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Signaling mechanisms of the epithelial-mesenchymal transition

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Abstract

The epithelial-mesenchymal transition (EMT) is an essential mechanism in embryonic development and tissue repair. EMT also contributes to the progression of disease, including organ fibrosis and cancer. EMT, as well as a similar transition occurring in vascular endothelial cells called endothelial-mesenchymal transition (EndMT), results from the induction of transcription factors that alter gene expression to promote loss of cell-cell adhesion, leading to a shift in cytoskeletal dynamics and a change from epithelial morphology and physiology to the mesenchymal phenotype. Transcription program switching in EMT is induced by signaling pathways mediated by transforming growth factor β (TGF-b) and bone morphogenetic protein (BMP), Wnt–β-catenin, Notch, Hedgehog, and receptor tyrosine kinases. These pathways are activated by various dynamic stimuli from the local microenvironment, including growth factors and cytokines, hypoxia, and contact with the surrounding extracellular matrix (ECM). We discuss how these pathways crosstalk and respond to signals from the microenvironment to regulate the expression and function of EMT-inducing transcription factors in development, physiology, and disease. Understanding these mechanisms will enable the therapeutic control of EMT to promote tissue regeneration, treat fibrosis, and prevent cancer metastasis.

Introduction

The epithelial-mesenchymal transition (EMT) is an important cellular mechanism in embryonic development, tissue repair, and disease. First described in the 1980s as a cellular phenomenon in the primitive streak of chick embryos, EMT governs many developmental processes such as gastrulation, neural crest development, somite dissociation, and palate and lip fusion (*1*, *2*). A similar process termed the endothelial-mesenchymal transition (EndMT) involves the transformation of vascular endothelial cells to mesenchymal cells, which regulates the formation of the valves and septa of the developing heart (*3*). In the adult, these developmental programming mechanisms can be awoken and often result in the development of various diseases. Both organ fibrosis and cancer metastasis are driven by EMT, the latter driven perhaps by the generation of cancer stem cells capable of colonizing

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other tissues to form secondary tumors (*4*, *5*). EndMT also has a role in fibrosis (*6*) and cancer progression (*7*) and is involved in the formation of stem-like cells that differentiate into osteoprogenitor cells during heterotopic ossification (*8*) and vascular calcification (*9*).

Epithelial cells form polarized sheets that are held together through various cell adhesion molecules, such as claudins and E-cadherin (*10*). Beneath this cell layer, the basement membrane anchors epithelial cells to the matrix surface and maintains apical-basal polarity through connections between intermediate filaments and hemidesmosomes. Adhesion to both the basement membrane and adjacent cells is critical for maintaining the epithelial phenotype (*10*). During EMT, cells lose these epithelial characteristics, gaining instead an invasive and migratory mesenchymal phenotype, which permits these cells to leave the tissue parenchyma and enter the systemic circulations during cancer metastasis (*11*). Hallmarks of EMT include the loss of expression or function of E-cadherin and reduced abundance of tight junction proteins [such as zona occludens 1 (ZO-1) and occludin] and cytokeratins, as well as concomitant increase in abundance of mesenchymal markers, such as vimentin, fibronectin, fibroblast specific protein 1 (FSP-1), α-smooth muscle actin (α-SMA), and N-cadherin (*12*). Loss of adherens junctions between cells triggers a change in cytoskeletal composition and an arrangement that alters cell polarity to form spindleshaped cells (Fig. 1). These newly formed mesenchymal cells invade their basal extracellular matrix (ECM) and migrate into underlying tissues along a secreted matrix of fibronectin (*12*).

Whereas EMT is a well-characterized process during development, its involvement in disease pathology is more readily debated (*13*). Many studies implicate EMT in the generation of cancer stem cells within primary tumors that may be capable of metastasizing (*5*). Additionally, the role of EMT in stemness has become a topic of particular interest, given the studies demonstrating that the production of induced pluripotent stem cells requires an initial mesenchymal-epithelial transformation (MET) (*14*, *15*). These topics are beyond the scope of this review, but have been recently reviewed elsewhere (*13*, *16*).

It is tempting to think of EMT and the reverse process of MET as existing as binary states. However, developing evidence suggests that EMT may be better described as a spectrum of partial EMT states. In the embryo, multiple rounds of EMT and MET are necessary to complete gastrulation and primitive streak formation, highlighting the reversibility of this process (*16*). Initial dedifferentiation to a mesenchymal phenotype enables cells to migrate and then undergo MET to give rise to multiple different cell types in the notochord, somites, primordia of the urogenital system, and the splanchnopleura and somatopleura. Development of the cardiac valves also requires EMT and MET (*16*).

However, in some cases, a partial EMT or MET is necessary, as is the case with differentiation and tubule formation during kidney development (*2*, *17*). In *Drosophila*, collective migration of groups of neural crest cells occurs without the loss of cell-cell contacts, suggesting that these cells have characteristics of both epithelial and mesenchymal cells (*18*). The flexible nature of the epithelial/mesenchymal state renders this process very elusive to observe.

Expression and activation of EMT-inducing transcription factors occurs in response to various signaling pathways, including those mediated by transforming growth factor β (TGF-β), bone morphogenetic protein (BMP), epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), Wnt, Sonic Hedgehog (Shh), Notch, and integrin signaling (*19–24*). These pathways signal through intracellular kinase cascades to induce transcription factors that activate the expression of EMT-associated genes. Some of these signals may be predominant in driving EMT at particular stages during reprogramming. Many EMT-inducing signals tend to be cell- and tissue type–specific, hinting that cells may integrate particular signals differently or react to extracellular molecules with differing sensitivities, depending on their microenvironment and cell state (*17*). Other signals from the ECM and hypoxic conditions have demonstrated their ability to induce EMT in various systems, and may also vary with tissue type and location within the parenchyma (*25*). Here, we describe many of the common signaling mechanisms that promote EMT, and we discuss how these intracellular cascades participate in crosstalk to integrate cues from the microenvironment and drive epithelial cell reprogramming.

EMT-Inducing Transcription Factors

Several transcription factors induce EMT. These include the zinc-finger binding transcription factors Snail1 and Snail2 (also known as Slug) and several other basic helix-loop-helix (bHLH) factors such as zinc finger E-box–binding homeobox 1 (ZEB1), ZEB2, and Twist (*26*, *27*). A T cell factor (TCF) transcription factor family member called lymphoid enhancer binding factor-1 (LEF-1) can directly induce EMT (*28*). These proteins bind to the promoter region of genes associated with cell-cell adhesion and repress their transcription, which is the key initiating step of EMT.

The Snail family of transcriptional repressors plays a critical role in regulating EMT. Snail1 and Snail2 bind the promoter of *CDH1*, encoding E-cadherin, to repress its transcription (*29*, *30*). Accumulation of Snail1 in the nucleus is associated with decreased E-cadherin abundance and the induction of metastatic phenotypes in breast cancer (*31*). Circulating tumor cells isolated from patients with metastatic hepatocellular carcinoma (HCC) were found to have 20 times more Snail1 than cells isolated from patients with nonmetastatic HCC (*32*). Snail2 also has a well-established role in the induction of EMT during cancer metastasis and is involved in gastrulation and the development and migration of the neural crest (*17*, *33*). During gastrulation in *Drosophila*, Snail cooperates with co-repressors CtBP (C-terminal binding protein) and Ebi to form a complex with the histone deacetylase HDAC3 (*34–36*). Together, these proteins trigger switching from E-cadherin to N-cadherin, the form found in mesenchymal cells (*35*). Genetic deletion of Snai2, which encodes Snail2, does not seem to perturb normal EMT during gastrulation in mice (*37*). Interestingly, in the chick embryo, Snail2 enhances the development and migration of the neural crest but not the trunk crest, in which overexpression of Snail2 has no effect (*38*). Overexpression of either Snail1 or Snail2 induces EMT and correlates with increased tumor metastasis in vivo, consistent with the theme of developmental reprogramming becoming reactivated in metastatic carcinomas in disparate ways, depending on location within the organism (*29*, *30*).

Twist1 and Twist2 belong to a bHLH transcription family and play an essential role in cancer metastasis (*27*). During determination of the ventral furrow in *Drosophila*, the expression of Twi and *Sna* (encoding Twist and Snail) are increased, and their respective protein products have central roles in invagination of ventral mesoderm and delamination of mesodermal cells (*39*). In human mammary cells, Twist1 binds the *SNAI2* promoter and stimulates its expression to induce EMT, consistent with reports of a higher abundance of Twist1 in metastatic mammary tumors compared with their less metastatic counterparts (*27*, *40*). Studies in a mouse model of atypical ductal hyperplasia, an early stage of primary breast tumor development, show increased expression of Twist1 (*41*). Regulation of transcription is not mediated by Twist1 alone; however, BMI1, a polycomb-group repressor complex protein, acts in a concerted fashion with Twist to repress E-cadherin and the cell cycle inhibitor $p16^{INK4\alpha}$ (*42*). Additionally, Twist1 is responsible for the expression of several microRNAs (miRNAs), which mediate inhibition of *HOXD1* expression and its downstream target genes (*43*). One of these targets is RhoC (Ras homolog gene family, member C), which has a well-established role in cytoskeletal reorganization and cancer metastasis (*44*).

The ZEB family of transcriptional repressors have an essential role in neural crest development and have attracted substantial interest as regulators of cancer progression (*45*). ZEB1 and ZEB2 interact with the bipartite E-box regions of DNA that flank the *CDH1* gene to repress its promoter activity (Fig. 2) (*46*, *47*). In the nucleus, ZEB1 cooperates with the deacetylase sirtuin 1 to modify histone H3 and reduce binding of RNA polymerase II at the *CDH1* promoter (*48*). Ectopic expression of ZEB proteins in mammary epithelial cells is sufficient to induce the dissociation of adherens junctions, presumably through suppressing the expression of genes encoding plakophilin-2 and ZO-3, both critical for maintaining the epithelial phenotype (*47*, *49*). They also increase the expression of genes encoding matrix metalloproteinases (MMPs), implicating ZEB1 and ZEB2 in various matrix remodeling mechanisms associated with EMT (*50*). These remodeling events may trigger the transmission of additional extracellular signals, which are discussed in later sections. The miR-200 family, a group of five miRNAs that share similar targeting sequences, inhibit the abundance of ZEB proteins on the *CDH1* promoter (*51*, *52*). In turn, ZEB1 and ZEB2 bind to the E-box promoters of miR-200, creating a reciprocal feedback loop controlling EMT (*51*, *53*). Interestingly, expression of miR-200 family members enhances the recolonization of metastatic cells, highlighting the role of this regulatory loop in maintaining the mesenchymal phenotype and demonstrating the reversibility of EMT (*54*).

LEF-1 is another key transcription factor that can directly induce EMT through its repression of E-cadherin (*55*). Overexpression of LEF-1 in colon carcinoma cell lines promotes EMT through its activation by β-catenin (*56*). Furthermore, inhibiting LEF-1 activity, either with a dominant-negative form of the protein or with targeted small interfering RNA, inhibits EMT in many systems (*28*, *57*, *58*).

Despite a host of other factors known to be involved in epithelial transformation, the repression of E-cadherin by the various transcription factors outlined above is often considered to be the critical event during EMT. This predominating idea is supported by the observation that genetic deletion of E-cadherin in mice results in the formation of migratory

lobular carcinomas (*59*), as well as long-standing evidence that loss of E-cadherin can be used as an indicator of carcinoma progression and poor prognosis in multiple tumor types (*60*). Furthermore, breast ductal carcinomas with low abundance of claudin (a critical component of tight junctions) are shown to have decreased expression of E-cadherin in addition to increased expression of EMTinducing transcription factors, such as Snail1, Twist, and ZEB2, thus linking the expression of E-cadherin with the classical EMT factors in a clinically relevant fashion (*61*). Although these studies seem to provide compelling evidence for a definitive link between E-cadherin and EMT, the loss of E-cadherin alone is not necessarily indicative of a migratory phenotype. Differential expression of *CDH1* within particular tissues has been reported, with particular sets of carcinomas lacking expression of *CDH1* during the early stage of the disease, resulting in cells with a static EMT phenotype (*62–65*).

Because numerous transcription factors are involved in the activation of EMT programs, it is perhaps not surprising that an array of phenotypes have been observed during epithelial cell reprogramming. Regulation of EMT-inducing transcription factors involves multiple layers of control that have been recently reviewed (*66*, *67*). Evolving evidence suggests that many of the EMT-inducing transcription factors act synergistically with one another and use common pathways, yet some studies show that inhibition of a single transcription factor is sufficient to block EMT (*68*).

TGF-β and BMP Signaling in EMT

TGF-β signaling is the most well-characterized pathway that is known to induce EMT, and acts through various intracellular messengers. Signaling is typically activated by the TGF-β superfamily of ligands, which include—among others—three isoforms of TGF-β (TGF-β1, 2, and 3) and six isoforms of BMP (BMP2 through BMP7). Most systems in which EMT is observed, including cancer and fibrosis, are regulated by TGF-β1 (*69*), whereas TGF-β2 primarily controls EndMT in heart development (*70*), and TGF-β3 mediates EMT in the developing palate (*28*). BMP2 and BMP4 induce EMT in cancer (*71–73*) and EndMT in the developing heart (*74*) as well as during heterotopic ossification (*8*). During development, an increasing gradient of BMP4 directs the patterning of the mesoderm along the mediolateral axis (*75*). BMP4 is highly increased in invasive epithelium compared with normal colonic mucosa, further highlighting the widespread reliance on this protein in several different tissues and its involvement in reactivating development processes (*76*, *77*). Conversely, BMP7 has been shown to counteract EMT in breast cancer (*78*) and fibrosis (*4*), and is the most common factor promoting an epithelial cell phenotype. In addition, BMP5 attenuates TGF-β–induced EMT, underscoring the specificity of particular isoforms of BMP in development and disease (*79*).

TGF-β signaling occurs through the formation of a heterotetrameric receptor complex composed of type I and type II TGF-β receptors (TGF-βRI and TGF-βRII). There are currently seven type I receptors and five type II receptors identified in mammals (*80–82*). Upon ligand binding, TGF-βRII trans-phosphorylates TGF-βRI, facilitating its kinase activity. Combinations of different receptors enable differential signaling to occur when activated by the same ligand (*82*). Varying combinations of type I and type II receptors

enable differential ligand binding to occur, thus enabling them to respond to various isoforms of the TGF-β superfamily (*82*, *83*). The TGF-β type III receptors β-glycan and endoglin, as well as accessory proteins (such as Cripto), can modulate ligand binding affinity at the membrane in epithelial and endothelial cells (*84*, *85*). BMP signaling occurs in a similar manner to TGF-β signaling, except that a specific type II BMP receptor is involved instead of TGF-βRII (*86*, *87*). Different BMP ligands can induce activation of various type I BMP receptors termed activin-like kinases (ALKs), which initiate the signaling cascade at the cell surface (*88*).

SMAD-dependent signaling

Phosphorylation of TGF-βRI by the Ser/Thr kinase activity of TGF-βRII creates a docking site in the Gly/Ser (GS)–rich domain of TGF-βRI, which recruits the transcription factors SMAD2 and SMAD3 [mothers against decapentaplegic homologs 2 and 3 (SMAD2/3)] (Fig. 3). SMADs are consequently phosphorylated at Ser residues in their C-terminal domain, facilitating formation of a complex with coactivator SMAD4. R-SMADs and SMAD4 contain conserved MH1 and C-terminal MH2 domains, which influence their ability to form complexes and bind to the minor groove of DNA (*80*, *81*, *89*). Phosphorylation of MH2 domains by TGF-βR1 is required for oligomerization of SMAD2/3 with SMAD4, and also enables the nuclear import of the R-SMAD/SMAD4 complex by exposing the nuclear localization signal. This signal permits binding of importins b1, 7, and 8 to the complex and their subsequent translocation into the nucleus (*90*–*92*). Additionally, Lys-rich localization sequences in the MAD homology 1 (MH1) domain of R-SMADs enable nuclear import of SMAD1 and SMAD3 (*93*). In addition to activating SMADs, TGF-β signaling is also capable of activating inhibitory SMAD proteins (SMAD6 and SMAD7), which bind to TGF-βRI and prevent the recruitment of effector SMADs (*80*, *81*, *89*). This system introduces a layer of control designed to prevent aberrant activation of this pathway. In BMP signaling, the basic pathway remains the same; however, BMP receptors make use of phosphorylated SMAD1/5/8 in place of SMAD2/3 complexes (*94*, *95*).

Inhibition of signaling may also be mediated by posttranslational degradation of SMADs. The HECT ubiquitin protesome E3 ligase family members Smurf1 and Smurf2 (SMAD ubiquitin regulatory factors 1 and 2) are capable of interacting with SMADs, targeting them for proteasome degradation and suppressing TGF-β signaling (*96*). Another E3 ligase, PIAS1 [protein inhibitor of activated STAT (signal transducer and activator of transcription), 1], is also inhibited by TGF-β signaling. Without the activity of PIAS1, the sumoylation of Ski-related novel protein N (SnoN) is decreased, eventually leading to the degradation of SnoN and enabling SMAD complexes to access and regulate target genes (*97*).

Once inside the nucleus, SMAD complexes bind regulatory elements and induce the transcription of key genes associated with EMT. Complexes of R-SMADs are capable of binding directly to the promoter of *SNAI1* to induce its transcription, and can form complexes with Snail1 to suppress the expression of genes encoding E-cadherin and occludin (*98*, *99*). Other factors directly influenced by the binding of R-SMADs include the ZEB transcription factors and the high mobility group factor HGMA2, which also regulates

the expression of *SNAI1*, *SNAI2*, and *TWIST* (Fig. 2) (*99*, *100*). R-SMAD/SMAD4 complexes can induce the transcription of the gene encoding the E3 ubiquitin ligase HDM2, which promotes the degradation of p53 (Fig. 2). Studies show that the loss of p53 function induces Snail1-mediated EMT by inhibiting the action of regulatory miRNAs, such as miR-34 and members of the miR-200 family that suppress the action of EMT-inducing transcription factors (*101–103*). TGF-β and the tumor suppressor p12 may also influence the expression of the gene encoding Twist2, which promotes EMT through suppression of E-cadherin (*104*).

TGF-β signaling can directly increase *LEF1* expression through SMADs and inhibit glycogen synthase kinase 3β (GSK-3β) through the phosphoinositide 3-kinase (PI3K)–Akt pathway, which enables β-catenin–dependent activation of LEF-1 to induce EMT (*58*, *105*). Additionally, induction of LEF-1 may occur in a β-catenin–independent manner through TGF-β3 by forming SMAD2/SMAD4/LEF-1 transcription complexes that suppress E-cadherin abundance during EMT in palate medial edge epithelial cells (*28*). Snail1 and Snail2 can indirectly increase the abundance of TGF-β3, which in turn stimulates *LEF1* expression (*57*).

SMAD-independent signaling

In addition to acting through SMAD proteins, signaling through TGF-β receptor complexes involves a number of SMAD-independent pathways most commonly observed in receptor tyrosine kinase (RTK) signaling. Multiple roles exist for PI3K-Akt signaling in controlling EMT. Mechanistically, it is likely that TGF-β activates PI3K machinery directly through its own receptors (*106*) or through trans-activation of EGF and PDGF receptors (*107*, *108*). Activation of PI3K and Akt signaling by TGF-β has been identified in multiple cell types (*106*, *109–111*). Activated PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP_2) to make phosphatidylinositol 3,4,5- trisphosphate (PIP_3), a phospholipid membrane protein that binds Akt (Fig. 4). Upon binding, Akt is phosphorylated by phosphoinositide-dependent kinase 1 (PDK1) (*112*). Mutations in p110α, the catalytic subunit of PI3K, are often present in tumors and cause increased PIP3 production and aberrant activation of Akt. Phosphorylation of Akt may also be induced through stimulation of integrins, which activate integrin-linked kinase (ILK) (*113*). Dephosphorylation of PIP3 is mediated by phosphatase and tensin homolog (PTEN), which is often absent in metastatic cancers (*114*).

Mammalian cells have three Akt isoforms, each with distinct and often opposing functions. Silencing Akt2 using RNA interference is correlated with loss of EMT phenotype and increased expression of E-cadherin (*115*). When activated, Akt2 phosphorylates heterogeneous nuclear ribonucleoprotein E1 (hnRNPE1), which, when not phosphorylated, binds to the 3′ UTR (untranslated region) of mRNAs and inhibits their translation. Phosphorylation of hnRNPE1 by Akt2 causes its dissociation from DAB2 and ILE1 mRNA (encoding disabled homolog 2 and interleukin-like EMT inducer, respectively), enabling translation of their respective protein products, which are then capable of promoting expression of EMT-inducing transcription factors (*116*, *117*). Akt also inhibits GSK-3β, which phosphorylates Snail1, marking it for degradation by β-transducin repeat– containing protein (β-TRCP) (*118*). In addition, Akt induces the transcription of *SNAI1* through the

activation of nuclear factor kB (NF-κB), inducing EMT in squamous cell carcinoma cells (*119*). Still other examples of PI3K signaling are found in the Src family kinases, which activate β-catenin and EMT in pancreatic carcinoma cells (*120*). These kinases also signal through STAT3 to affect transcriptional changes in epithelial cells (*121*). Src kinases are associated with integrin signaling, consistent with a role for ILK in activating Akt (*122*). Additionally, activation of Akt by TGF-β activates both mammalian target of rapamycin complex 1 (mTORC1) and mTORC2, both of which contribute to EMT in different ways: Motility and invasion, protein synthesis, and particularly cell size are controlled by mTORC1, whereas mTORC2 is essential for the phenotype transformation itself (*123*, *124*).

TGF-β1 is a potent activator of various other kinase pathways including extracellular signal–regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK). These pathways have been elucidated both independently of SMAD signaling and through regulation of SMAD complexes. MAPK kinase (MEK)–ERK signaling is implicated in the activation of EMT and in crosstalk with canonical SMAD signaling pathways. Early evidence from studies using dominant-negative SMADs suggests that MAPK pathways might be involved in TGF-β signaling independently of transcription (*125*), and that the activation of SMADs by TGF-βRI was not necessary for p38 MAPK activation (*126*). The oncogenic guanosine triphosphatase (GTPase) Ras activates ERK signaling (*127*), consistent with the observation that induction of EMT transcription factors, such as Twist1, requires concomitant Ras signaling to observe changes in phenotype (*128*). Activation of TGF-β receptors leads to Tyr phosphorylation of the adaptor protein Shc (Src homology 2 domain–containing transforming protein), permitting the GRB2 (growth factor receptor–bound 2)–SOS1 (son of sevenless homolog 1) complex to dock and activate Ras and the downstream ERK pathway (*129*). Abrogating the activation of ERK1 and ERK2 (ERK1/2) by inhibiting MEK1/2 inhibits the delocalization of E-cadherin and ZO-1, highlighting a necessary role for ERK signaling in EMT (*68*).

The JNK and p38 MAPK cascades are initiated by the E3 ligase member TRAF6. Association of TRAF6 with TGF-βRI causes activation of TGF-β–activated kinase 1 (TAK1), which is involved in EMT of mammary epithelial cells in a SMAD-independent manner (*130*). Depletion of TRAF6 in the mammary epithelium leaves TGF-β unable to activate p38 MAPK or JNK and induce EMT, but does not affect SMAD signaling (*130*, *131*). Furthermore, TGF-βRIs lacking the docking sites for SMADs are still capable of activating JNK signaling, underscoring the existence of multiple signaling pathways induced by TGF-β receptors (*132*). Activation of TAK1 is postulated to promote NF-κB signaling, which may lead to EMT (*133*). In addition to its role in mediating p38/JNK pathways, TRAF6 is also involved in stimulation of EMT by disrupting junction assembly at the plasma membrane (*130*). Close to the cell surface, the metalloproteinase TACE and protein kinase C (PKC) facilitate TRAF6-mediated cleavage of the intracellular portion of TGF-βRI, which may co-operate with nuclear SMAD complexes to induce transcription of *SNAI1* (*130*, *134*).

In addition to signaling through SMADs and other kinases that transmit signals to the nucleus, TGF-β induces changes proximally at the plasma membrane. Stimulation of TGF-β receptors leads to phosphorylation of Par6 by TGF-βRII and subsequent recruitment of

Smurf1. In addition to marking SMAD proteins for degradation, Smurf1 marks RhoA GTPase for degradation, and the loss of RhoA promotes EMT through changes in the organization of actin filaments and the loss of tight junctions and adherens junctions (*135*). It is suspected that a lack of RhoA activity induces *SNAI1* expression (*136*).

Interestingly, other studies have demonstrated that RhoA and Cdc42 (another Rho GTPase) are involved in the assembly of stress fibers and activation of p38 MAPK during the formation of lamellipodia, contrasting the role of RhoA in the regulation of epithelial polarity and motility as discussed above (*137*).

RTK Signaling in EMT

Various growth factors activate RTKs and through downstream signaling can induce EMT. The TGF-β receptors have kinase activity, and thus, many of the non-SMAD signaling cascades induced by TGF-β [such as Ras, PI3K, focal adhesion kinase (FAK), Src, and TAK] are also induced by RTK activation in response to growth factors, such as EGF, FGF, insulin growth factor (IGF), and PDGF (Fig. 4) (*23*, *107*, *108*, *138*). Growth factor binding to RTKs induces receptor dimerization and trans-phosphorylation of the intracellular domains, thus transducing the signal to intracellular cascades (*139*). In addition to participating in the signaling pathways discussed above, many of these ligands also increase cell proliferation and contribute to the formation of partial EMT (*140*).

FGF is involved in EMT in various ways. FGF-4 and FGF-8 play a role in controlling mesenchyme formation to mediate the patterning of the mesodermal layer during gastrulation (*141*). FGF-2 can induce EMT in tubularepithelial cells by increasing the expression of genes encoding vimentin and FSP-1 and inducing the activity of MMP-2 to increase cell motility (*142*). These results are consistent with observations that the abundance of FGF-2 is increased in fibrotic renal tissue (*143*). FGF-2 is also capable of inducing changes in the actin cytoskeleton through crosstalk between Rho GTPases and PI3K, which promotes the elongated mesenchymal phenotype associated with cell motility (*144*). Cell motility is also stimulated by EGF in an a_2 integrin–dependent manner in mammary epithelial cells (*145*). The additional observation that EGF dephosphorylates and inactivates FAK suggests that EGF-mediated inhibition of FAK facilitates cell detachment from the ECM to enable cell motility (*146*). How cell migration then proceeds through ECM-induced reactivation of FAK is discussed in a later section.

Similarly, overexpression of insulin-like growth factor 1 (IGF-1) in mammary epithelial cells leads to EMT, increased migration, and decreased abundance of E-cadherin through the activation of β-catenin (*138*). IGF-1 activates NF-κB to increase *SNAI1* expression in the mammary epithelium and increases ZEB1 expression through the activation of ERK (*138*, *147*). Interestingly, hepatocyte growth factor (HGF) is also involved in EMT through the induction of Snail1 or Snail2 in a cell type–dependent manner (*148*, *149*). Destabilization of desmosomes is also carried out by HGF, demonstrating the ability of RTKs to induce changes in multiple ways in response to ligand binding (*148*).

PDGF controls the expression of *CDH2* (encoding N-cadherin) in mesoderm by activating the PI3K pathway, providing directional information for primitive streak formation (*150*).

Inhibition of N-cadherin occurs primarily through the loss of phosphorylated Akt. Additionally, PDGF induces the phosphorylation of the nuclear RNA helicase p68, which promotes EMT through the nuclear translocation of β-catenin in a Wnt-independent manner in various epithelial cancer cell lines (*114*). Decreased MMP-2 activity and subsequent inhibition of mesenchymal cell migration is observed after ablation of the PDGF receptor, resulting in craniofacial and heart valve malformations (*151*).

In addition to promoting a migratory phenotype through FAK inhibition, as described above, EGF is capable of inducing EMT through repression of E-cadherin in a number of ways. Upon binding of EGF to its receptor, E-cadherin is internalized from the cell membrane, reducing cell-cell contacts and weakening the epithelial layer (*152*). In addition, EGF also induces the expression of *SNAI1* and TWIST, leading to repression of the *CDH1* promoter (*153*). During development, expression of the gene encoding the glycoprotein Cripto1 is associated with formation of the primitive streak and specification toward mesodermal and endodermal lineages (*154*). Consistent with this observation are data demonstrating an increase in the mesenchymal markers N-cadherin and vimentin when Cripto1 is expressed in mammary epithelium (*155*). Cripto1 is thought to act less like a ligand, relying instead on its ability to interact with Nodal and contribute to Wnt signaling (*156*, *157*). This is covered in more detail in subsequent sections of this review.

RTK signaling is not only involved in the induction of classical EMT transcription factors, but it also regulates the deposition of ECM, which provides important ligands for EMT and promotes an invasive phenotype. Pharmacological inhibition of JNK or PI3K impairs the production of type I collagen by mammary epithelial cells (*158*), and activation of p38 MAPK induces the production of b_1 or b_3 integrin subunits, which bind the ECM (*122*, *159*–*161*). ECM proteins induce various signaling mechanisms, and certain ECM proteins provide a suitable microenvironment for EMT, as outlined in subsequent sections of this review. The close relationship between the production of ECM and the induction of signaling pathways related to ECM binding further supports the viewpoint that EMT is a dynamic process that is continuously regulated to maintain the cell state. EGF signaling also mediates the production and secretion of proteolytic enzymes, such as MMP-2 and MMP-9, and activates ERK and ILK signaling. Withdrawal of EGF reverses cellular morphology back to the epithelial state and decreases the activity of the ILK and MEK-ERK pathways; however, inhibition of MEK and ILK abrogates cell migration and MMP production, but does not cause any change in cell morphology. This study demonstrates the ability of EGF, which may be produced by various sources within the tissue, to enact changes in epithelial cell reprogramming and basement membrane remodeling through multiple different mechanisms (*162*).

Even in a brief overview of the signaling pathways involving growth factors and RTKs, it is quickly apparent how many different extracellular signals can be involved in the regulation of EMT. Many of these growth factors act through similar or redundant pathways, yet the effects of activation by a particular ligand appear to be highly tissue- and cell type–dependent in some instances. This observation hints at the existence of an additional layer of control that may be present to integrate many cues from the extracellular space. For example, differential autocrine and paracrine signals in the surrounding tissue and the

biophysical functionality of the basement membrane may be integrated together to induce EMT in a particular way. A major source of TGF-β within tumors is the stromal fibroblasts, which provide extracellular signals that induce EMT (*163*). Once they have left the tissue and entered the bloodstream, many circulating cancer cells associate with platelet cells, which also provide a rich source of TGF-β (*164*). Whereas both environments provide a source of the necessary growth factors for maintenance of the mesenchymal phenotype, they surely also provide other soluble and nonsoluble cues that may become integrated with TGF-β and other factors to cause differential activation of cellular programs.

Wnt Signaling in EMT

Wnt signals are transduced across the plasma membrane by Frizzled and low-density lipoprotein receptor–related protein (LRP) receptors. In the absence of signaling, β-catenin is phosphorylated by a complex of GSK-3β, Axin, and the tumor suppressor adenomatous polyposis coli (APC), which sequesters β-catenin in the cytoplasm and marks it for proteasomal degradation (*165*). Activation of Frizzled by Wnt ligands results in phosphorylation of LRP6 by GSK-3β and the recruitment of Dishevelled (Dvl) and Axin to the plasma membrane. Unable to form a complex with Axin, GSK-3β cannot then phosphorylate β-catenin, enabling it to translocate to the nucleus. Restriction of GSK-3β to the cytoplasm after Wnt–β-catenin activation also prevents the destabilizing phosphorylation of Snail1 (*31*).

Nuclear β-catenin binds to members of the TCF/LEF family of transcription factors to promote EMT. During gastrulation, β-catenin forms a complex with LEF-1 to bind and inhibit the transcription of *CDH1* (Fig. 5) and induce EMT (*166*). Wnt signaling is critical for the formation of the primary axis during vertebrate embryogenesis: mouse embryos deficient in Wnt3 do not form and develop anterior-posterior neural patterning (*167*). Premature formation of the endoderm occurs as a result of stabilization of β-catenin during early development, emphasizing the importance of this pathway in development (*168*). In numerous cancers, Wnt signaling is inappropriately active (*24*, *169*) and directly induces *SNAI1* and *SNAI2* expression (*118*, *170*, *171*). The Wnt–GSK-3β–β-TRCP1 (β-transducin repeat–containing protein 1) axis induces the activity of Snail2, which promotes EMT and, by binding its promoter and recruiting a histone demethylase, inhibits the expression of *BRCA1* (encoding breast cancer 1, early onset). The loss of *BRCA1* is associated with aggressive basal-like breast cancer (*172*). Wnt-mediated induction of EMT through Snail2 is consistent with other reports of decreased E-cadherin and increased fibronectin after the accumulation of β-catenin in the nucleus (*173*, *174*). Wnt has also been linked to increased expression of TWIST in mammary epithelial cells (*175*). Mutations in APC and other components of the β-catenin signaling pathway have been identified in metastatic colorectal cancer, highlighting the importance of the Wnt pathway in EMT during cancer progression (*176*, *177*).

Several layers of crosstalk between the TGF-β and Wnt signaling pathways have been identified; for example, it is apparent that LEF-1 may be activated by binding to β-catenin or SMAD proteins (*178*, *179*). Additionally, activation of TGF-β in pancreatic carcinoma cells induces the expression of the genes encoding homeobox transcription factor CUTL1 and

downstream target Wnt-5A, a canonical activating Wnt ligand that induces EMT and mediates the invasive capacity of pancreatic tumor cells (*180*).

Notch Signaling in EMT

The Notch receptor is composed of an extracellular domain and an intracellular domain (NICD) that contains motifs for nuclear localization. Upon interaction with a neighboring Notch receptor, g-secretase and TACE cleave NICD, facilitating its translocation to the nucleus (*181*, *182*). The NICD binds to DNα-bound CSL [CBF1, Su(H), LAG1] transcription repressor complexes to activate the expression of genes encoding proteins involved in tumor development, such as NF-κB, Akt, and p21 (*182–184*). Notch signaling can regulate expression of *SNAI1* both directly (*185*, *186*) and indirectly through the induction of hypoxiα-inducible factor 1α (HIF-1α). Binding of HIF-1α to the promoter of *LOX* (encoding lysyl oxidase) leads to its transcription and subsequent LOX-mediated stabilization of Snail1 (*187*). Snail2 interacts with Notch and is essential for Notch-mediated repression of E-cadherin and β-catenin activation (*126*, *127*). Overexpression of Notch in endothelial cells results in loss of vascular endothelial (VE)– cadherin and subsequent EndMT (*185*). Additionally, inhibition of Notch1 in lung adenocarcinoma cells decreases their invasive phenotype and partially reverses EMT (*188*).

In addition to the direct effects mediated by its intracellular domain, Notch also indirectly regulates EMT through various signaling pathways, including NF-κB and β-catenin, and through the action of various regulatory miRNAs (*189*). Inhibition of Notch in pancreatic cancer cell lines decreases the DNA binding potential of NF-κB and lowers the expression of MMP9, which encodes a critical MMP involved in remodeling the ECM to facilitate extravasation of pancreatic cancer cells (*190*). Binding of the Notch ligand Jagged2 (JAG2) promotes EMT through the induction of GATα-binding protein 3, which inhibits the miR-200 family (*189*). Recent reports have highlighted a role for miR-200 in targeting JAG1 to regulate Notch activation in a feedback loop (*173*).

Hedgehog Signaling in EMT

Members of the Hedgehog (Hh) family include Shh, Desert Hedgehog (Dhh), and Indian Hedgehog (Ihh) (*191*, *192*). Hh ligands bind to patched homolog 1 (PTCH1) and PTCH2, which function to inhibit the activity of Smoothened (Smo) in the absence of ligand binding (*193*, *194*). Activation of PTCH1/2 releases Smo and initiates an intracellular cascade (*195*) that activates Gli family transcription factors, which promote transcription of target genes such as PTCH, WNT, and *SNAI1* (*24*, *196*). Expression of GLI1 in kidney epithelium results in the loss of E-cadherin and an increase in Shh signaling at the invasive front of neuroendocrine tumors (*196*, *197*). Additionally, Hh signaling induces TGF-β1 secretion to increase motility (*198*) and induces JAG2 expression, resulting in cleavage of the Notch intracellular domain (NICD) (*187*). Other known targets include secreted Frizzled-related protein 1 (SFRP1) (*199*), which modulates Wnt signaling in a concentration-dependent context, as well as FOX1 and FOX2, two mesenchymal forkhead transcription factors that control the intracellular accumulation of β-catenin and have been shown to limit Wnt signaling in the gut (*200*).

Oncogenic forms of Shh have been identified in basal cell carcinoma and are misregulated in prostate adenocarcinoma, esophageal and stomach cancer, and pancreatic adenocarcinoma (*201*). Disruption of Shh with cyclopamine, a steroidal alkaloid that binds and deactivates Smo (*202*), inhibits EMT in pancreatic cancer cell lines (*203*). Other studies have observed Hh-mediated inhibition of Wnt signaling through paracrine mechanisms involving local mesenchymal stromal cells (*204*). It is possible that the paracrine signaling from stromal cells in the microenvironment maintains an epithelial cell phenotype in the tumors. Those cells existing at the invasive front may be relatively free from such paracrine signaling, enabling EMT to occur. This once again underscores the dynamic nature of EMT regulation, relying on progressive gradients of different extracellular signals rather than an initial stimulus sufficient to induce and retain the mesenchymal phenotype indefinitely.

Matrix Signaling in EMT

Signaling mediated by the ECM is a critical regulator of epithelial cell fate, working both independently and in conjunction with the pathways described above. Not only does binding of epithelial cells to particular matrix elements initiate intracellular signaling cascades, but it also results in the remodeling of the matrix to activate additional signaling pathways that contribute to a migratory phenotype. EMT was initially discovered when isolated embryonic epithelium was incubated in three-dimensional gels of type I collagen in culture. Over time, the cells acquired a mesenchymal morphology and migrated along the collagen fibrils away from the implanted epithelial tissue (*205*). The abundance of type I and type III collagen is increased during EMT, and directly seeding epithelial cells on these matrices can induce EMT through various signaling pathways (*12*), summarized below.

During EMT, mesenchymal cells cut through the basal lamina of type IV collagen and laminin, laying down their own matrix of type I collagen and fibronectin. The formation of lamellipodia and extruding filopodia at the cell surface provides directional motility to the cell, and the release of MMPs degrades the matrix to facilitate cell invasion (*206*, *207*). The dissolution of epithelial junctions causes the formation of actin stress fibers and clustering of integrins at the migratory front of the cell (*208*) where MMPs are released. Unsurprisingly, EMT signaling pathways have been shown to induce expression of genes encoding MMP-2 and MMP-9, which cleave type IV collagen in the basal lamina to promote post-EMT invasion of underlying tissues (*7*, *12*). MMP-3 has also been shown to directly induce EMT through activation of Rac1 GTPase–ROS (reactive oxygen species) signaling, which promotes expression of *SNAI1* (*209*). Additionally, MMPs are capable of degrading E-cadherin in the cell membrane, disrupting adherens junctions, and weakening the epithelial sheet (*210*). This process enables cells to acquire a mesenchymal phenotype and migrate into the matrix.

At the cell membrane, integrins bind to matrix proteins and activate intracellular cascades that mediate EMT (Fig. 6) (*12*, *211*, *212*). The diversity in binding affinities of different integrin proteins enables cells to respond to a vast array of extracellular elements and mediate different signaling cascades in response to a changing matrix environment. As the composition of the ECM changes, so does the abundance of integrins at the cell surface, promoting the progression of EMT under the control of the pericellular environment. a_Vb_3

integrins facilitate Src-mediated phosphorylation of TGF-βRII, creating a docking site for ShcA and GRB2, which signal through the p38 MAPK pathway to induce EMT (*122*, *159*). Interactions between b_3 integrin subunit and TGF-βRII complexes may also be bridged by FAK as an essential mediator of EMT and metastasis in breast cancer (*213*, *214*). b_1 integrin subunits also function as critical mediators of the p38 MAPK pathway in addition to their role in JNK signaling and regulation of DAB2 expression (*161*, *215*). Other studies highlighting the crosstalk of integrins with TGF-β signaling show that phosphorylated β-catenin and SMAD2 form complexes through the cooperation of TGF-β and a_3b_1 integrin signaling, which promote EMT in vitro and in vivo in a model of lung fibrosis (*216*). The latent form of TGF-β contains an integrin-binding motif. The binding of latent TGF-β to integrins a_Vb_6 and a_Vb_8 induces the proteolytic release of the latency-associated peptide (LAP), hence activating TGF-β at the cell membrane; a_Vb_8 mediates this activation through the protease activity of membrane type 1 MMP (also known as MMP-14) (*217*, *218*). Release of LAP from TGF-β in the matrix further amplifies the EMT mechanism through the various signaling cascades activated by soluble TGF-β. This in turn promotes the synthesis of type I collagen and fibronectin, which contribute further to EMT (*140*). In this way, matrix remodeling not only changes the types of matrix proteins that interact with the cell membrane but also influences the soluble cytokine environment that affects EMT.

As they invade the basement membrane, mesenchymal cells synthesize a fibronectin matrix, which provides a track for migration of mesenchymal cells during EMT and maintains the mesenchymal phenotype (*219*). Its deposition in the ECM increases substrate rigidity, which is involved in progression of mammary tumors (*220*–*222*) and is controlled by SMADs and JNK signals activated by TGF-β (*132*, *223*). An increased amount of fibronectin in conjunction with activated Ras in mammary epithelial cells causes the replacement of a_6b_3 integrins with a_5b_1 integrins, which improves the migratory capacity of cells by increasing cell adhesion to fibronectin (*224*). Increased amounts of type I collagen and fibronectin in the matrix are also correlated with integrins switching to a high-affinity ligand-binding state, increasing signaling activity through FAK and ILK and promoting EMT (*225*, *226*).

The importance of type I collagen in EMT is evident in various cellular systems. Lack of type I collagen in mouse embryos results in abnormal craniofacial development and mandibular growth, highlighting the importance of proper matrix remodeling for the migration of mesenchymal cells during development (*227*). In the adult, type I collagen is associated with EMT in lung, breast, and pancreatic carcinomas, highlighting the importance of the matrix environment in metastasis as well (*158*, *228*, *229*). Induction of EMT is dependent on the interaction between type I collagen fibers and a_2b_1 integrin, which triggers the intracellular cascade (*230*). Type I collagen causes ILK-dependent phosphorylation of IkB (inhibitor of kB) to increase the abundance of nuclear-localized NF-κB, which promotes the expression of *SNAI1* and *LEF1* to induce EMT (*231*). Increased abundance of type I collagen also activates JNK pathways, consistent with studies demonstrating that pharmacological inhibition of JNK signaling abrogates type I collagen–mediated migration and metastasis of breast cancer cells (*232*, *233*). More recent studies highlight a ligand-independent role for collagen in promoting both canonical and noncanonical TGF-β signaling (*234*). The interaction of b_1 integrin subunits with type I collagen found in the

pericellular matrix is correlated with direct suppression of E-cadherin and indirect induction of N-cadherin (*233*, *235*).

Lastly, type I collagen is also shown to induce EMT through binding and activating the discoidin domain receptors DDR1 and DDR2. In cooperation with integrins, DDR1 promotes EMT through the JNK1–c-Jun pathway (*233*). The expression of both DDR2 and COL1A1 (which encodes type I collagen) is increased in response to TGF-β signaling, and DDR2 mediates type I collagen–induced EMT through activation of the NF-κB and LEF-1 transcription factors (*236*).

Hypoxia Signaling in EMT

Various systems in which EMT occurs, such as fibrosis and cancer, experience hypoxia as a result of ischemic conditions. Therefore, it should come as no surprise that low-oxygen tension is capable of enacting changes in cellular phenotype and cooperating with other pathways to induce EMT. Lack of oxygen inhibits prolyl hydroxylases, such as PHD2 and PHD3, which act to catalyze and degrade HIF-1α under normoxic conditions (Fig. 7). HIF-1α is a transcription factor that induces expression of a number of EMT-associated genes, such as TGF-β, TWIST, and *LOX* (*237*).

HIF-1α is capable of binding directly to the hypoxiα-responsive element of the TWIST1 promoter (*238*). These data are supported by studies in mouse models demonstrating similar phenotypes between HIF-1α mutants and Twist knockout mice (*239*, *240*). During hypoxiα-induced EMT, HDAC3 abundance is increased by HIF-1α. HDAC3 binds to the promoters of *CDH1* and JUP (encoding plakoglobin, also known as g-catenin) and cooperates with Snail1 to inhibit their transcription. Additionally, HDAC3 mediates the formation of histone methyltransferase complexes that are necessary to induce the expression of mesenchymal markers, such as vimentin and N-cadherin (*241*). In hepatocellular carcinoma cells, hypoxia facilitates EMT and enhances metastatic potential through the activation of β-catenin (*242*). Interestingly, hypoxia is able to reverse the inhibitory function of SMAD7 and convert it into a promoter of cell invasion, demonstrating the modular nature of these effector proteins and their flexibility to react to environmental cues (*243*).

Crosstalk of EMT Signaling Pathways

Given the vast number of processes in development that are controlled by EMT, it is not surprising that many overlapping pathways may be involved. Similarly, a wide array of extracellular signals (both soluble and matrix-bound) must be able to affect this process to carry out multiple rounds of EMT and MET in a spatially defined manner. Given the emerging evidence indicating the existence of partial EMT states, it is worthwhile revisiting the myriad of ways in which these different pathways interact with one another and postulating whether these interactions may be dependent on the existence of a particular cellular microenvironment or cell state.

Crosstalk between hypoxia and Notch signaling is a well-established phenomenon that contributes to EMT signaling. The expression of HIF-1α increases the abundance of LOX, an enzyme required for FAK activity and intercellular and cell-matrix adhesion (*244*). *LOX*

stabilizes the activity of Snail1 by deaminating trimethylated histone H3 Lys^4 in the *CDH1* promoter (*187*, *245*). Aberrant activation of Notch signaling by hypoxia can induce EMT during tumor progression. Not only does HIF-1α stimulate Notch signaling, but it also stabilizes nuclear NICD, which is involved in the induction of many EMT-associated genes (*246*, *247*). Additionally, hypoxia represses miR-34α, which inhibits the Notch signaling pathway (*248*).

TGF-β signaling is capable of interacting with various pathways through its ability to activate many different effector proteins. Notch and TGF-β signaling function together in endocardial cushion formation (*185*). Other development processes mediated by joint signals from TGF-β and other signaling pathways include gastrulation and neural crest delamination, which involve a triad of TGF-β, Wnt, and FGF signaling (*249*, *250*). As cellular junctions begin to disintegrate under the control of TGF-β, β-catenin accumulates in the nucleus, where it enhances signaling through complex formation with LEF-1 (*251*). LEF-1 is also capable of forming a complex with SMAD proteins to repress transcription of *CDH1* (*252*). TGF-β is capable of activating PI3K directly through its own receptors (*106*) or through trans-activation of EGF and PDGF receptors (*107*, *108*). Additionally, the ERK pathway is involved in crosstalk with TGF-β and other growth factors to induce EMT (*253*).

SMAD proteins often connect TGF-β and other signaling cascades. Some of the existing evidence regarding interactions with SMADs (and crosstalk with TGF-β signaling in general) seems to be conflicting, such as the observation that HGF inhibits TGF-β–induced EMT and prevents myofibroblast differentiation in renal fibrosis through the involvement of SMADs (*254*). This is surprising, given that TGF-β interacts positively with other growth factors, such as PDGF (*255*). The role of SMAD proteins is, in some cases, not clearly defined and can change in response to changes in cell environment (*243*).

RTK pathways are capable of signaling independently of SMADs. However, consistent lines of evidence highlight the crosstalk between SMAD and non-SMAD kinase pathways for EMT induction. Although PI3K signaling and SMAD signaling can act independently of each another, chemical inhibition of PI3K activity has been shown to abrogate EMT and reduce phosphorylation of SMAD2 (*106*). Both ATF-2 (activating transcription factor-2, a substrate of p38 MAPK pathways) and c-Jun (a substrate of JNK) are capable of interacting with SMADs (*80*, *81*, *89*). This convergence of pathways is consistent with observations that p38 MAPK and ERK1/2 are required for canonical TGF-β–induced EMT, despite the mechanistic ability of SMAD complexes to promote the induction of EMT-associated transcription factors (*126*, *253*, *256*).

One of the clearest examples of signal crosstalk occurs in positive feedback loops created through autocrine signaling and matrix remodeling. Snail1 cooperates with the transcription factor ETS1 downstream of MAPK to activate MMP expression (*257*). Additionally, binding of MMP3 induces the expression of RAC1 and increases the production of ROS and Snail1 (*258*, *259*), which activate EMT and lead to production of other MMPs, such as MMP-2 and MMP-9. These MMPs degrade the basement membrane, releasing latent TGF-β and enabling preferential binding to type I collagen and fibronectin (*17*). These ECM proteins activate intracellular EMT signaling cascades of their own, as previously discussed

(*225*, *226*). The synthesis of fibronectin is also associated with Wnt activation and accumulation of nuclear β-catenin, providing further evidence of multiple signaling pathways converging to produce ECM capable of supporting migration (*173*). It thus becomes clear that the process of epithelial reprogramming and acquiring an invasive phenotype is one that is continually fed back upon through signal crosstalk at various points within the cascade, particularly in interactions between the cell and its dynamic environment.

With so many pathways being activated at once, it is necessary to have effective mechanisms that integrate signals and dampen aberrant activation of EMT. GSK-3β exists at the intersection of many of these pathways and is responsible for mediating much of the crosstalk between pathways. For example, GSK-3β phosphorylates Snail1 and SMAD proteins and marks them for degradation, preventing aberrant activation of EMT. However, both Wnt and AKT inhibit the action of GSK-3β and thus stabilize Snail1 (*118*, *260*, *261*).

Concluding Remarks

EMT is a remarkable mechanism that is essential for proper embryogenesis, without which developmental defects or embryonic lethality can occur. Although essential for development, EMT can have detrimental consequences in the adult organism, promoting the progression of diseases, such as fibrosis and cancer metastasis. Identifying and understanding the signaling mechanisms that promote EMT may lead to novel therapeutic strategies to inhibit this cellular transformation in systems of human disease. Of particular importance is the recognition of the physiological context and dynamic nature of this process in the development of drugs that target EMT to combat disease because the effects of such drugs may need to be finely tuned for a particular tissue microenvironment. Moreover, many of the mechanisms that are involved in inducing EMT are also capable of maintaining the dormant state of circulating cancer cells (*140*). Great care must be taken not to inadvertently promote reestablishment of the epithelial phenotype in mesenchymal cells where they may induce the formation of secondary tumors. EMT is tightly regulated by particular cellular microenvironments; thus, careful investigation of the effects that these different extracellular contexts may have is necessary before designing novel therapeutics.

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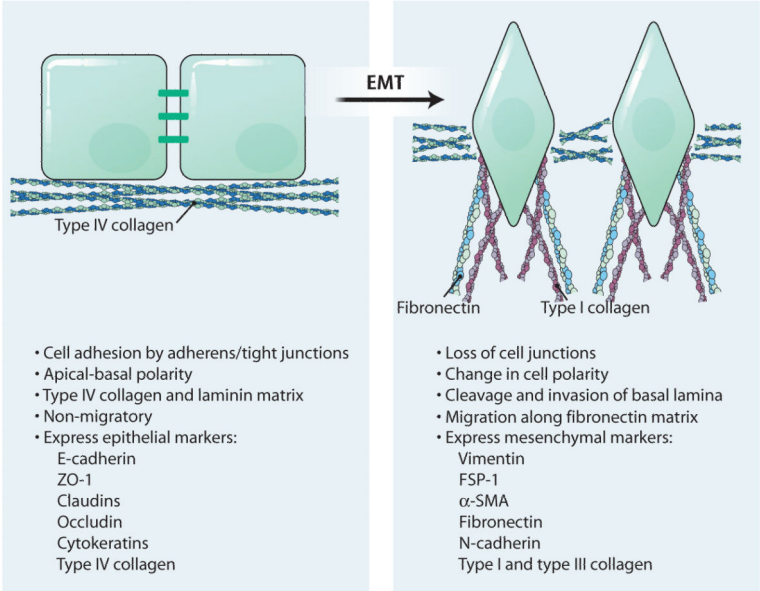

Fig. 1. Cellular changes associated with EMT

Epithelial cells demonstrate apical-basal polarity, show strong cell-cell adhesion through adherens junctions and tight junctions, and have a basal matrix consisting primarily of type IV collagen and laminin (**left**). Upon induction of EMT, the cells lose their adhesion and change morphology and acquire front end-to-back end polarity (**right**). These cells cleave and invade the basal lamina and migrate along a newly formed matrix of fibronectin and type I collagen. The abundance of epithelial biomarkers is reduced, whereas that of mesenchymal markers is increased.

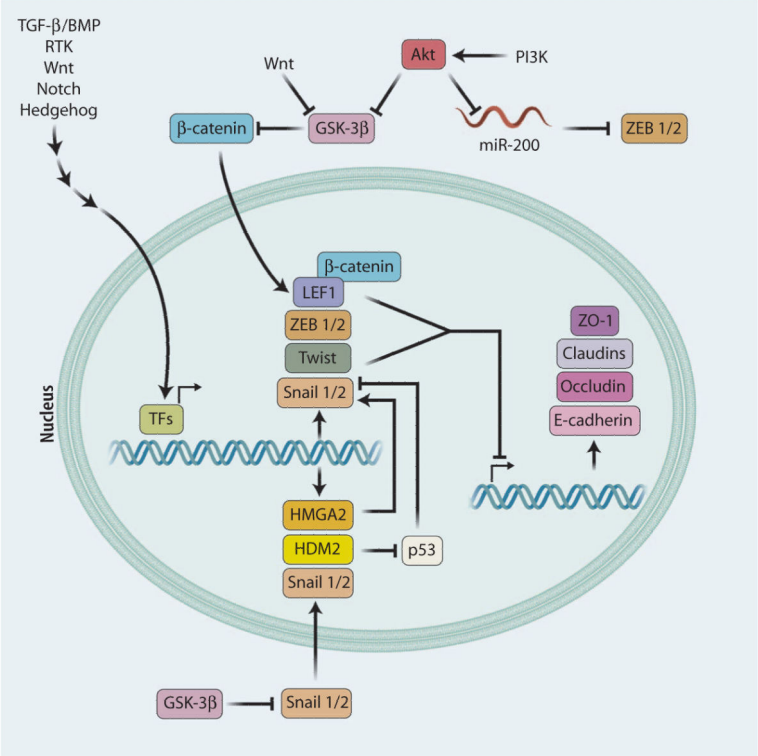

Fig. 2. EMT-inducing transcription factors

EMT is triggered by transcription factors that bind and inhibit the expression of genes encoding adherens junction and tight junction molecules, such as E-cadherin, ZO-1, claudins, and occludin. These transcription factors include Snail1/2, ZEB1/2, Twist, and LEF-1, the expression of which is induced by various signaling pathways. Several regulatory molecules can inhibit the function of these transcription factors. GSK-3β can inhibit β-catenin–induced activation of LEF-1 and can also inhibit the stability and nuclear translocation of Snail1/2. The miR-200 family of miRNAs can inhibit the expression of ZEB1/2. GSK-3β and miR-200 can be blocked by the kinase Akt, which is activated by most EMT signaling pathways.

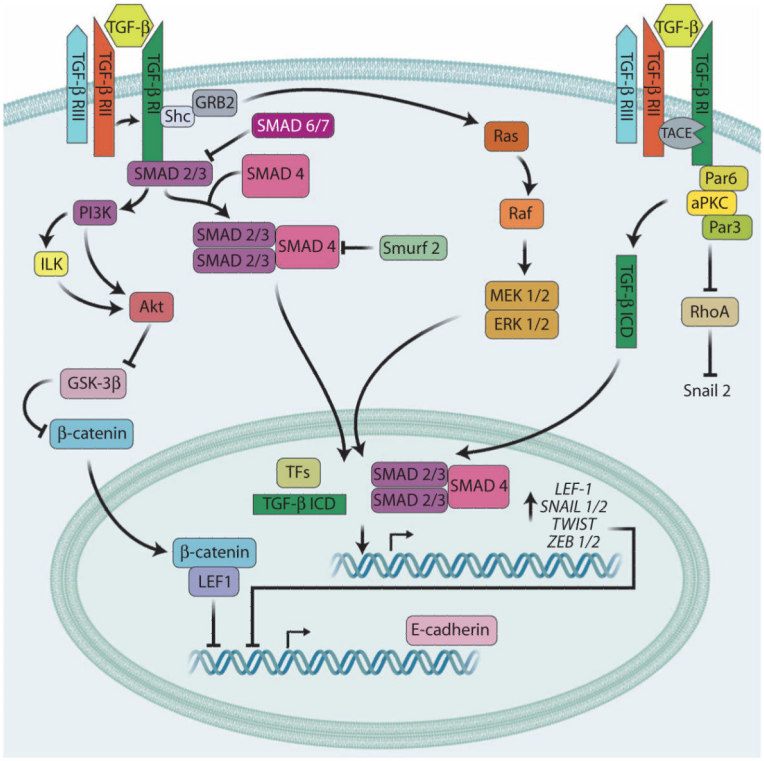

Fig. 3. TGF-β signaling in EMT

TGF-β ligands bind to their type II and type III receptors (TGF-βRII and TGF-βRIII), which causes recruitment and phosphorylation of the type I receptor (TGF-βRI). This activates various signaling pathways, including those mediated by SMAD2/3, Ras, and PI3K (simplified here), which activate transcription factors that induce the expression of genes encoding EMT-inducing transcription factors. At the surface of the cell, TACE cleaves the intracellular domain of TGF-β RI, which can then act as a transcriptional regulator to mediate EMT. Additionally, the Par3–Par6–aPKC (atypical PKC) complex also associates with TGF-βRs at the cell membrane and is involved in cytoskeletal remodeling to promote the mesenchymal phenotype. SMAD-independent pathways (such as through PI3K and ILK) can activate Akt, which in turn can inhibit the function of GSK-3β, a kinase that inhibits nuclear translocation of Snail and β-catenin. Inhibition of SMAD signaling is mediated by SMAD6/7, which prevent the binding and phosphorylation of SMAD2/3 at TGF-βRs, and by Smurf2, which is known to degrade the activated complex of SMAD2/3/4.

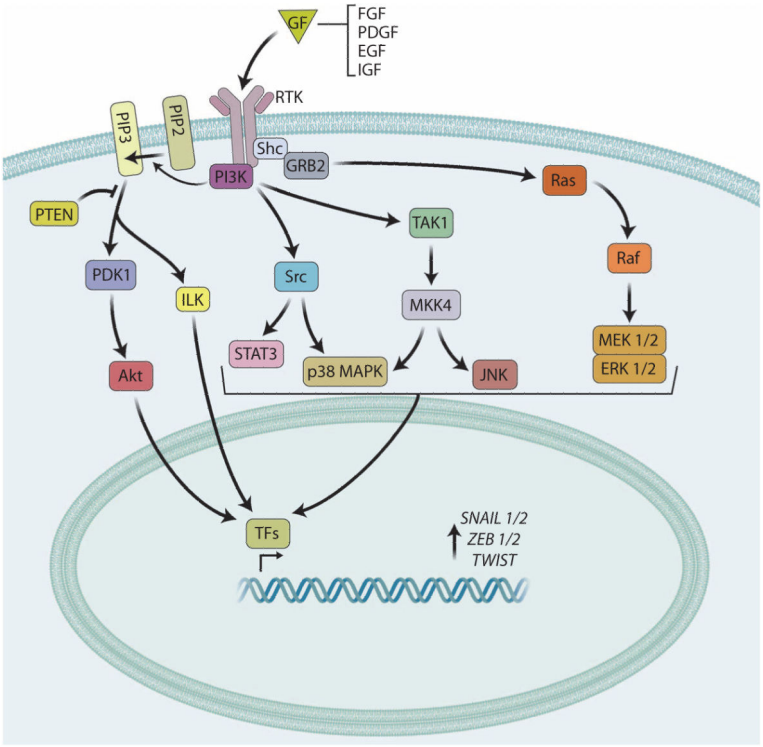

Fig. 4. RTK signaling in EMT

Growth factors (GFs), such as FGF, EGF, PDGF, or IGF, stimulate RTKs, which activate various signaling pathways (simplified here), including those mediated by Ras, PI3K, Src, and ILK. These signaling cascades activate transcription factors (TFs) that bind to the promoters of genes that encode EMT-inducing transcription factors, such as Snail1/2, ZEB1/2 and Twist, which induce EMT by inhibiting the expression genes encoding cell adhesion molecules. When present and functional, the tumor suppressor PTEN can suppress PI3K-mediated induction of EMT.

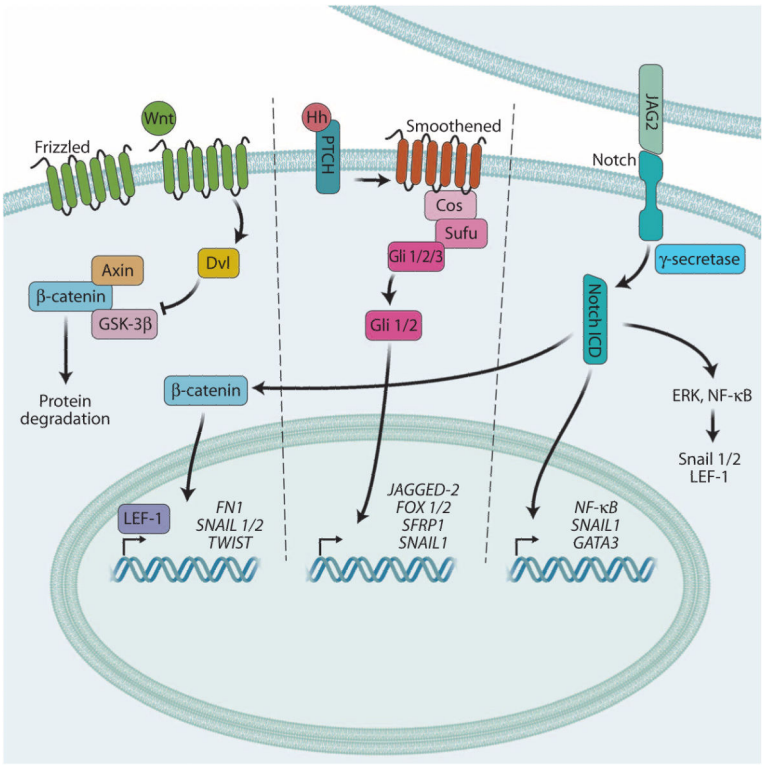

Fig. 5. Wnt, Notch, and Hedgehog signaling in EMT

Wnt ligands bind and activate Frizzled receptors, which promote Dvl-dependent inhibition of GSK-3β, a kinase that causes degradation of cytoplasmic β-catenin. This enables the accumulation and nuclear localization of β-catenin to activate the LEF-1 transcription factor, which promotes the expression of various EMT-associated genes. The intercellular interaction between JAG2 and its receptor Notch induces the g-secretase–mediated cleavage and release of the Notch ICD, which can directly activate target genes associated with EMT signaling. The Notch ICD can also stabilize cytoplasmic β-catenin and activate other pathways, like ERK and NF-κB, that induce the Snail1/2 and LEF-1 transcription factors. Hh signaling induces EMT-associated gene expression through activating Gli transcription factors.

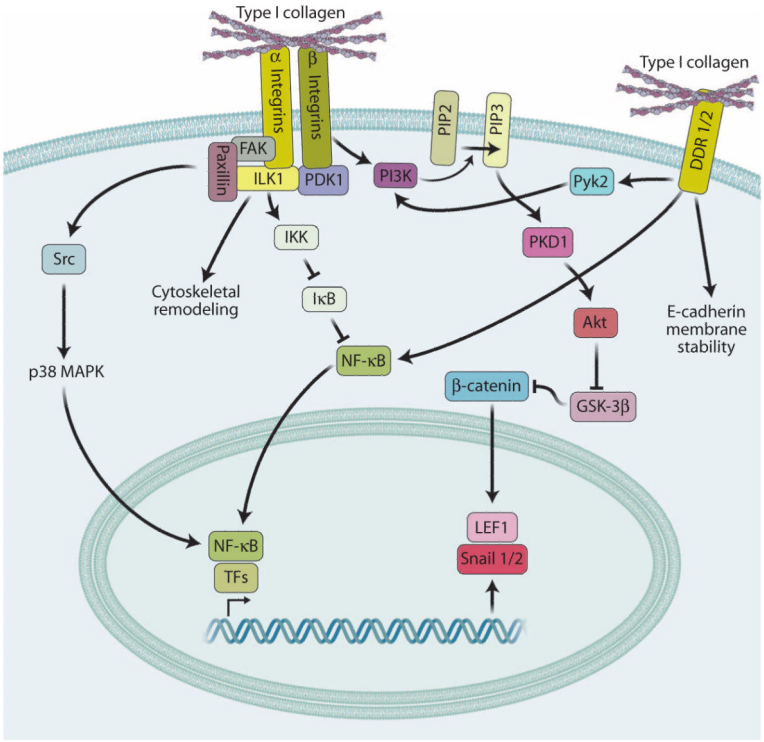

Fig. 6. Matrix signaling in EMT

Type I collagen induces EMT through integrin or DDR1/2 signaling. Both types of receptors activate NF-κB and other transcription factors (TFs) that promote expression of *SNAI1*/2 and *LEF1*. Other pathways simplified here, such as those mediated by PI3K, ILK, proline-rich tyrosine kinase 2 (PYK2)–PDK1, and FAK-paxillin, are activated by type I collagen through integrins and DDRs, ultimately promoting the stabilization and activity of the EMT-associated transcription factors Snail1/2 and LEF-1. DDR1 is also known to form complexes with E-cadherin at the cell surface, which are disrupted upon binding to type I collagen.

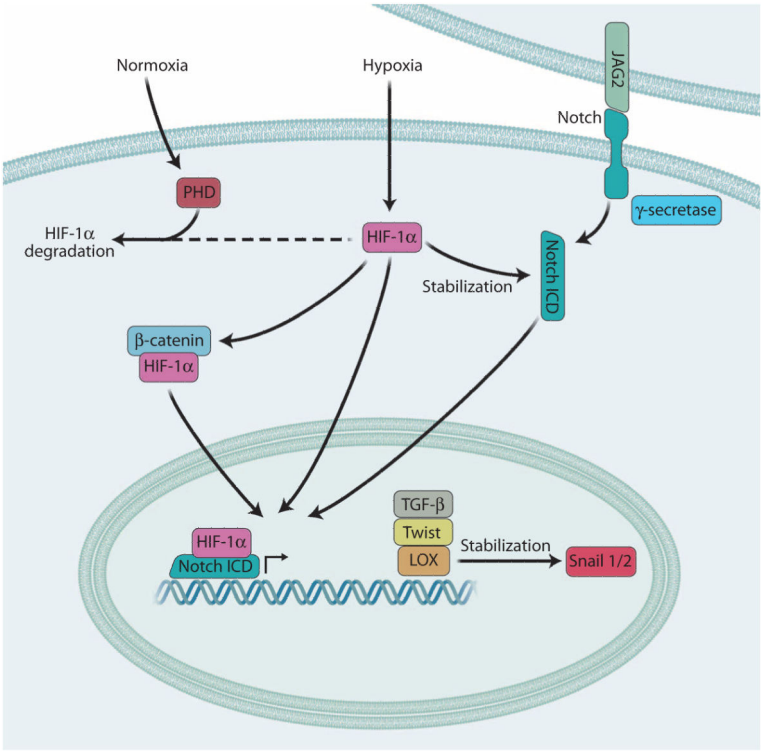

Fig. 7. Hypoxia signaling in EMT

Under normoxic conditions, prolyl hydroxylases (PHD) promote the degradation of the transcription factor HIF-1α. Under hypoxic conditions, PHDs are inactivated, thus enabling the accumulation and functional activation of HIF-1α, which induces the expression of genes associated with EMT, such as TGF-β, TWIST, and LOX, and the stabilization of the Snail1/2 transcriptional repressors. HIF-1α has also been shown to stabilize β-catenin as well as the Notch ICD after its JAG2-induced cleavage by g-secretase. Both β-catenin and the Notch ICD also promote the expression of EMT-associated genes.