β; TNF-α, tumor necrosis factor α; PAK-1, p21-activated kinase 2; PTH(rP)R, parathyroid hormone related peptide receptor; SCF, stem cell factor; SCP, small C-terminal domain phosphatase; UV, ultraviolet light; VEGF, vascular endothelial growth factor, YB-1, Y-box binding protein.

LOXL2 (Figure 5) (Peinado et al., 2007; Wu et al., 2009b). Snail1 protein stabilization is promoted by NFκB, which prevents its phosphorylation by GSK-3 and subsequent degradation (Wu et al., 2009a), whereas the formation of a ternary complex between wild-type p53, the ubiquitin ligase Mdm2, and Snail2 triggers its degradation (Wang et al., 2009a). Cap-independent translation of *Snail* mRNA is activated by the transcription/translation regulator Y-box binding protein 1 (YB-1), associated with breast cancer aggresiveness (Evdokimova et al., 2009).

Another breast cancer-associated protein that increases Snail nuclear translocation is the zinc transporter LIV1. LIV1 induces EMT during zebrafish development (Yamashita et al., 2004) and promotes invasive properties in tumor cells (Unno et al., 2009). Being pleiotropic proteins, Snail factors may require the cooperation of tissue-specific binding partners to regulate transcription. For example, Ajuba Lim proteins (Ajuba, LIMD1 and WTIP) act as corepressors of Snail1 and Snail2 during neural crest formation in *Xenopus* embryos (Langer et al., 2008). Snail can also bind to Smad proteins, which act as corepressors during TGFβ-induced EMT (Vincent et al., 2009). Interestingly, the levels of expression of the transcriptional coregulators CtBP and p300 are critical for SNAI1 and ZEB1 function in colon carcinoma (Peña et al., 2006). Furthermore, the enhancement of Wnt signaling promoted by Snail results both from an increase in cytoplasmic β-catenin due to the loss of E-cadherin and the disorganization of junctions and from its physical interaction with β-catenin, which promotes Wnt signaling in a direct manner (Stemmer et al., 2008). *Zeb* genes activation occurs frequently upon Snail activation, as Snail1 has been found to activate Zeb1. However, ZEB is active in some tumors that lack SNAIL expression (Peña et al., 2006), and thus the regulation of *Zeb* expression should be analyzed independently because the contribution of different EMT inducers is dependent on the cellular context. For instance, ZEB1 expression is important during colon cancer progression, whereas ZEB2 has been studied in ovarian, gastric, and pancreatic tumors, where it is associated with invasiveness and aggressive behavior (reviewed in Peinado et al., 2007).

The Kruppel-like factor 8 (KFL8) induces EMT and tumor invasion in breast cell lines after directly binding to the *E-cadherin* promoter through GT boxes (Figure 4). Indeed, there is an inverse correlation between E-cadherin and KFL8 expression in lymph node positive breast tumors (Wang et al., 2007b). Twist and Goosecoid also downregulate E-cadherin expression, albeit indirectly (Yang and Weinberg, 2008). FoxC2, which lies downstream of Twist, Snail, and Goosecoid, does not affect Cadherin expression, but rather it promotes its cytoplasmic localization, and its main role seems to be the induction of mesenchymal properties. Twist, Goosecoid, and FoxC2 are all associated with the metastatic potential (Figure 4) (Yang and Weinberg, 2008).

Regardless of the mechanism, E-cadherin repressors function as full EMT inducers in many cell contexts, regulating the expression of a variety of genes repressing the epithelial character and promoting the mesenchymal state. In addition, they repress epithelial cell polarity and cell division while promoting cell survival (Barrallo-Gimeno and Nieto, 2005; Peinado et al., 2007). Attenuation of cell proliferation favors invasion versus tumor growth, and resistance to cell death confers a selective advantage on embryonic migratory or cancer invasive cells to populate distant organs. Thus, rather than being strictly repressors of *E-cadherin* expression, they are regulators of the epithelial phenotype and of cell adhesion and movement (see Table S1 for a comprehensive list of targets and references). The EMT inducers that indirectly repress *E-cadherin* transcription frequently activate some of the direct repressors, and they also have multiple specific targets.

Loss of Cell Polarity in EMT

The loss of cell polarity is a crucial step for EMT. In epithelial cells, three protein complexes participate in establishing and maintaining apicobasal polarity (Par, Crumbs, and Scribble),

and components of the three are regulated by EMT inducers (see review by Moreno-Bueno et al., 2008, and Table S1 for additional references). SNAIL1 alters epithelial cell polarity by repressing the transcription of *Crumbs3* and abolishing the localization of both Par and Crumbs complexes at the junctions (Whiteman et al., 2008). Similarly, Zeb1 directly represses the transcription of cell polarity genes, including *Crumbs3*, *Pals1-associated tight junction proteins* (PATJ), and the member of the Scribble complex *Lethal giant larvae* (Lgl2) (Spaderna et al., 2008). TGFβ contributes to the loss of cell polarity during EMT in two ways, through the canonical pathway by inducing *Snail* and *Zeb* genes expression and through a noncanonical pathway that involves the downregulation of Par3 expression and the Par6-mediated degradation of RhoA and local alteration of the actin cytoskeleton (Ozdamar et al., 2005; Wang et al., 2008).

Proteases and the ECM Network

Snail and Zeb factors induce the expression of metalloproteases that can degrade the basement membrane, thereby favoring invasion (see Table S1 for references). Interestingly, some proteases are sufficient to induce EMT perhaps by triggering a positive regulatory feedback loop that stabilizes EMT. MMP3 triggers EMT by increasing the cellular levels of reactive oxygen species, which in turn induces Snail1 expression (Radisky et al., 2005). MMP13 also likely triggers EMT after being strongly induced by FGF1 (Billottet et al., 2008). Eplysin can also induce EMT through TGFβ activation (Illman et al., 2006). The transmembrane serine protease TMPRSS4 is overexpressed in colon carcinoma cell lines, in which it induces EMT after *Zeb* transcription and E-cadherin downregulation, promoting metastasis in nude mice (Jung et al., 2008). Finally, Periostin, an extracellular matrix protein secreted by osteoblasts, interacts with integrins and signals mainly via the PI3-K/Akt to promote EMT, invasion, and metastasis (Ruan et al., 2009).

Hierarchy and Cooperation

Cooperation between different transcription factors is a hallmark of EMT induction. The expression of Snail1, Snail2, Twist, and Id2 is controlled during TGFβ-induced EMT by the high-mobility group protein HMGA2, which behaves as an integrator of TGFβ signaling by using Snail1 as its downstream master effector (Thuault et al., 2008). Another factor that cooperates with TGFβ is the homeodomain-containing protein Six1, which works by increasing TGFβ signaling and induces EMT and metastasis when overexpressed in mammary gland epithelial cells (McCoy et al., 2009; Micalizzi et al., 2009). Conversely, the Mi-2/NuRD transcriptional complex behaves as a suppressor of breast tumor invasion and metastasis by incorporating into the complex the metastasis tumor antigen MTA3 or the lysine-specific demethylase LSD1, that prevent EMT by repressing *Snail* expression or by an epigenetic mechanism that inversely correlates with TGFβ signaling, respectively (Fujita et al., 2003; Wang et al., 2009b).

Interestingly, the *Snail1* and *Snail2* promoters contain AP1 and AP4, Smad and LEF responsive elements. There is increasing evidence of a hierarchy that controls the expression of these transcriptional regulators of EMT. Both in development and during carcinoma progression, *Snail1* is expressed at the onset of the transition, whereas *Snail2*, *Zeb* genes, *E47*, and *Twist* are subsequently induced to maintain the migratory mesenchymal state (Peinado et al., 2007). As such, Snail1 and Snail2 cooperate in primary tumor growth and in site-directed metastasis formation (Olmeda et al., 2008). Snail1 induces the expression of *Snail2* in fibrosis, and Snail1 upregulates the expression of Zeb proteins in carcinoma cells (Table S1). The control of *Zeb2* expression by Snail1 is mediated by the regulation of a natural antisense transcript in tumor cells (Figure 4). The natural antisense transcript prevents the splicing of an intron in the 5′ untranslated region of the *Zeb2* gene, increasing Zeb2 expression levels. This mechanism may be important in the maintenance of the mesenchymal state after EMT (Beltran et al., 2008).

New EMT Inducers

In addition to the many pathways triggered by membrane receptors, new intracellular molecules and external agents have recently been described as inducers of EMT. For instance, two tyrosine phosphatases, Pez and PRL3, both promote EMT. Pez is induced by TGFβ, and its expression is sufficient to trigger EMT in MDCK cells through the induction of both *Snail* and *Zeb* genes. Pez also induces the production of TGFβ, generating an autocrine activation loop (Wyatt et al., 2007). In embryos, Pez is expressed at sites of morphogenesis, partially associated with TGFβ3, making it likely to participate in the EMT that leads to the formation of cardiac valves (Wyatt et al., 2007). In turn, the dual specificity protein tyrosine phosphatase PRL3 induces EMT in a colon carcinoma line by activating PI3K/AKT. Stimulation of this pathway augments the degradation of PTEN and activates Snail1 (Wang et al., 2007a), possibly reinforcing PTEN repression given that PTEN is a direct target of Snail repression during radiation-induced apoptosis (Escriva et al., 2008). PRL3 also induces EMT through Src activation in a kidney cell line (Liang et al., 2007). The cytoplasmic kinase, Aurora-A, acting through the MAPK pathway, can induce EMT in nasopharyngeal tumor cells (Wan et al., 2008).

The mucin Podoplanin triggers EMT in MDCK cells by activating RhoA (Martin-Villar et al., 2006). By contrast, after inhibiting RhoA, podoplanin promotes collective MCF7 epithelial migration through the acquisition of filopodia and the loss of stress fibers. Podoplanin is expressed at the invasive front in an in vivo pancreatic tumor model, augmenting the frequency of high-grade tumors, although these invasive cells still retain E-cadherin expression (Wicki et al., 2006). Perhaps further analysis of the invasive front in human tumor specimens at the single-cell resolution will clarify the relationship between E-cadherin and podoplanin expression.

The L1 cell adhesion molecule, a member of the immunoglobulin superfamily, induces EMT in epithelial breast carcinoma cell lines by promoting adherens junction breakdown and the nuclear localization of β-catenin (Shtutman et al., 2006). Similarly, an interleukin-related molecule (ILEI) can induce EMT and metastatic properties in various cell lines; ILEI is overexpressed in tumors, where it correlates with metastasis and poor survival (Waerner et al., 2006). Likewise, interleukin-6 (IL-6) also promotes EMT in breast cancer cells, and Snail can induce IL-6 expression (Lyons et al., 2008; Sullivan et al., 2009),

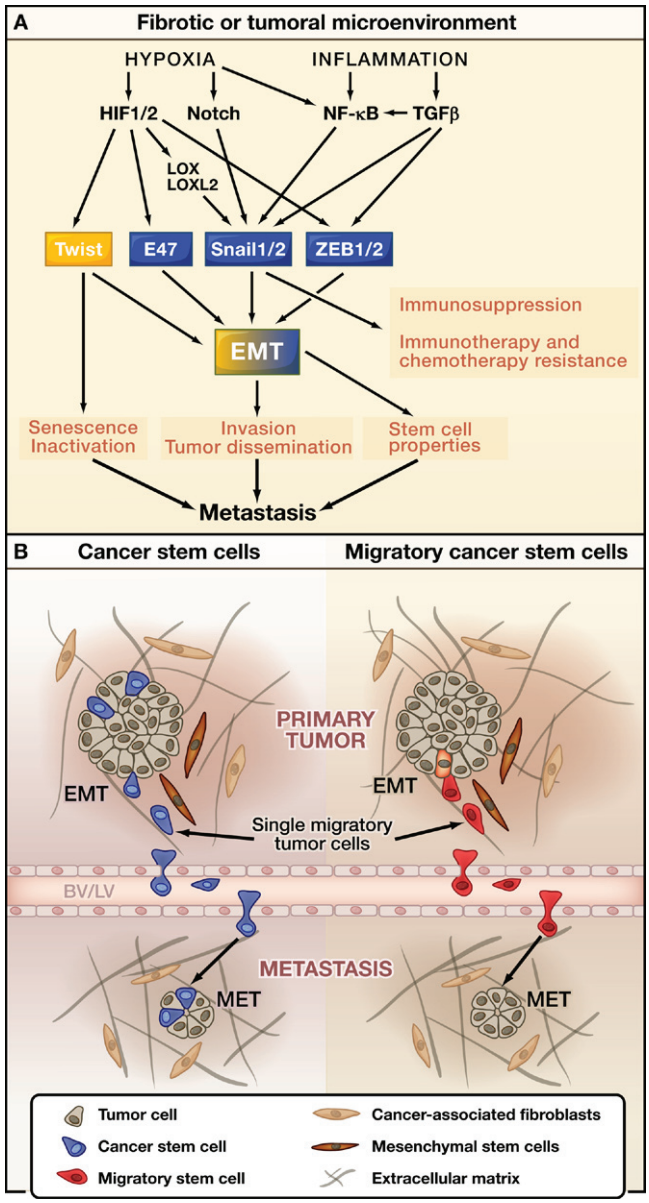

Figure 6. EMT and Tumor Progression

(A) EMT inducers as metastasis promoting agents. In the fibrotic or tumoral microenvironment hypoxia and inflammation favor the activation of EMT inducers. In addition to promoting tumor dissemination by inducing cell delamination and invasion of adjacent tissues, new facets of the EMT have been recently described that help to understand their implication in the metastatic potential. Both Twist and Snail confer stem cells properties, favoring the self-renewal of a small population of cells that can colonize and differentiate into secondary carcinomas. In addition, Twist also inactivates the cellular safeguard mechanism of cellular senescence triggered by oncogenes and Snail induces immunosuppression, immunoresistance, and chemoresistance.

(B) EMT is now thought to play a fundamental role in tumor progression and metastasis formation. Individual cells delaminate from primary tumors and migrate following the extracellular matrix network. Current research is actively analyzing the contributions of cancer-associated fibroblasts (CAF), including bone-marrow derived mesenchymal stem cells. Another challenge is to understand whether malignant migratory cells are cancer stem cells acting as tumor-initiating cells in the primary tumor (blue cells), if they are derived from somatic epithelial tumor cells that have undergone EMT to acquire stem cell-like properties (red cells), or some combination of these two possibilities. BV/LV, blood vessels/lymphatic vessels.

generating a regulatory loop that reinforces the relationship between EMT and inflammatory and immune responses (Wu et al., 2009a; López-Novoa and Nieto, 2009). Lastly, Thymosin β4, overexpressed in several cancers and involved in wound healing, can promote EMT in tumor cells through the activation of the integrin-linked kinase (ILK) pathway (Huang et al., 2007).

Complexity in EMT Signaling Pathways

Many signaling pathways trigger EMT in both embryonic development and in normal and transformed cell lines. The signaling pathways include those triggered by different members of the TGFβ superfamily, Wnts, Notch, EGF, HGF, FGF, HIF, and many others (Figure 5). As this has been the topic of a number of recent reviews (e.g., Thiery and Sleeman, 2006; Yang and Weinberg, 2008), here, we focus on the recent additions to this growing list.

The vast majority of the signaling pathways known to trigger EMT converge at the induction of the E-cadherin repressors, and in particular, of the *Snail* genes (Figures 4 and 5). A Ras-MAPK pathway activated by stimulation of different receptor tyrosine kinases can induce Snail1 and Snail2. Although HGF/SF usually activates MAPK-independent pathways, it can activate this pathway with the cooperation of the early growth response factor-1 (Egr-1), which binds directly to the SNAIL1 promoter, leading to its rapid induction and the execution of the EMT program (Grotegut et al., 2006). Snail1 can modulate the EMT response as it represses its own transcription (Peiro et al., 2006), while, conversely, Snail2 seems to be self-activated in avian neural crest (Sakai et al., 2006).

The NFκB pathway is also emerging as an important regulator of EMT in carcinoma cell lines and mesothelial fibrosis, acting through the induction of Snail1 transcription (Julien et al., 2007; Strippoli et al., 2008) and protein stabilization (Wu et al., 2009a). The importance of this pathway is evidenced by the blocking of EMT elicited by nondestructible IkB, a NFκB inhibitor (Huber et al., 2004).

TGFβ-induced Smad3 binds to myocardin-related transcription factors (MRTFs), and it is translocated to the nucleus in a Rho-dependent manner to activate Snail2 in MDCK cells. MRTFs also activate the transcription of actin filament remodeling proteins, fulfilling important aspects of EMT, including cell scattering and reorganization of cortical actin cytoskeleton into stress fibers (Morita et al., 2007), and they are important for the metastatic ability of tumor cells (Medjkane et al., 2009). These pathways are also at work in the conversion of kidney cells to myofibroblasts, with clear implications for renal fibrosis (Fan et al., 2007). In the renal system, the von Hippel-Lindau (VHL) tumor suppressor also negatively regulates the hypoxia-inducible factor-1 (HIF-1), and VHL loss is associated with renal clear cell carcinoma (RCC) and E-cadherin loss. Hypoxia induces Snail and EMT in many cellular and tumoral contexts (reviewed in López-Novoa and Nieto, 2009), although the cellular response may be associated with the concomitant induction of E47, Zeb factors, and, in particular, Twist, a direct target of HIF1-α that correlates with invasion and metastasis (Figure 6A) (Yang et al., 2008). TGFβ-induced EMT might be accelerated by additional mechanisms as occurs during gastrulation. E-cadherin

is endocytosed via clathrin-coated vesicle in EpH4 mammary cells expressing inducible Raf, followed by subsequent Snail1 activation (Janda et al., 2006).

Platelet-derived growth factor (PDGF) stimulates EMT in colon carcinoma cells through the nuclear translocation of β-catenin in a Wnt-independent manner (Yang et al., 2006c). A similar process may also be at work during EGF and TGFβ-induced EMT (Gotzmann et al., 2006). EGF is known to induce EMT by promoting E-cadherin endocytosis (Lu et al., 2003), but it can also induce the expression of both Snail and Twist, repressing E-cadherin transcription among other targets (Lee et al., 2008; Lo et al., 2007).

Vascular endothelial growth factor (VEGF) signaling promotes EMT in pancreatic and breast tumor cells by inducing *Snail* and *Twist* expression (Wanami et al., 2008; Yang et al., 2006a). Interestingly, Snail1 can induce VEGF expression in epithelial cells (Peinado et al., 2004), and they are coexpressed during peritoneal fibrosis (Zhang et al., 2008). Hence, a regulatory loop between angiogenesis and EMT may contribute to tumor progression.

Notch induces Snail1 during heart development (Timmerman et al., 2004) and is also required for neural crest induction and differentiation (Cornell and Eisen, 2005). Recent studies propose Snail2 as a target of Notch signaling (High et al., 2007; Leong et al., 2007; Niessen et al., 2008). Interestingly, Notch directly promotes Snail1 activation during hypoxia through the binding of its processed intracellular form to the *Snail* promoter and the activation of Lox2 expression by the hypoxia factor 1 (HIF-1), thereby stabilizing the Snail1 protein (Sahlgren et al., 2008).

The insulin growth factor receptor (IGFR) pathway induces EMT through the NFκB-Snail axis in mammary epithelial cells and by upregulating Zeb in prostate carcinoma cells (Graham et al., 2008; Kim et al., 2007). Similarly, a Sonic hedgehog-Gli-Snail1 pathway promotes EMT at the invasive front of neuroendocrine tumors (Fendrich et al., 2007), and it impairs the pregnancy-induced maturation of the mammary gland (Fiaschi et al., 2007). Prostaglandin E(2) acts in an autocrine or paracrine manner to induce both Snail and Zeb expression and EMT through the cyclooxygenase pathway (Dohadwala et al., 2006). In turn, Snail induces prostaglandin E(2) expression by repressing the prostaglandin dehydrogenase, generating a loop that may promote cancer progression (Mann et al., 2006).

New Players: Feedback Loops by MicroRNAs and Alternative Splicing

Recent studies have highlighted the importance of microRNA in the regulation of the epithelial phenotype by controlling EMT inducers (Figure 4) (reviewed in Cano and Nieto, 2008). MicroRNAs are noncoding single strands of ~22 nucleotides that exert a posttranscriptional control on gene expression by pairing their seed sequences (2–8 nucleotides at the 5′ end) to complementary sequences typically in the 3′ untranslated region of target mRNAs. This pairing results in the degradation of the target mRNA and/or the inhibition of its translation. MicroRNAs of the miR-200 family (as well as miR-205) have been shown to control EMT by downregulating the expression of the Zeb factors (Christoffersen et al., 2007; Gregory et al., 2008; Hurteau et al., 2007; Korpal et al., 2008; Park et al., 2008) and to control the metastatic ability of cancer cells. Indeed, forced expression of miR-200 family components inhibits the ability of lung adenocarcinoma cell lines to undergo EMT, invade, and metastasize (Gibbons et al., 2009).

In addition to the members of the miR200 family, miR-10b, miR-373, and miR-520c have also been implicated in the progression of breast carcinoma (Huang et al., 2008; Ma et al., 2007), and miR-21 is upregulated during TGFβ-induced EMT in tumor cell lines (Zavadil et al., 2007). Thus, a regulatory cascade involving microRNAs and EMT transcriptional regulators is likely to contribute significantly to the progression of carcinoma. This hypothesis has recently been validated as Zeb1 (and probably Snail1) can bind to the promoters of miR-141 and -200c and suppress their expression, thereby generating a regulatory loop that can reinforce the ability of Zeb1 to maintain a stable mesenchymal phenotype, as observed at the invasive front of colon carcinoma cells (Burk et al., 2008) (Figure 4). As it occurs in the regulation of EMT inducers by extracellular signals, microRNA are starting to emerge as regulators of gene transcription not only in cancer cells but also during development, perhaps reflecting an ancient role for these molecules. Indeed, they have been implicated in neuronal, muscle, and germline development (Stefani and Slack, 2008).

Alternative splicing can generate isoforms of the same gene with antagonistic functions, and recent studies confirm that this occurs during EMT. The invasion isoform of Mena (MenaINV) is specifically expressed in highly invasive tumor cells, and it facilitates cell invasion by stabilizing invadopodia (Philippar et al. 2008). Two RNA binding proteins, called ESRP1 and ESRP2 (epithelial splicing regulatory proteins 1 and 2), have recently been shown to control the splicing of epithelial-specific forms of EMT-associated genes including FGFR2, Mena, CD44, p120-catenin, and EPB41L5 (Warzecha et al., 2009a, 2009b). Whether the regulation of ESRPs expression is sufficient to regulate EMT or MET remains to be investigated.

EMT in Cancer: More Than Invasion

Although it is clear that EMT is involved in metastatic events in cancer, its participation in other events may be also highly relevant to tumor progression.

Resistance to Cell Death and Senescence

TGFβ can prevent the progression of incipient tumors and promote tumor invasion and evasion of immune surveillance at advanced stages (Massagué, 2008), directing apoptosis or survival plus EMT in many cell contexts. Interestingly, EMT is favored and apoptosis is inhibited when TGFβ acts on activated Ras-expressing mammary epithelial cells, and resistance to TGFβ-induced cell death is associated with hepatocytes undergoing EMT (Valdes et al., 2002). Similarly, exposure of breast tumor-derived NMuMG cells to TGFβ for several weeks generates cells that escape apoptosis and exhibit a sustained EMT (Gal et al., 2008). This model offers the opportunity to investigate the long-term effect of chronic TGFβ exposure during fibrosis in epithelial tissues and in cancer cells. Many cell lines undergo EMT in response to TGFβ over a period of days through a process that requires the activation of E-cadherin repressors. Indeed, TGFβ is a potent inducer of Snail expres-

sion, known to confer resistance to the cell death (reviewed in Barrallo-Gimeno and Nieto, 2005). This prosurvival activity can be extended to Twist, as it antagonizes the Myc-mediated proapoptotic effect in neuroblastoma (Puisieux et al., 2006).

The EMT process can also confer resistance to oncogene-induced premature senescence. Twist1 and Twist2 prevent cells from undergoing senescence induced by oncogenes by inhibiting p16/ink4a and p21/cip (Ansieau et al., 2008). Concomitantly, Twist proteins cooperate with activated Ras to trigger full EMT and promote invasion. Interestingly, Zeb1 also protects mouse embryonic fibroblasts from senescence (Liu et al., 2008). This suggests that abrogation of senescence may be a general mechanism associated with EMT (Figure 6A). Thus, constitutive expression of EMT inducers can maintain the mesenchymal and invasive phenotype while ensuring the survival of micrometastatic cells by suppressing two safeguard mechanisms against cancer: premature senescence and apoptosis.

Resistance to Chemotherapy and Immunotherapy

Tumors undergoing EMT may resist conventional chemotherapy, and, accordingly, colon carcinoma epithelial cell lines made resistant to oxaliplatin exhibit a mesenchymal morphology and express several markers of EMT (Yang et al., 2006b). The resistance of ovarian carcinoma epithelial cell lines to paclitaxel is also associated with the acquisition of EMT markers and loss of the epithelial phenotype (Kajiyama et al., 2007). Twist and one of its target genes are elevated in a subset of MCF7 or MDA-MB-434 cells selected for their invasive properties, and, having undergone EMT, they were also resistant to paclitaxel (Cheng et al., 2007). Moreover, the depletion of Twist can partially reverse multidrug resistance in breast cancer cells (Li et al., 2009). Similarly, Snail also confers resistance to paclitaxel, adriamycin, and radiation by antagonizing p53-mediated apoptosis (Kajita et al., 2004; Kurrey et al., 2009) (Figure 6A). Snail-expressing cells and the EMT process are also associated with resistance to dendritic cell immunotherapy (Kudo-Saito et al., 2009). Interestingly, forced expression of miR-200c, a negative regulator of EMT, restores chemotherapeutic sensitivity (Cochrane et al., 2009).

Immune Surveillance, Immunosuppression, and Inflammation

Tumors can escape immune surveillance by inducing tolerance or by modifying their phenotype through immunoediting. Indeed, Neu-driven tumors escape immune surveillance upon undergoing EMT (Knutson et al., 2006). Tumor relapse is observed in a Neu/ErBb2-inducible transgenic tumor model after removal of the inducer, indicating that tumors depend on continuous oncogenic signaling. However, all animals had residual foci that finally developed more aggressive new tumors of the EMT type (Moody et al., 2002). These two studies suggest that EMT may be involved in the acquisition of resistance to targeted therapies and that cells belonging to the foci of minimal residual disease acquired a mesenchymal phenotype. Snail1 expression is correlated with breast tumor recurrence (Moody et al., 2005), and Snail is associated with the activation of immunosuppressive cytokines, regulatory T cells, cytotoxic T lymphocytes resistance, and the generation of impaired dendritic cells (Kudo-Saito et al., 2009). Thus, Snail and very likely the EMT process in general, can accelerate cancer metastasis not only by increasing invasion, but also by acting on multiple immunosuppression and immunoresistance mechanisms, reflecting an alteration in the response of the host to the tumor. Thus, therapies directed to interfere with EMT might not only be anti-invasive but also able to restore immunocompetence in patients.

Inflammation is associated with the progression of cancer and fibrosis. Interestingly, recent studies point to the inflammation-induced EMT as critical to this connection (López-Novoa and Nieto, 2009). In the tumor microenvironment and in the course of organ fibrosis (such as occurs with kidney obstruction, diabetes, and glomerulonephritis) different mechanisms converge on the induction of NFκB, which increase the expression of EMT inducers and Snail in particular both at the transcriptional and translational levels (Figure 6A) (Julien et al., 2007; Strippoli et al., 2008; Wu et al., 2009a).

EMT Confers Stem Cell Properties

Recent evidence suggests that cells that undergo EMT acquire stem cell-like properties (Figure 6A) (Mani et al., 2008; Morel et al., 2008). Although further analyses are necessary to determine whether EMT in normal tissues leads to the production of normal stem cells, this intriguing concept is supported by studies on embryonic stem (ES) cells and mesenchymal stem cells. EMT is observed at the periphery of human ES cell clusters grown on matrigels (Eastham et al., 2007; Ullmann et al., 2007). These undifferentiated mesenchymal cells are characterized by a shift from E- to N-cadherin expression, the expression of Snail factors, vimentin, and metalloproteases. These cells also retain the expression of several totipotent transcription factors, including Oct-4 and Nanog, indicating that ES cells can adopt a mesenchymal phenotype without loosing their pluripotency.

Adult cells reprogrammed to pluripotency (induced pluripotent stem [iPS] cells) and mesenchymal stem cells share not only phenotypic features but also differentiation properties. Skin fibroblasts can give rise to hepatocyte-like cells, and these cells acquire an undifferentiated mesenchymal phenotype upon removal of growth/differentiation factors, remaining in an EMT state coexpressing mesodermal and endodermal markers (Lysy et al., 2007). Thus, mesenchymal status seems to be a condition to regain pluripotency.

Normal stem cells and cancer stem cells may share a mesenchymal phenotype that enhances their ability to preserve stemness, to retain migratory properties, and to respond to different stimuli during expansion and differentiation. Untransformed immortalized human mammary epithelial cells undergo EMT upon expression of Snail1 or Twist, or in the presence of TGFβ1. These cells adopt a mesenchymal phenotype, and, in addition to Twist and Snail, they also express FoxC2, Zeb factors, and N-cadherin. Upon transformation by activated Ras or Her2/neu, the subpopulation of CD44high/CD24low immortalized human mammary epithelial cells that possess stem-like properties increases with concommittant induction of EMT phenotype (Morel et al., 2008; Mani et al., 2008). Interestingly, Zeb1 is also able to confer stem cell-like properties, reinforcing the relationship between EMT and stemness (Wellner et al., 2009).

An issue that has emerged through the attempts to characterize a signature for the progression of primary tumors is the identity of tumor-initiating cells with inherent properties to sus-

tain the growth of the tumor. An important question that arises is whether such initiating cells are cancer stem cells. Direct support for the existence of cancer stem cells in carcinoma has come from mouse models of epithelial tumorigenesis and from initial data from patients (Visvader and Lindeman, 2008). However, some studies suggest that tumors are not necessarily initiated by rare cancer stem cells and that a significant percentage of individual human melanoma cells can efficiently form tumors (Kelly et al., 2007; Quintana et al., 2008). With the emergence of data indicating that EMT endows cells with stem cell-like properties, it will be important to determine whether the invasive cells disseminating from the primary tumor originate from resident stem cells or if they derive from somatic tumor cells that have undergone EMT. Regardless of their origin, such cells must undergo a full mesenchymal transition to become motile and invasive. Indeed, intravital two-photon microscopy demonstrates that these invasive cells delaminate from the primary tumor as individual cells and that they migrate in association with the extracellular matrix (Condeelis and Segall, 2003) (Figure 6B). In this process, cancer associated fibroblasts play an important role in tumor progression. A subpopulation of cancer-associated fibroblasts, the bone marrow-derived activated mesenchymal stem cells that are present in tumor stroma, instruct cancer cells in the primary tumor, enhancing their metastatic ability by promoting migration and extravasation (Figure 6B) (Karnoub et al., 2007). Interestingly, these mesenchymal stem cells in the stroma do not induce EMT, indicating that this process occurs independently in carcinoma cells. In pancreatic carcinoma, there is evidence of the existence of two different stem cell-like populations: one that maintains the growth of the primary tumor and another that produces metastatic growth (Hermann et al., 2007). It will be interesting to determine whether the latter have undergone EMT and whether these cells originate from resident cancer stem cells or other carcinoma cells (Figure 6B).

Together, these data indicate that EMT may not only be necessary for primary carcinoma to invade and disseminate, but also that these pioneer invasive cells with both a mesenchymal and a stem cell-like phenotype can generate a differentiated epithelial-like structure. This reversion to the differentiated phenotype through a process of MET is important for the formation of macrometastasis and thus to form the bulk of the secondary tumor mass. This hypothesis was previously formulated from an analysis of the progression of colon primary tumors and liver metastases, where it was proposed that cancer stem cells could acquire a mesenchymal phenotype, and thereby become migratory cancer stem cells that will form metastasis (Brabletz et al., 2005). Parallels can be found between these cells and the migratory embryonic cells with a mesenchymal phenotype that generate multiple differentiated cell types once they reach their destination.

Cancer Therapeutics that Target EMT Pathways

Regulating the activity of E-cadherin repressors may seem an obvious strategy to fight cancer progression. However, these inducers of the full EMT program are transcription factors, and are thus very difficult to target. RNA interference provides some hope in terms of specificity, but further development is needed to increase the stability of these reagents and the efficiency in cell targeting and intracellular delivery. An alternative would be the use of therapies based on negative regulators of EMT, but there is still little information or they are again transcription factors such as the recently described KLF17 transcription factor (Gumireddy et al., 2009). In addition, it is not known whether KLF17 is sufficient to revert the EMT by inducing a MET process. A strategy that is currently under way is to target the membrane receptors that transmit the extracellular signals that activate the EMT program.

Small-molecule inhibitors or antibodies directed against the EGFR, IGFR, PDGFR, cMET, TGFβR, and Endothelin type A receptor (ETAR) have been effective in preclinical and clinical trials. Although originally developed as inhibitors of cell proliferation or angiogenesis, it is likely that these molecules interfere with EMT (Chua et al., 2008). For instance, Cetuximab or panitumumab, two antibodies against EGFR, or erlotinib and gefitinib, two small molecules that act as competitive inhibitors of the EGFR kinase, are currently used clinically to treat advanced carcinoma. However, studies in cell lines show that not all cells expressing high levels of EGFR respond to erlotinib or gefitinib. Interestingly, there is a clear correlation between the EMT status of each cell line and the degree of response (Frederick et al., 2007; Thomson et al., 2005; Yauch et al., 2005). Restoration of E-cadherin can alleviate the resistance to kinase inhibitors (Witta et al., 2006), and a significant response was observed in a Phase 3 clinical trial for E-cadherin positive non-small-cell lung carcinoma (Herbst et al., 2005). Conversely, head and neck squamous cell carcinoma, pancreatic, colorectal, and bladder carcinoma that express EMT markers are more resistant to EGFR antagonists (Buck et al., 2007; Frederick et al., 2007; Shrader et al., 2007).

Other signaling pathways are also being targeted to interfere with EMT, including the use of neutralizing antibodies against TGFβ, which are in Phase 1 clinical trials for renal cell carcinoma and pulmonary fibrosis (Chua et al., 2008). Similarly, the inhibition of the Sonic hedgehog-Gli-Snail pathway with cyclopamine has been tested in xenograft mouse models of pancreatic carcinoma (Feldmann et al., 2007) and in gastrointestinal neuroendocrine tumors (Shida et al., 2006). The lysyl-oxidase propeptide reverses the Her-2/neu-induced EMT and the invasive phenotype, thereby providing a new target for Her-2/neu-driven breast cancers (Min et al., 2007). The strategies to target the IGF/IGFR pathway were recently reviewed, including the use of receptor-specific antibodies, kinase inhibitors, or activators of the AMP-activated protein kinase such as metformin (Pollak, 2008).

At initial stages of carcinoma development, there are autocrine mechanisms driven by EGFR and its cognate ligands. However, cells that have established a stable mesenchymal phenotype may utilize other receptor tyrosine kinases, or TGFβR autocrine or paracrine loops, and they may become refractory to EGFR inhibitors. There is evidence for a sustained activation of the PI3K-AKT pathway by IGFR, suggesting that combined targeted therapies may be useful during the initial phase of treatment. In addition, PDGFR activation may also be required for the sustained survival, growth, and invasion of mesenchymal-like carcinoma (Barr et al., 2008). Inhibition of

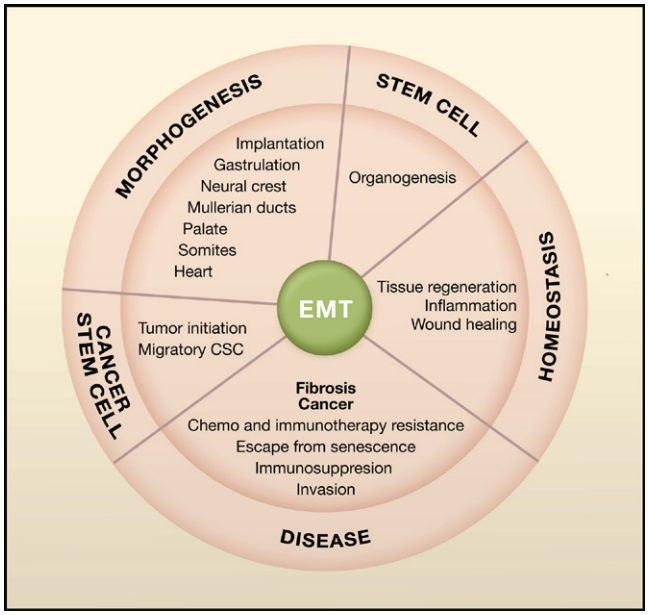

Figure 7. EMT at the Crossroads of Multiple Cellular Processes

the Src pathway by dasatinib is effective in mesenchymal and basal subtype breast carcinoma cell lines, probably because of the involvement of Src in signaling pathways controlling cell proliferation, differentiation, adhesion, motility, and survival (Finn et al., 2007). Thus, it would be interesting to examine whether dasatinib could overcome the refractiveness to EGFR inhibition by gefitinib and erlotinib.

Another approach to overcome refractoriness is to target directly the cancer stem cells. Recently, salinomycin was identified from a library consisting of 16,000 small molecules for its selective cytotoxity toward enriched breast cancer stem cells. This pioneer study provides a proof of principle that cancer stem cells exhibiting EMT features can be selectively targeted by drugs (Gupta et al., 2009).

Concluding Remarks

EMT is central to both physiological and pathological processes (Figure 7), and pathological EMT can be regarded as a reactivation of developmental programs in the adult. Given that EMT is controlled by a network of transcriptional regulators coupled to posttranscriptional and posttranslational modifications that amplify the initial signals, defining the gene regulatory networks operative during embryonic EMT will be fundamental to understanding those that govern EMT in cancer. The gene regulatory network may help assign molecular signatures to human tumors and pave the way for the design of improved specific therapies. Powerful imaging techniques together with lineage tracers developed to follow cell movements in vivo will be essential to study migratory cancer cells in animal models. In particular, these approaches will make it possible to determine whether these migratory cancer cells were stem cells in origin or somatic tumor cells that acquired stemness upon undergoing EMT. EMT also provides an explanation for the known associations between inflammation and fibrotic processes or cancer progression, and between cancer progression and immunosuppression. Finally, the notion that a mesenchymal state is required to maintain stemness opens new avenues to understand epithelial plasticity in health and disease. In addition, this standpoint may establish the foundations to develop targeted therapies aimed at reverting the mesenchymal state into an epithelial state and at restoring immunocompetence.

Supplemental Data

Supplemental Data include supplemental text and one table and can be found with this article online at http://www.cell.com/supplemental/S0092-8674(09)01419-6.

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