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Molecular mechanisms of epithelial–mesenchymal transition

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Abstract

The transdifferentiation of epithelial cells into motile mesenchymal cells, a process known as epithelial–mesenchymal transition (EMT), is integral in development, wound healing and stem cell behaviour, and contributes pathologically to fibrosis and cancer progression. This switch in cell differentiation and behaviour is mediated by key transcription factors, including SNAIL, zinc-finger E-box-binding (ZEB) and basic helix-loop-helix transcription factors, the functions of which are finely regulated at the transcriptional, translational and post-translational levels. The reprogramming of gene expression during EMT, as well as non-transcriptional changes, are initiated and controlled by signalling pathways that respond to extracellular cues. Among these, transforming growth factor- β (TGF β) family signalling has a predominant role; however, the convergence of signalling pathways is essential for EMT.

The idea that epithelial cells can downregulate epithelial characteristics and acquire mesenchymal characteristics arose in the early 1980s from observations made by Elizabeth Hay¹, who described epithelial to mesenchymal phenotype changes in the primitive streak of chick embryos. Initially described as ‘epithelial to mesenchymal transformation’, this differentiation process is now commonly known as epithelial–mesenchymal transition (EMT) to emphasize its transient nature; mesenchymal–epithelial transition (MET) describes the reverse process. The ability of epithelial cells to transition into mesenchymal cells and back, either partially or fully, illustrates an inherent plasticity of the epithelial phenotype. During EMT, epithelial cells lose their junctions and apical–basal polarity, reorganize their cytoskeleton, undergo a change in the signalling programmes that define cell shape and reprogramme gene expression; this increases the motility of individual cells and enables the development of an invasive phenotype^{2,3}. EMT is integral to development, and the processes underlying it are reactivated in wound healing, fibrosis and cancer progression^{3–5}. EMT and MET also regulate embryonic stem cell differentiation, induced pluripotency and cancer stem cell behaviour (BOX 1). Depending on the tissue and

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signalling contexts, epithelial cells may lose only some characteristics or may show some epithelial and mesenchymal properties; this can be considered as partial EMT. Epithelial plasticity, therefore, covers a range of changes in differentiation and cell behaviour, with full epithelial integrity at one end and EMT as the full realization of a plasticity response at the other end⁶. Cell culture models of EMT enable the complex molecular mechanisms that underlie it to be characterized, and they have revealed cooperation and crosstalk between signalling pathways and transcriptional, translational and post-translational regulation.

This Review describes the molecular processes that lead to EMT. It first outlines the main changes that occur in cells undergoing EMT, before focusing on mechanisms that direct changes in gene expression and the signalling pathways that control the initiation and progression of EMT.

EMT in development and disease

Epithelia are established as single cell layers or multilayer tissues with various functions. Epithelial cells show apical–basal polarity, adhere and communicate with each other through specialized intercellular junctions and are positioned on a basement membrane that helps to define their physiology; for example, through the interaction of basement membrane proteins with integrins. In this way, epithelia function as permeability barriers that delineate tissues and organs⁶. The transition of epithelial cells into mesenchymal cells, in development or pathologically, follows a common and conserved programme with hallmarks. However, it also has an inherent flexibility and some variation, which depends on the cell type, tissue context and signals that activate the EMT programme. Indeed, EMT has been assigned three distinct subtypes, which are dependent on the physiological context⁴. Furthermore, the plasticity of the epithelial phenotype enables cells to transition through multiple rounds of EMT and MET (FIG. 1).

In all tissue contexts, key events in EMT are the dissolution of the epithelial cell–cell junctions; loss of a pical–basal polarity and acquisition of a front–rear polarity; reorganization of the cytoskeletal architecture and changes in cell shape; downregulation of an epithelial gene expression signature and activation of genes that help to define the mesenchymal phenotype; increased cell protrusions and motility; and, in many cases, an ability to degrade extracellular matrix (ECM) proteins to enable invasive behaviour (FIG. 1). Additionally, cells that have undergone EMT acquire resistance to senescence and apoptosis³. Similarly to epithelial cells, endothelial cells can transition into a mesenchymal phenotype, and the mesenchymal transdifferentiation of epithelial or endothelial cells can result in the expression of myofibroblast genes (BOX 2).

Deconstructing cell junctions and polarity

Specialized cell surface protein complexes form epithelial cell–cell junctions that are essential for epithelial integrity. Vertebrate cells contact each other through subapical tight junctions, adherens junctions and desmosomes at lateral surfaces, and scattered gap junctions at lateral surfaces⁶ (FIG. 1). Upon the initiation of EMT, these junctions are deconstructed and the junction proteins are relocalized and/or degraded. The dissolution of tight junctions during EMT is accompanied by decreased claudin and occludin

expression, and the diffusion of zonula occludens 1 (ZO1; also known as TJP1) from cell–cell contacts⁶. During the destabilization of adherens junctions, epithelial cadherin (E-cadherin) is cleaved at the plasma membrane and subsequently degraded⁷. Consequently, β -catenin can no longer interact with E-cadherin and it is either degraded or protected from degradation (for example, in response to WNT signalling), so that it can act in transcription⁸. p120 catenin (also known as catenin δ 1) also accumulates in the nucleus and participates in transcription following a decrease in E-cadherin levels⁹. EMT initiation also disrupts desmosomes^{6,7}, and the integrity of gap junctions is compromised by decreased connexin levels¹⁰. As EMT progresses, the expression of junction proteins is transcriptionally repressed, which stabilizes the loss of epithelial junctions^{11,12}.

Instructed through contacts with the basement membrane, epithelial cells display apical–basal polarity, which is organized by polarity complexes that are physically and functionally integrated with the cell junction architecture. In vertebrate cells, partitioning-defective (PAR) complexes (comprising PAR6, PAR3 and atypical protein kinase C (aPKC)) and Crumbs complexes (comprising Crumbs (CRB), protein associated with Lin-7 1 (PALS1) and PALS1-associated tight-junction protein (PATJ)) localize apically in association with tight junctions and define the apical compartment; Scribble complexes (comprising Scribble (SCRIB), Discs large (DLG) and lethal giant larvae (LGL)) define the basolateral compartment¹³ (FIG. 1). Consequently, the dissolution of epithelial junctions during EMT confers a loss of apical–basal polarity⁶. In support of this association, decreased expression of E-cadherin in tumour cells prevents SCRIB from interacting with the lateral plasma membrane¹⁴, and decreasing SCRIB or E-cadherin expression reduces adhesion and increases cell motility¹⁵. After EMT is initiated, the expression of polarity complex proteins, such as CRB3 and LGL2, is repressed¹⁶, which further destabilizes the polarized phenotype.

Cytoskeletal changes and motility

Cells that undergo EMT reorganize their cortical actin cytoskeleton into one that enables dynamic cell elongation and directional motility^{2,7,17} (FIG. 1). New actin-rich membrane projections facilitate cell movement and act as sensory extensions of the cytoskeleton. These projections include sheet-like membrane protrusions called lamellipodia and spike-like extensions called filopodia at the edge of lamellipodia¹⁸. Actin-rich invadopodia exert a proteolytic function in ECM degradation, thus facilitating cell invasion^{18,19}. Finally, EMT is characterized by increased cell contractility and actin stress fibre formation. These dynamic changes in actin organization are probably mediated by regulatory proteins such as moesin²⁰, but the molecular mechanisms controlling F-actin dynamics during EMT remain to be elucidated.

RHO GTPases regulate actin dynamics and control actin rearrangement during EMT. Among these, RHOA promotes actin stress fibre formation, whereas RAC1 and CDC42 mainly promote the formation of lamellipodia and filopodia. The activation of RHO GTPases is tightly regulated by guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs)^{7,18}. Following RHO activation at the onset of EMT, the RHO-

associated kinase (ROCK) cooperates with the formin diaphanous 1 (DIA1) to promote actin polymerization. ROCK also induces myosin light chain phosphorylation, which enhances acto myosin contractility and activates LIM kinase (LIMK) to in activate the actin severing factor cofilin²¹. Following activation by RAC1 or CDC42, the kinase p21 activated kinase 1 (PAK1) activates targets that are involved in cell spreading and motility²². RHO GTPases also regulate the formation of cell–cell junction complexes and the stability of adherens junctions. Cytoplasmic p120 catenin, which is generated during EMT, represses RHO activity. This may facilitate the dissociation of cell junctions and activates RAC and CDC42 to induce the formation of membrane protrusions and cell motility²³.

The conversion from apical–basal polarity to front– rear polarity involves interplay between RHO GTPases and proteins that define apical–basal polarity^{24,25}. To achieve directional polarity, the PAR and Scribble complexes together with PATJ of the Crumbs complex relocate to the leading edge of the cell, where RAC1 and CDC42 activate actin polymerization and membrane protrusion formation. By contrast, RHOA localizes at the rear of the cell and promotes the disassembly of adhesion complexes and cell retraction²⁴. PI3K plays a central part in initiating front–rear polarity and participates in the recruitment of CDC42 and RAC GEFs to the leading edge²⁴. The front–rear polarity is maintained through feedback mechanisms that involve polarity proteins and small GTPases. For example, RAC1 activation at the leading edge stimulates PI3K, which recruits RAC GEFs. RHO GTPases also control the reorganization of the microtubule cytoskeleton. For instance, RAC1 promotes microtubule polymerization and is itself activated by it at the leading edge of cells, which sets up a positive feedback loop that participates in the reorganization of the microtubule cytoskeleton²⁶. Additionally, RAC1 promotes the clustering of integrins towards the front of the cell, and the localization of PAR3 and aPKC at the front of the cell requires PATJ, PALS1 and PAR3 (REF. 24). At the rear of the cell, RHOA signalling inhibits RAC1 activation and prevents formation of PAR complexes^{24,25}. The reorganization of the cytoskeletal architecture and polarity complexes, which result in cell shape changes, cell elongation, membrane protrusions and front–rear polarity, are essential in EMT and enable directional migration.

Overview of changes in gene expression

During EMT, cells downregulate the expression of epithelial proteins, including those that are part of cell junction complexes^{6,11} (FIGS 1,2). They also redirect their gene expression programme to promote changes in cytoskeletal architecture, to promote adhesion to mesenchymal cells and to alter the interaction of cells with the ECM^{7,12,17}. Variations in EMT-associated gene expression profiles are apparent and depend on cell and tissue type and on the degree of progression towards mesenchymal differentiation.

A hallmark of EMT is the downregulation of E-cadherin to reinforce the destabilization of adherens junctions. Additionally, the repression of genes encoding claudins and occludin, and desmoplakin and plakophilin, stabilizes the dissolution of apical tight junctions and desmosomes, respectively⁶. These changes in gene expression prevent the *de novo* formation of epithelial cell–cell junctions and result in the loss of the epithelial barrier function¹¹. The repression of genes encoding epithelial (or endothelial (BOX 1) cell junction proteins is

accompanied by the activation of genes, the protein products of which promote mesenchymal adhesion. Specifically, the downregulation of E-cadherin is balanced by the increased expression of mesenchymal neural cadherin (N-cadherin), which results in a 'cadherin switch' that alters cell adhesion^{7,27}. Through this switch, the transitioning cells lose their association with epithelial cells and acquire an affinity for mesenchymal cells through homotypic N-cadherin interactions; these interactions are weaker than homotypic E-cadherin interactions and facilitate cell migration and invasion²⁸. N-cadherin connects to the cytoskeleton through α -catenin and β -catenin and also interacts with p120 catenin, signalling mediators²⁹ and receptor tyrosine kinases (RTKs) such as platelet-derived growth factor (PDGF) and fibroblast growth factor receptors (FGFRs)^{7,30}. EMT also activates the expression of neural cell adhesion molecule (NCAM), another adhesion molecule that interacts with N-cadherin to modulate the activity of RTKs that are associated with it^{7,31}. NCAM interacts with the SRC family tyrosine kinase FYN to facilitate the assembly of focal adhesions, migration and invasion³².

Alterations in the expression of genes encoding cytoskeletal and polarity complex proteins also contribute to EMT. The intermediate filament composition changes with the repression of cytokeratin and the activation of vimentin expression⁶. Keratin and vimentin filaments regulate the trafficking of organelles and membrane-associated proteins, but they show differences in the proteins that they target to the membrane. For example, keratin, but not vimentin, directs E-cadherin to the membrane³³. Changes in intermediate filament composition also enable cell motility, possibly owing to the interaction of vimentin with motor proteins³⁴. To enable directional motility, cells undergoing EMT repress the expression of the apical Crumbs complex proteins Crumbs3 and PATJ, and of basolateral Scribble complex proteins, such as LGL2 (REFS 7,16,24).

Remodelling of the ECM and changes to cell interactions with the ECM are essential in the initiation and progression of EMT. Integrin complexes enable cells to receive signals from ECM proteins through interactions with signalling mediators, such as integrin-linked kinase (ILK), particularly interesting new Cys–His protein 1 (PINCH; also known as LIMS1) and parvin^{7,30}. As epithelial cells differentiate into mesenchymal cells, they do not interact with a basement membrane and communicate with a different ECM. Therefore, the cells downregulate some epithelial integrins, but activate the expression of others; some of these newly expressed integrins have key roles in EMT progression⁷. For example, EMT downregulates the expression of the epithelial $\alpha 6 \beta 4$ integrins that mediate contacts with the basement membrane through epigenetically silencing the gene encoding $\beta 4$ (REF. 35). Additionally, epithelial $\alpha 3 \beta 1$ integrin, which binds laminin but also associates with E-cadherin, is required for progression through EMT and integrates β -catenin and transforming growth factor- β (TGF β)–SMAD signalling³⁶. Increased $\alpha 5 \beta 1$ integrin expression during EMT increases cell adhesion to fibronectin, the expression of which is also activated during EMT and promotes cell migration^{37,38}. The increased expression of $\alpha 1 \beta 1$ or $\alpha 2 \beta 1$ integrins and their interactions with type I collagen facilitate the disruption of E-cadherin complexes and the nuclear translocation of β -catenin³⁹.

Changes to the integrin repertoire during EMT correlate with the increased expression of proteases, such as the matrix metalloproteinases MMP2 and MMP9, thus enhancing ECM

protein degradation and enabling invasion⁴⁰. MMPs additionally target some transmembrane proteins, which results in, for example, the release of the extracellular domain of E-cadherin, hence contributing to the loss of adherens junctions⁴⁰. Furthermore, MMP3 can induce EMT through increased expression and activity of RAC1B, which results in increased levels of cellular reactive oxygen species, leading to increased SNAIL1 expression⁴¹. Increased $\alpha\text{v}\beta 6$ integrin expression during EMT correlates with an increase in the expression of proteases, and the colocalization of these proteins in invadopodia mediates invasive cell behaviour^{7,19}. Also, $\alpha\text{v}\beta 3$ integrin, the expression of which is increased with EMT, contributes to pro-invasive functions at the leading edge of cancer cells⁴². Finally, localized ECM degradation by invading cells may release stored growth factors that then act on cells. Some proteases that mediate invasion and some integrins, such as $\alpha\text{v}\beta 6$, activate the differentiation factor TGF β that is stored in a latent form in the ECM⁴³. This exposes the cells to increased TGF β signalling, which promotes EMT (see below) and stimulates the expression of some ECM proteins, such as collagens and fibronectin, enhancing the remodelling of the ECM into a matrix with a different composition and properties^{2,3}.

Transcription factors driving EMT

The changes in gene expression that contribute to the repression of the epithelial phenotype and activation of the mesenchymal phenotype involve master regulators, including SNAIL, TWIST and zinc-finger E-box-binding (ZEB) transcription factors. Their expression is activated early in EMT, and they thus have central roles in development, fibrosis and cancer (FIG. 2). As these transcription factors have distinct expression profiles, their contributions to EMT depend on the cell or tissue type involved and the signalling pathways that initiate EMT. They often control the expression of each other and functionally cooperate at target genes¹¹, and additional transcription factors further define the EMT transcription programme and drive EMT progression. Together, the EMT transcription factors coordinate the repression of epithelial genes and the induction of mesenchymal genes, and often the same transcription factors direct both repression and activation¹².

SNAIL transcription factors

Of the three vertebrate SNAIL proteins, SNAIL1 (also known as SNAIL) and SNAIL2 (also known as SLUG) activate the EMT programme during development, fibrosis and cancer⁴⁴ (FIG. 2). They repress epithelial genes by binding to E-box DNA sequences through their carboxy-terminal zinc-finger domains^{11,45} (FIG. 2; TABLE 1). How SNAIL represses gene expression is well illustrated by the activity of SNAIL1 at the E-cadherin promoter^{46–52}. Upon binding of E-box sequences in the proximal promoter region of the E-cadherin gene, SNAIL1 recruits the Polycomb repressive complex 2 (PRC2), which contains the methyltransferases enhancer of zeste homologue 2 (EZH2), G9a and suppressor of variegation 3–9 homologue 1 (SUV39H1), the co-repressor SIN3A, histone deacetylases 1, 2 and/or 3, and the Lys-specific demethylase 1 (LSD1); all of these components coordinate histone modifications, specifically methylation and acetylation at histone H3 Lys 4 (H3K4), H3K9 and H3K27 (REFS 48–53). H3K9 methylation and H3K27 methylation mark repressive chromatin, and H3K4 methylation and H3K9 acetylation mark active chromatin. Repressive and active marks in ‘bivalent domains’, as seen in the E-cadherin promoter,

cooccur at many promoters in embryonic stem cells⁵⁴ and create a poised state for the promoter that enables timely activation while maintaining repression in the absence of differentiation signals. The bivalent control of the E-cadherin promoter may contribute to the reversible nature of EMT. In addition to repressing epithelial genes, SNAIL1 activates genes that contribute to the mesenchymal phenotype (TABLE 1). This may also involve bivalent domains with, for example, repressive H3K9 trimethylation and activating H3K18 acetylation, which enables mesodermal gooseoid expression in response to the TGF β -related Nodal⁵⁵.

Multiple signalling pathways cooperate in the initiation and progression of EMT (see below), and they often activate SNAIL1 expression. TGF β and WNT family proteins, Notch, and growth factors that act through RTKs, all activate SNAIL1 expression depending on the physiological context¹¹. SNAIL1 and SNAIL2 co operate with other transcription regulators to control gene expression. For example, SNAIL1 cooperates with ETS1, which is activated by MAPK, to activate MMP expression⁵⁶. It also cooperates with the SMAD3–SMAD4 complex to cause the TGF β -mediated repression of E-cadherin and occludin expression⁵⁷.

Post-translational modifications that are initiated by signalling pathways also control the localization and degradation, and thus the activity, of SNAIL1 (REF. 11). Glycogen synthase kinase-3 β (GSK3 β)-mediated SNAIL1 phosphorylation at two Ser-rich motifs in activates its transcriptional activity. Phosphorylation of Ser97 and Ser101 in the first motif facilitates the nuclear export of SNAIL1, and the subsequent phosphorylation of Ser108, Ser112, Ser116 and Ser120 in the second motif labels SNAIL1 for ubiquitin-mediated degradation⁵⁸ (FIG. 2). Several pathways increase SNAIL1 activity by influencing GSK3 β -mediated phosphorylation. The WNT and PI3K–AKT pathways inhibit SNAIL1 phosphorylation by GSK3 β ⁵⁹, and Notch and nuclear factor- κ B (NF- κ B) signalling disrupt the GSK3 β –SNAIL1 interactions^{60,61}; both mechanisms increase SNAIL1 stability. Conversely, small C-terminal domain phosphatase 1 (SCP1; also known as CTDSP1) antagonizes phosphorylation by GSK3 β and retains SNAIL1 in the nucleus where it represses transcription⁶². Additionally, PKD1 phosphorylates SNAIL1 at Ser11 to facilitate nuclear export⁶³, whereas SNAIL1 phosphorylation by PAK1 at Ser246 or large tumour suppressor 2 (LATS2) at Thr203 increases its nuclear retention and promotes EMT^{64,65}. The tumour suppressor p53 recruits SNAIL2 for degradation following its interaction with the ubiquitin ligase mouse double minute 2 (MDM2), consequently suppressing cancer invasion⁶⁶ (FIG. 2).

bHLH transcription factors

Homodimeric and heterodimeric basic helix–loop–helix (bHLH) transcription factors function as master regulators of lineage specification and differentiation. Among these, E12 and E47, TWIST1 and TWIST2, and inhibitor of differentiation (ID) proteins have key roles in EMT progression^{11,12}. As with SNAIL, TWIST expression downregulates epithelial gene expression and activates mesenchymal gene expression⁴⁵ (FIG. 2; TABLE 1). In cancer cells, TWIST1 represses E-cadherin and induces N-cadherin expression independently of SNAIL and probably through the association with other proteins^{45,67–69}. TWIST recruits the methyltransferase SET8 (also known as SETD8 in humans), which mediates H4K20

monomethylation, a histone mark that is associated with repression at E-cadherin promoters and with activation at N-cadherin promoters⁶⁹. In head and neck cancer cells, TWIST1 activates the expression of B lymphoma Mo-MLV insertion region 1 homologue (BMI1), a component of PRC1, and subsequently cooperates with BMI1 at E-box sequences to repress the expression of E-cadherin and the cell cycle inhibitor p16 (also known as INK4A; encoded by *CDKN2A*)⁶⁷. This cooperation involves recruitment of PRC2, which trimethylates H3K27 at the E-cadherin and *CDKN2A* gene promoters⁶⁷.

Diverse signalling pathways activate TWIST expression during development and tumorigenesis^{11,45}. Most notably, the transcription factor hypoxia-inducible factor 1 α (HIF1 α) induces TWIST expression under hypoxic conditions, thus promoting EMT and tumour cell dissemination⁶⁸. Additionally, mechanical stress induces Twist expression in *Drosophila melanogaster* epithelia, in a manner dependent on β -catenin⁷⁰. The activities of TWIST greatly depend on its dimer composition. TWIST1 and TWIST2 form homodimers, as well as heterodimers, with E12 or E47 to regulate E-box DNA binding and transcription regulation. ID proteins that cannot bind DNA associate with TWIST, E12 or E47, and hence inhibit TWIST function. Consequently, repression of ID gene expression by TGF β results in the derepression of TWIST (or other bHLH proteins) and increases its activity in EMT⁷¹. As with SNAIL, the stability of TWIST1 is regulated by phosphorylation; MAPKs phosphorylate TWIST1 at Ser68, protecting it from ubiquitin-mediated degradation and increasing its activity⁷².

ZEB transcription factors

The two vertebrate ZEB transcription factors, ZEB1 and ZEB2, bind regulatory gene sequences at E-boxes and can repress or activate transcription^{11,45}. ZEB-mediated transcriptional repression often involves the recruitment of a C-terminal-binding protein (CTBP) co-repressor; although in some cancer cells ZEB1 represses E-cadherin expression independently of CTBP by recruiting the Switch/sucrose nonfermentable (SWI/SNF) chromatin remodelling protein BRG1 (REF. 73). ZEB1 can also interact with the transcriptional coactivators p300/CBP-associated factor (PCAF; also known as KAT2B) and p300, which switches it from a transcriptional repressor to a transcriptional activator⁷⁴. Additionally, ZEB1 can recruit the Lys-specific demethylase 1 (LSD1), possibly linking it to histone demethylation in EMT⁷⁵. Therefore, like SNAIL and TWIST, ZEBs bind E-boxes and function as transcriptional repressors and activators, thereby repressing some epithelial junction and polarity genes and activating mesenchymal genes that define the EMT phenotype^{11,45} (FIG. 2).

ZEB expression often follows activation of SNAIL expression, consistent with SNAIL1 directly targeting the *ZEB1* gene. Additionally, TWIST1 cooperates with SNAIL1 in the induction of ZEB1 expression⁷⁶. ZEB expression is induced in response to TGF β and WNT proteins, and growth factors that activate RAS– MAPK signalling⁴⁵. The induction of ZEB expression by TGF β signalling involves ETS1, which is activated by MAPK signalling⁷⁷. ZEB expression is post-transcriptionally repressed by microRNAs (miRNAs) (see below), and the post-translational sumoylation of ZEB2 by PRC2 prevents its association with

CTBP and promotes its cytoplasmic localization, which attenuates ZEB2-mediated gene repression⁷⁸.

Novel transcription factors regulating EMT

In addition to the established EMT transcription factors, other transcription factors were recently shown to induce or regulate EMT, either in development or in cancer (TABLE 1). Several of these are forkhead box (FOX) transcription factors, which are defined by a DNA-binding forkhead domain⁷⁹. Others belong to the GATA family, which is characterized by a DNA-binding dual zinc-finger module and controls the differentiation of diverse cell lineages⁸⁰; they promote or are required for EMT by regulating the expression of genes that are required for epithelial cell junctions or polarity complexes⁸¹. Other inducers of EMT are SRY box (SOX) transcription factors⁸² that synergize with SNAIL1 or SNAIL2 in driving EMT and/or cell invasion^{83,84}. The regulation of these more recently identified EMT transcription factors, their roles in EMT and functional relationships with SNAIL, TWIST or ZEB transcription factors are not yet well defined. Regardless, it is clear that diverse transcription factors coordinate gene expression reprogramming during EMT, and that some of these are master regulators and others may have more restricted functions depending on the tissue context.

Regulating EMT at the RNA level

In addition to the direct effects of EMT transcription factors on gene expression, changes at the RNA level regulate EMT progression. The differential splicing of nascent RNAs into mRNAs that generate proteins with structural and functional differences and the miRNA-mediated degradation of gene transcripts define the activities of key proteins in EMT.

Alternative splicing in EMT

EMT is marked by extensive changes in the splicing of many mRNAs, which generates diverse protein isoforms in mesenchymal cells as compared to epithelial cells. The functional consequences of differential splicing in EMT are well illustrated by p120 catenin, the adhesion protein cluster of differentiation 44 (CD44), the RTK FGFR2 (REFS 85–88) and extensive isoform changes, as a result of alternative splicing, in various additional proteins that regulate EMT⁸⁹. Many of the changes in splicing result from the rapid downregulation, during EMT, of the expression of epithelial splicing regulatory protein 1 (ESRP1) and ESRP2, two RNA binding proteins that control the splicing of many gene transcripts. Their downregulation results in mesenchymal protein isoforms that help to define alterations in adhesion, motility and signalling pathways⁹⁰. Other changes in splicing result from the increased expression, during EMT, of RNA binding protein FOX1 homologue 2 (RBFOX2), another RNA binding protein that contributes to EMT and cell invasion^{89,91}. EMT is also frequently marked by increased expression and/or activity of the splicing factor Ser-Arg-rich splicing factor 1 (SRSF1), which promotes EMT by splicing the mRNA encoding the RTK receptor d'origine nantais 1 (RON1) and the GTPase RAC1 — further highlighting the key contributions of cell type-dependent splicing to this transdifferentiation^{92,93}. These changes in splicing impose another layer of complexity on the gene expression changes that occur during EMT.

miRNA-mediated control of EMT

Non-coding miRNAs that selectively bind mRNAs, thus inhibiting their translation or promoting their degradation, also regulate the epithelial phenotype and EMT⁹⁴. Some of these control the expression of EMT master transcription factors. For example, miR-29b and miR-30a repress SNAIL1 expression^{95,96}; hence, increased miR-29b expression can reverse EMT and decrease cell invasion⁹⁵. Additionally, miR-1 and miR-200b can repress SNAIL2 expression, and SNAIL2 represses the expression of miR-1 and miR-200b, resulting in a double-negative feedback mechanism⁹⁷. A similar feedback loop also occurs between miR-34 and SNAIL1 (REF. 98), and miR-203 and SNAIL1 (REF. 99).

Members of the miR-200 family and miR-205 repress the translation of *ZEB1* and *ZEB2* mRNAs¹⁰⁰, and double-negative feedback controls ZEB and miR-200 expression, with ZEB proteins repressing the expression of miR-200 miRNAs, and miR-200 suppressing ZEB expression¹⁰¹. During EMT, decreased miR-200 expression results in increased ZEB1 and ZEB2 levels and EMT progression¹⁰¹. Additionally, p53 represses liver carcinoma cell EMT by increasing the expression of miR-200 and miR-192 (a miR-215 homologue), which target and reduce ZEB1 and ZEB2 expression¹⁰². Finally, the expression of high mobility group A2 (HMGA2), which activates SNAIL1 and TWIST expression¹⁰³, is downregulated by the miRNA let-7 in a pancreas carcinoma model¹⁰⁴, and by miR-365 in lung adenocarcinoma cells¹⁰⁵.

In addition to controlling the expression of EMT transcription factors, miRNAs also target genes that help to define the epithelial or mesenchymal phenotype, such as those encoding adhesion junction and polarity complex proteins, and signalling mediators. For example, increased miR-9 expression, which occurs in mammary carcinomas, represses E-cadherin expression and promotes a mesenchymal phenotype with increased cell migration and invasion¹⁰⁶. N-cadherin expression is repressed by miR-194 (REFS 107,108), the expression of which is attenuated in advanced stage gastric cancer cells¹⁰⁷. Additionally, increased miR-194 expression in mesenchymal liver cancer cells results in decreased N-cadherin levels, cell migration and invasion¹⁰⁸. In TGF β -induced EMT, miR-491-5p represses PAR3 expression, which helps to destabilize tight junctions¹⁰⁹. miR-661 represses the expression of Nectin 1 (also known as poliovirus receptor-related protein 1), a protein that is involved in cell–cell adhesion, hence contributing to epithelial cell junction disassembly in SNAIL-expressing cancer cells¹¹⁰. Several miRNAs control the expression and/or activities of RHOA, and thus affect actin organization and tight junction stability. Among these, miR-155, which is expressed in response to TGF β , targets the mRNA encoding RHOA, resulting in the dissolution of tight junctions¹¹¹. TGF β also induces the expression of miR-24, which targets neuroepithelial cell-transforming 1A (NET1A), a RHO-GEF that activates RHOA, therefore promoting EMT through the disruption of adherens and tight junctions¹¹². Additionally, miR-31 reduces breast cancer cell dissemination by targeting RHOA expression¹¹³, and miR-124 decreases EMT, cell invasion and metastasis by targeting ROCK2, an effector of RHOA signalling¹¹⁴. Clearly, the regulated activities of miRNAs represent an extensive regulatory network that the cells impose on the gene expression programme to control EMT.

TGF β family proteins: inducers of EMT

The TGF β family comprises three TGF β s, two activins, many bone morphogenetic proteins (BMPs) and other homodimers and heterodimers of ligands that all act through binary combinations of transmembrane dual specificity kinase receptors (that is, receptors that act as Ser/Thr kinases, as well as Tyr kinases). In development, TGF β 1 and TGF β 2 expression is associated with EMT-like events in the formation of endocardial cushions¹¹⁵, whereas TGF β 3 drives EMT that mediates palate fusion¹¹⁶. Postnatally, TGF β 1 induces EMT in wound healing, fibrosis and cancer. For example, increased levels of TGF β 1 have been linked to EMT in mesangial cells before kidney fibrosis¹¹⁷, biliary epithelial cells before liver disease progression to hepatic fibrosis¹¹⁸ and fibroblasts from patients with pulmonary fibrosis¹¹⁹. Cardiac fibrosis through endothelial–mesenchymal transition (EndMT) also results from increased TGF β signalling¹²⁰. In carcinomas, increased expression and activation of TGF β 1 promotes an epithelial plasticity response that may progress to EMT, a prerequisite for cancer cell invasion and dissemination^{3,4,121}. For example, squamous carcinomas convert into invasive spindle cell carcinomas in response to increased expression of activated TGF β 1 (REF. 122).

Other TGF β family proteins also regulate EMT, primarily in development. Nodal and vegetal 1 (VG1) participate in EMT during cell ingression to form mesoderm in mouse and *Xenopus laevis* embryos, respectively¹²³. BMP signalling cooperates with WNT and FGF signalling to induce neural crest delamination¹²⁴, and it also participates in EMT that is required in heart cushion formation¹²⁵. Additionally, anti-Müllerian hormone mediates EMT in coelomic epithelium during Müllerian duct regression¹²⁶. In pathological contexts, BMP signalling is involved in both EMT and MET^{127,128}. BMP2, BMP4 and BMP7 promote EMT and invasiveness in pancreatic cancer cells, which is apparent by the loss of E-cadherin expression and an increase in MMP2 expression¹²⁷. In renal fibrosis, BMP7 induces MET of renal fibroblasts with decreased type I collagen secretion and restored E-cadherin expression¹²⁸. Finally, TGF β and BMP signalling induce endothelial cells to acquire mesenchymal stem cell-like properties that contribute to fibrodysplasia ossificans progressiva, in which heterotypic ossification occurs as a result of activating mutations in the gene encoding activin receptor-like kinase 2 (ALK2; also known as ACTR1), a type I TGF β and BMP receptor¹²⁹.

TGF β signalling through SMADs in EMT

Binding of TGF β family proteins to tetrameric cell surface receptor complexes enables the ‘type II’ TGF β family receptors to phosphorylate and thus to activate the ‘type I’ transmembrane kinases, which then phosphorylate the C termini of the intracellular signalling effectors — that is, SMADs. In response to TGF β , which generally acts through complexes of T β RII (type II; also known as TGFR2) and T β RI (type I; also known as TGFR1) receptors, the receptor-activated SMAD2 and/or SMAD3 combine with SMAD4 to form trimeric SMAD complexes; SMAD1 and/or SMAD5 interact with SMAD4 in response to BMPs. Following their translocation into the nucleus, the SMAD complexes combine with DNA-binding transcription factors at regulatory gene sequences and activate or repress transcription by interacting with transcriptional coactivators and/or transcriptional co-

repressors^{130,131} (FIG. 3). SMAD activation is negatively regulated by inhibitory SMADs (SMAD6 and SMAD7), which compete with SMAD2 and SMAD3, or SMAD1 and SMAD5, for binding to the type I receptors¹³².

Dominant-negative forms of T β R II or T β R I , and pharmacological inhibition of the kinase activity of T β R I , block TGF β -induced EMT in many cell types^{122,133,134}, whereas a constitutively activated T β R I protein initiates EMT¹³⁵. Expression of inactive SMAD2, SMAD3 or SMAD4, as well as decreased SMAD4 or increased SMAD7 expression, prevent TGF β -induced EMT^{133,136,137}. Interestingly, although SMAD3 drives gene expression that initiates EMT, SMAD2 may antagonize TGF β -induced EMT^{138–140}; accordingly, SMAD2-deficient keratinocytes undergo EMT and promote tumour formation owing to the increased activity of SMAD3–SMAD4 (REF. 138).

In response to TGF β , SMAD complexes not only activate the expression, but also increase the activity of EMT transcription factors. TGF β induces SNAIL1 expression through SMAD3-dependent transcription¹³⁸, whereas SNAIL2 expression is induced indirectly by the SMAD3-induced expression of myocardin-related transcription factor (MRTF)¹⁴¹. SMAD3–SMAD4 also cooperates with SNAIL1 in response to TGF β , and thus relays the TGF β -activated repression of the genes encoding E-cadherin and occludin⁵⁷. TGF β also induces the expression of ZEB1, which is further controlled by MAPK signalling⁷⁷, and SMAD3–SMAD4 complexes interact with ZEB1 and ZEB2 to mediate TGF β -regulated gene expression^{77,142,143}. SMAD3–SMAD4 complexes also interact with activating transcription factor 3 (ATF3) to repress ID1 expression, hence enhancing TWIST expression and activity⁷¹. Finally, SMAD3 and SMAD4 also activate the expression of HMGA2 in TGF β -induced EMT¹⁴⁴.

Other changes in gene expression during EMT occur without directly requiring EMT transcription factors and are controlled by TGF β -activated SMADs^{140,145}. SMADs directly activate the expression of some mesenchymal genes; for example, those encoding fibronectin, vimentin and collagen αI ^{146,147}. Moreover, the association of Nodal-induced SMAD complexes with the PHD-Bromo domain protein tripartite interaction motif 33 (TRIM33) mediates its interactions with histone marks, which leads to the activation of mesodermal goosecoid expression⁵⁵.

Non-SMAD signalling in EMT

Complementing its signalling through SMADs, TGF β also induces signalling through RHO-like GTPases, PI3K and MAPK pathways^{148,149}, which also contribute to EMT^{45,137,150} (FIG. 3). Activation of RHO, RAC and CDC42 GTPases drives actin reorganization, lamellipodia and filopodia formation¹⁸ (see above). Early in EMT, PAR6 interacts with TGF β receptors at tight junctions. Following TGF β stimulation, PAR6 is phosphorylated by T β R II and recruits the E3 ubiquitin ligase SMAD ubiquitylation regulatory factor 1 (SMURF1), which locally mediates RHOA ubiquitylation and degradation as the tight junctions dissolve¹⁵¹. TGF β also induces RHOA activation¹⁵², partly by promoting the expression of GEFs¹⁵³, and the activation of ROCK and LIMK^{152,154}. Consequently, decreased RHOA expression or the chemical inhibition of ROCK prevents the actin reorganization that is required for TGF β -induced EMT^{152,154,155}. Finally, RHOA activation

also contributes to changes in gene expression, and mammalian epithelial cells that are unable to express a RHOA GEF show reduced α -smooth muscle actin (α SMA) expression in response to TGF β ¹⁵⁶.

In epithelial cells undergoing EMT, TGF β activates AKT through PI3K, which results in the activation of mammalian TOR complex 1 (mTORC1) and mTORC2 (REFS 134,157,158). Pharmacological inhibition of PI3K prevents TGF β -induced EMT¹⁵⁷, illustrating the essential role of the PI3K–AKT pathway in this process. In TGF β -induced EMT, mTORC1 contributes to the increased cell size, protein synthesis, motility and invasion that take place¹³⁴, whereas mTORC2 is required for the transition from an epithelial to a mesenchymal phenotype¹⁵⁸. Inhibition of AKT decreases the level of SNAIL1 expression^{158,159}, attenuating the repression of E-cadherin and the activation of MMP9 expression, and mTORC2 inhibition impedes invasive behaviour and metastatic potential¹⁵⁸. Phosphorylation of GSK3 β by AKT inhibits its activity, stabilizing SNAIL1 to repress the expression of several genes, including the gene encoding E-cadherin^{58,160}. Finally, TGF β induces the AKT-mediated phosphorylation of heterogeneous nuclear ribonucleoprotein E1 (hnRNPE1; also known as PCBP1), which results in the release of hnRNPE1 from the 3' untranslated regions of disabled 2 (*DAB2*) and interleukin (IL)-like EMT inducer (*ILEI*; also known as *FAM3C*) mRNA, thus derepressing their translation and enabling EMT¹⁶¹ (FIG. 3).

TGF β proteins also activate the ERK, p38 and JUN N-terminal kinase (JNK) MAPK pathways^{148,149}, probably as a result of the dual specificity kinase activities of TGF β receptors¹⁶². As dual specificity kinases generally have much lower Tyr phosphorylation activity than Ser/Thr phosphorylation activity, the TGF β receptors induce a much lower level of MAPK activation than growth factor-activated RTKs¹⁶². TGF β induces ERK MAPK signalling through the adaptor protein SRC homology 2 domain-containing-transforming A (SHCA; also known as SHC1), which associates with the T β RI receptor and is phosphorylated at Tyr and Ser by T β RI in response to TGF β (FIG. 4). The phosphorylation of SHCA on Tyr provides a docking site for growth factor receptor-bound protein 2 (GRB2) and son of sevenless (SOS), and initiates the RAS–RAF–MEK–ERK (also known as MAPKK) MAPK pathway¹⁶³. TGF β -induced p38 MAPK and JNK activation results from the association of the ubiquitin ligase TNF receptor-associated factor 6 (TRAF6) with the TGF β receptor complex, which activates TAK1, a kinase upstream of p38 MAPK and JNK^{164,165}. Although the contributions of these MAPK pathways to TGF β -induced EMT remain to be defined, inhibition of ERK or p38 MAPK kinase activity represses TGF β -induced EMT^{166,167}. ERK MAPK signalling increases TGF β -induced transcription, leading to increased repression of E-cadherin and the activation of N-cadherin and MMP expression^{168,169}. Additionally TGF β -induced activation of ERK5 MAPK stabilizes SNAIL1 by inhibiting GSK3 β , thus increasing SNAIL1 activity¹⁷⁰. Activation of TGF β -activated kinase 1 (TAK1; also known as MAP3K7) and p38 MAPK are required for TGF β -induced expression of the transcription factor ATF2 and consequently for its cooperation with SMAD3–SMAD4 in TGF β -induced gene expression¹⁷¹. Finally, JNK activates c-JUN, a component of the activator protein 1 (AP1) complex, hence mediating TGF β -induced transcription in cooperation with SMAD3–SMAD4 (REF. 172) (FIG. 4).

Tyrosine kinase receptors induce EMT

In contrast to TGF β family proteins, growth factors that act through transmembrane RTKs often stimulate cell proliferation. Ligand binding induces the autophosphorylation of these receptors on Tyr; this enables them to activate the PI3K–AKT pathway, and the ERK MAPK, p38 MAPK and JNK pathways (the roles of which in EMT were discussed above) and SRC signalling. Growth factors that act through RTKs can induce partial or full EMT. In fact, the induction of EMT by RTKs was uncovered before TGF β was found to induce EMT^{2,173}.

PI3K–AKT and ERK MAPK activation by growth factors

The activation of EMT by RTKs highlights the roles of the PI3K–AKT and ERK MAPK pathways in this transdifferentiation process. As with TGF β -induced EMT, signalling through AKT and mTORC2 is essential for RTK-induced EMT^{158,174}. ERK MAPK pathway activation, not only by growth factors but also by mutations in the genes encoding RAS or RAF that occur in cancer cells, also contributes to EMT. RAS and RAF signalling activate the expression of SNAIL1 and/or SNAIL2, and induce the activation of RHO-GTPases, therefore promoting cell motility and invasive behaviour in cancer-associated EMT¹⁷⁵. Activation of the 90 kDa ribosomal protein S6 kinase by ERK MAPK contributes to the invasive properties of epithelial and carcinoma cells by inducing the expression of the transcription factor FOS-related antigen 1 (FRA1)¹⁷⁶. Of note, cells in culture exhibit autocrine TGF β signalling, and RTK signalling induces TGF β 1 expression, thus enhancing autocrine TGF β signalling. These results position growth factor activity in the context of the cell response to TGF β family signalling.

FGF signalling and EMT

FGFs induce mesenchymal characteristics in epithelial cells. FGF1-induced EMT of bladder carcinoma cells is accompanied by SNAIL2 expression, the destabilization of desmosomes, and the expression of α 2 β 1 integrin and MMP13 (REFS 177–179). FGFs act as EMT inducers in development, for example, in gastrulation. Mouse embryos lacking FGFR1 show aberrant mesoderm formation in the primitive streak and maintain E-cadherin and β -catenin at the cell surface¹⁸⁰. In FGF8-deficient mouse embryos, cells in the primitive streak fail to migrate away to generate mesoderm¹⁸¹. Later in development, FGF signalling is required for neural crest cell migration, and studies in *X. laevis* have revealed roles for fgf2 and fgf8 in the expression of neural crest cell markers¹²⁴.

Hepatocyte growth factor signalling and EMT

Hepatocyte growth factor (HGF), which acts through the RTK c-MET (also known as HGFR), was identified as ‘scatter factor’, as it was seen to convert kidney epithelial cells into migratory fibroblast-like cells¹⁷³. HGF induces the expression of SNAIL1 or SNAIL2, which depends on cell type, to drive EMT^{178,182}. The induction of SNAIL expression by HGF requires the ERK MAPK pathway and involves binding of the transcription factor early growth response 1 (EGR1) to the SNAIL gene promoter¹⁸². HGF also induces desmosome destabilization and the repression of the gene encoding desmoplakin¹⁷⁸. The c-MET receptor was found to associate with E-cadherin¹⁸³, but the role of this interaction in

EMT remains to be clarified. Finally, in development, HGF participates in EMT in the formation of somites and the endocardial cushion¹⁸⁴.

Insulin-like growth factor 1 and EMT

Insulin-like growth factor 1 (IGF1) induces EMT in some cell culture models. IGF1 receptor activation causes EMT in mammary epithelial cells, resulting in loss of E-cadherin, and gain of N-cadherin, vimentin and fibronectin¹⁸⁵. In these cells, increased SNAIL1 expression in response to IGF1 depends on NF- κ B¹⁸⁵. In other cells, however, IGF1-induced EMT was accompanied by ZEB1 expression that was dependent on activation of the ERK MAPK pathway¹⁸⁶. Also, the PI3K–AKT pathway is required for IGF1-induced EMT¹⁸⁷. Interestingly, in one cell system, AKT1 and AKT2 act differently, with downregulation of AKT1 expression stimulating EMT, and this induction is blocked following downregulation of AKT2 expression¹⁸⁷. Finally, the IGF1 receptor forms complexes with E-cadherin and α v integrin¹⁸⁸, as was also seen with c-MET¹⁸³, and IGF1-induced disruption of these complexes promotes cell migration¹⁸⁸.

Epidermal growth factors and EMT

In cell culture, epidermal growth factor (EGF) induces the endocytosis of E-cadherin, as well as SNAIL1 or TWIST expression, which leads to a reduction in E-cadherin levels^{189,190}. EGF also induces EMT in epithelial explants, resulting in increased cell motility and MMP2- and MMP9-mediated proteolysis that is dependent on ILK kinase and the ERK MAPK pathway¹⁹¹. Activation of human EGF receptor 2 (HER2; also known as ERBB2) in mammary epithelial cells induces tumours with properties of EMT that are able to escape immune surveillance^{192,193}. The EGF-related Cripto1 (also known as TDGF1) may also have a role in EMT during development. Its expression correlates with primitive streak formation, and mesoderm and endoderm specification, whereas a decrease in its expression confers defects in mesoderm formation¹⁹⁴. Cripto1 may also have a role in mammary gland morphogenesis¹⁹⁴. Not only does its expression increase with mammary development, but adding Cripto1 to, or overexpressing it in, cultured mammary epithelial cells triggers a fibroblast morphology, decreases the level of E-cadherin and increases the levels of N-cadherin, vimentin and SNAIL expression¹⁹⁵. Increased Cripto1 expression has also been associated with the increased motility and invasion of tumour cells, and tumour progression¹⁹⁴. Rather than acting as a ligand, the activity of Cripto1 in EMT may relate to its role as Nodal co-receptor and its functions in WNT signalling^{196,197}.

Platelet-derived growth factor in EMT

In colon adenocarcinoma cells, platelet-derived growth factor (PDGF) induces the dissolution of adherens junctions, the nuclear localization of β -catenin and the repression of E-cadherin expression¹⁹⁸. Ablation of the PDGF receptor α -subunit results in craniofacial and heart valve anomalies owing to defective mesenchymal cell migration and decreased MMP2 activity¹⁹⁹. The PDGF-related growth factor vascular endothelial growth factor (VEGF), an inducer of angiogenesis, also induces EMT. VEGF induces SNAIL expression in breast cancer cells, partly by inhibiting GSK3 β activity²⁰⁰, as well as EMT that correlates with SNAIL1, SNAIL2 and Twist expression in pancreas carcinoma cells²⁰¹. Additionally, SNAIL1 increases VEGF expression, possibly conferring feedback amplification of the

EMT response²⁰². VEGF-induced EMT may link EMT or EndMT with angiogenesis, which could greatly benefit cancer progression and invasion.

Other extracellular signals regulate EMT

Other differentiation factors activate or regulate EMT, although the underlying mechanisms are less well-defined (FIG. 4). In canonical WNT signalling, the binding of WNT ligands to Frizzled receptors results in the inhibition of GSK3 β , hence preventing β -catenin phosphorylation, ubiquitylation and degradation, and enabling β -catenin to regulate gene expression⁸. Inhibition of the GSK3 β kinase increases SNAIL stability, thus promoting EMT⁵⁸. WNT signalling has roles in developmental EMT. WNT3-deficient mouse embryos do not form a primitive streak and mesoderm²⁰³, while ectopic WNT8C expression induces the formation of multiple primitive streaks²⁰⁴. Canonical WNT signalling also enables the generation and proliferation of neural crest precursors²⁰⁵, whereas GSK3 β -independent WNT signalling is required for neural crest cell migration²⁰⁶. WNT signalling also promotes EMT associated with cancer progression, and β -catenin-mediated gene expression is increased in cells at the invasive front of colorectal tumours where EMT occurs²⁰⁷.

In Hedgehog (HH) signalling, ligand binding to patched (PTC) receptors activates glioma (GLI) family transcription factors²⁰⁸. During development, cells in the ventral somite region transition into mesenchyme to form the sclerotome, where sonic HH (SHH) induces the expression of sclerotomal cell markers²⁰⁹. Ectopic expression of GLI1 in rat kidney epithelial cells induces SNAIL1 expression, the loss of E-cadherin²¹⁰, increased SHH expression and signalling associated with increased SNAIL1 expression in epithelial cancers, including in the invasive front of neuroendocrine tumours²¹¹.

Notch signalling also participates in developmental EMT and possibly in EMT during cancer progression. Interactions of Delta-like or Jagged ligands with Notch receptors initiate signalling through the proteolytic release of the Notch intracellular domain that then regulates target gene expression²¹². In development, Notch signalling is required for EMT during endocardial cushion formation, and reduced SNAIL1 expression together with defective cushion formation occurs in Notch1-deficient mice²¹³. Additionally, the Notch intracellular domain directly activates SNAIL2 expression²¹⁴. Notch1 inhibition partially reverts EMT in lung adenocarcinoma cells and decreases their invasive behaviour²¹⁵.

Additional factors in the tissue or tumour microenvironment facilitate the initiation of EMT. For example, the hypoxic conditions in growing tumours facilitate EMT by inducing expression of the transcription factor HIF1 α , which activates TWIST expression and thus initiates EMT that leads to an invasive phenotype⁶⁸. HIF1 α also induces SNAIL1 expression in endothelial cells and ovarian carcinoma cells, resulting in the loss of vascular endothelial cadherin (VE-cadherin) or E-cadherin, respectively^{216,217}. The release of inflammatory cytokines, besides TGF β 1, by immune cells, endothelial cells and cancer-associated fibroblasts contributes to EMT. For example, IL-6 promotes EMT of breast cancer cells in culture, which correlates with a decrease in E-cadherin levels, an increase in N-cadherin, vimentin, SNAIL1 and TWIST levels, and cell invasion²¹⁸. Ectopic TWIST expression in these cells induces IL-6 expression and activates the transcription factor signal

transducer and activator of transcription 3 (STAT3)²¹⁸, which then activates SNAIL1 expression²¹⁹. The transcription factor brachyury promotes EMT in tumour cells, partly by increasing the secretion of IL-8, which in turn maintains the mesenchymal phenotype of tumour cells by increasing brachyury expression²²⁰. The mechanisms through which inflammatory cytokines other than TGF β contribute to EMT remain to be defined, although some cytokines may induce NF- κ B signalling to directly activate SNAIL1 or ZEB expression²²¹. Considering the link between inflammation and fibrosis, these inflammatory cytokines may also contribute to EMT in fibrosis.

Crosstalk of signalling pathways in EMT

As is apparent from this overview, growth and differentiation factors, and other extracellular cues, can activate a differentiation response in epithelial cells that leads to EMT. Distinct pathways have predominant roles in the initiation and progression of EMT and regulate changes in morphology and gene expression that convert epithelial cells into motile mesenchymal cells. EMT-inducing extracellular factors often activate several pathways, and the expression of EMT transcription factors can be activated by different pathways, strongly suggesting the presence of signalling cooperation and the convergence of these pathways on common targets during EMT (FIG. 4). It should be noted that the studies on signalling in EMT use cell culture systems in which key pathways show basal autocrine activation, which is in part defined by the culture conditions.

Cooperation between signalling pathways in EMT is seen *in vivo* as well as in cell culture. For example, WNT signalling cooperates with FGF and TGF β family members to regulate EMT during gastrulation²²² and neural crest cell delamination¹²⁴, whereas Notch and TGF β signalling regulate endocardial cushion formation²¹³. Synergy between TGF β signalling and RTK signalling, activated by, for example, the EGF-related TGF α or FGF, is apparent in EMT of cancer cells. Indeed, TGF β facilitates EGF- or FGF-induced EMT in cancer cell models, thus increasing the epithelial to mesenchymal gene expression switch^{86,169,223}, and ERK MAPK pathway activation in response to growth factors or mutant RAS increases TGF β -induced EMT¹³⁷. Cooperation between signalling pathways can also inhibit EMT. For example, HGF, which can induce EMT¹⁷³, can inhibit TGF β -induced EMT and myofibroblast differentiation in renal fibrosis by promoting the expression of the SMAD transcriptional co-repressor ski-related novel protein N (SNON)²²⁴. Additionally phosphorylation of SMADs by ERK MAPK can attenuate their nuclear translocation and transcriptional activities in mink lung epithelial cells¹³¹.

WNT and TGF β signalling seem to cooperate primarily to regulate gene expression changes during EMT. Destabilization of adherens junctions in response to TGF β enables β -catenin to accumulate in the nucleus and feed into canonical WNT signalling². In palate medial-edge epithelial cells, TGF β 3 induces the formation of a complex between SMAD2, SMAD4 and lymphoid enhancer-binding factor 1 (LEF1), which binds E-cadherin gene sequences and represses E-cadherin gene expression to promote EMT¹⁴⁶. In this way, the interactions of LEF or T cell factor (TCF) transcription factors with TGF β - or BMP-activated SMAD complexes may have key roles in the regulation of gene expression during EMT. Additionally, GSK3 β , which is inhibited by WNT signalling, integrates signalling pathways

and controls EMT. GSK3 β -mediated SNAIL1 phosphorylation increases the cytoplasmic retention and degradation of SNAIL1, and phosphorylation of GSK3 β by AKT promotes GSK3 β ubiquitylation and degradation, hence stabilizing SNAIL1 and enhancing the repression of E-cadherin expression⁵⁸. GSK3 β -mediated phosphorylation also decreases the stability of TGF β -and BMP-activated SMADs and, thus, attenuates SMAD-mediated responses^{225,226}. Also, PDGF induces the phosphorylation of a nuclear p68 (also known as DDX5) RNA helicase, which interacts with β -catenin to promote its nuclear accumulation independently of WNT signalling¹⁹⁸.

Finally, functional crosstalk between TGF β and Notch signalling has been reported. Inactivation of the genes encoding Notch1 or J κ -recombination signal-binding protein (RBPjk), a target of Notch signalling, impedes endocardial EMT in mouse embryos, which is accompanied by reduced SNAIL1 expression, maintenance of VE-cadherin expression in endocardial cells, and impaired TGF β 2 and T β RI expression^{213,227}. Conversely, TGF β can induce, through SMAD3, the expression of the Notch signalling target hairy and enhancer of split-related with YRPW motif 1 (HEY1) in several EMT cell models²²⁸.

Concluding remarks

Epithelial plasticity responses need to be seen as a spectrum of changes and transitions, depending on the tissue and signalling context. The process of MET, although less well characterized than EMT, illustrates the reversible nature of this transition. The initiation and progression of EMT involve distinct signalling pathways and signalling crosstalk. TGF β has emerged as a potent inducer of EMT, not only through SMAD-mediated changes in gene expression and the activation of EMT transcription factors but also through signalling pathways that are not mediated by SMADs. Quantifying the extent of EMT and the degree of its progression may be challenging, as the expression of EMT markers often depends on the cell type and initiating signalling pathway in question. Furthermore, characterizing EMT *in vivo* is impeded by its transient and reversible nature. Recent advances in imaging, including intravital techniques, will increase our knowledge of how the cellular environment controls EMT. Our understanding of EMT gained from cell culture systems, and from development and cancer, may enable us to target EMT associated with fibrosis and cancer progression.

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Glossary

Apical–basal polarity	The unequal distribution of proteins and other materials between the apical side (facing the exterior) and the basal side (facing the interior) in epithelial cells.
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CD44^{hi}CD24^{low} phenotype	The profile of the cell surface expression of two cell surface proteins, cluster of differentiation 44 (CD44) and CD24, which correlates with epithelial and carcinoma stem cell properties and behaviour.
Front–rear polarity	Unequal distribution of proteins between the front of a migrating cell, defined by the leading edge of the membrane, and the rear, where adhesion complexes disassemble. Front–rear polarity is required for directional migration.
Tight junctions	Adhesion protein complexes between adjacent epithelial or endothelial cells. Composed of integral membrane proteins, including claudins and occludin, and the cytoplasmic proteins zonula occludens 1 (ZO1), ZO2 and ZO3, which link the transmembrane proteins to the actin cytoskeleton and signalling proteins.
Adherens junctions	Form an adhesion belt between cells and provide homophilic interactions between epithelial cadherin (E-cadherin) molecules on opposing cell surfaces. The cytoplasmic proteins β -catenin and p120 catenin associate with E-cadherin and with the actin cytoskeleton through actin-associated proteins such as α -catenin.
Desmosomes	Characterized by a ‘patch’ that holds cells on opposing lateral cell surfaces together, using similar protein complexes to adherens junctions. Desmosomal cadherin, desmogleins and desmocollins associate with plakoglobins and plakophilins underneath the plasma membrane, and connect to intermediate filaments through desmoplakin.
Gap junctions	Protein complexes that connect neighbouring cells and enable the passage of ions and small molecules between them through hemichannels formed by hexamers of connexin proteins. They also provide adherence.
Apical compartment	The membrane-associated compartment at the apical side of a polarized epithelial cell.
Basolateral compartment	The membrane-associated compartment of an epithelial cell combining both the lateral sides that mediate cell–cell interactions and the basal side that enables the cell to interact with the underlying basement membrane.
Cortical actin cytoskeleton	The organization of the actin bundles and associated proteins underneath the membranes of epithelial cells that mediate cell–cell and cell– extracellular matrix interactions.
Actin stress fibre	Dynamic structures of actin filaments and associated proteins that have important roles in cell motility and contractility.

Endocardial cushion	An accumulation of cells, mostly arising from endothelial cells, in the primordial heart that will give rise to the valves and septa of the heart.
Fibrodysplasia ossificans progressiva	Very rare progressive connective tissue disease resulting in the gradual ossification of fibrous tissue, either spontaneously or in response to damage. It is caused by an activating mutation in the activin receptor type 1 (<i>ACVRI</i>) gene that encodes the transforming growth factor- β family type I receptor activin receptor-like kinase 2 (<i>ALK2</i>).
Guanine nucleotide exchange factors	(GEFs). Proteins that facilitate the exchange of GDP for GTP in the nucleotide-binding pocket of a GTP-binding protein.
GTPase-activating proteins	(GAPs). Proteins that inactivate small GTPases by stimulating them to hydrolyse GTP into GDP.
Guanine nucleotide dissociation inhibitors	(GDIs). Cytosolic proteins that bind to prenylated RAB proteins in their inactive, GDP-bound state. They are involved in RAB GTPase delivery to, and removal from, membranes.
Coelomic epithelium	The epithelium that lines the surface of the body wall and the abdominal organs.
Sclerotome	Part of the somite in vertebrate development that gives rise to the vertebrae and much of the skull.

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Box 1 | EMT, MET and stem cells

Epithelial–mesenchymal transition (EMT) and mesenchymal–epithelial transition (MET) have been closely linked to ‘stemness’ in development and cancer. The pluripotent embryonic stem (ES) cells in the inner mass of the blastocyst have epithelial characteristics²²⁹. In gastrulation, pluripotent epithelial epiblast cells ingress to form, through EMT, the primary mesoderm¹²³. EMT thus represents an initial differentiation event in the generation of the three germ layers from pluripotent cells. Illustrating the importance of EMT in early differentiation, ES cell or epiblast cell colonies in culture give rise to peripheral cells with a mesenchymal phenotype, as judged by loss of epithelial cadherin (E-cadherin) expression and the expression of vimentin and neural cadherin (N-cadherin)^{229,230}.

Conversely, the reprogramming of fibroblasts into induced pluripotent stem (iPS) cells requires the transition from a mesenchymal phenotype to an epithelial phenotype. This reprogramming recapitulates an MET process as it involves the repression of mesenchymal genes, including some that encode transcription factors with a role in EMT, and the activation of epithelial genes encoding epithelial cell junction proteins^{231,232}. Consistent with the role of transforming growth factor- β (TGF β) family proteins in EMT, TGF β receptor inhibitors increase, whereas TGF β decreases, the efficiency of iPS cell generation²³³. Furthermore, MET represents an initiation step that is required for progression towards pluripotency and which depends on, and is promoted by, bone morphogenetic protein (BMP) signalling²³⁴. As is the case in EMT, microRNAs (miRNAs) regulate MET, which is required for iPS cell reprogramming. Indeed, MET is promoted by the BMP-induced expression of the epithelial miR-200 family of miRNAs and miR-205 that repress zinc-finger E-box-binding 1 (ZEB1) and ZEB2 expression^{100,234}. Additionally, downregulation of TGF β receptor type II (T β RII) and RHOC expression by miR-302 or miR-372 increases reprogramming efficiency²³⁵.

EMT has also been associated with epithelial and carcinoma stem cell properties. Expression of SNAIL or TWIST in mammary epithelial cells induces a mesenchymal cell population marked with a CD44^{hi}CD24^{low} phenotype, which is similar to that observed in epithelial stem cells²³⁶. The correlation of EMT with stemness extends to carcinomas. These contain a subpopulation of self-renewing tumour-initiating cells, known as cancer stem cells (CSCs), which efficiently generate new tumours. In mammary carcinomas, induction of EMT promotes the generation of CD44^{hi}CD24^{low} CSCs that are able to form mammospheres, and similarly defined CSCs isolated from tumours express EMT markers²³⁶. Consistent with the reversible nature of EMT, differentiated cancer cells can transition into CSCs, and vice versa, enabling oncogenic mutations that arose in differentiated cancer cells to integrate through EMT into CSCs. As EMT promotes cell invasion that leads to tumour cell dissemination, this scenario enables CSCs with new oncogenic mutations to clonally expand, following invasion, dissemination and MET in secondary tumours^{237,238}.

In cancer, both EMT and CSC generation have been associated with TGF β signalling. For example, breast cancer CSCs show higher levels of TGF β 1 and T β RII expression than the more differentiated cells, and inhibition of TGF β signalling in CSCs

reestablishes an epithelial phenotype²³⁹. Also, WNT and Notch signalling are associated with CSCs. Colon CSCs show a high level of WNT signalling, with nuclear β -catenin at the invasive cancer front and in scattered tumour cells^{240,241}. Notch signalling contributes to the generation of CSCs in other cancers²⁴², including pancreatic adenocarcinomas²⁴³, and the inhibition of Notch signalling suppresses EMT and CSCs in a xenograft model²⁴⁴. As is the case in EMT- and MET-based cell reprogramming, miRNAs contribute to the generation and maintenance of CSCs. For example, the miR-106b-25 cluster induces EMT and tumour-initiating characteristics in breast cancer by repressing SMAD7 to increase TGF β signalling²⁴⁵. However, it also promotes MET and iPS cell reprogramming by targeting T β RII, possibly reflecting context-dependent differences in its functions²⁴⁶.

Box 2 | EndMT and the expression of smooth muscle actin

Similarly to epithelial cells, endothelial cells can transition to a mesenchymal phenotype, a process known as endothelial-mesenchymal transition (EndMT)^{247,248}. EndMT is developmentally required in endocardial cushion formation, and contributes to fibroblast accumulation in mouse models of renal, pulmonary and cardiac fibrosis²⁴⁹. EndMT also provides a major source of cancer-associated fibroblasts that contribute to carcinoma progression²⁵⁰. Additionally, endothelial cells generate chondrocytes and osteoblasts in heterotypic bone formation, which characterizes fibrodysplasia ossificans progressiva²⁵¹.

During EndMT, as in epithelial-mesenchymal transition (EMT), endothelial cells downregulate the expression of cell junction proteins, specifically vascular endothelial (VE)-cadherin and cluster of differentiation 31 (CD31; also known as PECAM1) (components of adherens junctions)¹²⁰, and the endothelial tight junction protein claudin 5 (REFS 248,252). The transcription programmes and signalling pathways that drive EndMT share extensive similarities with EMT. SNAIL transcription factors are induced in EndMT and directly repress the expression of the genes encoding VE-cadherin and CD31, while activating the expression of invasion-associated genes and the migratory phenotype^{213,248}. In heart development, SNAIL1, SNAIL2 and TWIST1 cooperate with the transcription factors hairy/enhancer-of-split related with YRPW motif protein 1 (HEY1) and HEY2 downstream of Notch signalling, to drive EndMT in a signalling crosstalk that is balanced by bone morphogenetic protein 2 (BMP2) and NOTCH1 (REF. 253). Disturbing this balance leads to septal or valve malformation²⁵³.

As in EMT, transforming growth factor- β (TGF β) family signalling through SMADs induces EndMT, which further emphasizes the similarities between EMT and EndMT. TGF β activates the expression of SNAIL, which drives EMT and EndMT, and represses the expression of VE-cadherin, CD31 and claudin 5 (REF. 248). A dominant-negative form of SMAD4 blocks TGF β -induced EndMT, which correlates with decreased SNAIL expression, attenuated decrease in VE-cadherin and CD31 expression, and increased fibroblast-specific protein 1 (FSP1) and α -smooth muscle actin (α SMA) expression²⁵⁴. As TGF β 2 and BMP4 can induce EndMT through the activin receptor-like kinase 2 (ALK2) receptor¹²⁹, SMAD1 and/or SMAD5 may have important roles in controlling endothelial plasticity. The epidermal growth factor (EGF) receptor human EGF receptor 2 (HER2) also participates in EndMT during heart valve formation, in an ERK MAPK signalling-dependent manner²⁵⁵.

EMT and EndMT can generate mesenchymal cells that express α SMA, as seen in myofibroblasts, and these processes have been named EMyT and EndMyT^{248,256,257}. It is, however, unclear whether functional myofibroblasts seen in fibrosis or cancer derive from epithelial or endothelial cells. In EMyT, the transcription factor serum response factor (SRF) and myocardin family transcription factors, including myocardin and myocardin-related transcription factors (MRTFs; also known as MKLs), drive expression of the myofibroblast markers α SMA and smooth muscle protein 22 α (SM22 α ; also known as transgelin). Zinc-finger E-box-binding 1 (ZEB1) and SMAD3 interact with SRF to activate α SMA expression¹⁴², and SMAD3 interacts with myocardin to activate

SM22 α expression²⁵⁸. In response to TGF β , SMAD3 also induces expression and nuclear import of MRTFs, and cooperates with MRTFs to induce myofibroblast differentiation, partly by inducing the expression of SNAIL2 (REFS 141,259). In kidney epithelial cells, SMAD3 has a biphasic role in TGF β -induced EMT; it drives EMT at an early stage of differentiation, but it is degraded at a later stage. Persistent SMAD3 expression interferes with SRF–MRTF complex formation, thus repressing α SMA expression²⁶⁰. In addition, β -catenin, which is released from junctions, antagonizes the inhibitory effect of SMAD3 on the MRTF–SRF complex, which in turns increases α SMA expression²⁶¹.

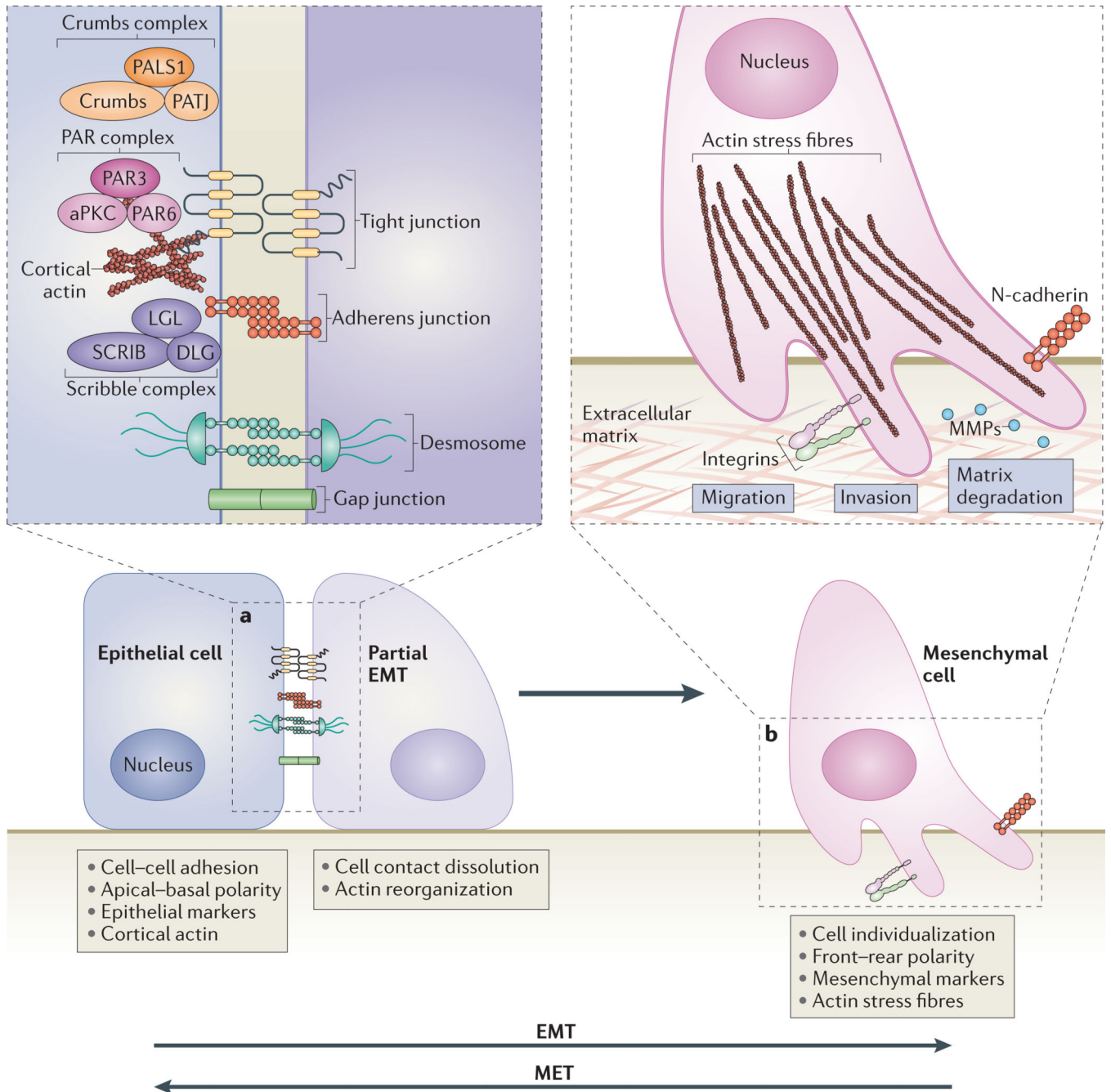


Figure 1. Cellular events during EMT

a | The first steps of epithelial–mesenchymal transition (EMT) are the disassembly of epithelial cell–cell contacts — that is, tight junctions, adherens junctions, desmosomes and gap junctions — and the loss of cell polarity through the disruption of the Crumbs, partitioning defective (PAR) and Scribble (SCRIB) polarity complexes. The expression of epithelial genes is repressed, concomitantly with the activation of mesenchymal gene expression. **b** | Next, the epithelial actin architecture reorganizes, and cells acquire motility and invasive capacities by forming lamellipodia, filopodia and invadopodia, and by

expressing matrix metalloproteinases (MMPs) that can degrade extracellular matrix (ECM) proteins. The process of mesenchymal–epithelial transition (MET) enables the cells that have undergone EMT to revert to the epithelial state. Three types of EMT can be discerned depending on the physiological tissue context⁴. Type 1 EMT occurs in embryogenesis and organ development²⁶², type 2 EMT is important for tissue regeneration and organ fibrosis^{4,263}, and type 3 EMT is associated with cancer progression⁴ and cancer stem cell properties²³⁷. MET also contributes to development (for example, kidney development²⁶⁴) and the generation of metastatic carcinomas^{238,265}. Consecutive rounds of EMT and MET occur during development^{262,264}. In primary EMT, ectodermal or epithelial cells without prior history of EMT differentiate into mesenchymal cells. In secondary EMT, cells that have already undergone EMT and reverted to the epithelial state initiate a new EMT process^{3,262}. Similarly, following dissemination, cancer cells can revert through MET to an epithelial state, leading to the formation of secondary carcinomas with phenotypes similar to the primary tumour²⁶⁵. aPKC, atypical protein kinase C; DLG, discs large; LGL, lethal giant larvae; N-cadherin, neural cadherin; PALS1, protein associated with Lin-7 1; PATJ, PALS1-associated tight-junction protein.

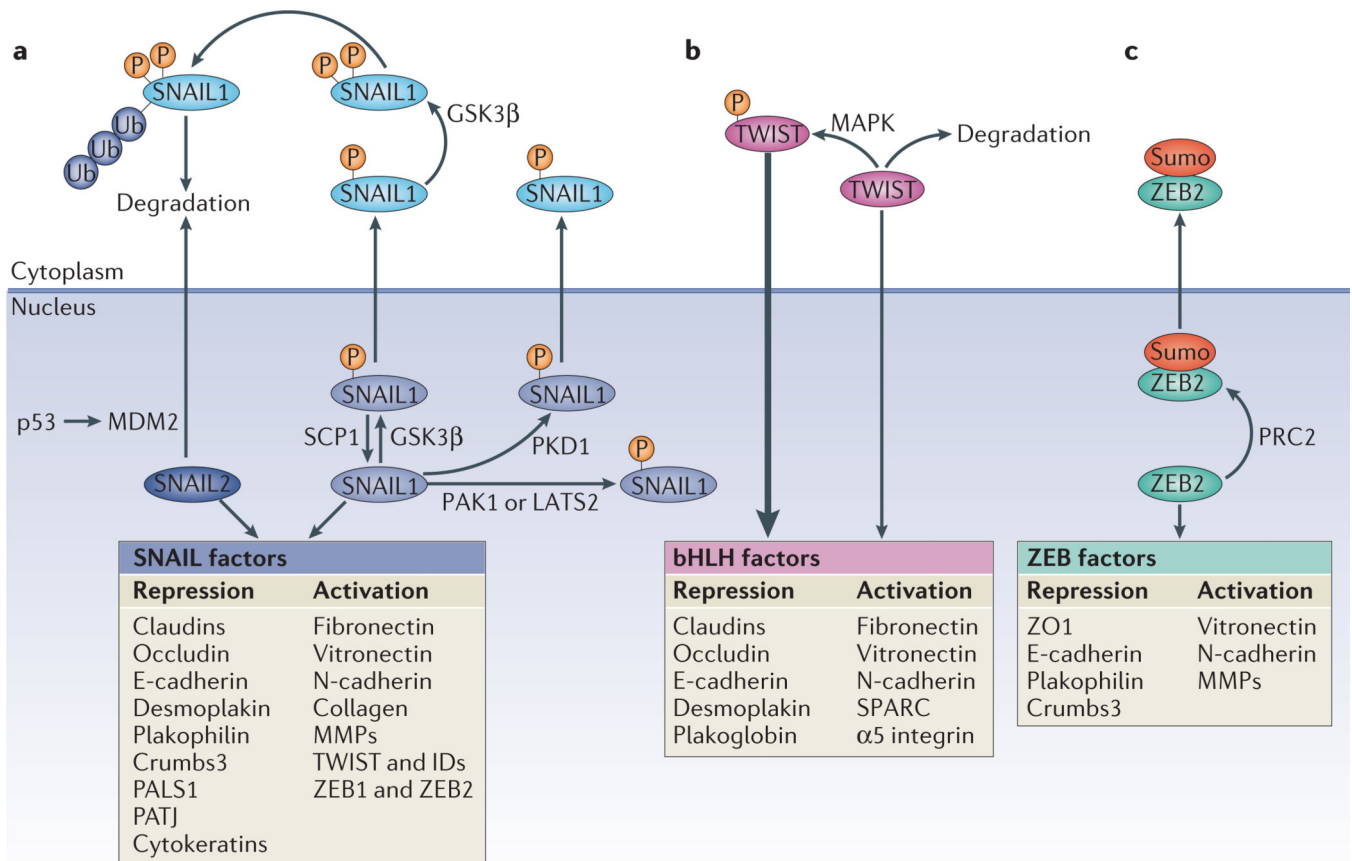


Figure 2. Roles and regulation of major EMT transcription factors

Epithelial–mesenchymal transition (EMT) is driven by SNAIL, zinc-finger E-box-binding (ZEB) and basic helix–loop–helix (bHLH) transcription factors that repress epithelial marker genes and activate genes associated with the mesenchymal phenotype. Post-translational modifications regulate their activities, subcellular localization and stability. **a** | Glycogen synthase kinase-3 β (GSK3 β) phosphorylates (P) SNAIL1 at two motifs; phosphorylation of the first motif facilitates the nuclear export of SNAIL1, and phosphorylation of the second motif enables the ubiquitin (Ub)-mediated degradation of SNAIL1. Phosphorylation of SNAIL1 by protein kinase D1 (PKD1) also leads to its nuclear export. Conversely, phosphorylation of SNAIL1 by p21 activated kinase 1 (PAK1) or large tumour suppressor 2 (LATS2), or dephosphorylation of SNAIL1 by small C-terminal domain phosphatase 1 (SCP1) promotes the nuclear retention of SNAIL1 and enhances its activity. SNAIL2 is degraded as a result of its p53-mediated recruitment to the p53–mouse double minute 2 (MDM2) complex. **b** | TWIST is phosphorylated by the MAPK p38, JUN N-terminal kinase (JNK) and ERK, which protects it from degradation, and thus promotes its nuclear import and functions. **c** | ZEB2 is sumoylated (Sumo) by Polycomb repressive complex 2 (PRC2) and subsequently exported from the nucleus, which reduces its activity as a transcription factor. E-cadherin, epithelial cadherin; ID, inhibitor of differentiation; MMP, matrix metalloproteinase; N-cadherin, neural cadherin; PALS1, protein associated with Lin-7 1; PATJ, PALS1-associated tight-junction protein; SPARC, secreted protein acidic and rich in Cys; ZO1, zonula occludens 1.

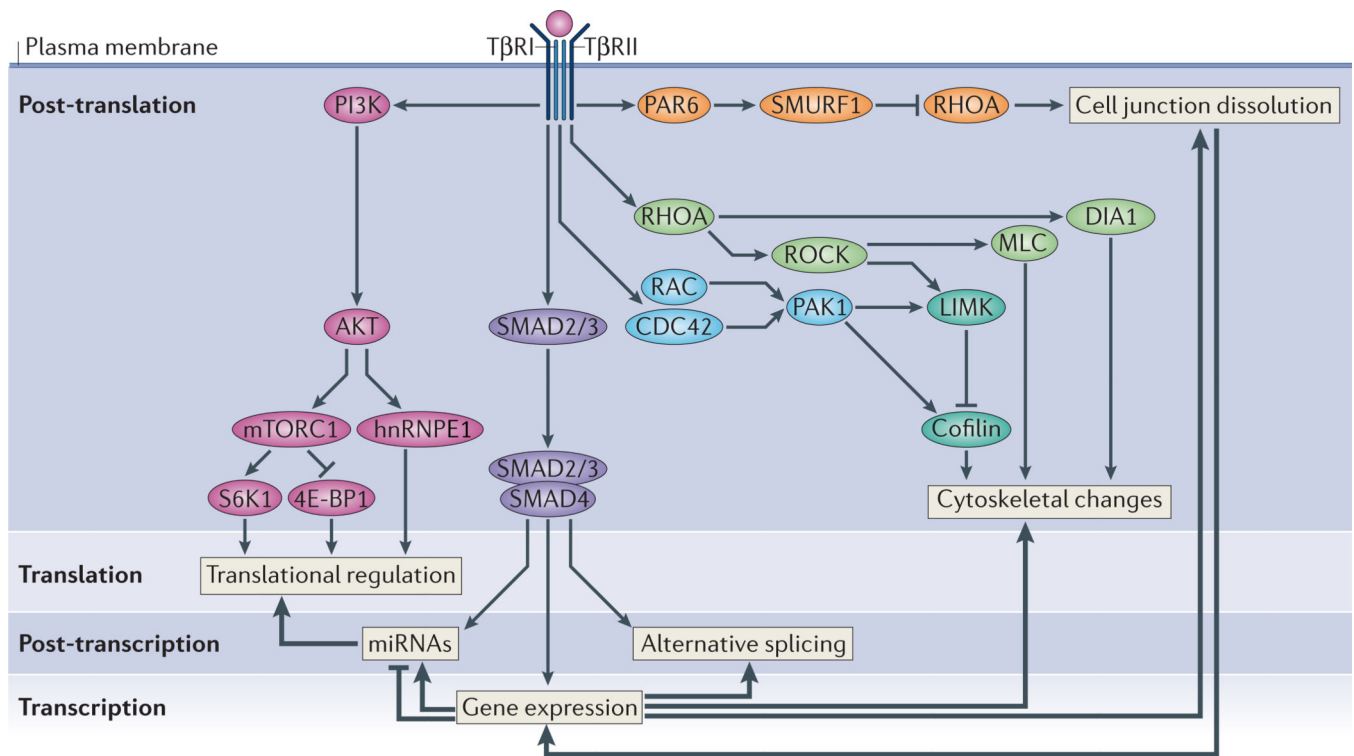


Figure 3. Molecular mechanisms of TGFβ-induced EMT

The initiation of, and progression through, epithelial-mesenchymal transition (EMT) are regulated at the transcriptional, post-transcriptional, translational and post-translational levels. Transforming growth factor-β (TGFβ) induces EMT by acting at several of these levels and through SMAD-mediated and non-SMAD signalling. TGFβ signals through a tetrameric complex of type I and type II receptors (TβRI and TβRII) to activate SMAD2 and SMAD3, which then combine with SMAD4. The trimeric SMAD complex translocates into the nucleus and cooperates with transcription regulators in the repression or activation of target genes. TGFβ–SMAD signalling activates the expression of EMT transcription factors, and SMAD complexes cooperate with these transcription factors to increase their transcriptional activities. TGFβ also induces the expression of microRNAs (miRNAs) that repress the expression of epithelial proteins, and EMT transcription factors can repress the expression of miRNAs that target mesenchymal components, thus promoting EMT. TGFβ signalling also decreases the expression of the epithelial splicing regulatory proteins (ESRPs), which leads to a differential splicing programme following EMT. TGFβ can also induce non-SMAD signalling pathways that contribute to EMT. It activates PI3K–AKT–mammalian TOR complex 1 (mTORC1) signalling, which increases translation and cell size; active AKT also derepresses the translation of specific mRNAs by phosphorylating heterogeneous nuclear ribonucleoprotein E1 (hnRNPE1). TGFβ also increases cell junction dissolution and induces cytoskeletal changes by regulating RHO–GTPases. TGFβ induces TβRII association with a TβRI-partitioning defective 6 (PAR6) complex at tight junctions, which enables TβRII to phosphorylate PAR6; this results in the recruitment of the E3 ubiquitin ligase SMAD ubiquitylation regulatory factor 1 (SMURF1), RHOA ubiquitylation and degradation, and the loss of tight junctions. TGFβ also induces RHOA activity; this

promotes actin reorganization by leading to the activation of diaphanous (DIA1) and also RHO-associated kinase (ROCK), which phosphorylates myosin light chain (MLC) to activate LIM kinase (LIMK) and thus inhibit cofilin. RAC and CDC42 also participate in cytoskeletal changes through p21 activated kinase 1 (PAK1) and direct the formation of lamellipodia and filopodia. 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; S6K1, ribosomal S6 kinase 1.

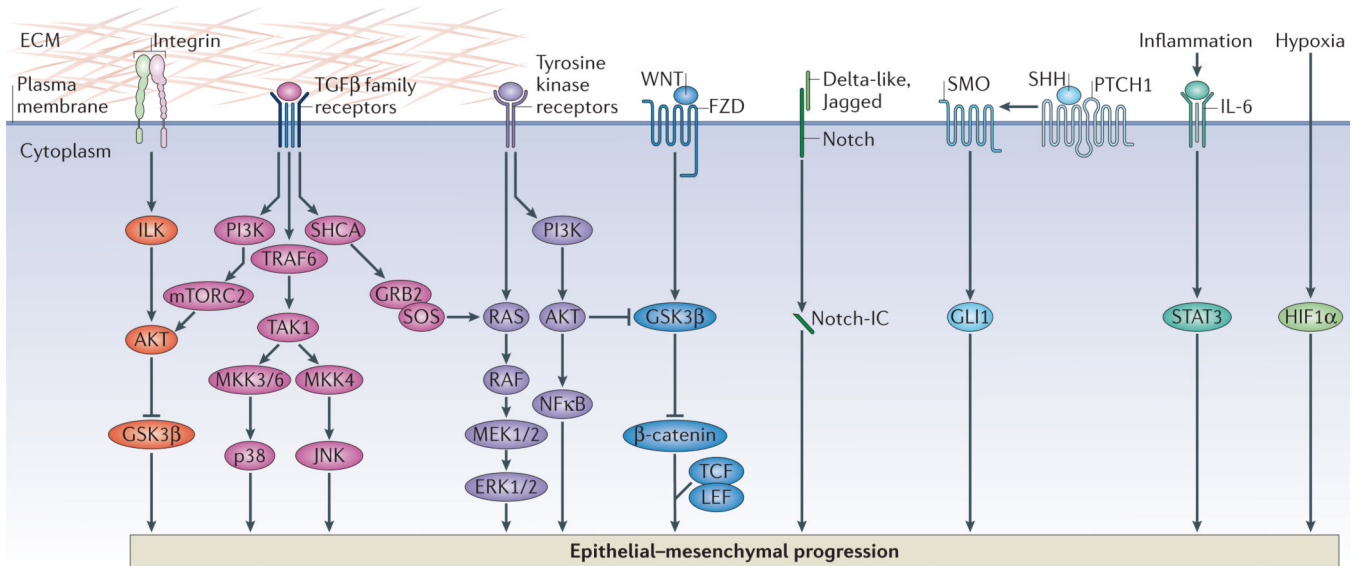


Figure 4. Signalling pathways involved in EMT

Epithelial-mesenchymal transition (EMT) progression is regulated by signalling pathways that can cooperate to induce full EMT responses. In addition to promoting EMT through SMAD proteins, transforming growth factor- β (TGF β) can activate the PI3K–AKT, ERK MAPK, p38 MAPK and JUN N-terminal kinase (JNK) pathways. T β RI phosphorylates the adaptor protein SRC homology 2 domain-containing-transforming A (SHCA), which creates a docking site for growth factor receptor-bound protein 2 (GRB2) and son of sevenless (SOS) and initiates the RAS-RAF-MEK-ERK MAPK pathway. TGF β -induced p38 MAPK and JNK activation results from the association of TNF receptor-associated factor 6 (TRAF6) with the TGF β receptor complex, which activates TGF β -activated kinase 1 (TAK1) and p38 MAPK and JNK as a result. Several growth factors that act through receptor tyrosine kinases (RTKs), including epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF), can induce EMT. The RAS-RAF-MEK-ERK MAPK signalling cascade represents a major pathway that is activated by RTKs in response to growth factors. Once activated ERK1 and ERK2 MAPK can facilitate EMT by increasing the expression of EMT transcription factors and regulators of cell motility and invasion, such as RHO GTPases and the 90 kDa ribosomal protein S6 kinase. Other signalling pathways, such as the WNT, Notch and Hedgehog (HH) pathways, also participate in EMT. WNT signalling promotes EMT by inhibiting glycogen synthase kinase-3 β (GSK3 β) to stabilize β -catenin, which translocates to the nucleus to engage the transcription factors lymphoid enhancer-binding factor 1 (LEF) and T cell factor (TCF) and promote a gene expression programme that favours EMT. In HH signalling, glioma 1 (GLI1) can induce SNAIL1 expression, and the intracellular domain of Notch can activate SNAIL2 expression; thus HH and Notch signalling promote a decrease in epithelial cadherin (E-cadherin) levels. The cell microenvironment also regulates EMT. During inflammation and in cancer, interleukin-6 (IL-6) can promote EMT through Janus kinase (JAK)-signal transducer and activator of transcription 3 (STAT3)-induced SNAIL1 expression. Hypoxia in the tumour environment can promote EMT through hypoxia-inducible factor 1 α (HIF1 α), which activates the expression of TWIST. EMT

responses can be increased through crosstalk and cooperation between distinct pathways. For example, RTK- or integrin-induced AKT activation can induce SNAIL expression through nuclear factor- κ B (NF- κ B) and stabilize SNAIL and β -catenin by inhibiting GSK3 β , thus cooperating with WNT signalling. TGF β signalling can also increase EMT responses initiated by growth factors such as FGF or EGF. ECM, extracellular matrix; FZD, frizzled; ILK, integrin-linked kinase; MKK, MAPK kinase; mTORC2, mammalian TOR complex 2; Notch-IC, intracellular fragment of Notch; PTCH1, patched 1; SHH, sonic HH; SMO, smoothed.

Table 1

EMT transcription factors, their direct targets and the signalling pathways that induce their activity

Transcription factor	Downregulated expression during EMT	Upregulated expression during EMT	Regulatory signalling pathways	Refs
SNAIL1 and SNAIL2	E-cadherin, claudins, occludin, Crumbs3, PALS1, PATJ, cytokeratins, desmoplakin and plakophilin	Fibronectin, N-cadherin, collagen, MMP15, MMP2, MMP9, TWIST, ID1, ID2, ZEB1 and ZEB2	TGF β -SMAD3, WNT- β -catenin, Notch, PI3K-AKT, NF- κ B, EGF and FGF	11,45,59,60,61,66,159,185
TWIST1	E-cadherin, claudins, occludin, desmoplakin and plakoglobin	Fibronectin, N-cadherin and α 5 integrin	MAPK	11,45,67,68,72
ZEB1 and ZEB2	E-cadherin, ZO1, Crumbs3 and plakophilin	N-cadherin and MMPs	TGF β -SMAD3, WNT- β -catenin and RAS-MAPK	11,45,73-75,77,142,143
FOXD3	Unknown	Unknown	β 1 integrin and laminin	266
FOXC2	E-cadherin	Fibronectin, vimentin, N-cadherin and α SMA	TGF β -SMAD3	267
FOXF1	E-cadherin, claudin1, occludin, desmoglein1 β , desmoglein2, desmocollin2 and desmoplakin	Fibronectin and N-cadherin	Unknown	268
FOXQ1	E-cadherin	Fibronectin, N-cadherin and vimentin	Unknown	269
FOXO3A	E-cadherin	SNAIL1	AKT	270
FOXA1	E-cadherin	Fibronectin, vimentin and SNAIL1	TGF β , HGF and AKT	271
FOXA2	E-cadherin and ZO1	Fibronectin, vimentin, N-cadherin, SNAIL1 and SNAIL2	TGF β , HGF and AKT	271
Serpent, GATA4 and GATA6	E-cadherin, Crumbs and claudins	N-cadherin and MMP1	Unknown	81
HMGA2	E-cadherin	SNAIL1, SNAIL2 and TWIST	TGF β -SMAD3	103
SOX9	Unknown	SNAIL2	BMPs and PKA	83
KLF8	E-cadherin	MMP9	Unknown	272
CBFA-KAP1	Unknown	FSP1	Unknown	273
ZNF703 (also known as Zeppo1 in mice)	E-cadherin	Vimentin, N-cadherin, SNAIL1 and cytokeratin	RHO-GTPase	274
PRX1	E-cadherin	Vimentin and laminin	BMP2 and TGF β	275

α SMA, α -smooth muscle actin; BMPs, bone morphogenetic proteins; CBFA, CArG-binding factor-A; E-cadherin, epithelial cadherin; EGF, epidermal growth factor; EMT, epithelial-mesenchymal transition; FGF, fibroblast growth factor; FOX, forkhead box; FSP1, fibroblast-specific protein 1; HGF, hepatocyte growth factor; HMGA2, high mobility group A2; ID, inhibitor of differentiation; KAP1, KRAB-associated protein 1 (also known as TIF1 β); KLF8, Krueppel-like factor 8; MMP, matrix metalloproteinase; N-cadherin, neural cadherin; NF- κ B, nuclear factor- κ B; TGF β , transforming growth factor- β ; PALS1, protein associated with Lin-7 1; PATJ, PALS1-associated tight-junction protein; PKA, protein kinase A; PRX1, paired-related homeobox 1; SOX, SRY box; ZNF703, zinc-finger 703; ZO1, zonula occludens 1; ZEB, zinc-finger E-box-binding.