Chapter 6

Molecular Organization of Cells

Jon D. Ahlstrom
University of Utah and George E. Wahlen VAMC, Salt Lake City, Utah, USA

Chapter Outline

INTRODUCTION

Multicellular tissues exist in one of two types of cellular arrangements, epithelial or mesenchymal. Epithelial cells adhere tightly to each other at their lateral surfaces and to an organized extracellular matrix (ECM) at their basal domain, thereby producing a sheet of cells resting on a basal lamina with an apical surface. Mesenchymal cells, in contrast, are individual cells with a bipolar morphology that are held together as a tissue within a three-dimensional ECM (see Figure 6.1). The conversion of epithelial cells into mesenchymal cells, an "epithelial–mesenchymal transition" (EMT), is central to many aspects of embryonic morphogenesis, adult tissue repair, as well as a number of disease states (Thiery et al., 2009; Hay, 2005; Nieto, 2011). The reverse process whereby mesenchymal cells coalesce into an epithelium is a "mesenchymal–epithelial transition" (MET). Understanding the molecules that regulate this transition between epithelial and mesenchymal states offers important insights into how cells and tissues are organized.

The early embryo is structured as one or more epithelia. An EMT allows the rearrangements of cells to create additional morphological features. Well-studied examples of EMTs during embryonic development includes gastrulation in *Drosophila* (Baum et al., 2008), the emigration of primary mesenchyme cells (PMCs) in sea urchin embryos (Shook and Keller, 2003), and gastrulation in amniotes (reptiles, birds, and mammals) at the primitive streak (Hay, 2005). EMTs also occur later in vertebrate development, such as the emigration of neural crest cells from the neural tube (Sauka-Spengler and Bronner-Fraser, 2008), the formation of the sclerotome from epithelial somites, and during palate fusion (Hay, 2005). The reverse process of MET is likewise crucial to development, and examples include the condensation of mesenchymal cells to form the notochord and somites (Thiery et al., 2009), kidney tubule formation from nephrogenic mesenchyme (Schmidt-Ott et al., 2006), and the creation of heart valves from cardiac mesenchyme (Nakajima et al., 2000). In the adult organism, EMTs and METs occur during wound healing and tissue remodeling (Hay, 2005; Kalluri and Weinberg, 2009). The conversion of neoplastic epithelial cells into invasive cancer cells has long been considered an EMT process (Thiery et al., 2009). However, there are also examples of tumor cells that have functional cell–cell adhesion junctions, yet are still migratory and invasive as

Handbook of Stem Cells, Two-Volume Set. DOI: http://dx.doi.org/10.1016/B978-0-12-385942-6.00006-8

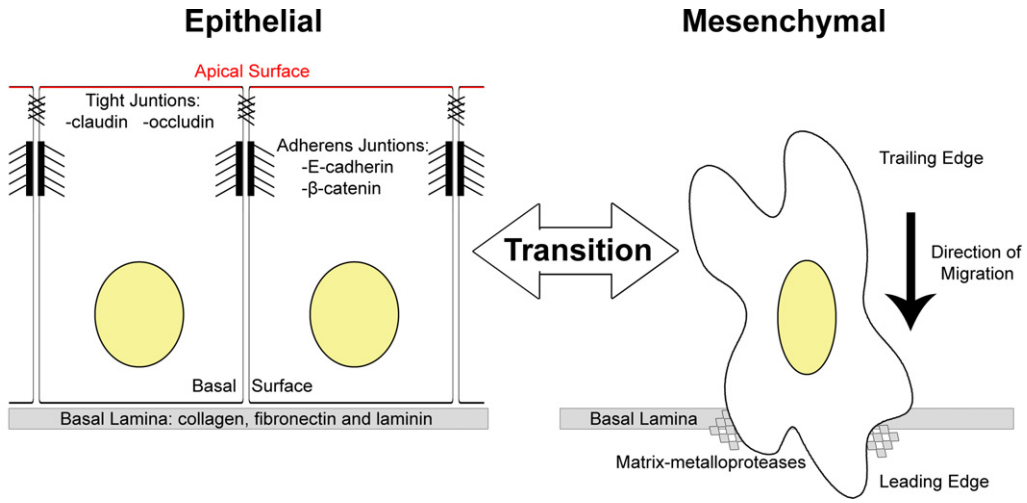

FIGURE 6.1 **Epithelial vs. mesenchymal.** Epithelial cells adhere tightly together by tight junctions and adherens junctions localized near the apical surface. Epithelial cells also have a basal surface that rests on a basal lamina (ECM). Mesenchymal cells in contrast do not have well-defined cell−cell adhesion complexes, have front-end/back-end polarity instead of apical/basal polarity, and mesenchymal cells are characterized by their ability to invade the basal lamina.

a group (Rørth, 2009). This "collective migration" also occurs during development (Nieto, 2011). Hence, there is debate about whether an EMT model accurately describes all epithelial metastatic cancers. Similarly, the fibrosis of cardiac, kidney, lens, and liver epithelial tissue has also long been categorized as an EMT event (Hay, 2005; Iwano et al., 2002). However, recent research in the kidney *in vivo* shows that the myofibroblasts induced following kidney injury are derived from mesenchymal pericytes, rather than the proximal epithelial cells (Humphreys et al., 2010). Therefore, the origin of the cells that contribute to fibrotic tissue scarring (epithelial or otherwise) may need to be carefully re-examined.

The focus of this chapter is on the molecules that regulate the organization of cells into epithelium or mesenchyme. We will first discuss the cellular changes that occur during an EMT, including changes in cell−cell and cell−ECM adhesions, changes in cell polarity, and the stimulation of invasive cell motility. Then we will consider the molecules and mechanisms that control the EMT or MET, including the structural molecules, transcription factors and signaling pathways that regulate EMTs.

MOLECULES THAT ORGANIZE CELLS

The conversion of an epithelial sheet into individual migratory cells and back again requires the coordinated changes of many distinct families of molecules.

Changes in Cell−Cell Adhesion

Epithelial cells are held together by specialized cell−cell junctions, including adherens junctions, desmosomes, and tight junctions (Giepmans and van Ijzendoorn, 2009). These junctions are localized in the lateral domain near the apical surface and establish the apical polarity of the epithelium. In order for an epithelial sheet to produce individual mesenchymal cells, cell−cell adhesions must be disrupted. The principal transmembrane proteins that mediate cell−cell adhesions are members of the cadherin superfamily (Stepniak et al., 2009). E-cadherin and N-cadherin are classical cadherins that interact homotypically through their extracellular IgG domains with like-cadherins on adjacent cells. Cadherins are important mediators of cell−cell adhesion. For example, misexpression of E-cadherin is sufficient for promoting cell−cell adhesion and assembly of adherens junctions in fibroblasts (Nagafuchi et al., 1987). In epithelial cancers (carcinomas), E-cadherin acts as a tumor suppressor (Thiery, 2002). In a mouse model for β-cell pancreatic cancer, the loss of E-cadherin is the rate-limiting step for transformed epithelial cells to become invasive (Perl et al., 1998). Although the loss of cadherin-mediated cell−cell adhesion is necessary for an EMT, the loss of cadherins is not always sufficient to generate a complete EMT *in vivo*. For example, the neural tube epithelium in mice expresses N-cadherin, but in the N-cadherin knockout mouse an EMT is not induced in the neural tube (Radice et al., 1997). Hence, cadherins are essential for maintaining epithelial integrity, and the loss of cell−cell adhesion due to the reduction of cadherin function is an important step for an EMT.

One characteristic of an EMT is "cadherin switching." Often, epithelia that express E-cadherin will downregulate E-cadherin expression at the time of the EMT, and express different cadherins such as N-cadherin (Christofori, 2003). Cadherin switching may promote motility. For instance, in

mammary epithelial cell lines, the misexpression of N-cadherin is sufficient for increased cell motility, and blocking N-cadherin expression results in less motility. However, the misexpression of N-cadherin does not result in the complete loss of epithelial morphology. Hence, cadherin switching may be necessary for cell motility, but cadherin switching alone is not sufficient to bring about a complete EMT (Maeda et al., 2005).

There are several ways that cadherin expression and function are regulated. Transcription factors that are central to most EMTs such as Snail-1, Snail-2, Zeb1, Zeb2, Twist, and E2A, all bind to E-boxes on the *E-cadherin* promoter and repress the transcription of *E-cadherin* (De Craene et al., 2005). Post-transcriptionally, the E-cadherin protein is ubiquitinated by the E3-ligase, Hakai, which targets E-cadherin to the proteasome (Fujita et al., 2002). E-cadherin turnover at the membrane is regulated by either caveolae-dependent endocytosis or clathrin-dependent endocytosis (Bryant and Stow, 2004), and p120-catenin prevents endocytosis of E-cadherin at the membrane (Xiao et al., 2007). E-cadherin function can also be disrupted by matrix metalloproteases, which degrade the extracellular domain of E-cadherin (Egeblad and Werb, 2002). Some or all of these mechanisms may occur during an EMT to disrupt cell−cell adhesion.

In summary, cell−cell adhesion is maintained principally by cadherins, and changes in cadherin expression are typical of an EMT.

Changes in Cell−ECM Adhesion

Altering the way that a cell interacts with the ECM is also important in EMTs. For example, at the time that sea urchin PMCs ingress, the cells have increased adhesiveness for ECM (Shook and Keller, 2003). Cell−ECM adhesion is mediated principally by integrins. Integrins are transmembrane proteins composed of two non-covalently linked subunits, α and β, that bind to ECM components such as fibronectin, laminin, and collagen. The cytoplasmic domain of integrins links to the cytoskeleton and interacts with signaling molecules. Changes in integrin function are required for many EMTs, including neural crest emigration (Delannet and Duband, 1992), mouse primitive streak formation (Hay, 2005), and cancer metastasis (Desgrosellier and Cheresh, 2010). However, the misexpression of integrin subunits is not sufficient to bring about a full EMT *in vitro* (Valles et al., 1996) or *in vivo* (Carroll et al., 1998).

The presence and function of integrins is modulated in several ways. For example, the promoter of the *integrin β6* gene is activated by the transcription factor Ets-1 during colon carcinoma metastasis (Bates, 2005). Most integrins can also cycle between "On" (high affinity) or "Off" (low affinity) states. This "inside-out" regulation of integrin adhesion occurs at the integrin cytoplasmic tail (Hood and Cheresh, 2002). In addition to integrin activation, the "clustering" of integrins on the cell surface also affects the overall strength of integrin−ECM interactions. The increased adhesiveness of integrins due to clustering, known as avidity, can be activated by chemokines, and is dependent on RhoA and phosphatidylinositol 3′ kinase (PI3K) activity (Hood and Cheresh, 2002).

In summary, changes in ECM adhesion are required for an EMT. Cell−ECM adhesions are maintained by integrins, and integrins have varying degrees of adhesiveness dependent upon the presence, activity, or avidity of the integrin subunits.

Changes in Cell Polarity and Stimulation of Cell Motility

Cellular polarity is defined by the distinct arrangement of cytoskeletal elements and organelles in epithelial versus mesenchymal cells. Epithelial polarity is characterized by cell−cell junctions found near the apical−lateral domain (non-adhesive surface), and a basal lamina opposite to the apical surface (adhesive surface). Mesenchymal cells in contrast do not have apical/basal polarity, but rather front-end/back-end polarity, with actin-rich lamellipodia and Golgi localized at the leading edge (Hay, 2005). Molecules that establish cell polarity include Cdc42, PAK1, PI3K, PTEN, Rac, Rho, and the PAR proteins (McCaffrey and Macara, 2009; Moreno-Bueno et al., 2008). Changes in cell polarity help to promote an EMT. In mammary epithelial cells, the activated TGF-β receptor II causes Par6 to activate the E3 ubiquitin ligase Smurf1, and Smurf1 then targets RhoA to the proteasome. The loss of RhoA activity results in the loss of cell−cell adhesion and epithelial cell polarity (Ozdamar et al., 2005).

In order for mesenchymal cells to leave the epithelium, they must become motile. Many of the same polarity (Crumbs, PAR, and Scribble complexes), structural (actin, microtubules), and regulatory molecules (Cdc42, Rac1, RhoA) that govern epithelial polarity are also central to cell motility (Nelson, 2009). Cell motility mechanisms also vary depending on whether the environment is 2D or 3D (Friedl and Wolf, 2010). Many mesenchymal cells express the intermediate filament vimentin, and vimentin may be responsible for several aspects of the EMT phenotype (Mendez et al., 2010).

In short, a wide variety of structural, polarity, and regulatory molecules must be reassigned as cells transition between epithelial polarity and mesenchymal migration.

Invasion of the Basal Lamina

In most EMTs the emerging mesenchymal cells must penetrate a basal lamina which consists of ECM

components such as collagen type IV, fibronectin, and laminin. The basal lamina functions to stabilize the epithelium and is a barrier to migratory cells (Erickson, 1987). One mechanism that mesenchymal cells use to breach the basal lamina is to produce enzymes that degrade it. Plasminogen activator is one protease associated with a number of EMTs, including neural crest emigration (Erickson, 1987) and the formation of cardiac cushion cells during heart morphogenesis (McGuire and Alexander, 1993). The type II serine protease, TMPRSS4, also promotes an EMT and metastasis when overexpressed *in vitro* and *in vivo* (Jung et al., 2007). Matrix-metalloproteases (MMPs) are also important for many EMTs. When MMP-2 activity is blocked in the neural crest EMT, neural crest emigration is inhibited, but not neural crest motility (Duong and Erickson, 2004). In mouse mammary cells, MMP-3 overexpression is sufficient to induce an EMT *in vitro* and *in vivo* (Sternlicht et al., 1999). Misexpressing MMP-3 in cultured cells induces an alternatively spliced form of Rac1 (Rac1b), which then causes an increase in reactive oxygen species (ROS) intracellularly, and Snail-1 expression. Either Rac1b activity or ROS are necessary and sufficient for an MMP-3-induced EMT (Radisky et al., 2005). Hence, a number of extracellular proteases are important to bring about an EMT.

While epithelial cells undergoing an EMT do eventually lose cell−cell adhesion, apical−basal polarity, and gain invasive motility, the EMT program is not necessarily ordered or linear. For example, in a study where neural crest cells were labeled with cell-adhesion or polarity markers and individual live cells were observed undergoing the EMT in slice culture, neural crest cells changed epithelial polarity either before or after the complete loss of cell−cell adhesion, or lost cell−cell adhesions either before or after cell migration commenced (Ahlstrom and Erickson, 2009). Therefore, while an EMT does consist of several distinct phases, these steps may occur in different orders or combinations, some of which (e.g., the complete loss of cell−cell adhesion) may not always be necessary.

In summary, changes in a wide range of molecules are needed for an EMT as epithelial cells lose cell−cell adhesion, change cellular polarity, and gain invasive cell motility.

THE EMT TRANSCRIPTIONAL PROGRAM

At the foundation of every EMT or MET program are the transcription factors that regulate the gene expression required for these cellular transitions. While many of the transcription factors that regulate EMTs have been identified, the complex regulatory networks are still incomplete. Here we review the transcription factors that are known to promote the various phases of an EMT. Then we examine how these EMT transcription factors themselves are regulated at the promoter and post-transcriptional levels.

Transcription Factors that Regulate EMTs

The Snail family of zinc-finger transcription factors, including Snail-1 and Snail-2 (formerly Snail and Slug), are direct regulators of cell−cell adhesion and motility during EMTs (De Craene et al., 2005; Barrallo-Gimeno and Nieto, 2005). The knockout of *Snail-1* in mice is lethal early in gestation, and the presumptive primitive streak cells that normally undergo an EMT still retain apical/basal polarity, adherens junctions, and express E-cadherin mRNA (Carver et al., 2001). Snail-1 misexpression is sufficient for breast cancer recurrence in a mouse model *in vivo*, and high levels of *Snail-1* predict the relapse of human breast cancer (Moody et al., 2005). Snail-2 is necessary for the chicken primitive streak and neural crest EMTs (Nieto et al., 1994). One way that Snail-1 or Snail-2 causes a decrease in cell−cell adhesion is by repressing the *E-cadherin* promoter (De Craene et al., 2005). This repression requires the mSin3A co-repressor complex, histone deacetylases, and components of the Polycomb 2 complex (Herranz et al., 2008). Snail-1 is also a transcriptional repressor of the tight junction genes *Claudin* and *Occludin* (De Craene et al., 2005) and the polarity gene *Crumbs3* (Whiteman et al., 2008). The misexpression of Snail-1 and Snail-2 further leads to the transcription of proteins important for cell motility such as fibronectin, vimentin (Cano et al., 2000), and RhoB (Del Barrio and Nieto, 2002). Further, Snail-1 promotes invasion across the basal lamina. In Madin−Darby Canine Kidney (MDCK) cells, the misexpression of Snail-1 represses laminin (basement membrane) production (Haraguchi et al., 2008) and indirectly upregulates *mmp-9* transcription (Jorda et al., 2005). Snail and Twist also make cancer cells more resistant to senescence, chemotherapy, apoptosis, and endow cancer cells with "stem cell" properties (Thiery et al., 2009). Hence, Snail-1 or Snail-2 are necessary and sufficient for bringing about many of the steps of an EMT, including loss of cell−cell adhesion, changes in cell polarity, gain of cell motility, invasion of the basal lamina, and increased proliferation and survival.

Other zinc-finger transcription factors important for EMTs are zinc-finger E-box-binding homeobox 1 (Zeb1, also known as δEF1), and Zeb2 (also known as Smad-interacting protein-1, Sip1). Both Zeb1 and Zeb2 bind to the *E-cadherin* promoter and repress transcription (De Craene et al., 2005). Zeb1 can also bind to and repress the transcription of the polarity proteins Crumbs3, Pals1-associated tight junction proteins (PATJ), and Lethal giant larvae 2 (Lgl2) (Spaderna et al., 2008). Zeb2 is structurally similar to Zeb1, and Zeb2 overexpression is sufficient to

downregulate E-cadherin, dissociate adherens junctions, and increase motility in MDCK cells (Comijn et al., 2001).

The Lymphoid Enhancer-binding Factor/ T-Cell Factor (LEF/TCF) transcription factors also play an important role in EMTs. For instance, the misexpression of Lef-1 in cultured colon cancer cells reversibly causes the loss of cell−cell adhesion (Kim et al., 2002). LEF/TCF transcription factors directly activate genes that regulate cell motility, such as the L1 adhesion molecule (Gavert et al., 2005), and the *fibronectin* gene (Gradl et al., 1999). LEF/TCF transcription factors also upregulate genes required for basal lamina invasion, including *mmp-3* and *mmp-7* (Gustavson et al., 2004).

Other transcription factors that have a role in promoting EMTs are the class I bHLH factors E2-2A and E2-2B (Sobrado et al., 2009), the forkhead box transcription factor FOXC2 (Mani et al., 2007), the homeobox protein Goosecoid (Hartwell et al., 2006), and the homeoprotein Six1 (Micalizzi et al., 2009; McCoy et al., 2009).

To summarize, transcription factors that regulate an EMT often do so by directly repressing cell adhesion and epithelial polarity molecules, and by upregulating genes required for cell motility and basal lamina invasion.

Regulation at the Promoter Level

Given the importance of the Snail, Zeb, and LEF/TCF transcription factors in orchestrating the various phases of an EMT, it is essential to understand the upstream events that regulate these EMT-promoting transcription factors.

The activation of *Snail-1* transcription in *Drosophila* requires the transcription factors Dorsal (NF-κB) and Twist (De Craene et al., 2005). The human *Snail-1* promoter also has functional NF-κB sites (Barbera et al., 2004) and blocking NF-κB reduces *Snail-1* transcription (Vargha et al., 2008). Additionally, a region of the *Snail-1* promoter is responsive to integrin-linked kinase (ILK) (De Craene et al., 2005), and ILK can activate Snail-1 expression via poly-ADP-ribose polymerase (PARP) (Lee et al., 2006). In mouse mammary epithelial cells, high mobility group protein A2 (HMGA2) and Smads activate *Snail-1* expression, and subsequently *Snail-2*, *Twist*, and *Id2* transcription (Thuault et al., 2008). For *Snail-2* expression, myocardin-related transcription factors (MRTFs) interact with Smads to induce *Snail-2* (Morita et al., 2007) and MRTFs may play a role in metastasis (Medjkane et al., 2009) and fibrosis (Fan et al., 2007). There are also several *Snail-1* transcriptional repressors. In breast cancer cell lines, metastasis-associated protein 3 (MTA3) binds directly to and represses the transcription of *Snail-1* in combination with the Mi-2/NuRD complex (Fujita et al., 2003), as also does lysine-specific demethylase (LSD1) (Wang et al., 2009). The Ajuba LIM proteins (Ajuba, LIMD1, and WTIP) are additional transcriptional corepressors of the Snail family (Langer et al., 2008).

The transcription of LEF/TCF genes such as *Lef-1* are activated by Smads (Nawshad and Hay, 2003). The misexpression of Snail-1 results in the transcription of *δEF-1* and *Lef-1* through a yet unknown mechanism (De Craene et al., 2005).

Post-Transcriptional Regulation of EMT Transcription Factors

The activity of EMT transcription factors is also regulated post-transcriptionally, where alternative splicing, translational control, protein stability (targeting to the proteasome), and nuclear localization can all regulate an EMT.

One newly discovered layer of EMT regulation is the epithelial- or mesenchymal-specific expression of alternatively spliced transcripts. For example, epithelial splicing regulatory proteins 1 and 2 (ESRP1 and ESRP2) are two RNA binding proteins that regulate the epithelial-specific expression of many molecules that are important to an EMT such as Rho regulators, integrins, and collagen (Warzecha et al., 2010). Blocking ESRP1 and 2 expression changes the pattern of alternatively spliced transcripts and causes cultured epithelial cells to upregulate vimentin and fibronectin, to reduce E-cadherin at cell−cell contacts, and to increase protease activity (Warzecha et al., 2010).

Non-coding RNAs are also emerging as important regulators of EMTs. In a breast cancer model, Myc activates the expression of microRNA-9 (miR-9), and miR-9 directly binds to and represses the *E-cadherin* promoter (Ma et al., 2010). Members of the miR-200 family repress the translation of *Zeb1*, and the expression of these miR-200 family members are repressed by Snail-1. Additionally, *Zeb2* transcription can be activated by naturally occurring RNA antisense transcripts (Beltran et al., 2008). It is not yet known if there are non-coding RNAs that regulate Snail family members. However, the Y-box binding protein-1 (YB-1) is important for the selective activation of *Snail-1* translation (Evdokimova et al., 2009).

Protein stability is another layer of EMT control. Snail-1 is phosphorylated by GSK-3β and targeted for destruction (Zhou et al., 2004). Therefore, the inhibition of GSK-3β activity by Wnt signaling may have multiple roles in an EMT, leading to the stabilization of both β-catenin and Snail-1. Some proteins that prevent GSK-3β-mediated phosphorylation (and thus promote Snail-1 activation) are lysyl-oxidase-like proteins LOXL2, LOXL3 (Peinado et al., 2007), and ILK (Delcommenne et al., 1998). A Snail-1-specific phosphatase (Snail-1 activator) is C-terminal domain phosphatase (SCP) (Wu et al., 2008). Snail-2 is targeted for degradation by the direct action of p53 and the ubiquitin ligase Mdm2 (Wang et al., 2009).

In addition to protein translation and stability, the function of Snail-1 also depends upon nuclear localization mediated by Snail-1's nuclear localization sequence. The phosphorylation of human Snail-1 by p21-activated kinase 1 (Pak1) promotes the nuclear localization of Snail-1 (and therefore Snail-1 activation) in breast cancer cells (Yang et al., 2005). In zebrafish, LIV-1 promotes the translocation of Snail-1 into the nucleus (Yamashita et al., 2004). Snail-1 also contains a nuclear export sequence (NES) that is dependent on the calreticulin (CalR) nuclear export pathway (Dominguez et al., 2003). This NES sequence is activated by the phosphorylation of the same lysine residues targeted by GSK-3β, which suggests a mechanism whereby phosphorylation of Snail-1 by GSK-3β results in the export of Snail-1 from the nucleus and subsequent degradation.

LEF/TCF activity is also regulated by other proteins. β-catenin is required as a co-factor for LEF/TCF-mediated activation of transcription, and Lef-1 can also associate with co-factor Smads to activate the transcription of additional EMT genes (Labbe et al., 2000). In colon cancer cells, Thymosin β4 stabilizes ILK activity (Huang et al., 2006).

In summary, EMT transcription factors such as Snail-1, Zeb1, and Lef-1 are regulated by a variety of mechanisms, both at the transcriptional level and post-transcriptional level by alternative splicing, non-coding RNA translation control, protein degradation, nuclear localization, and co-factors such as β-catenin.

MOLECULAR CONTROL OF THE EMT

The initiation of an EMT or MET is a tightly regulated event during development and tissue repair because deregulation of cellular organization is disastrous to the organism. A variety of external and internal signaling mechanisms coordinate the complex events of the EMT, and these same signaling pathways are often disrupted or reactivated during disease. EMTs or METs can be induced by either diffusible signaling molecules or ECM components. Below we discuss the role of signaling molecules and ECM in triggering an EMT, and then present a summary model for EMT induction.

Ligand-Receptor Signaling

During development, five main ligand-receptor signaling pathways are employed, namely TGF-β, Wnt, RTK, Notch, and Hedgehog. These pathways, among others, all have a role in triggering EMTs. While the activation of a single signaling pathway can be sufficient for an EMT, in most cases an EMT or MET is initiated by multiple signaling pathways acting in concert.

TGF-β Pathway

The transforming growth factor-beta (TGF-β) superfamily includes TGF-β, activin, and bone morphogenetic protein (BMP) families. These ligands operate through receptor serine/threonine kinases to activate a variety of signaling molecules including Smads, MAPK, PI3K, and ILK. Most of the EMTs studied to date are induced in part, or solely, by TGF-β superfamily members (Zavadil and Bottinger, 2005). During embryonic heart development, TGF-β2 and TGF-β3 have sequential and necessary roles in activating the endocardium to invade the cardiac jelly and from the endocardial cushions (Camenisch et al., 2002). In the avian neural crest, BMP4 induces *Snail-2* expression (Liem Karel et al., 1995). In the EMT that transforms epithelial tissue into metastatic cancer cells, TGF-β acts as a tumor suppressor during early stages of tumor development, but as a tumor/EMT inducer at later stages (Zavadil and Bottinger, 2005; Cui et al., 1996). TGF-β signaling may combine with other signaling pathways to induce an EMT. For example, in cultured breast cancer cells, activated Ras and TGF-β induce an irreversible EMT (Janda et al., 2002), and in pig thyroid epithelial cells, TGF-β and epidermal growth factor (EGF) synergistically stimulate the EMT (Grande et al., 2002).

One outcome of TGF-β signaling is to immediately change epithelial cell polarity. In a TGF-β-induced EMT of mammary epithelial cells, TGF-βR II directly phosphorylates the polarity protein, Par6, leading to the dissolution of tight junctions (Ozdamar et al., 2005). TGF-β signaling also regulates gene expression through the phosphorylation and activation of Smads. Smads are important co-factors in the stimulation of an EMT. For example, Smad3 is necessary for a TGF-β-induced EMT in lens and kidney tissue *in vivo* (Roberts et al., 2006). The Smad3/4 also complexes with Snail-1 and co-represses the promoters of cell−cell adhesion molecules (Vincent et al., 2009). Further, TGF-βR I directly binds to and activates PI3K (Yi et al., 2005), which in turn activates ILK and downstream pathways.

ILK is emerging as an important positive regulator of EMTs (Larue and Bellacosa, 2005). ILK interacts directly with growth factor receptors (TGF-β, Wnt, or RTK), integrins, the actin skeleton, PI3K, and focal adhesion complexes. ILK directly phosphorylates Akt and GSK-3β, and results in the subsequent activation of transcription factors such as AP-1, NF-κB, and Lef-1. Overexpression of ILK in cultured cells causes the suppression of GSK-3β activity (Delcommenne et al., 1998), translocation of β-catenin to the nucleus, activation of Lef-1/β-catenin transcription factors, and the downregulation of E-cadherin (Novak et al., 1998). Inhibition of ILK in cultured colon cancer cells leads to the stabilization of GSK-3β activity, decreased nuclear β-catenin localization, the suppression of *Lef-1* and *Snail-1* transcription, and reduced invasive

behavior of colon cancer cells (Tan et al., 2001). ILK activity also results in Lef-1-mediated transcriptional upregulation of MMPs (Gustavson et al., 2004). Hence, ILK (inducible by TGF-β signaling) is capable of orchestrating most of the major events in an EMT, including the loss of cell−cell adhesion and invasion across the basal lamina.

Wnt Pathway

Many EMTs or METs are also regulated by Wnt signaling. Wnts signal through seven-pass transmembrane proteins of the Frizzled family, which activates G-proteins, PI3K, inhibits GSK-3β, and promotes nuclear β-catenin signaling. For example, during zebrafish gastrulation, Wnt11 activates the GTPase Rab5c, which results in the endocytosis of E-cadherin (Ulrich et al., 2005). Wnt6 signaling is sufficient for increased transcription of *Snail-2* in the avian neural crest (Garcia-Castro et al., 2002). Snail-1 expression increases Wnt signaling (Stemmer et al., 2008), which suggests a positive feedback loop.

One of the downstream signaling molecules activated by Wnt signaling is β-catenin. β-catenin is a structural component of adherens junctions. Nuclear β-catenin is also a limiting factor for the activation of LEF/TCF transcription factors. β-catenin is pivotal for regulating most EMTs. Interfering with nuclear β-catenin signaling blocks the ingression of sea urchin PMCs (Logan et al., 1999), and in β-catenin mouse knockouts, the primitive streak EMT does not occur, and no mesoderm is formed (Huelsken et al., 2000). β-catenin is also necessary for the EMT that occurs during cardiac cushion development (Liebner et al., 2004). In breast cancer, β-catenin expression is highly correlated with metastasis and poor survival (Cowin et al., 2005), and blocking β-catenin function in tumor cells inhibits invasion *in vitro* (Wong and Gumbiner, 2003). It is unclear if β-catenin overexpression alone is sufficient for all EMTs. If β-catenin is misexpressed in cultured cells, it causes apoptosis. However, the misexpression of a stabilized form of β-catenin in mouse epithelial cells *in vivo* results in metastatic skin tumors (Gat et al., 1998).

Signaling by RTK Ligands

The receptor tyrosine kinase (RTK) family of receptors and the growth factors that activate them also regulate EMTs or METs. Ligand binding promotes RTK dimerization and activation of the intracellular kinase domains by autophosphorylation of tyrosine residues. These phosphotyrosines act as docking sites for intracellular signaling molecules, which can activate signaling cascades such as Ras/MAPK, PI3K/Akt, JAK/STAT, or ILK. Below we cite a few examples of RTK signaling in EMTs and METs.

Hepatocyte growth factor (HGF, also known as scatter factor) acts through the RTK c-met. HGF is important for the MET in the developing kidney (Woolf et al., 1995). HGF signaling is required for the EMT that produces myoblasts (limb muscle precursors) from somite tissue in the mouse (Thiery, 2002). In epithelial cells, HGF causes an EMT through MAPK and early growth response factor-1 (Egr-1) signaling (Grotegut et al., 2006).

Fibroblast growth factor (FGF) signaling regulates mouse primitive streak formation (Ciruna and Rossant, 2001). FGF signaling also stimulates cell motility and activates MMPs (Suyama et al., 2002; Billottet et al., 2008).

Epidermal growth factor (EGF) promotes the endocytosis of E-cadherin (Lu et al., 2003). EGF can also increase Snail-1 activity via the inactivation of GSK3-β (Lee et al., 2008) and EGF promotes increased *Twist* expression through a JAK/STAT3 pathway (Lo et al., 2007).

Insulin growth factor (IGF) signaling induces an EMT in breast cancer cell lines through the activation of Akt2 and suppression of Akt1 (Irie et al., 2005). In prostate cancer cells, IGF-1 promotes Zeb-1 expression (Graham et al., 2008). In fibroblast cells, constitutively activated IGF-IR increases NF-κB activity and Snail-1 levels (Kim et al., 2007). In several cultured epithelial cell lines, IGFR1 is associated with the complex of E-cadherin and β-catenin, and the ligand IGF-II causes the redistribution of β-catenin from the membrane to the nucleus, activation of the transcription factor TCF-3, and a subsequent EMT (Morali et al., 2001).

Another RTK known for its role in EMTs is the ErbB2/HER-2/Neu receptor, whose ligand is heregulin/neuregulin. Overexpression of HER-2 occurs in 25% of human breast cancers, and the misexpression of HER-2 in mouse mammary tissue *in vivo* is sufficient to cause metastatic breast cancer (Muller et al., 1988). Herceptin® (antibody against the HER-2 receptor) treatment is effective in reducing the recurrence of HER-2-positive metastatic breast cancers. HER-2 signaling activates *Snail-1* expression in breast cancer through an unknown mechanism (Moody et al., 2005). The RTK Axl is also required for breast cancer carcinoma invasiveness (Gjerdrum et al., 2010).

Vascular endothelial growth factor (VEGF) signaling promotes Snail-1 activity by suppression of GSK3-β (Wanami et al., 2008), and results in increased levels of *Snail-1*, *Snail-2*, and *Twist* (Yang et al., 2006). Snail-1 can also activate the expression of VEGF (Peinado et al., 2004). In summary, RTK signaling is important for many EMTs.

Notch Pathway

The Notch signaling family also regulates EMTs. When the Notch receptor is activated by its ligand Delta, an intracellular portion of the Notch receptor ligand is cleaved and transported to the nucleus where it regulates target genes. Notch1 is required for cardiac endothelial cells to undergo

an EMT to make cardiac cushions, and the role of Notch may be to make cells competent to respond to TGF-β2 (Timmerman et al., 2004). In the avian neural crest EMT, Notch signaling is required for the induction and/or maintenance of *BMP4* expression (Endo et al., 2002). Similarly, Notch signaling is required for the TGF-β-induced EMT of epithelial cell lines (Zavadil et al., 2004), and Notch promotes *Snail-2* expression in cardiac cushion cells (Niessen et al., 2008) and cultured cells (Leong et al., 2007).

Hedgehog Pathway

The hedgehog pathway is also involved in EMTs. Metastatic prostate cancer cells express high levels of hedgehog and *Snail-1*. If prostate cancer cell lines are treated with the hedgehog-pathway inhibitor, cyclopamine, levels of *Snail-1* are decreased. If the hedgehog-activated transcription factor, Gli, is misexpressed, *Snail-1* expression increases (Karhadkar et al., 2004).

Additional Signaling Pathways

Other signaling pathways that activate EMTs include inflammatory signaling molecules, lipid hormones, ROS species, and hypoxia. Interleukin-6 (inflammatory and immune response) can promote Snail-1 expression in breast cancer cells (Sullivan et al., 2009), and Snail-1 in turn can activate Il-6 expression (Lyons et al., 2008), providing a link between inflammation and EMTs (López-Novoa and Nieto, 2009). The lipid hormone prostaglandin E2 (PGE2) induces Zeb1 and Snail activity in lung cancer cells (Dohadwala et al., 2006), and Snail-1 can also induce PGE2 expression (Mann et al., 2006). ROS species can also activate EMTs by PKC and MAPK signaling (Yang et al., 2008). Hypoxia is important for initiating EMTs during development (Dunwoodie, 2009) and disease (López-Novoa and Nieto, 2009), often through hypoxia-inducible factor-1 (HIF-1), which directly activates *Twist* expression (Yang et al., 2008). Hypoxia also activates lysyl oxidases (LOX), which stabilize Snail-1 expression (Sahlgren et al., 2008) by inhibiting GSK-3β activity (Peinado et al., 2005).

In addition to diffusible signaling molecules, extracellular matrix molecules also regulate EMTs or METs. This was first dramatically demonstrated when lens or thyroid epithelium was embedded in collagen gels, and then promptly underwent an EMT (Hay, 2005). Integrin signaling appears to be important in this process (Zuk and Hay, 1994), and involves ILK-mediated activation of NF-κB, Snail-1, and Lef-1 (Koshikawa et al., 2000). Other ECM components that regulate EMTs include hyaluronan (Camenisch et al., 2002), the gamma-2 chain of laminin 5 (Koshikawa et al., 2000), periostin (Ruan et al., 2009), and podoplanin (Martin-Villar et al., 2006; Wicki et al., 2006). In summary, a variety of diffusible signals and ECM components can stimulate EMTs or METs.

A Model for EMT Induction

Many of the experimental studies on EMT mechanisms are piecework, and while great progress has been made in discovering EMT pathways, the entire signaling network

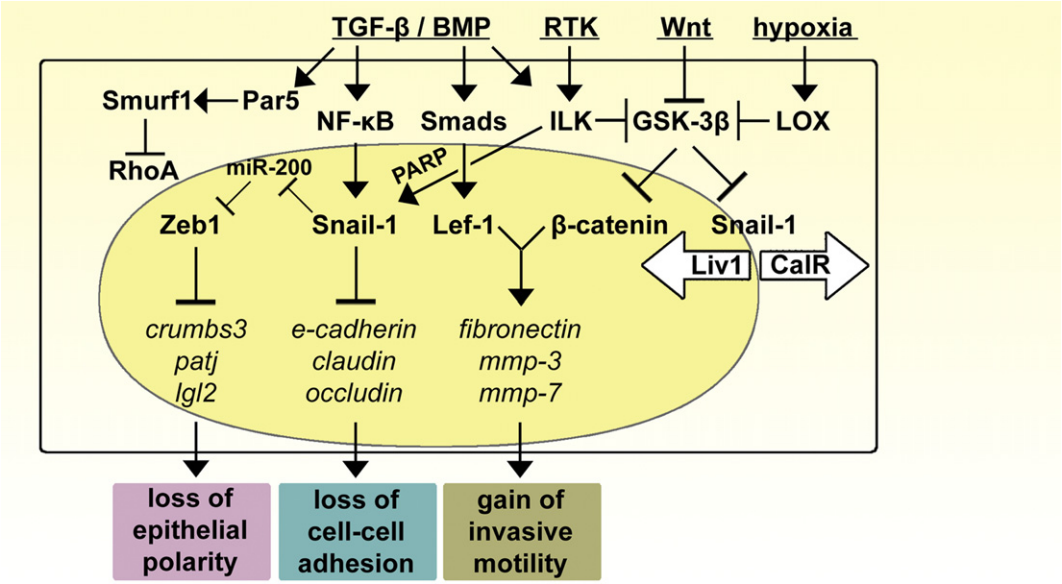

FIGURE 6.2 **Induction of an EMT.** This figure summarizes some of the important molecular pathways that bring about an EMT. Many of the signaling pathways converge on the activation of Snail-1 and nuclear β-catenin signaling to change gene expression, which results in the loss of epithelial cell polarity, the loss of cell−cell adhesion, and increased invasive cell motility.

is still incomplete. Figure 6.2 summarizes many of the various signaling mechanisms, although in actuality only a few of the inductive pathways will be utilized for a particular EMT. From experimental evidence to date, it appears that many of the EMT signaling pathways converge on ILK, the inhibition of GSK-3β, and stimulation of nuclear β-catenin signaling to activate Snail and LEF/TCF transcription factors. Snail, Zeb, and LEF/TCF transcription factors then act on a variety of targets to suppress cell−cell adhesion, induce changes in cell polarity, stimulate cell motility, and promote invasion of the basal lamina.

CONCLUSION

Over the past 20+ years since the term "EMT" was coined (Greenburg and Hay, 1982), important insights have been made in this rapidly expanding field of research. EMT and MET events occur during development, tissue repair, and disease, and many molecules that regulate the various EMTs or METs have been characterized, thanks in large part to the advent of cell culture models. However, the EMT regulatory network as a whole is still incomplete. The improved understanding of EMT and MET pathways in the future will lead to novel therapeutic targets for the treatment of disease and more effective strategies for tissue engineering.

REFERENCES

Ahlstrom, J.D., Erickson, C.A., 2009. The neural crest epithelial-mesenchymal transition in 4D: a "tail" of multiple non-obligatory cellular mechanisms. Development 136, 1801−1812.

Barbera, M.J., Puig, I., Dominguez, D., Julien-Grille, S., Guaita-Esteruelas, S., Peiro, S., et al., 2004. Regulation of Snail transcription during epithelial to mesenchymal transition of tumor cells. Oncogene 23, 7345−7354.

Barrallo-Gimeno, A., Nieto, M.A., 2005. The Snail genes as inducers of cell movement and survival: implications in development and cancer. Development 132, 3151−3161.

Bates, R.C., 2005. Colorectal cancer progression: Integrin alphavbeta6 and the epithelial-mesenchymal transition (EMT). Cell Cycle 4, 1350−1352.

Baum, B., Settleman, J., Quinlan, M.P., 2008. Transitions between epithelial and mesenchymal states in development and disease. Semin. Cell Dev. Biol. 19, 294−308.

Beltran, M., Puig, I., Peña, C., García, J.M., Álvarez, A.B., Peña, R., et al., 2008. A natural antisense transcript regulates Zeb2/Sip1 gene expression during Snail1-induced epithelial−mesenchymal transition. Genes Dev. 22, 756−769.

Billottet, C., Tuefferd, M., Gentien, D., Rapinat, A., Thiery, J.P., Broët, P., et al., 2008. Modulation of several waves of gene expression during FGF-1 induced epithelial−mesenchymal transition of carcinoma cells. J. Cell. Biochem. 104, 826−839.

Bryant, D.M., Stow, J.L., 2004. The ins and outs of E-cadherin trafficking. Trends Cell Biol. 14, 427−434.

Camenisch, T.D., Molin, D.G.M., Person, A., Runyan, R.B., Gittenberger-de Groot, A.C., McDonald, J.A., et al., 2002. Temporal and distinct TGFβ ligand requirements during mouse and avian endocardial cushion morphogenesis. Dev. Biol. 248, 170−181.

Camenisch, T.D., Schroeder, J.A., Bradley, J., Klewer, S.E., McDonald, J.A., 2002. Heart-valve mesenchyme formation is dependent on hyaluronan-augmented activation of ErbB2-ErbB3 receptors. Nat. Med. 8, 850−855.

Cano, A., Perez-Moreno, M.A., Rodrigo, I., Locascio, A., Blanco, M.J., del Barrio, M.G., et al., 2000. The transcription factor Snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat. Cell Biol. 2, 76−83.

Carroll, J.M., Luetteke, N.C., Lee, D.C., Watt, F.M., 1998. Role of integrins in mouse eyelid development: studies in normal embryos and embryos in which there is a failure of eyelid fusion. Mech. Dev. 78, 37−45.

Carver, E.A., Jiang, R., Lan, Y., Oram, K.F., Gridley, T., 2001. The mouse snail gene encodes a key regulator of the epithelial−mesenchymal transition. Mol. Cell. Biol. 21, 8184−8188.

Christofori, G., 2003. Changing neighbours, changing behaviour: cell adhesion molecule-mediated signalling during tumour progression. EMBO J. 22, 2318−2323.

Ciruna, B., Rossant, J., 2001. FGF signaling regulates mesoderm cell fate specification and morphogenetic movement at the primitive streak. Dev. Cell 1, 37−49.

Comijn, J., Berx, G., Vermassen, P., Verschueren, K., van Grunsven, L., Bruyneel, E., et al., 2001. The two-handed E Box binding Zinc Finger protein SIP1 downregulates E-cadherin and induces invasion. Mol. Cell 7, 1267−1278.

Cowin, P., Rowlands, T.M., Hatsell, S.J., 2005. Cadherins and catenins in breast cancer. Curr. Opin. Cell Biol. 17, 499−508.

Cui, W., Fowlis, D.J., Bryson, S., Duffie, E., Ireland, H., Balmain, A., et al., 1996. TGFβ1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice. Cell 86, 531−542.

De Craene, B., van Roy, F., Berx, G., 2005. Unraveling signalling cascades for the Snail family of transcription factors. Cell Signal. 17, 535−547.

Del Barrio, M.G., Nieto, M.A., 2002. Overexpression of Snail family members highlights their ability to promote chick neural crest formation. Development 129, 1583−1593.

Delannet, M., Duband, J.L., 1992. Transforming growth factor-beta control of cell−substratum adhesion during avian neural crest cell emigration in vitro. Development 116, 275−287.

Delcommenne, M., Tan, C., Gray, V., Rue, L., Woodgett, J., Dedhar, S., 1998. Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. Proc. Natl. Acad. Sci. U.S.A. 95, 11211−11216.

Desgrosellier, J.S., Cheresh, D.A., 2010. Integrins in cancer: biological implications and therapeutic opportunities. Nat. Rev. Cancer 10, 9−22.

Dohadwala, M., Yang, S.-C., Luo, J., Sharma, S., Batra, R.K., Huang, M., et al., 2006. Cyclooxygenase-2-dependent regulation of E-cadherin: prostaglandin E2 induces transcriptional repressors ZEB1 and Snail in non-small cell lung cancer. Cancer Res. 66, 5338−5345.

Dominguez, D., Montserrat-Sentis, B., Virgos-Soler, A., Guaita, S., Grueso, J., Porta, M., et al., 2003. Phosphorylation regulates the

subcellular location and activity of the Snail transcriptional repressor. Mol. Cell. Biol. 23, 5078–5089.

Dunwoodie, S.L., 2009. The role of hypoxia in development of the mammalian embryo. Dev. Cell 17, 755–773.

Duong, T.D., Erickson, C.A., 2004. MMP-2 plays an essential role in producing epithelial–mesenchymal transformations in the avian embryo. Dev. Dyn. 229, 42–53.

Egeblad, M., Werb, Z., 2002. New functions for the matrix metalloproteinases in cancer progression. Nat. Rev. Cancer 2, 161–174.

Endo, Y., Osumi, N., Wakamatsu, Y., 2002. Bimodal functions of Notch-mediated signaling are involved in neural crest formation during avian ectoderm development. Development 129, 863–873.

Erickson, C.A., 1987. Behavior of neural crest cells on embryonic basal laminae. Dev. Biol. 120, 38–49.

Evdokimova, V., Tognon, C., Ng, T., Ruzanov, P., Melnyk, N., Fink, D., et al., 2009. Translational activation of Snail1 and other developmentally regulated transcription factors by YB-1 promotes an epithelial-mesenchymal transition. Cancer Cell 15, 402–415.

Fan, L., Sebe, A., Peterfi, Z., Masszi, A., Thirone, A.C.P., Rotstein, O.D., et al., 2007. Cell contact-dependent regulation of epithelial–myofibroblast transition via the Rho-Rho kinase-phospho-myosin pathway. Mol. Biol. Cell 18, E06–07-0602.

Friedl, P., Wolf, K., 2010. Plasticity of cell migration: a multiscale tuning model. J. Cell Biol. 188, 11–19.

Fujita, N., Jaye, D.L., Kajita, M., Geigerman, C., Moreno, C.S., Wade, P.A., 2003. MTA3, a Mi-2/NuRD complex subunit, regulates an invasive growth pathway in breast cancer. Cell 113, 207–219.

Fujita, Y., Krause, G., Scheffner, M., Zechner, D., Leddy, H.E.M., Behrens, J., et al., 2002. Hakai, a c-Cbl-like protein, ubiquitinates and induces endocytosis of the E-cadherin complex. Nat. Cell Biol. 4, 222–231.

Garcia-Castro, M.I., Marcelle, C., Bronner-Fraser, M., 2002. Ectodermal Wnt function as a neural crest inducer. Science 297, 848–851.

Gat, U., DasGupta, R., Degenstein, L., Fuchs, E., 1998. De novo hair follicle morphogenesis and hair tumors in mice expressing a truncated β-catenin in skin. Cell 95, 605–614.

Gavert, N., Conacci-Sorrell, M., Gast, D., Schneider, A., Altevogt, P., Brabletz, T., et al., 2005. L1, a novel target of β-catenin signaling, transforms cells and is expressed at the invasive front of colon cancers. J. Cell Biol. 168, 633–642.

Giepmans, B.N., van Ijzendoorn, S.C., 2009. Epithelial cell–cell junctions and plasma membrane domains. Biochim. Biophys. Acta 1788, 820–831.

Gjerdrum, C., Tiron, C., Høiby, T., Stefansson, I., Haugen, H., Sandal, T., et al., 2010. Axl is an essential epithelial-to-mesenchymal transition-induced regulator of breast cancer metastasis and patient survival. Proc. Natl. Acad. Sci. U.S.A. 107, 1124–1129.

Gradl, D., Kuhl, M., Wedlich, D., 1999. The Wnt/Wg signal transducer β-catenin controls fibronectin expression. Mol. Cell. Biol. 19, 5576–5587.

Graham, T.R., Zhau, H.E., Odero-Marah, V.A., Osunkoya, A.O., Kimbro, K.S., Tighiouart, M., et al., 2008. Insulin-like growth factor-I-dependent up-regulation of ZEB1 drives epithelial-to-mesenchymal transition in human prostate cancer cells. Cancer Res. 68, 2479–2488.

Grande, M., Franzen, A., Karlsson, J.O., Ericson, L.E., Heldin, N.-E., Nilsson, M., 2002. Transforming growth factor-β and epidermal growth factor synergistically stimulate epithelial to mesenchymal transition (EMT) through a MEK-dependent mechanism in primary cultured pig thyrocytes. J. Cell. Sci. 115, 4227–4236.

Greenburg, G., Hay, E.D., 1982. Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. J. Cell Biol. 95, 333–339.

Grotegut, S., von Schweinitz, D., Christofori, G., Lehembre, F., 2006. Hepatocyte growth factor induces cell scattering through MAPK/Egr-1-mediated upregulation of Snail. EMBO J. 25, 3534–3545.

Gustavson, M.D., Crawford, H.C., Fingleton, B., Matrisian, L.M., 2004. Tcf binding sequence and position determines β-catenin and Lef-1 responsiveness of MMP-7 promoters. Mol. Carcinog. 41, 125–139.

Haraguchi, M., Okubo, T., Miyashita, Y., Miyamoto, Y., Hayashi, M., Crotti, T.N., et al., 2008. Snail regulates cell–matrix adhesion by regulation of the expression of integrins and basement membrane proteins. J. Biol. Chem. 283, 23514–23523.

Hartwell, K.A., Muir, B., Reinhardt, F., Carpenter, A.E., Sgroi, D.C., Weinberg, R.A., 2006. The Spemann organizer gene, Goosecoid, promotes tumor metastasis. Proc. Natl. Acad. Sci. U.S.A. 103, 18969–18974.

Hay, E.D., 2005. The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create it. Dev. Dyn. 233, 706–720.

Herranz, N., Pasini, D., Diaz, V.M., Franci, C., Gutierrez, A., Dave, N., et al., 2008. Polycomb complex 2 is required for E-cadherin repression by the Snail1 transcription factor. Mol. Cell. Biol. 28, 4772–4781.

Hood, J.D., Cheresh, D.A., 2002. Role of integrins in cell invasion and migration. Nat. Rev. Cancer 2, 91–100.

Huang, H.C., Hu, C.H., Tang, M.C., Wang, W.S., Chen, P.M., Su, Y., 2006. Thymosin B4 triggers an epithelial–mesenchymal transition in colorectal carcinoma by upregulating integrin-linked kinase. Oncogene 26, 2781–2790.

Huelsken, J., Vogel, R., Brinkmann, V., Erdmann, B., Birchmeier, C., Birchmeier, W., 2000. Requirement for beta-catenin in anterior–posterior axis formation in mice. J. Cell Biol. 148, 567–578.

Humphreys, B.D., Lin, S.-L., Kobayashi, A., Hudson, T.E., Nowlin, B.T., Bonventre, J.V., et al., 2010. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. Am. J. Pathol. 176, 85–97.

Irie, H.Y., Pearline, R.V., Grueneberg, D., Hsia, M., Ravichandran, P., Kothari, N., et al., 2005. Distinct roles of Akt1 and Akt2 in regulating cell migration and epithelial–mesenchymal transition. J. Cell Biol. 171, 1023–1034.

Iwano, M., Plieth, D., Danoff, T.M., Xue, C., Okada, H., Neilson, E.G., 2002. Evidence that fibroblasts derive from epithelium during tissue fibrosis. J. Clin. Invest. 110, 341–350.

Janda, E., Lehmann, K., Killisch, I., Jechlinger, M., Herzig, M., Downward, J., et al., 2002. Ras and TGFβ cooperatively regulate epithelial cell plasticity and metastasis: dissection of Ras signaling pathways. J. Cell Biol. 156, 299–314.

Jorda, M., Olmeda, D., Vinyals, A., Valero, E., Cubillo, E., Llorens, A., et al., 2005. Upregulation of MMP-9 in MDCK epithelial cell line in response to expression of the Snail transcription factor. J. Cell. Sci. 118, 3371–3385.

Jung, H., Lee, K.P., Park, S.J., Park, J.H., Jang, Y.S., Choi, S.Y., et al., 2007. TMPRSS4 promotes invasion, migration and metastasis of human tumor cells by facilitating an epithelial–mesenchymal transition. Oncogene 27, 2635–2647.

Kalluri, R., Weinberg, R.A., 2009. The basics of epithelial−mesenchymal transition. J. Clin. Invest. 119, 1420−1428.

Karhadkar, S.S., Steven Bova, G., Abdallah, N., Dhara, S., Gardner, D., Maitra, A., et al., 2004. Hedgehog signalling in prostate regeneration, neoplasia and metastasis. Nature 431, 707−712.

Kim, H.-J., Litzenburger, B.C., Cui, X., Delgado, D.A., Grabiner, B.C., Lin, X., et al., 2007. Constitutively active type I insulin-like growth factor receptor causes transformation and xenograft growth of immortalized mammary epithelial cells and is accompanied by an epithelial-to-mesenchymal transition mediated by NF-kB and Snail. Mol. Cell. Biol. 27, 3165−3175.

Kim, K., Lu, Z., Hay, E.D., 2002. Direct evidence for a role of β-catenin/LEF-1 signalling pathway in induction of EMT. Cell Biol. Int. 26, 463−476.

Koshikawa, N., Giannelli, G., Cirulli, V., Miyazaki, K., Quaranta, V., 2000. Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5. J. Cell Biol. 148, 615−624.

Labbe, E., Letamendia, A., Attisano, L., 2000. Association of Smads with lymphoid enhancer binding factor 1/T cell-specific factor mediates cooperative signaling by the transforming growth factor-beta and Wnt pathways. Proc. Natl. Acad. Sci. U.S.A. 97, 8358−8363.

Langer, E.M., Feng, Y., Zhaoyuan, H., Rauscher III, F.J., Kroll, K.L., Longmore, G.D., 2008. Ajuba LIM proteins are Snail/Slug corepressors required for neural crest development in Xenopus. Dev. Cell 14, 424−436.

Larue, L., Bellacosa, A., 2005. Epithelial−mesenchymal transition in development and cancer: role of phosphatidylinositol 3′ kinase//AKT pathways. Oncogene 24, 7443−7454.

Lee, J.M., Dedhar, S., Kalluri, R., Thompson, E.W., 2006. The epithelial−mesenchymal transition: new insights in signaling, development, and disease. J. Cell Biol. 172, 973−981.

Lee, M.-Y., Chou, C.-Y., Tang, M.-J., Shen, M.-R., 2008. Epithelial−mesenchymal transition in cervical cancer: correlation with tumor progression, epidermal growth factor receptor overexpression, and Snail up-regulation. Clin. Cancer Res. 14, 4743−4750.

Leong, K.G., Niessen, K., Kulic, I., Raouf, A., Eaves, C., Pollet, I., et al., 2007. Jagged1-mediated Notch activation induces epithelial-to-mesenchymal transition through Slug-induced repression of E-cadherin. J. Exp. Med. 204, 2935−2948.

Liebner, S., Cattelino, A., Gallini, R., Rudini, N., Iurlaro, M., Piccolo, S., et al., 2004. β-Catenin is required for endothelial−mesenchymal transformation during heart cushion development in the mouse. J. Cell Biol. 166, 359−367.

Liem Karel, F.J., Tremml, G., Roelink, H., Jessell, T.M., 1995. Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. Cell 82, 969−979.

Lo, H.-W., Hsu, S.-C., Xia, W., Cao, X., Shih, J.-Y., Wei, Y., et al., 2007. Epidermal growth factor receptor cooperates with signal transducer and activator of transcription 3 to induce epithelial−mesenchymal transition in cancer cells via up-regulation of TWIST gene expression. Cancer Res. 67, 9066−9076.

Logan, C.Y., Miller, J.R., Ferkowicz, M.J., McClay, D.R., 1999. Nuclear beta-catenin is required to specify vegetal cell fates in the sea urchin embryo. Development 126, 345−357.

López-Novoa, J.M., Nieto, M.A., 2009. Inflammation and EMT: an alliance towards organ fibrosis and cancer progression. EMBO Mol. Med. 1, 303−314.

Lu, Z., Ghosh, S., Wang, Z., Hunter, T., 2003. Downregulation of caveolin-1 function by EGF leads to the loss of E-cadherin, increased transcriptional activity of β-catenin, and enhanced tumor cell invasion. Cancer Cell 4, 499−515.

Lyons, J.G., Patel, V., Roue, N.C., Fok, S.Y., Soon, L.L., Halliday, G.M., et al., 2008. Snail up-regulates proinflammatory mediators and inhibits differentiation in oral keratinocytes. Cancer Res. 68, 4525−4530.

Ma, L., Young, J., Prabhala, H., Pan, E., Mestdagh, P., Muth, D., et al., 2010. miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nat. Cell Biol. 12, 247−256.

Maeda, M., Johnson, K.R., Wheelock, M.J., 2005. Cadherin switching: essential for behavioral but not morphological changes during an epithelium-to-mesenchyme transition. J. Cell Sci. 118, 873−887.

Mani, S.A., Yang, J., Brooks, M., Schwaninger, G., Zhou, A., Miura, N., et al., 2007. Mesenchyme Forkhead 1 (FOXC2) plays a key role in metastasis and is associated with aggressive basal-like breast cancers. Proc. Natl. Acad. Sci. U.S.A. 104, 10069−10074.

Mann, J.R., Backlund, M.G., Buchanan, F.G., Daikoku, T., Holla, V.R., Rosenberg, D.W., et al., 2006. Repression of prostaglandin dehydrogenase by epidermal growth factor and Snail increases prostaglandin E2 and promotes cancer progression. Cancer Res. 66, 6649−6656.

Martin-Villar, E., Megias, D., Castel, S., Yurrita, M.M., Vilaro, S., Quintanilla, M., 2006. Podoplanin binds ERM proteins to activate RhoA and promote epithelial−mesenchymal transition. J. Cell. Sci. 119, 4541−4553.

McCaffrey, L.M., Macara, I.G., 2009. Widely conserved signaling pathways in the establishment of cell polarity. Cold Spring Harb. Perspect. Biol. 1, a001370.

McCoy, E.L., Iwanaga, R., Jedlicka, P., Abbey, N.-S., Chodosh, L.A., Heichman, K.A., et al., 2009. Six1 expands the mouse mammary epithelial stem/progenitor cell pool and induces mammary tumors that undergo epithelial−mesenchymal transition. J. Clin. Invest. 119, 2663−2677.

McGuire, P.G., Alexander, S.M., 1993. Inhibition of urokinase synthesis and cell surface binding alters the motile behavior of embryonic endocardial-derived mesenchymal cells in vitro. Development 118, 931−939.

Medjkane, S., Perez-Sanchez, C., Gaggioli, C., Sahai, E., Treisman, R., 2009. Myocardin-related transcription factors and SRF are required for cytoskeletal dynamics and experimental metastasis. Nat. Cell Biol. 11, 257−268.

Mendez, M.G., Kojima, S.-I., Goldman, R.D., 2010. Vimentin induces changes in cell shape, motility, and adhesion during the epithelial to mesenchymal transition. FASEB J. 24, 1838−1851.

Micalizzi, D.S., Christensen, K.L., Jedlicka, P., Coletta, R.D., Barón, A.E., Harrell, J.C., et al., 2009. The Six1 homeoprotein induces human mammary carcinoma cells to undergo epithelial−mesenchymal transition and metastasis in mice through increasing TGF-B signaling. J. Clin. Invest. 119, 2678−2690.

Moody, S.E., Perez, D., Pan, T.-chi, Sarkisian, C.J., Portocarrero, C.P., et al., 2005. The transcriptional repressor Snail promotes mammary tumor recurrence. Cancer Cell 8, 197−209.

Morali, O.G., Delmas, V., Moore, R., Jeanney, C., Thiery, J.P., Larue, L., 2001. IGF-II induces rapid beta-catenin relocation to the nucleus during epithelium to mesenchyme transition. Oncogene 20, 4942−4950.

Moreno-Bueno, G., Portillo, F., Cano, A., 2008. Transcriptional regulation of cell polarity in EMT and cancer. Oncogene 27, 6958−6969.

Morita, T., Mayanagi, T., Sobue, K., 2007. Dual roles of myocardin-related transcription factors in epithelial mesenchymal transition via slug induction and actin remodeling. J. Cell Biol. 179, 1027–1042.

Muller, W.J., Sinn, E., Pattengale, P.K., Wallace, R., Leder, P., 1988. Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. Cell 54, 105–115.

Nagafuchi, A., Shirayoshi, Y., Okazaki, K., Yasuda, K., Takeichi, M., 1987. Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA. Nature 329, 341–343.

Nakajima, Y., Yamagishi, T., Hokari, S., Nakamura, H., 2000. Mechanisms involved in valvuloseptal endocardial cushion formation in early cardiogenesis: roles of transforming growth factor (TGF)-beta; and bone morphogenetic protein (BMP). Anat. Rec. 258, 119–127.

Nawshad, A., Hay, E.D., 2003. TGFβ3 signaling activates transcription of the LEF1 gene to induce epithelial mesenchymal transformation during mouse palate development. J. Cell Biol. 163, 1291–1301.

Nelson, W.J., 2009. Remodeling epithelial cell organization: transitions between front-rear and apical–basal polarity. Cold Spring Harb. Perspect. Biol. 1, a000513.

Niessen, K., Fu, Y., Chang, L., Hoodless, P.A., McFadden, D., Karsan, A., 2008. Slug is a direct Notch target required for initiation of cardiac cushion cellularization. J. Cell Biol. 182, 315–325.

Nieto, M.A., 2011. The ins and outs of the epithelial to mesenchymal transition in health and disease. Annu. Rev. Cell Dev. Biol. 27, 1–30.

Nieto, M.A., Sargent, M.G., Wilkinson, D.G., Cooke, J., 1994. Control of cell behavior during vertebrate development by Slug, a zinc finger gene. Science 264, 835–839.

Novak, A., Hsu, S.-C., Leung-Hagesteijn, C., Radeva, G., Papkoff, J., Montesano, R., et al., 1998. Cell adhesion and the integrin-linked kinase regulate the LEF-1 and β-catenin signaling pathways. Proc. Natl. Acad. Sci. U.S.A. 95, 4374–4379.

Ozdamar, B., Bose, R., Barrios-Rodiles, M., Wang, H.-R., Zhang, Y., Wrana, J.L., 2005. Regulation of the polarity protein Par6 by TGFβ peceptors controls epithelial cell plasticity. Science 307, 1603–1609.

Peinado, H., Del Carmen Iglesias-de la Cruz, M., Olmeda, D., Csiszar, K., Fong, K.S., Vega, S., et al., 2005. A molecular role for lysyl oxidase-like 2 enzyme in Snail regulation and tumor progression. EMBO J. 24, 3446–3458.

Peinado, H., Marin, F., Cubillo, E., Stark, H.-J., Fusenig, N., Nieto, M.A., et al., 2004. Snail and E47 repressors of E-cadherin induce distinct invasive and angiogenic properties in vivo. J. Cell. Sci. 117, 2827–2839.

Peinado, H., Olmeda, D., Cano, A., 2007. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat. Rev. Cancer 7, 415–428.

Perl, A.-K., Wilgenbus, P., Dahl, U., Semb, H., Christofori, G., 1998. A causal role for E-cadherin in the transition from adenoma to carcinoma. Nature 392, 190–193.

Radice, G.L., Rayburn, H., Matsunami, H., Knudsen, K.A., Takeichi, M., Hynes, R.O., 1997. Developmental defects in mouse embryos lacking N-cadherin. Dev. Biol. 181, 64–78.

Radisky, D.C., Levy, D.D., Littlepage, L.E., Liu, H., Nelson, C.M., Fata, J.E., et al., 2005. Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature 436, 123–127.

Roberts, A.B., Tian, F., Byfield, S.D., Stuelten, C., Ooshima, A., Saika, S., et al., 2006. Smad3 is key to TGF-β-mediated epithelial-to-mesenchymal transition, fibrosis, tumor suppression and metastasis. Cytokine Growth Factor Rev. 17, 19–27.

Rørth, P., 2009. Collective cell migration. Annu. Rev. Cell Dev. Biol. 25, 407–429.

Ruan, K., Bao, S., Ouyang, G., 2009. The multifaceted role of periostin in tumorigenesis. Cell. Mol. Life Sci. 66, 2219–2230.

Sahlgren, C., Gustafsson, M.V., Jin, S., Poellinger, L., Lendahl, U., 2008. Notch signaling mediates hypoxia-induced tumor cell migration and invasion. Proc. Natl. Acad. Sci. U.S.A. 105, 6392–6397.

Sauka-Spengler, T., Bronner-Fraser, M., 2008. A gene regulatory network orchestrates neural crest formation. Nat. Rev. Mol. Cell Biol. 9, 557–568.

Schmidt-Ott, K.M., Lan, D., Hirsh, B.J., Barasch, J., 2006. Dissecting stages of mesenchymal-to-epithelial conversion during kidney development. Nephron Physiol. 104, 56–60.

Shook, D., Keller, R., 2003. Mechanisms, mechanics and function of epithelial–mesenchymal transitions in early development. Mech. Dev. 120, 1351–1383.

Sobrado, V.R., Moreno-Bueno, G., Cubillo, E., Holt, L.J., Nieto, M.A., Portillo, F., et al., 2009. The class I bHLH factors E2-2A and E2-2B regulate EMT. J. Cell. Sci. 122, 1014–1024.

Spaderna, S., Schmalhofer, O., Wahlbuhl, M., Dimmler, A., Bauer, K., Sultan, A., et al., 2008. The transcriptional repressor ZEB1 promotes metastasis and loss of cell polarity in cancer. Cancer Res. 68, 537–544.

Stemmer, V., de Craene, B., Berx, G., Behrens, J., 2008. Snail promotes Wnt target gene expression and interacts with beta-catenin. Oncogene 27, 5075–5080.

Stepniak, E., Radice, G.L., Vasioukhin, V., 2009. Adhesive and signaling functions of cadherins and catenins in vertebrate development. Cold Spring Harb. Perspect. Biol. 1, a002949.

Sternlicht, M.D., Lochter, A., Sympson, C.J., Huey, B., Rougier, J.P., Gray, J.W., et al., 1999. The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. Cell 98, 137–146.

Sullivan, N.J., Sasser, A.K., Axel, A.E., Vesuna, F., Raman, V., Ramirez, N., et al., 2009. Interleukin-6 induces an epithelial–mesenchymal transition phenotype in human breast cancer cells. Oncogene 28, 2940–2947.

Suyama, K., Shapiro, I., Guttman, M., Hazan, R.B., 2002. A signaling pathway leading to metastasis is controlled by N-cadherin and the FGF receptor. Cancer Cell 2, 301–314.

Tan, C., Costello, P., Sanghera, J., Dominguez, D., Baulida, J., de Herreros, A.G., et al., 2001. Inhibition of integrin linked kinase (ILK) suppresses beta-catenin-Lef/Tcf-dependent transcription and expression of the E-cadherin repressor, snail, in APC−/− human colon carcinoma cells. Oncogene 20, 133–140.

Thiery, J.P., 2002. Epithelial–mesenchymal transitions in tumour progression. Nat. Rev. Cancer 2, 442–454.

Thiery, J.P., Acloque, H., Huang, R.Y.J., Nieto, M.A., 2009. Epithelial–mesenchymal transitions in development and disease. Cell 139, 871–890.

Thuault, S., Tan, E.J., Peinado, H., Cano, A., Heldin, C.-H., Moustakas, A., 2008. HMGA2 and Smads co-regulate SNAIL1 expression during induction of epithelial-to-mesenchymal transition. J. Biol. Chem. 283, 33437–33446.

Timmerman, L.A., Grego-Bessa, J., Raya, A., Bertran, E., Perez-Pomares, J.M., Diez, J., et al., 2004. Notch promotes epithelial–mesenchymal transition during cardiac development and oncogenic transformation. Genes Dev. 18, 99–115.

Ulrich, F., Krieg, M., Schotz, E.-M., Link, V., Castanon, I., Schnabel, V., et al., 2005. Wnt11 functions in gastrulation by controlling cell cohesion through Rab5c and E-Cadherin. Dev. Cell 9, 555–564.

Valles, A.M., Boyer, B., Tarone, G., Thiery, J.P., 1996. Alpha 2 beta 1 integrin is required for the collagen and FGF-1 induced cell dispersion in a rat bladder carcinoma cell line. Cell Adhes. Commun. 4, 187–199.

Vargha, R., Bender, T.O., Riesenhuber, A., Endemann, M., Kratochwill, K., Aufricht, C., 2008. Effects of epithelial-to-mesenchymal transition on acute stress response in human peritoneal mesothelial cells. Nephrol. Dial. Transplant. 23, 3494–3500.

Vincent, T., Neve, E.P.A., Johnson, J.R., Kukalev, A., Rojo, F., Albanell, J., et al., 2009. A SNAIL1-SMAD3/4 transcriptional repressor complex promotes TGF-beta mediated epithelial–mesenchymal transition. Nat. Cell Biol. 11, 943–950.

Wanami, L.S., Chen, H.-Y., Peiró, S., García de Herreros, A., Bachelder, R.E., 2008. Vascular endothelial growth factor-A stimulates Snail expression in breast tumor cells: implications for tumor progression. Exp. Cell Res. 314, 2448–2453.

Wang, S.-P., Wang, W.-L., Chang, Y.-L., Wu, C.-T., Chao, Y.-C., Kao, S.-H., et al., 2009. p53 controls cancer cell invasion by inducing the MDM2-mediated degradation of Slug. Nat. Cell Biol. 11, 694–704.

Wang, Y., Zhang, H., Chen, Y., Sun, Y., Yang, F., Yu, W., et al., 2009. LSD1 is a subunit of the NuRD complex and targets the metastasis programs in breast cancer. Cell 138, 660–672.

Warzecha, C.C., Jiang, P., Amirikian, K., Dittmar, K.A., Lu, H., Shen, S., et al., 2010. An ESRP-regulated splicing programme is abrogated during the epithelial–mesenchymal transition. EMBO J. 29, 3286–3300.

Whiteman, E.L., Liu, C.J., Fearon, E.R., Margolis, B., 2008. The transcription factor snail represses Crumbs3 expression and disrupts apico-basal polarity complexes. Oncogene 27, 3875–3879.

Wicki, A., Lehembre, F., Wick, N., Hantusch, B., Kerjaschki, D., Christofori, G., 2006. Tumor invasion in the absence of epithelial–mesenchymal transition: Podoplanin-mediated remodeling of the actin cytoskeleton. Cancer Cell 9, 261–272.

Wong, A.S.T., Gumbiner, B.M., 2003. Adhesion-independent mechanism for suppression of tumor cell invasion by E-cadherin. J. Cell Biol. 161, 1191–1203.

Woolf, A.S., Kolatsi-Joannou, M., Hardman, P., Andermarcher, E., Moorby, C., Fine, L.G., et al., 1995. Roles of hepatocyte growth factor/scatter factor and the met receptor in the early development of the metanephros. J. Cell Biol. 128, 171–184.

Wu, Y., Evers, B.M., Zhou, B.P., 2008. Small C-terminal Domain Phosphatase enhances Snail activity through dephosphorylation. J. Biol. Chem. 284, M806916200.

Xiao, K., Oas, R.G., Chiasson, C.M., Kowalczyk, A.P., 2007. Role of p120-catenin in cadherin trafficking. Biochim. Biophys. Acta 1773, 8–16.

Yamashita, S., Miyagi, C., Fukada, T., Kagara, N., Che, Y.-S., Hirano, T., 2004. Zinc transporter LIVI controls epithelial–mesenchymal transition in zebrafish gastrula organizer. Nature 429, 298–302.

Yang, A.D., Camp, E.R., Fan, F., Shen, L., Gray, M.J., Liu, W., et al., 2006. Vascular endothelial growth factor receptor-1 activation mediates epithelial to mesenchymal transition in human pancreatic carcinoma cells. Cancer Res. 66, 46–51.

Yang, M.-H., Wu, M.-Z., Chiou, S.-H., Chen, P.-M., Chang, S.-Y., Liu, C.-J., et al., 2008. Direct regulation of TWIST by HIF-1alpha promotes metastasis. Nat. Cell Biol. 10, 295–305.

Yang, Z., Rayala, S., Nguyen, D., Vadlamudi, R.K., Chen, S., Kumar, R., 2005. Pak1 phosphorylation of Snail, a master regulator of epithelial-to-mesenchyme transition, modulates Snail's subcellular localization and functions. Cancer Res. 65, 3179–3184.

Yi, J.Y., Shin, I., Arteaga, C.L., 2005. Type I transforming growth factor beta receptor binds to and activates phosphatidylinositol 3-kinase. J. Biol. Chem. 280, 10870–10876.

Zavadil, J., Bottinger, E.P., 2005. TGF-β and epithelial-to-mesenchymal transitions. Oncogene 24, 5764–5774.

Zavadil, J., Cermak, L., Soto-Nieves, N., Bottinger, E.P., 2004. Integration of TGF-β/Smad and Jagged1/Notch signalling in epithelial-to-mesenchymal transition. EMBO J. 23, 1155–1165.

Zhou, B.P., Deng, J., Xia, W., Xu, J., Li, Y.M., Gunduz, M., et al., 2004. Dual regulation of Snail by GSK-3β-mediated phosphorylation in control of epithelial–mesenchymal transition. Nat. Cell Biol. 6, 931–940.

Zuk, A., Hay, E.D., 1994. Expression of β1 integrins changes during transformation of avian lens epithelium to mesenchyme in collagen gels. Dev. Dyn. 201, 378–393.