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Signaling pathway cooperation in TGF-β-induced epithelial-mesenchymal transition

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Abstract

Transdifferentiation of epithelial cells into cells with mesenchymal properties and appearance, i.e. epithelial-mesenchymal transition (EMT), is essential during development, and occurs in pathological contexts, such as in fibrosis and cancer progression. Although EMT can be induced by many extracellular ligands, TGF-β and TGF-β-related have emerged as major inducers of this transdifferentiation process in development and cancer. Additionally, it is increasingly apparent that signaling pathways cooperate in the execution of EMT. This update summarizes the current knowledge of the coordination of TGF-β-induced Smad and non-Smad signaling pathways in EMT, and the remarkable ability of Smads to cooperate with other transcription-directed signaling pathways in the control of gene reprogramming during EMT.

Through “epithelial-mesenchymal transition” (EMT), epithelial cells transdifferentiate into mesenchymal cells, either partially or fully. Also endothelial cells use similar mechanisms to convert into mesenchymal cells, and this process is often named EndMT. Combining EMT with the reciprocal reversion of mesenchymal cells to an epithelial phenotype, i.e. “mesenchymal-epithelial transition” (MET), allows cell populations to transition through several rounds of EMT in development, e.g. in dorsal somite cells that arise through MET from early mesoderm and then differentiate into dermal mesenchyme and myoblasts [1]. EMT has been classified as three types depending on the physiological context. Type 1 EMT occurs in development, while type 2 EMT is seen in wound healing, inflammation and fibrosis. In cancer, type 3 EMT enables carcinoma cell invasion and dissemination, has been linked to cancer stem cell properties of some carcinomas, and contributes to the tumor stroma through conversion of epithelial and endothelial cells [2]. However, underlying these three types of EMT is a common transdifferentiation program with inherent variability depending on cell type and context. Key events in EMT are (1) dissolution of epithelial cell-cell junctions with loss of apical-basal polarity and acquisition of front-rear polarity, (2) reorganization of the cytoskeletal architecture with changes in cell shape and increased cell motility, (3) reprogramming of gene expression resulting in repression of epithelial gene expression and activation of genes that help define the mesenchymal phenotype. “Master” transcription factors, such as Snail1 or Snail2/Slug, ZEB1 or ZEB2, and Twist, drive this

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reprogramming, which often results in a switch in cadherin expression, a switch in integrin repertoire and, in many cases, expression of metalloproteases that degrade extracellular matrix (ECM) proteins, thus enabling invasive behavior [3,4].

Many EMT inducers, few pathways

Various secreted factors can induce or are required for EMT. Fibroblast growth factors (FGFs) and hepatocyte growth factor (HGF), which act through receptor tyrosine kinases (RTKs), were among the first reported EMT inducers conferring cell dispersion. Other ligands that activate RTKs or receptor-associated tyrosine kinases also induce EMT, and integrin signaling through tyrosine kinases can contribute to activation of the EMT program [1,4]. Other EMT inducers execute more direct changes in gene expression during EMT. TGF-β family proteins that act through Smad transcription factors, Wnts acting through β-catenin and TCF/LEF transcription factors, and Hedgehog proteins that activate Gli proteins, also induce or are required for EMT in diverse contexts. Aiming to provide a unifying framework for the induction and regulation of EMT, it is logical to postulate that EMT requires the cooperation of signaling pathways that coordinately direct changes in cell-cell and cell-matrix interactions, cyto-architectural remodeling, and gene reprogramming. This cooperation may depend on distinct ligands activating complementary pathways, while some ligands activate several of these, and thus depend less on additional autocrine or paracrine factors.

Among the EMT inducers, TGF-β receives substantial attention, largely because of its potency in inducing EMT in cell culture and its roles in cancer-associated EMT, while TGF-β family proteins direct EMT during development. Consequently, TGF-β-induced EMT has been better characterized than EMT in response to other inducers, and often serves as paradigm for analyses of this process. In TGF-β-induced EMT, Smads induce gene reprogramming by directly activating the expression of EMT transcription factors, and then cooperating with these in the control of target genes [4,5]. The functional dependence of Smads on interactions with DNA binding transcription factors additionally enables cooperation with other pathways at the level of gene expression [6]. TGF-β additionally induces non-Smad signaling, leading to activation of Rho GTPases, MAP kinase (MAPK) pathways and the PI3 kinase-Akt-mTOR pathway, similarly to, albeit to a lower level than RTKs [7,8]. These instruct non-transcription changes and cooperate with Smad-mediated gene expression during EMT, yet also directly regulate the stabilities and activities of Smads [7,8]. The roles of Smads in gene reprogramming, microRNA-mediated control and differential mRNA splicing during EMT have recently been extensively reviewed [9,10]. This update focuses on the control of EMT by non-Smad pathways that are activated by TGF-β family proteins (Fig. 1), and crosstalk of Smads with other transcription-directed signaling pathways (Fig. 2).

Roles of Rho, Rac and Cdc42 GTPases in EMT

In EMT, Rho, Rac and Cdc42 GTPases direct changes in epithelial cell junctions, redirect the apical-basal polarity into a front-rear polarity, and orchestrate the cytoskeletal organization that enables lamellipodia and filipodia formation [11,12]. As with EMT

inducers that act through RTKs, TGF-β proteins induce changes in RhoA activity as receptor-proximal events that do not require protein synthesis (Fig. 1).

The integrity of the epithelial cell junctions is coupled to protein complexes that maintain apical-basal polarity, with the Par and Crumbs complexes linked to apical tight junctions, and the Scribble complex localized at lateral adherens junctions [11,12]. Rho activation results in dissolution of epithelial junctions, and loss of cell contacts and apical-basal polarity. Cdc42 regulates tight junction integrity through association with the Crumbs complex [13], yet can also control polarity, in association with the Par complex, through effects on Rac GEFs that activate Rac1 [14]. TGF-β-induced EMT involves RhoA degradation at tight junctions, resulting from Par6 phosphorylation by the type II TGF-β receptor at tight junctions and RhoA ubiquitylation [15], complementing the downregulation of expression of epithelial junction proteins [4,9,10]. As the cells acquire front-rear polarity, Par and Scribble complexes, and Patj of the Crumbs complex, localize at the leading edge, where Rac1 and Cdc42 promote actin reorganization and membrane protrusions, and Rac1 drives integrin clustering [9,11,12]. At the trailing edge, RhoA enables cell de-adhesion, inhibits Rac and prevents Par complex formation [11,12].

Upon RhoA activation, the Rho-associated kinase, ROCK, cooperates with the formin mDIA1 to promote actin polymerization, e.g. in actin stress fibers and lamellipodia. ROCK also induces myosin light chain phosphorylation, which enhances actomyosin contractility and contributes to cell retraction, and activates LIM kinase (LIMK) to inactivate the actin severing cofilin [16]. ROCK and LIMK activation were shown to be required in TGF-β- and BMP-induced EMT [17,18]. Rac1 and Cdc42 activation induce lamellipodia and filipodia formation, and resultant PAK1 activation promotes cell spreading and motility [19].

Rho GTPase-mediated control of actin polymerization also connects to changes in gene expression that are required for cell motility. Nuclear actin binds ribonucleoprotein complexes and participates in chromatin remodeling [20]. RhoA and actin also control the activities of the transcription factor SRF and its co-activators, the myocardin-related transcription factors (MRTFs) [21], which, among other genes, activate the gene encoding α-smooth muscle actin (α-SMA) [21], a myofibroblast protein often expressed in EMT. TGF-β-activated Smads also cooperate with MRTFs in the control of α-SMA expression [22].

Interactions with GAPs, GEFs, and GDIs, and polarity complexes, and mutual interactions control the activities of Rho GTPases in EMT, with Rho and Rac activities often correlating inversely. TGF-β induces the expression of the Rho-associated GEF-H1, which enhances RhoA activity and contributes to α-SMA expression [23]. Depletion of GEF-H1, thus decreasing RhoA activity, was shown to attenuate mesenchymal marker and increase E-cadherin expression in liver carcinomas [24]. Integrin stimulation of the focal adhesion kinase, FAK, can control Rho GEFs and GAPs, and, consequently, actin dynamics and cell membrane protrusions [25]. Src activation can promote phosphorylation and degradation of the Rac GEF Tiam1, leading to adherens junction disassembly and Erk MAPK activation [26]. Binding of RhoA to p120-catenin, which associates with E-cadherin, inhibits RhoA activity, leading to enhanced Rac and Cdc42 activities and cell motility [27]. The

transcription corepressor ZNF703 enhances p120-catenin expression, leading to decreased RhoA and increased Rac1 activity, thus contributing to EMT and cell motility [28]. TGF-β also induces the expression of the RhoA GEF NET1A, leading to RhoA activation, but long-term TGF-β treatment activates miR-24 expression, which inhibits NET1A expression, thus promoting adherens and tight junction disruption, and EMT [29]. Finally, activation of RhoC during EMT of colon carcinoma cells enhances cell migration, suggesting that cell migration may require RhoC [30].

The PI3K-Akt-mTOR pathway in EMT

Like EMT inducers that act through RTKs or membrane-associated tyrosine kinases, TGF-β family proteins activate the PI3K-Akt pathway, leading to mTOR activation and enhanced protein synthesis [31]. PI3K or Akt inhibition arrests EMT [32,33], indicating an essential role of this pathway in EMT.

Although encoded by distinct genes, the roles of Akt1 and Akt2, which are generally expressed in epithelial cells, are usually not distinguished, but some studies note intriguing differences. In one study, silencing Akt1 but not Akt2 expression in IGF-1- or EGF-stimulated epithelial cells cooperates with the enhanced Erk MAPK signaling in promoting EMT and cell motility, whereas Akt2 controlled primarily cell proliferation and survival [34]. In another context, however, silencing Akt1 expression was seen to enhance TGF-β-induced EMT [35], and silencing of Akt2 has been shown to attenuate the increased migration and invasion of cells that underwent EMT upon expression of Twist [36].

Akt2 activation in response to TGF-β has also been linked to selective translational control in EMT. Phosphorylation of hnRNPE1, a selective RNA binding protein, by Akt2 reverses translation inhibition, and thus induces expression, of Dab2 and ILEI, which are required for TGF-β-induced EMT [37,38]. This role of Akt2 may be initiated by TGF-β-induced Tyr phosphorylation of ShcA that enables recruitment of the p85 regulatory subunit of PI3K and FAK, resulting in Akt2 phosphorylation [39].

Downstream from Akt, mTOR activation results in increased protein synthesis, cell size and migration. Selective inactivation of mTOR complex 1, which controls protein synthesis, confers decreased cell size, without affecting the EMT phenotype and gene expression, but impairs migration and invasion [40]. In contrast, mTOR complex 2 inactivation blocks TGF-β-induced EMT without apparent effect on non-induced epithelial cells [32]. In another system, mTOR complex 1 is required for E-cadherin downregulation and EMT transcription factor expression, perhaps dependent on GSK3β phosphorylation and inhibition by Akt [41]. The inability of cells to transition through EMT when mTOR is inactivated may relate in part to impaired RhoA and/or Rac1 activation, affecting cytoskeletal remodeling [32,33]. Additionally, Akt destabilizes adherens junction complexes through phosphorylation of the nectin-associated protein afadin [42].

Akt activation also impacts Smad activation in response to TGF-β, and, thus, Smad-mediated transcription responses in EMT. For example, association of Akt with unphosphorylated Smad3 can sequester Smad3, thus attenuating TGF-β-induced Smad activation [43]. Considering the key roles of Smads in TGF-β-induced EMT, this attenuation

is expected to inhibit the expression and activity of EMT transcription factors. However, TGF-β was shown to inhibit insulin-induced Akt-Smad3 association, thus enhancing Smad activation [44]. Additionally, since phosphorylation of Smads by GSK3β leads to ubiquitylation and degradation, inactivation of GSK3β by Akt may enhance Smad-mediated transcription in EMT.

Besides targeting the Smads, Akt targets the EMT transcription factors themselves. Snail1 is phosphorylated by GSK3β, leading to its ubiquitylation and degradation, and inhibition of GSK3β by Akt stabilizes Snail1, thus enhancing EMT [45,46]. Similarly, GSK3β phosphorylates and destabilizes NF-κB, and Akt activation enhances NF-κB-mediated contributions to EMT [47]. Akt also phosphorylates Twist1, enhancing its activity and promoting Twist1-mediated expression of TGF-β2, which then promotes EMT [48]. In HER2-induced EMT, Akt was shown to phosphorylate heat shock factor-1, which activates Snail2 expression, thus promoting EMT [49]. Finally, the induction of Akt2 expression by Twist [36] provides yet another level of crosstalk between the PI3K-Akt pathway and EMT transcription factors.

MAP kinase pathways control EMT

TGF-β family proteins induce MAPK pathways, but their activation levels are weaker than in response to many RTK ligands. Erk1/2 MAPK signaling in response to TGF-β is initiated by ShcA phosphorylation on Tyr by the type I TGF-β receptor [50], whereas activation of p38 MAPK and/or JNK results from the recruitment of the E3 ubiquitin ligases TRAF4 or TRAF6 to the TGF-β receptor complex and subsequent activation of the TAK1 kinase [51-53]. Initiation of EMT is often accompanied by activation of Erk1/2 MAPK, Erk5 MAPK, p38 MAPK and/or JNK, and their upstream kinases (Fig. 1).

Pharmacological inhibition of Erk1/2 MAPK activation prevents both TGF-β- and HGF-induced EMT [54]. In response to HGF, Erk1/2 MAPK activates the expression of the transcription factor EGR-1, which induces Snail1 expression and EMT [55]. In IGF-1-induced EMT, activation of ZEB1 expression requires the Erk1/2 MAPK pathway [56], whereas, in radiation-induced EMT, the Erk1/2 MAPK pathway induces GSK3β phosphorylation, thus attenuating GSK3β-mediated decrease in Snail1 activity [57]. Finally, in cells with Ras- or Raf-induced EMT, the Erk-activated ribosomal S6 kinase RSK induces gene expression, in part dependent on the transcription factor Fra1, that contributes to increased motility and invasion [58]. Consequently, activation of the Erk1/2 MAPK allows scenarios of transcriptional cooperation of TGF-β-activated Smads with c-Jun and/or Fra1.

Additionally, direct phosphorylation of receptor-activated Smads by Erk MAPK allows Erk MAPK to control the nuclear translocation of Smad complexes and repress or enhance TGF-β- or BMP-induced gene responses [59]. This crosstalk appears to depend on the nature of the Smad and Smad target genes [11, 60], and their control by other pathways and functionally interacting proteins in transcription complexes. Thus, Smad and Erk MAPK signaling cooperate to control gene reprogramming during EMT.

Some studies specifically implicate Erk2 as key effector in EMT. EMT induced by oncogenic Ras requires Erk2, but not Erk1, with Erk2 acting in part through activation of

expression of Fra1 and its target genes, thus inducing ZEB1/2 expression [61]. Supporting a key role of Erk2, TGF-β-induced EMT in prostate cancer cells expressing activated Ras requires activation of MEK1, and not MEK2, and Erk2, as well as c-Myc, which is phosphorylated by Erk2 [62]. Furthermore, EMT of colon cancer cells, resulting from increased expression of PLAC8, a protein involved in colon cancer, was shown to correlate with and depend on Erk2 phosphorylation [63].

In parallel with the Erk1/2 MAPK pathway, TGF-β and EMT inducers that act through RTKs, activate MEK5, leading to Erk5 MAPK (Bmk1) signaling [64]. In keratinocyte wound healing, Erk5 is required for Snail2 expression and motility [65], whereas, in TGF-β-treated hepatocytes, Erk5 stabilizes Snail1 through GSK3β inactivation, promoting EMT [66]. Conversely, silencing Erk5 expression was shown to enhance Akt/GSK3β signaling, Snail1 expression and EMT [67]. These data suggest that in some contexts, Erk5 MAPK may antagonize Erk2 MAPK in the control of EMT.

Like many cytokines, TGF-β family proteins induce p38 MAPK activation, and inactivation of p38 MAPK prevents TGF-β-induced EMT [68]. In gastrulation, E-cadherin downregulation, EMT and migration of mesoderm from the primitive streak are defective when p38 MAPK activation is impaired [69]. In TGF-β-induced EMT of lung alveolar cells, p38 MAPK and Smads cooperate in gene reprogramming, with distinct gene expression changes impaired when p38 MAPK is inactivated [70]. p38 MAPK can cooperate with Smad3/4 at TGF-β target genes through the transcription factor ATF2 [71]. Inhibition of p38 MAPK in some cancer cells allows for E-cadherin re-expression and reversal to an epithelial phenotype, and may play a role following cancer dissemination [72]. Conversely, however, p38 MAPK activation attenuates E-cadherin downregulation during EMT of mesothelial cells, by suppressing NF-κB signaling, allowing p38 MAPK to act as “brake” in the control of EMT. In that system, p38 MAPK activation promotes Snail1 and represses Twist1 expression [73]. The differential control of EMT by p38 MAPK may depend on the activation of converging signaling pathways and cell type. In how far Smad1 or Smad3 phosphorylation by p38 MAPK [59] contributes to EMT remains to be appreciated.

Lastly, EMT induced by TGF-β requires JNK activation, which in tracheal epithelial cells has been attributed to JNK1 and not JNK2 [74]. Further supporting a role of JNK activation in EMT, JNK deficiency in $p53^{-/-}$ mouse embryonic fibroblasts promotes an epithelial phenotype [75]. At the molecular level, JNK may contribute to EMT through phosphorylation, and consequent stabilization, of Twist1 [76], and phosphorylation of Smad1 and Smad3, resulting in enhanced Smad-mediated responses [77]. The control of EMT by JNK signaling, and differential roles of JNK1 and JNK2 require further studies.

Smads enable transcriptional crosstalk with other transcription-directed EMT pathways

In TGF-β- or BMP-induced EMT, the cells coordinate Smad-directed gene expression with non-transcription effects of non-Smad signaling pathways. Additionally, these pathways target Smads for phosphorylation or other modifications, and thus define their function [7,8,59]. Finally, in the nucleus, the activated Smad complexes cooperate with DNA binding

transcription factors and coregulators at regulatory gene sequences [4,6], thus providing a platform for functional integration of Smads with other EMT pathways in the control of gene expression (Fig. 2).

Wnt proteins induce gene expression changes using β-catenin and TCF/LEF transcription factors, and “non-canonical” signaling that activates Rho GTPases and MAPK pathways [78]. In development, Wnts control or are required for EMT, e.g. in gastrulation, neural crest cell delamination and heart valve development, in which also TGF-β family proteins drive EMT. Pathologically, Wnts are implicated in EMT in fibrosis, e.g. in diabetic nephropathy, and carcinomas, again contexts in which TGF-β induces EMT. In both developmental and pathological EMT, TGF-β/BMP proteins and Wnts were shown to cooperate, e.g. in cardiac development [79] and generation of cancer stem cells with EMT properties [80].

Upon Wnt signaling, TCF/LEF transcription factors activate genes that contribute to EMT, and TGF-β- and BMP-activated Smads were shown to cooperate with β-catenin or TCF/LEF in the control of gene expression, thus enabling interdependent transcriptional crosstalk. For example, TGF-β-induced EndMT and endocardial EMT require β-catenin, with convergence of Wnt/β-catenin and Smad signaling [81]. In pulmonary epithelial cells, β-catenin is required for TGF-β-induced EMT, and cooperates with Smad3 in the control of α-SMA expression [82]. Similarly, TGF-β-induced EMT of kidney epithelial cells depends on association of Smad3 with β-catenin [83].

At another level, GSK3β mediates crosstalk between Wnt and TGF-β/BMP signaling. GSK3β phosphorylates proteins with key roles in EMT, including β-catenin, and Snail, ZEB and Twist, thus targeting them degradation [80]. Deactivation of GSK3β in response to Wnts not only activates Wnt target gene expression in EMT, but also stabilizes EMT transcription factors [78,84]. Since GSK3β targets Smad1 for degradation, Wnt signaling additionally stabilizes Smad1, thus enhancing BMP signaling [85], and promotes Wnt cooperation with BMP signaling. Such cooperation controls cardiac progenitor cell migration [79], and directs segmental patterning in Drosophila [86]. Wnts may similarly cooperate with TGF-β signaling, since GSK3β also phosphorylates Smad3, thus attenuating TGF-β signaling [87,88]. Finally, Wnts induce the expression of some TGF-β/BMP ligands, and vice versa [89], enabling reciprocal control of ligand production.

The versatility of receptor-activated Smad association with transcription factors also enables TGF-β/BMP cooperation with Notch signaling. Notch induces or is required for EMT in several contexts, including in cardiac development and carcinomas, and integrates with TGF-β/Smad signaling in EMT. Stimulation of Notch by Delta-like or Jagged ligands induces the release of the Notch intracellular domain (NICD) from the membrane, which then acts as transcription cofactor with RBPJ/CSL [90]. The NICD has been shown to associate with Smad1 and Smad3, resulting in coordinate control of target genes [91-93]. In EndMT, TGF-β-induced Smad complexes and Notch-activated RBPJ/CSL complexes cooperate at target genes [94,95], and Smad4 cooperation with RBPJ/CSL controls N-cadherin expression [96]. Additionally, TGF-β can induce expression of the Notch ligand Jagged1, and the transcription factor Hey1, downstream from Notch, and silencing either gene, or Notch inactivation, prevents TGF-β-induced EMT [97]. Through this crosstalk,

Notch signaling is required for a subset of TGF-β-induced gene responses in epithelial cells, including some that control EMT [97,98], and controls the duration and amplitude of TGF-β target gene responses [99]. The integration of TGF-β/BMP and Notch signaling in EMT has been well studied in heart valve development, where Notch signaling through Hey represses BMP2 expression and signaling [94], controls Smad expression [94], and is required for TGF-β-induced EMT [100].

Hedgehog, in particular Sonic Hedgehog (Shh), signaling can also lead to, or is required for, EMT in carcinomas. Hedgehog ligands act through Gli transcription factors [101] that can induce EMT-associated changes in gene expression [102]. Furthermore, PI3K-Akt signaling was shown to be required for Shh-induced EMT [103]. Conversely, however, silencing of Gli1 promotes EMT of pancreas carcinoma cells [104], suggesting attenuation of EMT by Shh/Gli1 signaling. Association of Gli1 with Smad4 allows for direct Shh/Gli1 cooperation with TGF-β/Smad signaling in the control of TGF-β target genes [105]. Whether combinatorial targeting allows for crosstalk in the control of EMT-associated gene reprograming remains to be shown. TGF-β-induced expression of Gli1 and Gli2 [106] allows for additional crosstalk with Hedgehog signaling of relevance in EMT.

The transcription coactivators TAZ and YAP regulate cell proliferation and differentiation, and are controlled by the Hippo pathway [107]. TAZ defines mesenchymal cell differentiation [108], and increased TAZ levels, frequently observed in cancers, promote EMT [109-111] and cancer stem cell generation, and cancer progression [110,111]. TAZ associates with TGF-β-induced Smad complexes [112], while YAP forms complexes with BMP- and TGF-β-activated Smads [113,114]. TAZ and YAP facilitate nuclear translocation of Smads and thus contribute to efficient regulation of TGF-β target genes [112,113]. They also cooperate with Smads in the control of target gene expression, and this integration is facilitated through interactions with components of the Mediator complex [112,113,115]. Differential TAZ or YAP binding with Smad complexes and cooperation of Smads with diverse DNA-binding transcription factors set the stage for differential regulation of Smad-mediated and EMT-associated gene reprogramming by the Hippo pathway. Finally, Hippo signaling controls overall Wnt signaling, while also exhibiting differential effects at Wnt target genes through interactions of TAZ or YAP with β-catenin and Wnt-activated transcription complexes [114,116]. The regulation of TAZ and YAP by Hippo signaling may control the cooperation of TGF-β/Smad signaling with Wnt signaling in EMT.

As is apparent from these examples, Smads enable functional crosstalk with signaling and transcription pathways of importance for the reprogramming of gene expression in EMT. These observations could be extended with additional examples of functional interactions, as, for example, Smads functionally interact with NFκB, an effector of inflammatory cytokines that also contributes to EMT [117]. TGF-β-activated Smads also associate with, and potentiate the activities of the hypoxia-induced transcription factor HIF-1α, which plays a central role in hypoxia-induced EMT in tumors [118]. This extensive versatility of Smad-mediated control over other pathways, together with the activation of non-Smad signaling pathways, allows TGF-β/BMP signaling to act as a critical inducer of the EMT process.

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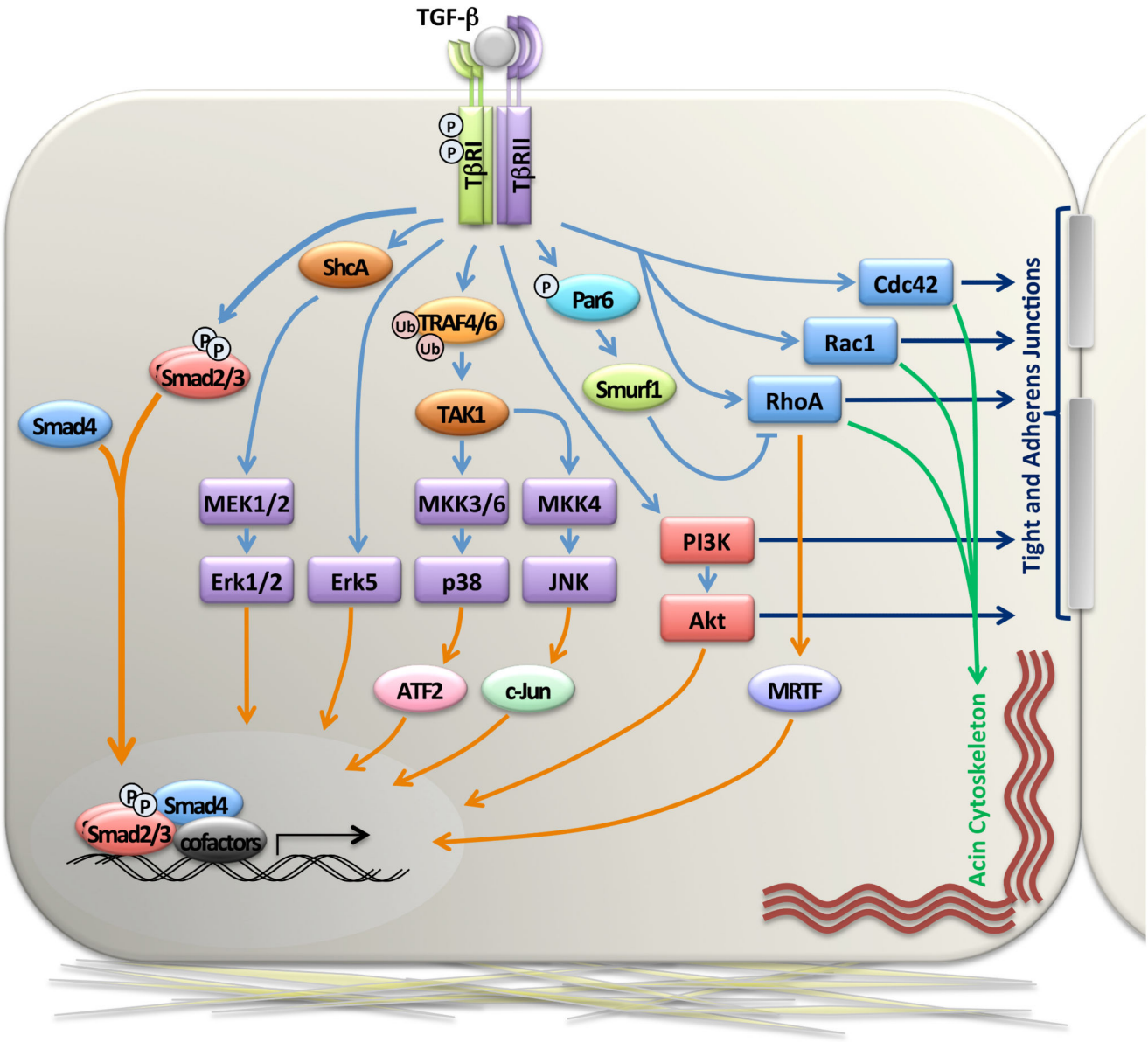

Figure 1.

TGF-β-activated non-Smad pathways in epithelial-mesenchymal transition. In addition to the well-established Smad signaling pathway that controls target gene transcription during EMT, TGF-β family proteins also activate non-Smad pathways. These pathways have non-transcriptional roles in EMT, including dissolution of epithelial junctions, cytoskeletal reorganization and motility, and translational control. They also target Smads and thus help define their functions, while also controling the expression and activation of transcription factors, with which Smad complexes cooperate in the control of gene expression.

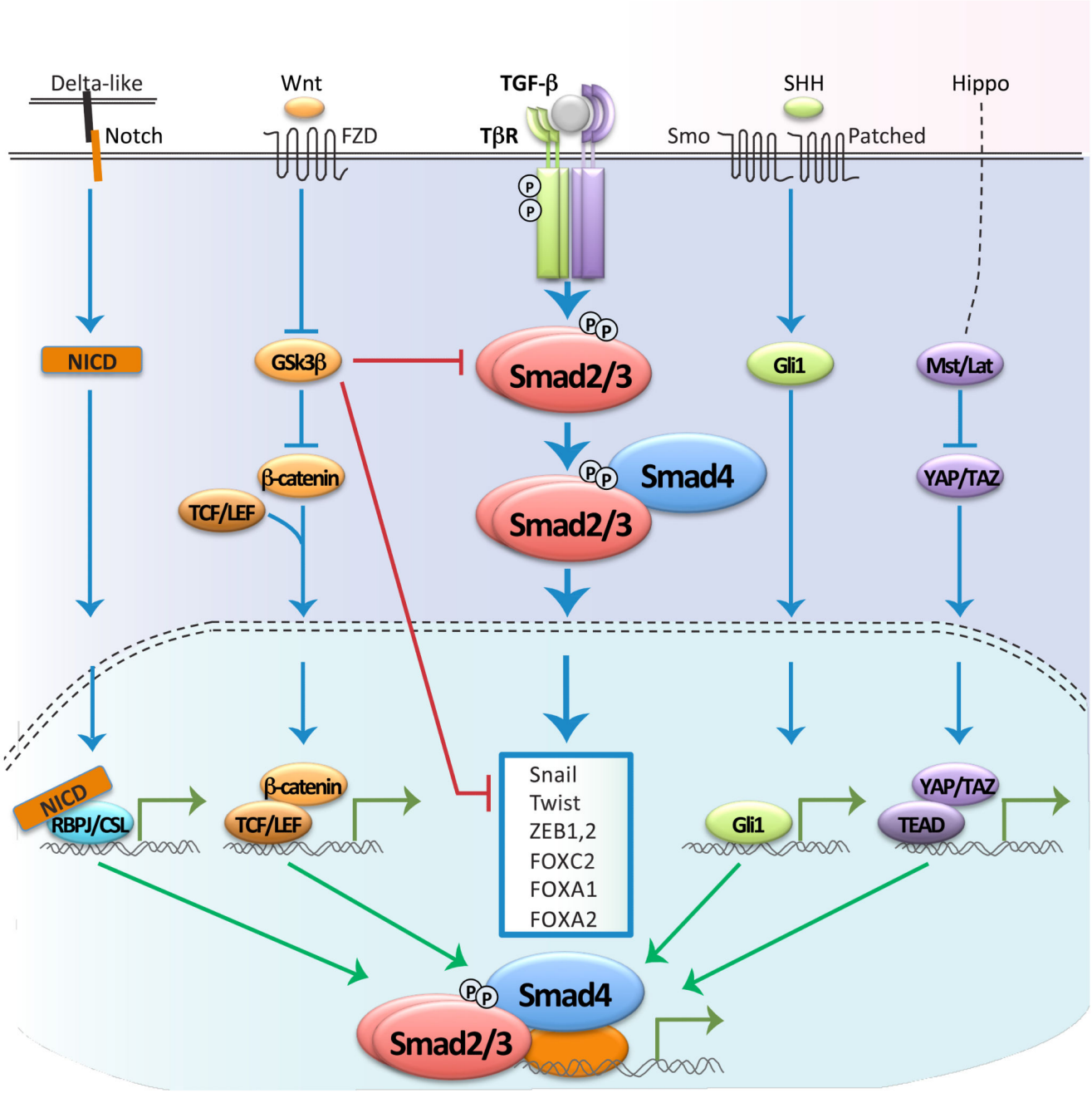

Figure 2.

Smad complexes can cooperate with transcription pathways in the control of gene expression in epithelial-mesenchymal transition. The Notch, Hedgehog, Wnt and Hippo signaling pathways direct transcriptional activation or repression of target genes by their respective effectors. TGF-β/BMP-activated Smad complexes control target gene transcription in cooperation with an extensive array of DNA binding transcription factors and coactivators and corepressors. This versatility enables the Smad complexes to associate and functionally cooperate with transcription effectors of Notch, Hedgehog, Wnt and Hippo

signaling, and, thus, to coordinately control gene reprogramming in EMT. The genes encoding EMT transcription factors are known to be directly activated by TGF-β-activated Smad complexes and may represent examples of such coordinate control. In addition to the pathways shown and discussed, Smads can also coordinately control gene expression with IκB/NFκB, STAT transcription factors and an array of other transcription factors that are respond to signaling pathways.