

# TGF- $\beta$ -induced epithelial to mesenchymal transition

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During development and in the context of different morphogenetic events, epithelial cells undergo a process called epithelial to mesenchymal transition or transdifferentiation (EMT). In this process, the cells lose their epithelial characteristics, including their polarity and specialized cell-cell contacts, and acquire a migratory behavior, allowing them to move away from their epithelial cell community and to integrate into surrounding tissue, even at remote locations. EMT illustrates the differentiation plasticity during development and is complemented by another process, called mesenchymal to epithelial transition (MET). While being an integral process during development, EMT is also recapitulated under pathological conditions, prominently in fibrosis and in invasion and metastasis of carcinomas. Accordingly, EMT is considered as an important step in tumor progression. TGF- $\beta$  signaling has been shown to play an important role in EMT. In fact, adding TGF- $\beta$  to epithelial cells in culture is a convenient way to induce EMT in various epithelial cells. Although much less characterized, epithelial plasticity can also be regulated by TGF- $\beta$ -related bone morphogenetic proteins (BMPs), and BMPs have been shown to induce EMT or MET depending on the developmental context. In this review, we will discuss the induction of EMT in response to TGF- $\beta$ , and focus on the underlying signaling and transcription mechanisms.

**Keywords:** TGF- $\beta$ , EMT, Smad, transcription, non-Smad signaling

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## EMT: loss of epithelial and acquisition of mesenchymal characteristics

Epithelial cells typically form sheets, tubes or vesicles, in which the cells establish an apical-basal polarity. Their apical and basolateral cell surfaces possess a distinct appearance and serve different functions. In tube- or vesicle-like structures, the apical side is exposed to the lumen, whereas the basolateral surface rests on a basement membrane. Epithelial cells intimately associate with each other through laterally located, specialized cell-cell contact structures, i.e. tight junctions, adherens junctions and desmosomes [1].

Tight junctions are membrane fusions at the lateral side close to the apical surface that provide intercellular sealing and protect against paracellular diffusion, and separate the distinct functions of the apical and basolateral surfaces [2]. Occludin and claudins are important com-

ponents of the intercellular tight junction strands, while the cytoplasmic components Zonula Occludens (ZO)-1, -2, -3 and p120 are integral to an undercoat structure. The cytoplasmic tails of ZO proteins attach to actin filaments, thus contributing to the strength and integrity of tight junctions [2]. Par3 and Par6, key components of the polarity protein complex that maintains the apical compartment, are recruited to tight junctions through their PDZ domains [3, 4]. One of the early events in EMT is the disassembly of tight junctions, which results in the redistribution of ZO proteins, claudins and occludin, concomitant with disruption of the polarity complex and initiation of cytoskeleton reorganization [1].

Adherens junctions are located adjacent to the tight junctions in the basolateral surface compartments of epithelial cells, and connect to cytoskeletal microfilaments. Similarly to the tight junctions, adherens junctions form a belt-like structure at the lateral interface of epithelial cells [5]. The transmembrane adhesion receptor E-cadherin, which characterizes this type of epithelial junction, engages in homotypic interactions through its ectodomain, thus allowing E-cadherin proteins to span the intercellular space between neighboring cells at op-

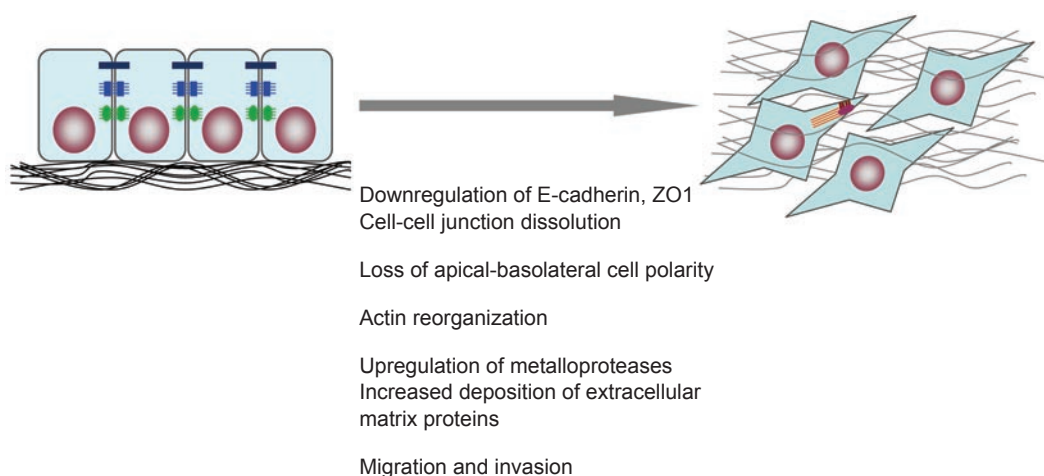
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posing adherens junctions. The cytoplasmic domains of E-cadherin bind tightly to  $\beta$ -catenin, a cytoplasmic protein that interacts with  $\alpha$ -catenin, which in turn anchors to the actin cytoskeleton, either directly or indirectly via actin-binding proteins  $\alpha$ -actinin and vinculin [5, 6]. During EMT, the adherens junction complexes disassemble and the actin cytoskeleton reorganizes from an epithelial cortical alignment associated with cell-cell junctions into actin stress fibers that are anchored to focal adhesion complexes [1]. Loss of E-cadherin is considered as a hallmark event of EMT that initiates a series of signaling events and major cytoskeletal reorganization. However, loss of E-cadherin should not be considered as the sole pivotal event in EMT, since blocking E-cadherin expression by transfection of antisense RNA does not induce a full EMT [7]. Furthermore, forced E-cadherin expression is insufficient to restore the epithelial phenotype in spindle carcinoma cells and does not reverse induced EMT [8-12].

Also located at the lateral side are the desmosomes, which provide additional strength for intercellular adhesion. Desmosomes are structurally similar to adherens junctions, containing transmembrane cadherins and linker proteins that connect the cadherins to the intermediate filament cytoskeleton. However, unlike the adherens junctions, they are organized as individual patches and not as a belt [13, 14]. The desmosome cadherins are paired transmembrane proteins composed of a desmoglein and a desmocollin. Their cytoplasmic tails interact with the Armadillo family proteins, plakophilin and plakoglobin, which also associate with desmoplakins, thus providing a link to keratin intermediate filaments.

Decrease in desmoplakin expression has been reported in various EMT settings and downregulation of the expression of desmosome components is apparent in microarray analyses that evaluate changes in gene expression associated with EMT [11, 15-17]. Hence, dissolution of the desmosomes accompanies the EMT process, but how this change fits into the sequential events resulting in EMT and whether it has a causative effect in EMT remain unknown.

Concomitant with the loss of epithelial cell-cell contact structures and actin reorganization, cells undergoing EMT acquire a mesenchymal identity (Figure 1). The mesenchymal phenotype is apparent from the expression of mesenchymal cytoskeletal proteins, such as vimentin, and the increased deposition of extracellular matrix proteins, including collagens and fibronectin. These extracellular matrix components stimulate integrin signaling and induce the formation of focal adhesion complexes, which facilitate cell migration [18, 19]. The generally observed downregulation of E-cadherin also promotes the assembly of focal adhesion through activation of focal adhesion kinase [20]. Furthermore, decreased expression of E-cadherin during EMT is accompanied by increased expression of N-cadherin, which renders the cell more motile and invasive [21-23]. These different events result in a loss of apical-basal polarity that is critical to the maintenance of epithelial morphology as well as the function of the epithelial sheet. Furthermore, the loss of specialized cell-cell contact structures facilitates the increased migration, a defining behavioral property of cells that have undergone EMT [24]. With the loss of apical-basal polarity, cells acquire a front-back polar-



**Figure 1** Epithelial-mesenchymal transition (EMT) occurs when epithelial cells lose their epithelial cell characteristics, including dissolution of cell-cell junctions, i.e. tight junctions (black), adherens junctions (blue) and desmosomes (green), and loss of apical-basolateral polarity, and acquire a mesenchymal phenotype, characterized by actin reorganization and stress fiber formation (red), migration and invasion.

ity that allows them to migrate in a directional fashion. The increased expression and activity of extracellular proteases, such as matrix metalloproteinases, allow the cells to degrade extracellular matrix proteins, thus allowing the migration to translate into an invasive behavior, whereby the cells delaminate and escape from their epithelial structures [25].

This complex and multifaceted process that defines EMT results from a plexus of changes in transcriptional regulation, in which epithelial gene expression is repressed and expression of mesenchymal genes is activated. In fact, several families of transcription factors, including the Snail family, ZEB family and basic helix-loop-helix (bHLH) family, act in concert to control the EMT process [26]. These changes in the transcription program are complemented by non-transcriptional changes that help define the changes in cytoskeletal organization and cell shape and the interaction of the cell with its environment.

EMT can be induced or regulated by various growth and differentiation factors, including TGF- $\beta$ , growth factors that act through receptor tyrosine kinases, such as fibroblast growth factor, hepatic growth factor and platelet derived growth factor, and Wnt and Notch proteins [25]. Among these, TGF- $\beta$  has received much attention as a major inducer of EMT during embryogenesis, cancer progression and fibrosis. How TGF- $\beta$  induces this orchestrated program of transcriptional changes and integrates these changes with the non-transcriptional regulation will be discussed below.

## EMT in development and pathology

The induction of EMT by TGF- $\beta$  was first recognized in cell culture. Upon TGF- $\beta$  treatment, epithelial cells changed from cuboidal to an elongated spindle shape, and showed decreased expression of epithelial markers and enhanced expression of mesenchymal markers fibronectin and vimentin [17]. These changes were accompanied by increased motility. Consistent with their binding to the same receptor complexes, TGF- $\beta$ 1, TGF- $\beta$ 2 and TGF- $\beta$ 3 share the capacity to induce EMT in epithelial cells [17, 27, 28]. Subsequent studies have demonstrated an involvement of TGF- $\beta$  and TGF- $\beta$ -related proteins in EMT in normal development and in pathological processes.

In triploblastic metazoans, EMT first occurs at gastrulation and allows the formation of a third germ layer, called the mesoderm, between the ectoderm and the endoderm [29]. While lower vertebrates acquire those internal layers through movement of epithelial cell sheets, EMT is an integral process in gastrulation in higher

vertebrates [29]. BMPs are believed to participate in the EMT associated with gastrulation initiation and mesoderm patterning [30]. At a later stage in embryogenesis, neural crest cells, arising from the dorsal part of the neural tube, undergo EMT and migrate to contribute as different cell types to the formation of various tissues, including bones and smooth muscle [31]. In cardiogenesis, TGF- $\beta$  has been shown to play a key role in the EMT that occurs in the atrioventricular canal and the outflow tract region. Accordingly, the expression of TGF- $\beta$ 1 and TGF- $\beta$ 2 increases at the onset of EMT in the atrioventricular canal endothelium and myocardium, respectively [32]. In mice, TGF- $\beta$ 1 null embryos present severe cardiac abnormalities including defective atrioventricular junction. Moreover, TGF- $\beta$ 2-deficient mice present atrioventricular and outflow tract defects. Finally, blocking TGF- $\beta$  activity using antibodies or antisense oligonucleotides inhibits EMT in chicken atrioventricular explants and TGF- $\beta$ 2-induced EMT in mouse explant cultures [33, 34]. TGF- $\beta$  signaling has also been implicated in the EMT that is associated with palatal morphogenesis. EMT occurs to induce mesenchyme continuity during the fusion of the two palatal shelves with the nasal septum, and high TGF- $\beta$ 3 expression is apparent at these sites [35]. TGF- $\beta$ 3 null mice present a cleft palate, resulting from the lack of fusion of the two palatal shelves [36]. At a later developmental stage, EMT occurs in the coelomic epithelium during the second phase of Müllerian duct regression upon signaling by anti-Müllerian hormone, a member of the TGF- $\beta$  family [37].

While EMT is spatially and temporally highly regulated in normal development, EMT also occurs in pathological contexts in the adult organism, specifically in cancer progression and fibrosis. In cancer, the epithelial tumor cells become more invasive after undergoing EMT and access the circulatory system through intravasation, resulting in dissemination of cancer cells to distal loci from the primary tumor. Consequent metastatic colonization of secondary sites by cancer cells then involves the reverse MET process [38]. Although it has been difficult to observe EMT during cancer development, presumably a result of its transient and reversible nature, recent progress in *in vivo* imaging techniques may facilitate the study of this process [25]. Several lines of evidence implicate increased TGF- $\beta$  signaling as a key effector of EMT in cancer progression and metastasis. Chemical carcinogenesis studies *in vivo* showed that transgenic expression of activated TGF- $\beta$ 1 correlates with the conversion of squamous into more invasive spindle cell carcinomas [39]. Cancer cells often increase their production of active TGF- $\beta$ , which not only triggers EMT and allows the cells to become invasive, but also enhances angiogenesis

in close proximity to the tumor microenvironment, providing an exit route for migratory mesenchymal cells [40].

EMT also occurs following tissue injury and contributes to organ fibrosis. In renal fibrosis, renal interstitial fibroblasts derive not only from mesenchymal stem cells in the bone marrow, but also from proximal tubular kidney epithelial cells that undergo EMT [41]. Several observations indicate that TGF- $\beta$  is causally associated with this process. Abnormally high levels of TGF- $\beta$  are expressed in renal fibrotic sites of patients with kidney diseases. Moreover, transgenic mice with increased expression of TGF- $\beta$ 1 develop renal fibrosis [42].

TGF- $\beta$  has also been shown to play a key role in pulmonary and hepatic fibrosis, not only through its ability to attract fibroblasts and to stimulate their proliferation, but also through induction of EMT in alveolar epithelial cells and transdifferentiation of quiescent hepatic stellate cells into myofibroblasts respectively [43, 44]. Finally, the recently described endothelial to mesenchymal transition may contribute to TGF- $\beta$ -induced cardiac fibrosis [45].

### TGF- $\beta$ -activated Smad signaling in EMT

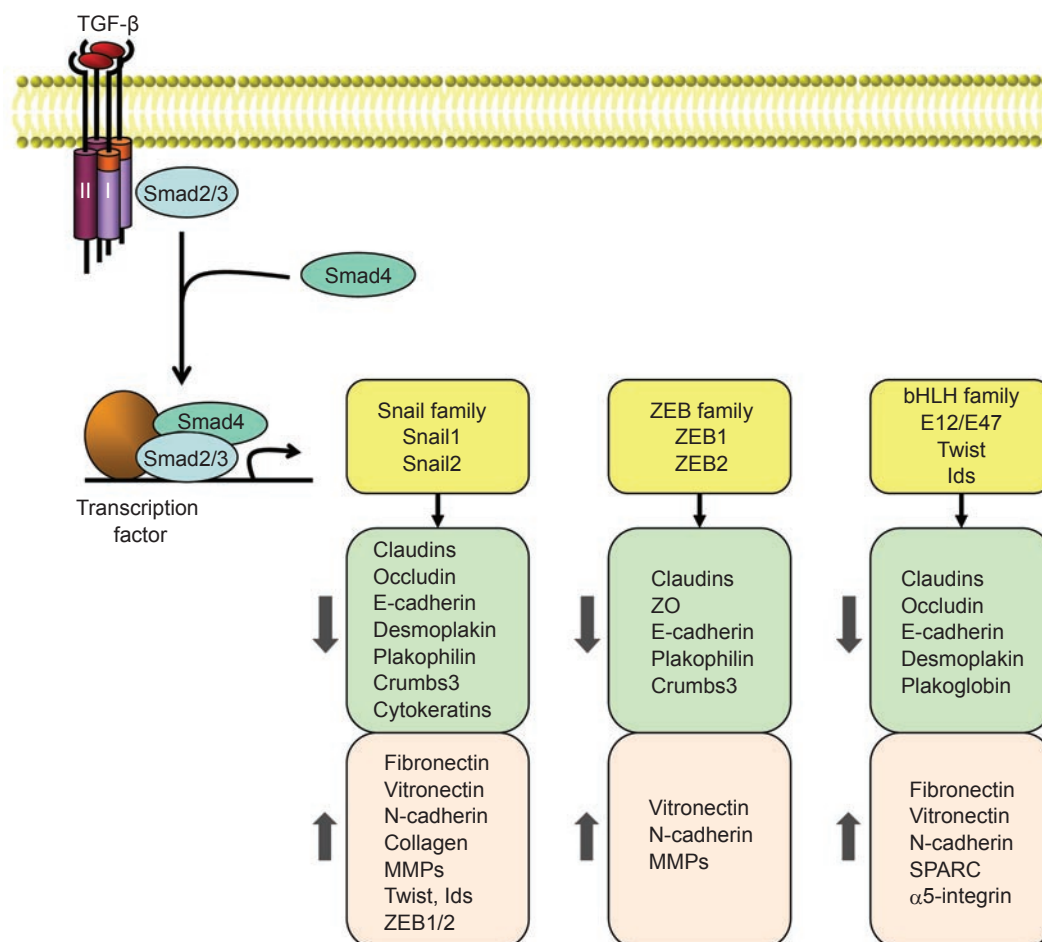
The dissection of the signaling mechanisms that are activated in response to TGF- $\beta$  and lead to EMT has been largely conducted in cells in culture, predominantly using the NMuMG, MDCK and HaCaT cell lines as model systems. Parallel studies have allowed an evaluation of the role of TGF- $\beta$  signaling components in EMT *in vivo*. TGF- $\beta$  signals through a heteromeric complex of two type I and two type II transmembrane serine-threonine kinase receptors. In response to TGF- $\beta$ , the type II receptor kinases phosphorylate the type I receptors, which then leads to activation of the cellular responses to TGF- $\beta$  [46]. Dominant negative interference with the TGF- $\beta$  type II receptor function reverses EMT in colon cancer cells in culture and inhibits EMT in skin and mammary cancer models *in vivo* [47, 48]. Furthermore, loss of the TGF- $\beta$  type II receptor has been associated with decreased EMT at cancer loci in a mouse skin carcinoma model [49]. The key role of the type I receptor in TGF- $\beta$ -induced EMT is also revealed in cell culture and *in vivo* studies. Indeed, expression of an activated version of the T $\beta$ RI receptor ALK-5, the major TGF- $\beta$  type I receptor, or of ActRIB/ALK-4, the major type I receptor for activin and nodal, recapitulates TGF- $\beta$ -induced EMT in NMuMG cells [27, 28], while dominant negative forms of either type I receptor block TGF- $\beta$ -induced EMT [28]. Inhibition of T $\beta$ RI function using a chemical inhibitor also blocks EMT and promotes an enhanced epithelial phenotype in cell culture [50, 51], while interference with T $\beta$ RI func-

tion *in vivo* suppresses EMT and decreases mesenchymal differentiation during cardiac valve formation [52].

TGF- $\beta$ -induced activation of the receptor complex leads to activation of Smad2 and Smad3 through direct C-terminal phosphorylation by T $\beta$ RI. Phosphorylated Smad2 and Smad3 then form trimers with Smad4, and translocate into the nucleus, where they associate and cooperate with DNA binding transcription factors to activate or repress target gene transcription (Figure 2). Consequently, Smad2 and Smad3 function in cooperation with Smad4 as TGF- $\beta$ -induced transcription regulators. In contrast, the inhibitory Smad6 and Smad7 inhibit activation of the receptor-regulated Smads [46].

Various studies have explored the roles of TGF- $\beta$ -activated Smads in EMT. Increased expression of Smad2 or Smad3 with Smad4 induces EMT, or enhances the induction of EMT by the activated form of T $\beta$ RI, in NMuMG cells [27, 28], whereas expression of dominant negative versions of Smad2 or Smad3 blocks TGF- $\beta$ -induced EMT in this cell system [28]. Consistent with the pivotal role of Smad3 in EMT, renal tubular epithelial cells deficient in Smad3 fail to undergo EMT in response to TGF- $\beta$  or mechanical stress [53], and keratinocytes derived from Smad3<sup>-/-</sup> mice show reduced migration in response to TGF- $\beta$  [54]. Compared with Smad3, Smad2 may play an antagonistic role in the EMT process *in vivo*. Loss of Smad2 is frequently noted in human skin cancer patients, and Smad2 deficiency in keratinocytes promotes EMT and accelerates skin tumor formation. This has been explained by increased binding of the Smad3/4 complex to the promoter of the Snail gene and by increased Snail expression in the absence of Smad2, thus enhancing the progression of EMT [55]. Similarly, Smad2<sup>-/-</sup> hepatocytes appear mesenchymal and migrate faster than wild-type cells, while Smad3<sup>-/-</sup> hepatocytes retain their epithelial characteristics [56]. However, expression of activated Smad2 promotes spindle tumor cell invasion, and a dominant negative form of Smad2 inhibits it, suggesting that Smad2 may promote EMT *in vivo* [57]. Similarly to Smad3, Smad4 is indispensable for EMT. RNA interference-mediated knockdown of Smad4 expression or expression of a dominant negative mutant of Smad4 results in preserved E-cadherin expression [22, 28, 58, 59], suppression of fibrotic type I collagen synthesis *in vitro* [59], and decreased bone metastasis *in vivo* [22]. Furthermore, genetic ablation of Smad4 leads to preservation of epithelial markers and a lower degree of EMT in adenocarcinoma [60]. Conversely, the inhibitory Smads function as negative regulators of and thus repress TGF- $\beta$ -induced EMT. Smad6 controls the timing and extent of EMT during cardiac valve formation [61, 62], while increased expression of Smad7 blocks TGF- $\beta$ -





**Figure 2** Transcriptional regulation of EMT induced by TGF- $\beta$ . In response to TGF- $\beta$ , Smad2 and 3 are activated, and form complexes with Smad4, which then regulate transcription of target genes through interactions with other DNA binding transcription factors. In the induction of EMT, the activated Smads mediate transcriptional regulation through three families of transcription factors, resulting in repression of epithelial marker gene expression and activation of mesenchymal gene expression.

induced EMT in multiple tissues [28, 63-66].

### Transcriptional regulation of EMT

Consistent with the important roles of Smads, the loss of epithelial markers and acquisition of mesenchymal features are achieved through a well-orchestrated transcription program that involves three families of transcription factors, the Snail, ZEB and bHLH families (Figure 2). Their expression is induced in response to TGF- $\beta$ , either through a Smad-dependent mechanism (in the case of Snail proteins) or indirectly through activation of other transcription factors or relief of repression. Upon activation, these transcription factors in turn repress epithelial marker gene expression and concomitantly activate mesenchymal gene expression. Additionally,

these factors elaborate tissue-specific or developmental stage-specific functions associated with their distinct expression profiles [26].

#### *Snail family transcription factors*

The Snail transcription factors share extensive structural similarity, containing a characteristic C-terminal domain with four to six zinc fingers that mediates sequence-specific DNA binding to E-box elements C/A(CAGGTG) [67]. Three Snail family proteins have been identified in vertebrates: Snail1 (first described as Snail), Snail2 (also known as Slug) and a more recently characterized Snail3. Snail proteins function as transcription repressors and their activities depend on the zinc finger domain and an N-terminal SNAG (Snail/Gfi) domain.

Induction of Snail1 expression has been noted in all

EMT processes that have been studied [67, 68], and increased Snail1 levels have been correlated with more invasive tumor types [15, 69]. Snail2 is more broadly expressed than Snail1, and its expression appears in some cases unrelated to EMT [68, 70]. Both genes are induced in response to TGF- $\beta$  in cells that undergo TGF- $\beta$ -induced EMT. Furthermore, TGF- $\beta$  induces Snail1 expression in skin [71] and palate development [72], in mesothelial cells during pathological fibrosis [73], in cultured hepatocytes [59], and in multiple epithelial cell lines [74], as well as during heart development [75]. The induction of Snail1 expression in response to TGF- $\beta$  is mediated by Smad3; Smad3 binds to the Snail1 promoter and activates its transcription [55, 76]. Renal tubular epithelial cells deficient in Smad3 expression fail to activate Snail1 expression upon TGF- $\beta$  treatment [53], and interference with Smad4 expression attenuates TGF- $\beta$ 1-induced Snail1 expression in epithelial cell lines [22, 58, 59]. Smad3 also mediates TGF- $\beta$ -induced expression of Snail2 in MDCK cells, whereby Smad3 forms a complex with myocardin-related transcription factors and binds to the Snail2 promoter to activate transcription [77].

Induction of EMT by hepatocyte growth factor (HGF) [78], fibroblast growth factor (FGF), or epidermal growth factor (EGF), which all act through the Ras-MAPK or PI3K-Akt pathway, also results in the induction of Snail expression [68]. Furthermore, the TGF- $\beta$ -Smad pathway also cooperates with Ras, Notch and Wnt signaling in inducing Snail expression in development and in tumor metastasis [68]. Additionally, post-translational modification defines Snail subcellular localization, stability and transcription activity [26, 68].

Several lines of evidence highlight the key roles of Snail transcription factors in the execution of the EMT program [15, 69]. Ectopic expression of Snail1 or Snail2 suppresses E-cadherin and plakoglobin expression and enhances vimentin and fibronectin expression, leading to a full EMT phenotype, whereas silencing of Snail expression reverses this process [15, 69, 79-81]. Snail1-deficient mouse embryos are unable to complete the EMT process and form a defective mesodermal layer that maintains E-cadherin expression, causing the mutant embryos to die at gastrulation [82]. Silencing of Snail2 expression in chick embryos results in mesodermal malformation and neural crest emigration failure [83].

Substantial efforts have gone into defining the targets of the Snail transcription factors. Snail1 and 2 both repress the expression of the epithelial marker gene *CDH1* that encodes E-cadherin. At the E-cadherin promoter, Snail1 binds to E-box elements, recruits a complex consisting of HDAC1, HDAC2 and mSin3A, and thus represses gene transcription [15, 69, 81]. Snail2 simi-

larly represses E-cadherin through binding to the same E-box elements, but recruits a different combination of co-repressors, i.e. HDAC1/3 and CTBP [70, 80, 81]. The expression of Snail proteins appears to inversely correlate with E-cadherin expression, and silencing of the Snail1 gene can restore E-cadherin levels [69, 81]. However, forced expression of E-cadherin is unable to counteract Snail1-induced EMT [11, 12], suggesting an involvement of additional Snail target genes in the elaboration of EMT. In fact, Snail proteins repress a spectrum of genes involved in maintaining epithelial structure and function, including genes encoding claudins and occludin, major transmembrane components of tight junctions. Snail1 represses the expression of claudin-3, -4 and -7 at their promoters [11, 84], whereas Snail1 and Snail2 both repress the expression of claudin-1 and occludin [84-86]. In contrast, Snail1 expression does not dramatically decrease the levels of cytoplasmic components of tight junctions, such as ZO-1 and p120, but their distribution is altered from peripheral localization to a diffused cytoplasmic pattern [84]. Snail proteins regulate the expression of desmosome proteins as well. Ectopic expression of Snail1 results in decreased desmoplakin and plakophilin levels, and causes disorganized plakoglobin distribution [11, 15, 16, 87]. Furthermore, Snail1 disrupts epithelial polarity by repressing the expression of Crumbs3, which in complex with two other proteins, PALS1 and PATJ, is required for the maintenance of apical-basal polarity. Snail1 binds to the E-box element in the Crumbs3 promoter and inhibits Crumbs3 expression, leading to disassembly of the Crumbs complex [88]. However, Snail1 expression does not affect the Par complex, another important polarity complex [88]. Snail1 expression also results in decreased expression of a subset of cytokeratins, i.e. cytokeratin 17, 18, 19 and 20, thus affecting the epithelial cytoskeletal organization. Cytokeratins 17 and 18 have been identified as direct targets of Snail1 by chromatin immunoprecipitation [11, 84, 89]. Recent microarray analyses have unveiled additional genes encoding epithelial or basal lamina proteins that are down-regulated by Snail1, suggesting an even broader range of regulation of the epithelial program by Snail proteins [90].

Other Snail target genes show a more tissue-restricted distribution and are hence associated with tissue-specific EMT processes. One such target of Snail1 is HNF-1 $\beta$ , a transcription factor that is expressed in kidney epithelial cells and activates the expression of the kidney-specific epithelial marker cadherin-16. Through direct repression of HNF-1 $\beta$  transcription, Snail1 suppresses cadherin-16 expression and induces EMT in cell culture, reminiscent of the development of kidney fibrosis *in vivo* [91, 92]. In hepatocytes, Snail1 represses another HNF transcription

factor, HNF-4 $\alpha$ , resulting in loss of epithelial markers and expression of mesenchymal proteins [93]. Snail proteins also repress the expression of mucin-1, a transmembrane protein on the apical surface of pulmonary airway epithelium that serves to hydrate, lubricate and protect the epithelium [94].

While repressing epithelial gene expression, Snail proteins activate the expression of the mesenchymal proteins fibronectin [15, 79], vitronectin [15, 79] and N-cadherin [90], the extracellular matrix proteins collagen type III and V [90], and proteins involved in migration and invasion, such as RhoB, plasminogen activator inhibitor-1 and matrix metalloproteinases [68, 79]. These effects of Snail may be indirect and involve other transcription factors, such as Ets-1 which has been proposed to mediate the induction of MMP-2 expression [95] or Sp-1 and Ets-1 which are thought to be partially responsible for the upregulation of MMP-9 expression [96]. Snail1 also regulates the expression of multiple actin-modulating proteins to facilitate the rearrangement of actin filaments from cortical distribution to stress fibers anchored to focal adhesions [11]. Consistent with the indirect induction of gene expression by Snails, Snail transcription factors induce the expression of other EMT-related transcription factors, such as Twist and Ids in MDCK cells [90], and ZEB1 and ZEB2 in squamous carcinoma cell lines [89, 95].

In addition to regulating the expression of epithelial or mesenchymal genes, Snail also regulates genes required for cell survival [68], which are frequently intertwined with EMT during embryonic development or in pathological conditions. Finally, in some cases Snail1 and Snail2 cooperate in the control of the transcription network that regulates EMT. For example, in breast tumors, Snail1 expression correlates with mesenchymal transition and metastasis, and Snail2 expression associates with the repression of tumor suppressor gene BRCA2 [68].

#### *ZEB family transcription factors*

Two ZEB family transcription factors are known in vertebrates: ZEB1, also known as  $\delta$ EF1 or AREB6, and ZEB2, also known as Smad-interacting protein 1 (SIP1). They have two zinc-finger clusters at each end, whose simultaneous binding to bipartite E-boxes mediates the interaction with regulatory DNA sequences. The central region contains a Smad-interaction domain, a homeodomain and a CTBP binding domain. Repression of gene transcription by ZEB1 or ZEB2 is mediated by repressor motifs in the central homeodomain and through recruitment of CTBP as a co-repressor. However, interaction of ZEB1 with co-activators PCAF and p300 switches ZEB1 function from repression to activation [26, 97]. ZEB

proteins are expressed during development in various tissues, including the central nervous system, the heart, skeletal muscle and haematopoietic cells. They partially compensate for each other in these tissues, although in other cases, such as during neural crest emigration, and in lymphocytes, ZEB proteins exhibit distinct expression patterns and do not functionally compensate [26].

TGF- $\beta$  signaling induces the expression of ZEB proteins during EMT through an indirect mechanism mediated in part by Ets-1 [21]. ZEB proteins then interact with Smad3 and directly repress the expression of epithelial marker genes, possibly by recruiting the co-repressor CTBP [97, 98]. ZEB1 is also induced by TGF- $\beta$  during smooth muscle differentiation, in which it synergistically interacts with Smad3 and SRF to transactivate the genes encoding smooth muscle  $\alpha$ -actin and smooth muscle myosin heavy chain [99]. As is in the case of the Snails, the expression of ZEB proteins is not only activated by TGF- $\beta$  but also by other growth factors that activate Ras-MAPK signaling and by Wnt/ $\beta$ -catenin signaling [26]. The expression of ZEB factors is also post-transcriptionally repressed by microRNAs, miR-200 family and miR-205 [100-102]. This group of microRNAs is dramatically downregulated in cells undergoing EMT, allowing expression of ZEB transcription factors. Thus, forced expression of miR-200 family is sufficient to block TGF- $\beta$ -induced EMT [100-102]. In addition, ZEB2 is subject to post-translational regulation, whereby Pc2-mediated sumoylation impairs its repressor activity [103].

The induction of ZEB proteins is necessary for the downregulation of E-cadherin expression and promotion of cell migration [21, 104-106]. ZEB proteins directly repress E-cadherin expression independently of the Snail transcription factors in mouse mammary epithelial cells [21, 106]. Concomitantly, they confer a delocalization of  $\beta$ -catenin in a cell context-dependent manner and promote cell migration [104, 107]. Besides its effects on adherens junctions, ZEB2 directly represses the expression of the tight junction proteins claudin-4 and ZO-3 [107]. ZEB2 also suppresses the expression of the desmosome protein plakophilin-2 [107] and induces the expression of the mesenchymal proteins vimentin [108], N-cadherin [107] and matrix metalloproteinase-2 [95] through as yet unknown mechanism(s). Both ZEB proteins promote cell migration and induce invasion [104, 107, 109]. ZEB1 has also been implicated in the downregulation of epithelial polarity through the direct repression of Crumbs3 expression; depletion of ZEB1 restores the expression of Crumbs3 and PATJ, major components of the Crumbs3 polarity complex [109, 110]. ZEB1 also directly represses the expression of mucin-1 [89].

### *Helix-loop-helix family factors*

The HLH family is a large family of transcription factors controlling a wide array of developmental and pathological processes. The basic structure of HLH family members includes two parallel  $\alpha$ -helices linked by a loop required for dimerization. HLH factors are divided into seven categories based on their tissue distribution, dimerization capability and DNA-binding specificity [111]. Among these, the class I proteins E12 and E47, class II proteins Twists and class V proteins Ids are involved in the elaboration of EMT. The class I proteins E12 and E47 form homodimers or heterodimers with class II proteins, and the class II proteins Twists (Twist1 and Twist2) always heterodimerize with class I proteins. In contrast to these, the class V Id proteins (Id1, Id2, Id3 and Id4) lack the basic domain and thus are incapable of DNA binding. They function as dominant negative inhibitors through high affinity binding to class I proteins [26, 111].

E12 and E47 are encoded by alternative splicing products of the E2A gene [26, 111]. They directly repress E-cadherin expression through DNA binding at the E-box element in the proximal promoter. Ectopic expression of E12 or E47 represses E-cadherin and plakoglobin expression, induces vimentin and fibronectin expression, and promotes migration and invasion [80, 112, 113]. It remains unclear whether E12 and E47 form homodimers or heterodimerize with other HLH factors for E-cadherin repression. However, their repression of E-cadherin transcription can be antagonized by Id factors, which interact with E2A proteins [113]. The expression of Id1, Id2 and Id3 is repressed in response to TGF- $\beta$ , which in the case of Id1, occurs through the rapid activation of expression of the transcription repressor ATF3 by TGF- $\beta$  and the subsequent binding of an ATF3/Smad3/Smad4 complex to the Id1 promoter [114, 115]. Consequently, loss of Id protein expression correlates with a decrease in E-cadherin expression, and ectopic expression of Id2 or Id3 dose-dependently blocks TGF- $\beta$ -induced repression of E-cadherin expression, inhibits TGF- $\beta$ -induced ZO-1 delocalization and represses TGF- $\beta$ -induced smooth muscle actin expression [113, 114]. Besides E-cadherin, E47 also represses desmoplakin expression and induces the expression of N-cadherin, SPARC and  $\alpha$ 5-integrin [90].

The HLH protein Twist1 is a major regulator of mesoderm formation in *Drosophila* and neural tube closure in mice, suggesting its involvement in developmental EMT [116]. Mutation of the gene encoding Twist1 in human has been associated with Saethre-Chotzen syndrome, which is characterized by cleft palate and possibly caused by an inhibition of EMT [117, 118]. Conversely, both Twist1 and Twist2 expression are upregulated in a

large fraction of human tumors [119]. Ectopic expression of Twist1 or Twist2 decreases E-cadherin, occludin and claudin-7 expression, increases vimentin and N-cadherin expression, and enhances migration and invasion [9, 10]. In addition, Twist1 and Twist2 both synergize with H-Ras to induce a full EMT [10].

### *HMGA2*

HMGA2 (high mobility group A2) is another downstream effector of TGF- $\beta$  during EMT. HMGA2 belongs to a group of transcription factors that bind AT-rich DNA sequences to form nucleoprotein complexes. HMGA2 is expressed at high levels during embryogenesis and at low levels in adulthood, and is expressed aberrantly in some transformed cells or cancer tissues. TGF- $\beta$  induces a drastic increase in HMGA2 expression through a Smad3/Smad4-dependent mechanism, and ectopic HMGA2 expression is able to induce the expression of Snail1/2 and Twist1 [120].

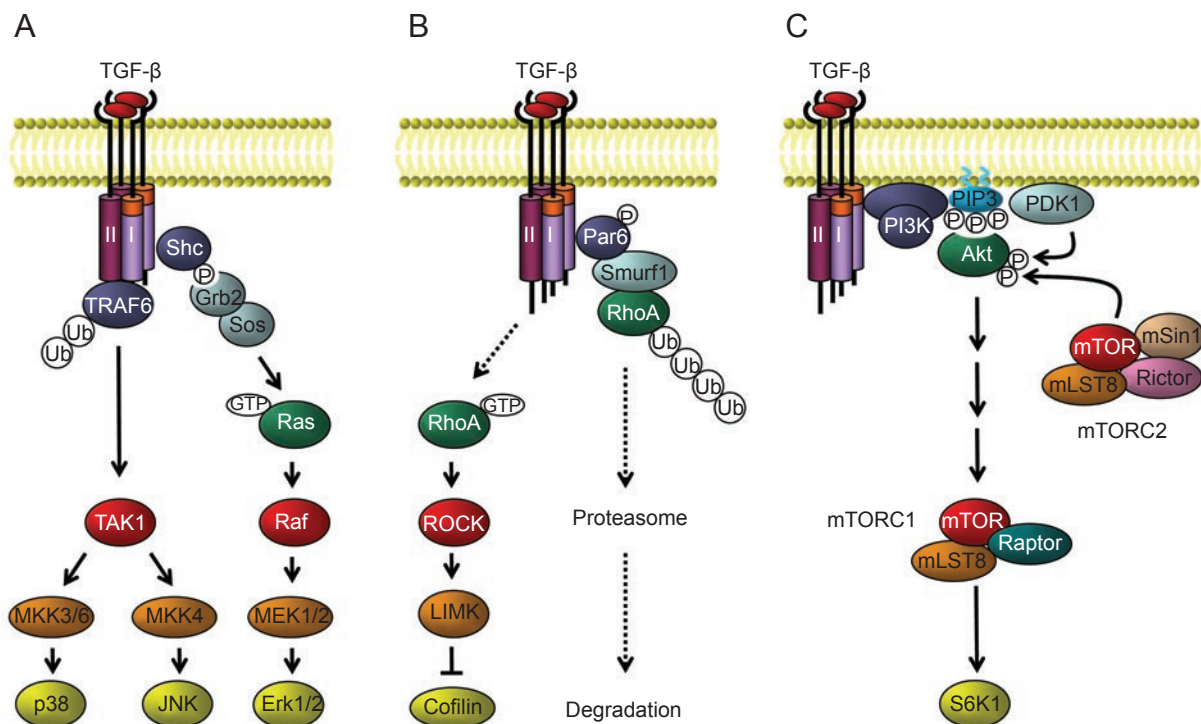
### **Non-Smad signaling in TGF- $\beta$ -induced EMT**

TGF- $\beta$  also elicits signaling responses through pathways that are generally considered as important effector pathways for tyrosine kinase receptors in response to ligands that do not belong to the TGF- $\beta$  family. The rapid activation of these non-Smad signaling pathways by TGF- $\beta$  often follows similar kinetics as Smad signaling, and attenuation of Smad signaling does not generally affect the activation of these pathways (Figure 3). In addition, non-Smad signaling responses to TGF- $\beta$  can also occur with delayed kinetics and are then often indirect, presumably as a result of Smad-mediated changes in gene expression [121]. Direct activation of non-Smad signaling pathways by TGF- $\beta$  occurs through interactions of signaling mediators either directly with the T $\beta$ RII and/or T $\beta$ RI receptors or through adaptor proteins. Among the non-Smad signaling responses, activation of Erk MAP kinases, Rho GTPases and the PI3 kinase/Akt pathway in response to TGF- $\beta$  has been linked to TGF- $\beta$ -induced EMT through their regulation of distinct processes, such as cytoskeleton organization, cell growth, survival, migration or invasion [28, 121]. Treatment of cells with chemical inhibitors that selectively or specifically block one or several of these pathways dramatically affects the induction of the EMT phenotype and downstream transcriptional responses by TGF- $\beta$ , strongly suggesting that activation of non-Smad signaling complements Smad signaling in the elaboration of the EMT response.

### *Ras and MAP kinase activation in TGF- $\beta$ -induced EMT*

Growth factor signaling through tyrosine kinase re-





**Figure 3** Non-Smad signaling in response to TGF- $\beta$ . **(A)** TGF- $\beta$  activates p38 MAP kinase and JNK MAP kinase signaling through the activation of TAK1 by receptor-associated TRAF6, and Erk MAP kinase signaling through recruitment and phosphorylation of Shc by the T $\beta$ RII receptor. **(B)** Activation of RhoA in response to TGF- $\beta$  and induction of ubiquitin-mediated RhoA degradation at tight junctions. **(C)** TGF- $\beta$  induces PI3-kinase signaling, leading to the activation of Akt-mTOR signaling, and consequently to increased translation.

ceptors leads to MAP kinase signaling, and MAP kinase pathways have been shown to regulate TGF- $\beta$ -induced Smad signaling. Erk MAP kinases phosphorylate R-Smads in their linker region thus inhibiting their nuclear translocation and decreasing their activities [122]. Other reports, however, support a cooperation between Erk MAP kinase signaling and TGF- $\beta$ /Smad signaling in TGF- $\beta$ -induced gene expression [123-125]. TGF- $\beta$ , acting through its distinct class of receptors, is also capable of directly activating MAP kinase signaling pathways, although the activation levels are generally much lower than that by tyrosine kinase receptors. The activation of Erk1 and Erk2 MAP kinases in response to TGF- $\beta$  is initiated by Ras, leading to activation of Raf and MEK1/2 kinases, following a similar activation cascade as in response to growth factors [121, 126]. This signaling pathway is linked to the receptor complex by ShcA. In response to TGF- $\beta$ , ShcA associates with and is phosphorylated on tyrosine by T $\beta$ RII, thus offering a docking site for the recruitment of Grb2 and Sos, and allowing the Shc/Grb2/Sos complex to initiate Ras activation upstream from the kinase cascade [126]. In addition to this role of Shc, the interaction of the integrin  $\alpha$ v $\beta$ 3 with

T $\beta$ RII was shown to result in tyrosine phosphorylation of T $\beta$ RII by Src, leading in turn to association of Shc and Grb2 with the T $\beta$ RII receptor [127].

Various observations support a cooperation of the Ras-Erk MAP kinase pathway with TGF- $\beta$  signaling in the induction of EMT. Increased Ras-Erk MAP kinase signaling, *e.g.* in response to growth factor stimulation or due to expression of mutant Ras, enhances TGF- $\beta$ -induced EMT, as apparent by the morphological changes and downregulation of E-cadherin expression [124, 128-130]. Consistent with this cooperation, blocking the kinase function of MEK1/2 using a chemical inhibitor, thus resulting in inactivation of the Erk1/2 MAP kinases, inhibits TGF- $\beta$ -induced EMT [131]. Since Erk1/2 MAP kinases target various transcription factors, this cooperation with TGF- $\beta$  signaling may occur at the level of gene expression, which would be consistent with the key role of Smads in activating the EMT process. Accordingly, activation of MEK/Erk MAP kinase signaling enhances TGF- $\beta$ -induced transcription responses, leading to downregulation of E-cadherin, upregulation of N-cadherin and upregulation of matrix metalloproteinase expression [128, 130]. In addition, this cooperation may result in part from

the increased expression of Snail2 leading to downregulation of E-cadherin expression [132], and oncogenic Ras hyperactivity was shown to result in enhanced TGF- $\beta$  signaling concomitant with increased autocrine TGF- $\beta$  secretion, phosphorylation and nuclear accumulation of Smads, and TGF- $\beta$ -specific transcriptional responses [124]. *In vivo*, ectopic expression of an activated version of Smad2 enhances the metastatic potential of cells expressing activated Ras [57]. Additionally, activation of Erk5 MAP kinase, another member of Erk family, which can also occur in response to TGF- $\beta$ , stabilizes Snail1 expression [133]. Taken together, these results suggest that activation of Erk MAP kinase signaling contributes to and may even be required for TGF- $\beta$ -induced EMT. Therefore, the induction of this signaling pathway by TGF- $\beta$  itself may be important in the EMT response.

TGF- $\beta$  treatment also induces activation of p38 MAP kinase. In fact, TGF- $\beta$  was shown to induce phosphorylation of p38 MAP kinase [134, 135]. The observation that a chemical inhibitor of p38 MAP kinase prevents TGF- $\beta$ -induced EMT in mammary epithelial cells without affecting Smad phosphorylation suggests a role of p38 MAP kinase in TGF- $\beta$ -induced EMT [134, 135]. This may be explained by the activation of the transcription factor ATF-2 by p38 MAP kinase, since ATF-2 mediates TGF- $\beta$ -induced transcription responses [136].

Activation of JNK MAP kinase, which occurs in response to TGF- $\beta$  in several cell systems, has also been shown to be required for TGF- $\beta$ -induced EMT. Indeed, epithelial cells deficient in JNK1 expression, and keratinocytes transfected with antisense oligonucleotides targeting JNK, were unable to undergo EMT upon TGF- $\beta$  treatment [137, 138]. Moreover, a chemical inhibitor of JNK blocks the TGF- $\beta$ -induced increase in fibronectin, vimentin and  $\alpha$ -smooth muscle actin expression, and decrease in E-cadherin expression observed during EMT [137, 139]. This requirement may reflect the role of JNK in cytoskeleton organization, cell migration and invasion through regulation of matrix metalloproteinase expression [140]. JNK also activates c-Jun, a component of the transcription complex AP-1 that cooperates with TGF- $\beta$ -induced Smads in mediating TGF- $\beta$ -induced transcription responses, such as plasminogen activator like-urokinase (uPA) expression, and plays an important role in EMT [137].

Consistent with the activation of p38 MAP kinase in response to TGF- $\beta$ , TGF- $\beta$  has been shown to induce phosphorylation of p38 MAP kinase, as well as upstream kinases that mediate p38 activation, i.e. MKK3 and MKK6, and TAK1, which can activate MKK3 and MKK6 [137, 138]. In addition, TAK1 has been shown to be required for JNK MAPK activation in response

to TGF- $\beta$  signaling through activation of MKK4 [141, 142]. The activation of TAK1 was reported to require the interaction of the ubiquitin ligase TRAF6 with the TGF- $\beta$  receptor complex followed by a consequent direct activation of TAK1 by TRAF6 [143, 144].

#### *Activation of Rho-like GTPases in TGF- $\beta$ -induced EMT*

Rho-like GTPases, comprised of the Rho, Rac and Cdc42 subfamilies of proteins, are key regulators of cytoskeleton organization, cell migration and gene regulation. TGF- $\beta$  has been found to induce activation of Rho, Rac and Cdc42 in different cell systems, however, most studies have focused on the role of RhoA and its effector kinase ROCK in TGF- $\beta$ -induced EMT. During embryonic chick heart development, RhoA is necessary for normal EMT in the atrioventricular canal and antibodies against TGF- $\beta$  lead to a dramatic reduction of RhoA mRNA [145]. TGF- $\beta$  induces increased levels of active GTP-RhoA, which in lens epithelial cells occurs in a biphasic manner within the first 30 minutes and then after 1 to 2 days [146]. The activation of RhoA in response to TGF- $\beta$  in turn results in activation of ROCK, which induces the formation of actin stress fibers [147]. Moreover, TGF- $\beta$  can activate LIM kinase, a downstream target of ROCK, which inactivates the actin-depolymerizing factor cofilin. Downregulation of LIM kinase expression using siRNA inhibits TGF- $\beta$ -induced actin reorganization in fibroblasts [148]. Chemical inhibition of ROCK activity or downregulating Rho mRNA levels using antisense oligonucleotides results in inhibition of TGF- $\beta$ -induced actin reorganization and  $\alpha$ -smooth muscle actin expression that accompany EMT, without affecting Smad activation [145, 146, 149]. Complementary to these observations, TGF- $\beta$  also directly regulates RhoA activity at the tight junctions of epithelial cells. At these junctions, Par6 was found to interact with T $\beta$ RI and TGF- $\beta$  induces the association of T $\beta$ RII with the T $\beta$ RI/Par6 complex, permitting T $\beta$ RII to phosphorylate Par6 at a defined serine [150]. This event in turn recruits the E3 ubiquitin ligase Smurf1, resulting in enhanced RhoA ubiquitination by Smurf1 and degradation at tight junctions. This Smad-independent, TGF- $\beta$ -induced mechanism thus participates in the disassembly of the junctions during TGF- $\beta$ -mediated EMT, yet contrasts with the overall activation of RhoA by TGF- $\beta$ , which may be explained by the spatio-temporal regulation of Rho during EMT.

#### *Activation of PI3 kinase/Akt signaling in TGF- $\beta$ -induced EMT*

Similarly to various growth factors that act through tyrosine kinase receptors, TGF- $\beta$  has been shown to rapidly activate PI3 kinase, leading to activation of the Akt

kinase, in diverse cell systems [151–156]. Consistent with this observation, the regulatory subunit of PI3 kinase was found to interact with the T $\beta$ RII and T $\beta$ RI receptors, and the PI3 kinase activity increases upon TGF- $\beta$  stimulation [157]. Akt is a central regulator of several pathways involved in cell survival, cell size control and cell migration. Downstream from Akt, mTOR is activated resulting in activation of S6 kinase 1, which in turn regulates the translational machinery and confers increased cell size [158].

Activation of the PI3 kinase/Akt pathway by TGF- $\beta$  plays a major role in EMT. Inhibitors of PI3 kinase and Akt, or a dominant negative form of Akt, were found to inhibit TGF- $\beta$ -induced morphological transition,  $\alpha$ -smooth muscle actin expression and E-cadherin down-regulation [151, 159]. We found that during EMT, TGF- $\beta$  induces activation of the mTOR/S6 kinase 1 pathway through PI3 kinase and Akt, resulting in increased protein synthesis and cell size. Furthermore, rapamycin, an inhibitor of mTOR, inhibits the increased cell migration and invasion that are associated with TGF- $\beta$ -induced EMT, but does not block the phenotypic changes characteristic of EMT [50].

During EMT, activation of S6 kinase 1 is required for the induction of Snail1 expression whereas Snail2 expression is not affected [160]. The observation that S6 kinase 1 regulates gene transcription in addition to its effects on protein translation requires further mechanistic characterization. In a lens epithelial cell EMT model, it has been shown that the Smad-activated expression of Snail1 is required for Akt activation via PI3 kinase [76]. Furthermore, blocking the PI3 kinase/Akt pathway inhibits TGF- $\beta$ -induced Smad2 but not Smad3 phosphorylation in a mammary gland epithelial cell line, demonstrating crosstalk and feedback loops between Smad and PI3 kinase/Akt signaling that are not yet well understood [50, 151].

### Other mechanisms of signaling crosstalk in TGF- $\beta$ -induced EMT

As illustrated above, TGF- $\beta$  signaling activates non-Smad pathways that are also activated by tyrosine kinase receptors or other receptor types in response to their respective ligands. These pathways cooperate with TGF- $\beta$ /Smad signaling in the execution of the plethora of responses that constitute TGF- $\beta$ -induced EMT. Thus, activation of the Ras-Erk MAP kinase pathway, p38 MAP kinase and JNK signaling, as well as Rho GTPase signaling and the PI3 kinase/Akt pathway all enhances and contributes to TGF- $\beta$ -induced EMT. The activation of these pathways by TGF- $\beta$ , albeit at a lower level than in

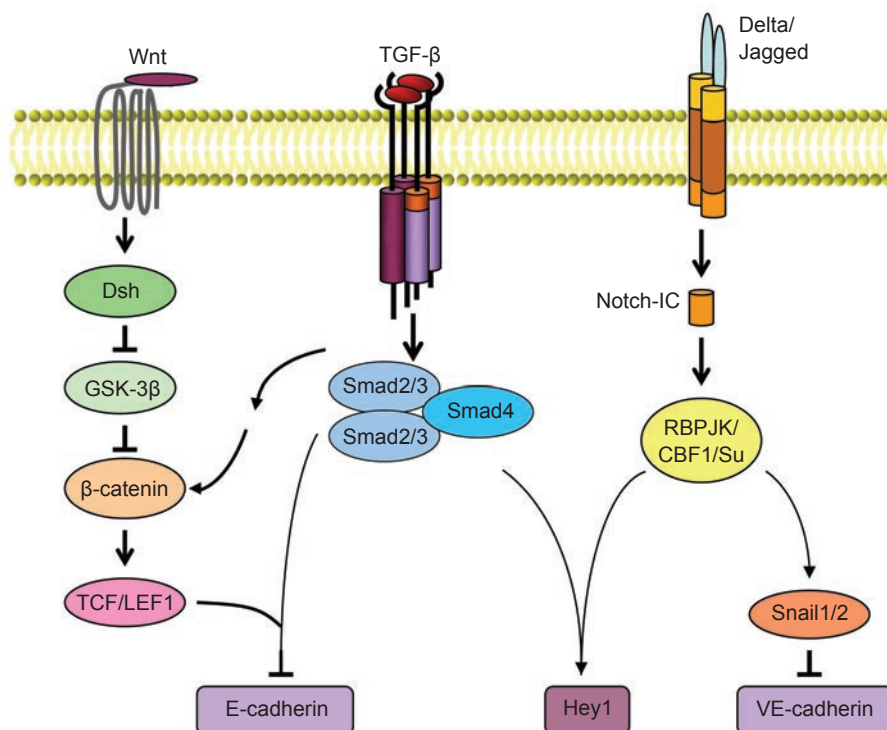
response to ligands such as growth factors, strongly suggests that TGF- $\beta$  signaling provides an inherent complementarity that supports the initiation of the EMT process. Other families of ligands activate signaling pathways that are specific to these classes of ligands and receptors, and are not activated by TGF- $\beta$  family ligands. Some of these, specifically Wnt signaling and Notch signaling, also cooperate with TGF- $\beta$  signaling in the elaboration of the EMT response (Figure 4).

### Wnt signaling

Secreted Wnt proteins do not induce EMT *per se*, but their “canonical” signaling pathway which controls target gene transcription cooperates with TGF- $\beta$  signaling to control EMT in some tissues. The canonical Wnt signaling pathway is mediated by  $\beta$ -catenin, which also functions as a component of adherens junctions and links E-cadherin to the cytoskeleton. In the absence of Wnt signaling, GSK-3 $\beta$  phosphorylates  $\beta$ -catenin and targets it for ubiquitination and degradation, thus maintaining cytoplasmic  $\beta$ -catenin at a low level. Activation of Wnt signaling inhibits GSK-3 $\beta$ , leading to accumulation of cytoplasmic  $\beta$ -catenin. This allows the formation of complexes of  $\beta$ -catenin with TCF or LEF1, two closely related transcription factors, which then translocate into the nucleus and regulate target gene transcription in response to Wnt ligands.

Crosstalk between TGF- $\beta$ /Smad signaling and Wnt signaling have been documented in developmental and pathological events. The mechanistic basis for this crosstalk has been attributed to interactions of activated Smad complexes with the Wnt-activated transcription factor complexes, either with TCF or LEF1 or with  $\beta$ -catenin [161–163]. In the context of EMT, it has been reported that Smad2 and Smad4 form a complex with LEF1 at the E-cadherin promoter, resulting in transcriptional repression in palate medial-edge epithelial cells. Smad4 and LEF1 are also required for the upregulation of mesenchymal markers vimentin and fibronectin, as well as for the acquisition of migratory behavior that characterizes cells that have undergone EMT [163].

During cardiac morphogenesis, which involves EMT induced by TGF- $\beta$ ,  $\beta$ -catenin is an indispensable component downstream of TGF- $\beta$ . TGF- $\beta$ 2 induces endocardial cells to undergo EMT and invade the cardiac jelly to form the cardiac cushion, which will give rise to atrio-ventricular valves and part of the septum. Mouse embryos that lack  $\beta$ -catenin expression showed defective septum formation, suggesting an impaired EMT process. In fact,  $\beta$ -catenin deficient endocardial cells showed dramatically reduced actin stress fiber formation and failed to invade *ex vivo* [162]. The molecular basis for the role



**Figure 4** Signaling crosstalk between the TGF- $\beta$ -activated Smad pathway and the Wnt- and Notch-activated signaling pathways during EMT. TGF- $\beta$  signaling interacts with the Wnt pathway at multiple levels, by regulating the activity of  $\beta$ -catenin, or through interactions of Smad complexes with  $\beta$ -catenin and/or TCF/LEF1. TGF- $\beta$  signaling crosstalks with the Notch pathway, through interactions of Smad complexes with RBPJK/CBF1/Su to regulate EMT-related gene transcription.

of  $\beta$ -catenin in TGF- $\beta$  signaling remains to be elucidated.

#### Notch signaling

Notch signaling occurs in a highly localized manner, when receptors and ligands, both membrane-bound, interact through cellular contacts between neighboring cells. Four receptors (Notch1-4) and two families of ligands (Delta or Delta-like and Serrate or Jagged) have been identified. Notch signaling has been shown to contribute to EMT in both tumor progression and cardiac development. When a ligand binds to the Notch receptor, Notch undergoes two proteolytic cleavage events, first an extracellular cleavage by TACE, a protease from the ADAM family, and then an intracellular cleavage by the  $\gamma$ -secretase complex, to liberate the Notch intracellular domain (NIC) from its membrane anchoring. Upon activation, the NIC enters the nucleus, where it binds to the transcription factor RBPJK/CBF1/Su(H) and converts it from a repressor to an activator for target genes, including the Hes and Hey family genes.

Signaling crosstalk between Notch and TGF- $\beta$  pathways has been demonstrated, and may be associated with tumor progression. In epithelial cells, Hey1, a well-

known target gene of Notch signaling, was found to be required for TGF- $\beta$ -induced EMT and migration. TGF- $\beta$  induces a biphasic expression of Hey1: the immediate-early induction is Smad3-dependent and Notch-independent, whereas the delayed induction is Notch-dependent, and mediated by Smad3 and Erk-induced Jagged1 expression [164].

Notch signaling has also been shown to function in the TGF- $\beta$ -mediated EMT that occurs during endocardial cushion formation. Activation of Notch1 was shown to induce Snail1 expression in mice and Snail2 expression in chicks, which then lead to repression of VE-cadherin expression and promote endocardial cell migration and invasion into the cardiac jelly. Mutations in the Notch pathway components Notch1 or RBPJK cause reduction in TGF- $\beta$ 2 and Snail1 expression, accompanied by persistent VE-cadherin expression [165, 166].

#### Perspectives

The phenotypic changes during EMT – the loss of epithelial cell-cell junctions, gain of stress fibers and invasive properties – are shared among diverse cell types, but



the molecular underpinnings often diverge among tissues from different origins. This multi-tasking is achieved by TGF- $\beta$  proteins through a complex network of effectors. TGF- $\beta$  proteins activate both Smad and non-Smad signals, which crosstalk with various signal transduction pathways at multiple levels to provide context-dependent outcomes. Hence, context-specific effects can be generated by distinct strength and duration of Smad and non-Smad pathway activation, changes in levels of interacting protein partners and availability of repressors/activators. Although our understanding of the molecular mechanism of EMT has significantly advanced during the past decade, much work is needed to define the transcriptional regulatory networks and the key target genes that drive EMT in a context-specific way.

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