OPEN

Experimental & Molecular Medicine (2014) 46, e108; doi:10.1038/emm.2014.44

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REVIEW

Role of the epithelial–mesenchymal transition and its effects on embryonic stem cells

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The epithelial–mesenchymal transition (EMT) is important for embryonic development and the formation of various tissues or organs. However, EMT dysfunction in normal cells leads to diseases, such as cancer or fibrosis. During the EMT, epithelial cells are converted into more invasive and active mesenchymal cells. E-box-binding proteins, including Snail, ZEB and helix–loop–helix family members, serve as EMT-activating transcription factors. These transcription factors repress the expression of epithelial markers, for example, E-cadherin, rearrange the cytoskeleton and promote the expression of mesenchymal markers, such as vimentin, fibronectin and other EMT-activating transcription factors. Signaling pathways that induce EMT, including transforming growth factor-β, Wnt/glycogen synthase kinase-3β, Notch and receptor tyrosine kinase signaling pathways, interact with each other for the regulation of this process. Although the mechanism(s) underlying EMT in cancer or embryonic development have been identified, the mechanism(s) in embryonic stem cells (ESCs) remain unclear. In this review, we describe the underlying mechanisms of important EMT factors, indicating a precise role for EMT in ESCs, and characterize the relationship between EMT and ESCs.
Experimental & Molecular Medicine (2014) **46,** e108; doi:10.1038/emm.2014.44; published online 1 August 2014

INTRODUCTION

The epithelial–mesenchymal transition (EMT) is important for generating multiple tissues during organismal development. This process is particularly essential for the gastrulation of metazoans and neural crest delamination of vertebrates.[1] EMT is also involved in wound healing. However, EMT dysfunction leads to pathological conditions, including carcinogenesis and fibrosis. The most critical difference between embryonic and tumorigenic EMT is that genetically abnormal cells are employed during EMT for tumorigenesis, and these cells lose sensitivity to normal growth regulatory signals.[2] During EMT, polarized epithelial cells are converted into mesenchymal cells. Therefore, epithelial cells lose characteristics that enable differentiation, including cell–cell adhesion, apical–basal polarity and motility dysfunction, and obtain mesenchymal properties, such as motility, invasiveness and apoptotic resistance.[3] The results of studies conducted since the 2007 EMT conference held in Poland have suggested several characteristics for the classification of EMT; consequently, three types of EMT were proposed at Cold Spring Harbor Laboratories in 2008.[3] Type 1 EMT affects embryo formation, implantation, organ development and the generation of various cell types. Type 2 EMT promotes wound healing, tissue regeneration and fibrosis, and participates in inflammation.[3,4] Type 3 EMT is involved in cancer progression and metastasis.[3,4]

Embryonic stem cells (ESCs) are acquired from the inner cell mass of early blastocysts. Accordingly, these cells differentiate into multiple cell types, and can be used to treat diseases through the generation of tissues and organs.[5] Mouse and human ESCs have been used for EMT-related research,[6] and many differences in the cellular properties of these cells have also been identified (Table 1). For example, human ESCs grow more slowly and have a flattened morphology, different gene expression profile and distinct pluripotent and differentiation signals compared with murine ESCs.[7–9] Therefore, terms such as 'primed' and 'naïve' have been used to classify these cell types. Naïve cells can be more undifferentiated than primed cells, as naïve cells can be harvested from the inner cell mass of early blastocysts in mouse ESCs, whereas primed cells have been identified in the epiblast of late blastocysts in mouse ESCs. Naïve cells can be subcultured as single cells, forming round colonies. In addition, naïve stem cells possess two active X chromosomes and express stage-specific embryonic

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Received 27 December 2013; revised 4 March 2014; accepted 10 April 2014

Table 1 Features of naïve and primed stem cell types

Naïve state	*Primed state*
Small, round, dome-shaped colony	Flattened, monolayer colony morphology
LIF, BMP4 signaling pathway dependent	FGF and activin/nodal signaling pathway
Cell surface marker: SSEA-1	SSEA-4, TRA 1-60 and TRA 1-81
Two active X chromosomes in females	X chromosome inactivation in females
Mouse embryonic stem cells	Mouse epiblast stem cells

Abbreviations: FGF, fibroblast growth factor; LIF, leukemia inhibitory factor; SSEA, stage-specific embryonic antigen.

antigen-1 on the cell surface.[10] The mechanism(s) underlying EMT during cancer or embryonic development have been elucidated. However, the corresponding mechanism(s) of ESCs remains unclear. In this review, we highlight the functions of important EMT factors to determine the precise role for EMT in ESCs and characterize the relationship between EMT and ESCs.

KEY EMT SIGNALING MOLECULES

E-cadherin

Cadherins are calcium ion-dependent glycoproteins expressed on the cell surface. These proteins are involved in cell–cell adhesion and interaction. The cadherin family is divided into type 1 and type 2.[11] E-cadherin is a cadherin family member that possesses a single-pass transmembrane domain, and this protein is primarily detected in epithelial cells. Although the region of E-cadherin that participates in cell–cell adhesion is unknown, the histidine-alanine-valine domain might have an important role in cell–cell interaction.[11,12] E-cadherin has two preserved domains: the β-catenin-binding domain and p120-binding domain. The β-catenin-binding domain promotes the interaction between the actin cytoskeleton and E-cadherin. This interaction is achieved through the cytoplasmic cell adhesion complex, which comprises epithelial protein lost in neoplasm, β-catenin and α-catenin.[11,13] As decreased E-cadherin expression on the cell surface might be involved in tumor progression and metastasis, E-cadherin is considered a repressor of tumor progression and metastasis.[11] Decreased E-cadherin expression breaks down cell–cell contact and increases EMT induction, resulting in tumor motility. For example, in colorectal carcinoma, which expresses STAT3, the expression of E-cadherin was decreased. However, the induction of small interfering RNA for STAT3 increased E-cadherin expression, thereby decreasing the invasive properties and expression of vimentin and N-cadherin in these cells.[14] E-cadherin has also been associated with transcriptional repressive pathways through the E-box-binding proteins, Snail and Slug, and matrix metalloproteinase-7 (MMP-7) and MMP-13. For example, MMP-7 and MMP-13 can remove the extracellular E-cadherin domain, and the deleted soluble ectodomain might suppress E-cadherin activity in neighboring cells.[11,15,16]

Transcription factors that regulate EMT

A number of transcription factors, including Snail, ZEB and helix–loop–helix (HLH) family members, regulate EMT.[17–19] In vertebrates, the Snail family comprises three members: Snail 1, Snail 2 and Snail 3.[20] Snail 1 is also called 'Snail,' and Snail 2 is also known as 'Slug,' and these proteins suppress the expression of epithelial genes, such as E-cadherin and plakoglobin, and also activate the expression of mesenchymal proteins, including N-cadherin and fibronectin. The activation of the Snail family depends on a conserved zinc finger domain and an N-terminal snail/Gfi domain.[16,20,21] In addition, the C-terminal region attaches to the E-box, represses the expression of target genes associated with epithelial cell markers and activates mesenchymal protein expression.[20,21] Other transcription factors, including Ets-1 and specificity protein 1,[22,23] are also involved in this process. The Snail family members are important factors for EMT regulation. In colon cancer (DLD1), cells transfected with Snail and Slug show increased β-catenin –T-cell factor-4 transcription complex formation.[24] This complex increases the expression of transforming growth factor (TGF)-β3, resulting in the TGF-β3-induced upregulation of lymphoid enhancer factor-1 gene expression, which induces the EMT response. In addition, TGF-β1 and 2 stimulate EMT signaling pathways through Snail and Slug.[24] Snail blocks epithelial markers, that is, cytokeratin-18, Muc-1, desmoplakin and E-cadherin, thereby increasing the expression of fibronectin and vimentin. It upregulates Rho-GTPase during gastrulation, resulting in cytoskeletal changes.[16] In lung cancer, Slug acts as a metastasis-promoting gene, demonstrating that slug downregulates E-cadherin expression, upregulates MMP-2 and increases angiogenesis.[16]

HLH family members also influence the EMT process.[20] This protein family can be divided into seven groups. Class 1 HLH proteins include E12 and E47, class 2 HLH proteins include twist and class 5 HLH proteins include Ids. These three HLH groups, classes 1, 2, 5, are involved in EMT induction. E12 or E47 inhibit the expression of E-cadherin and plakoglobin and increase the production of vimentin and fibronectin.[20] Ids repress transcription through different molecules, such as TGF-β, associated with the repression of E-cadherin expression.[20] Ids do not bind DNA, but rather bind to E12 or E47, acting as negative inhibitors.[20,25] The ectopic expression of twist represses the expression of E-cadherin, occludin and claudin-7 and facilitates the expression of vimentin and N-cadherin to enhance cancer cell migration and invasion.[26] Twist forms a heterodimer with E12 and E47, and this dimer regulates transcription through DNA binding.[27]

In vertebrates, the ZEB family includes ZEB1 (deltaEF1 or AREB6) and ZEB2 (smad-interacting protein 1, SIP1).[18] ZEB family proteins possess a zinc finger cluster at each end, which interacts with DNA. A repressor motif in the central homeodomain and the recruitment of C-terminal-binding protein as a co-repressor mediate transcriptional repression through ZEB1 and ZEB2.[20] However, the interaction between ZEB, PCAF and p300 converts ZEB1 from a repressor to an

activator. These proteins reduce epithelial marker expression and increase mesenchymal marker expression during the induction of EMT.[25] ZEB is activated through other signaling molecules, such as TGF-β, and growth factors that activate Ras/mitogen-activated protein kinase; in addition, this protein is repressed through microRNA 200 and microRNA 250 family members.[17,28,29]

Signaling pathways that influence EMT

Several pathways regulate the EMT, including pathways involving TGF-β, Wnt, receptor tyrosine kinase (RTK), hedgehog, tumor necrosis factor-α and Notch.[30] TGF-β is an important molecule for the induction of EMT during metastasis and embryogenesis.[31–33] TGF-β was shown to have two actions: tumor suppression and tumor promotion. TGF-β acts as a tumor repressor during early tumor growth and induces cell growth arrest and apoptosis. During late tumor growth, TGF-β initiates cancer progression and metastasis through smad-dependent or smad-independent signaling pathways.[21,34] For example, the exposure of mouse keratinocytes overexpressing TGF-β to chemical carcinogens results in the TGF-β-mediated suppression of skin tumor formation during the initial stages of cancer progression, while TGF-β acts as a tumor promoter during the late stages of cancer progression.[35] The Smad-dependent signaling pathway regulates EMT through the expression of snail, ZEB and twist.[20] These transcriptional regulators repress the expression of epithelial markers, such as E-cadherin, plakoglobin and occludin, and also activate mesenchymal markers, including vimentin, fibronectin and N-cadherin.[20,36] TGF-β signaling involves ligand binding to TGF receptors (TβR1 and TβR2). Smad2/3 is subsequently activated and forms a complex with smad4. Smad 2/3/4 complexes translocate to the nucleus and interact with other transcription factors to regulate the expression of target genes.[37] Smad-independent pathways include phosphoinositide 3-kinase/Akt, Ras, mitogen-activated protein kinase and Rho-like GTPase pathways.[38] These pathways upregulate the expression of snail, twist and ZEB. TGF-β also promotes EMT through interactions with Wnt and Notch.[20]

The Notch pathway is initiated through interactions between the Notch receptor and ligands on adjacent cells. Four Notch receptors (1–4) and five ligands (Dll-1, Dll-3, Dll-4, Jagged-1 and Jagged-2) exist in mammals.[39,40] Notch signaling is initiated through ligand binding to an adjacent receptor. Subsequently, γ-secretase cleaves the intramembrane Notch receptor. The released Notch intracellular domain translocates to the nucleus and interacts with C-protein-binding factor 1/Suppressor of Hairless/Lag-1[40,41] and acts as an activator of target genes, including Hes and Hey. Although studies have suggested that Notch signaling is insufficient to completely induce EMT and crosstalk with other signaling molecules might therefore be required,[21] the Notch signaling pathway has been considered an important regulator for the induction of EMT. Notch activation morphologically, phenotypically and functionally converts epithelial cells into mesenchymal-type cells.[42] This change has been associated with the downregulation of epithelial markers, including E-cadherin, Tei1, Tei2 and platelet–endothelial epithelial cell adhesion molecule-1, and the upregulation of mesenchymal markers, such as α-smooth muscle actin (SMA) and fibronectin.[42] It has been reported that snail and slug are induced through Notch signaling to promote EMT, which has an important role during both embryonic development and tumor progression.[43,44]

Wnt signaling is also important for diverse cell functions via canonical (β-catenin) or noncanonical pathways.[21] The formation of the Wnt–Fz–LRP complex through the binding of ligands, that is, wnt1 and wnt3, to their receptors, Frizzled (Fz) and LRP 5/6, initiates the canonical pathway. Without the Wnt signaling pathway, cytoplasmic β-catenin forms a complex with Axin, adenomatous polyposis coli, glycogen synthase kinase-3β and Ck1.[45] When the cell receives Wnt signals, a complex is formed between LRP5/6 and Fz. These structures affect β-catenin stabilization, nuclear translocation and protein accumulation. In the nucleus, β-catenin forms a complex with T-cell factor/lymphoid enhancer factor, thereby initiating the expression of Wnt target genes.[45,46] During EMT, smad2 and smad 4 influence Wnt signaling to repress E-cadherin expression in medial-edge epithelial cells. Lymphoid enhancer factor-1 has also been associated with mesenchymal marker expression.[20,47] Other studies have suggested that wnt3 promotes EMT through the increased expression of N-cadherin, twist and slug and the decreased expression of E-cadherin in the trastuzumab-insensitive cells, SKBR3/100-8 and BT474/100-2.[48] In addition, previous studies have shown that the activation of Wnt signaling is important for the induction of EMT in breast and prostate cancers.[49,50]

RTK signaling alone does not induce EMT. Therefore, interplay with other signaling molecules might be required.[21] Growth factors, such as hepatocyte growth factor, epidermal growth factor and fibroblast growth factor, bind to their respective receptors and activate extensive crosstalk networks that affect EMT.[21] These growth factors switch signals through the structural activation of RTK, which activates TGF-β and integrin signaling pathways to alter EMT. Three activities occurring downstream of Ras might be required for the induction of EMT: smad2 activation, phosphoinositide 3-kinase/Akt pathway initiation and Raf/mitogen-activated protein kinase signaling pathway.[30]

EMT IN ESCS

Few studies examining the role(s) of EMT in human and mouse ESCs have been published.[51,52] In most ESCs, the transformation of epithelial cells into mesenchymal cells might occur during ESC differentiation. In monolayer cultures, differentiation of human ESCs involves (1) the conversion from E-cadherin to N-cadherin, (2) increased vimentin expression, (3) the increase of repression molecules of E-cadherin, such as Snail and Slug, and (4) increased gelatinase activity and cellular motility. In undifferentiated human ESCs, the abrogation of E-cadherin-mediated cell–cell contact increases cellular motility and induces actin

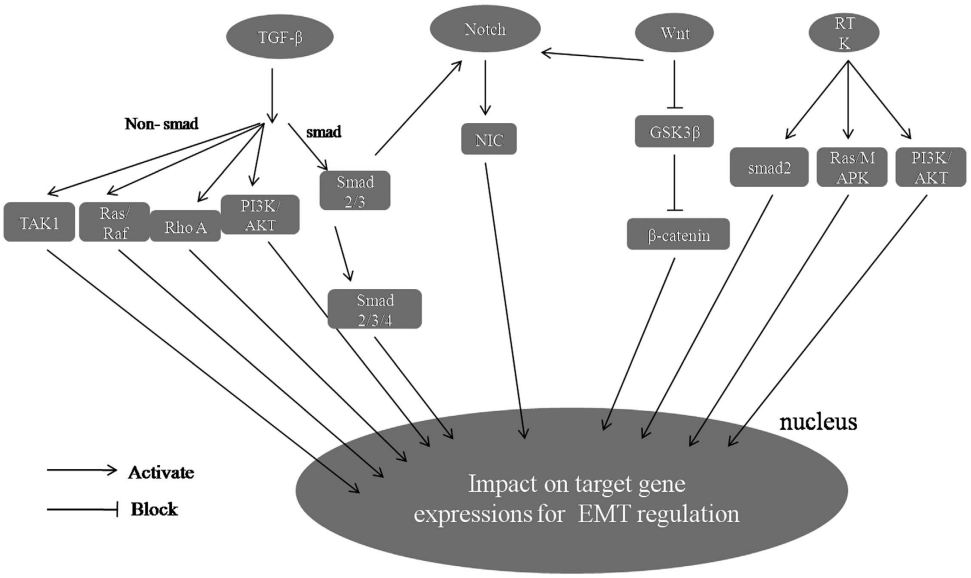

Figure 1 Signaling pathways that induce epithelial–mesenchymal transition (EMT). Transforming growth factor (TGF)-β might affect both Smad-dependent and -independent pathways. Smad2 and Wnt activate the Notch signaling pathway. The Notch receptor is cleaved, and subsequently the Notch intracellular domain (NIC) translocates into the nucleus. The role of this protein shifts from that of a repressor to an activator through binding to DNA-binding proteins, such as CBF1, Su (H) and LAG-1. Wnt signaling is initiated through the inhibition of glycogen synthase kinase (GSK)-3β. β-Catenin subsequently enters the nucleus and forms a complex with lymphoid enhancer factor/ T-cell factor to control the expression of target genes. Receptor tyrosine kinase (RTK) has a critical role in the induction of these intermediates. PI3K, phosphoinositide 3-kinase.

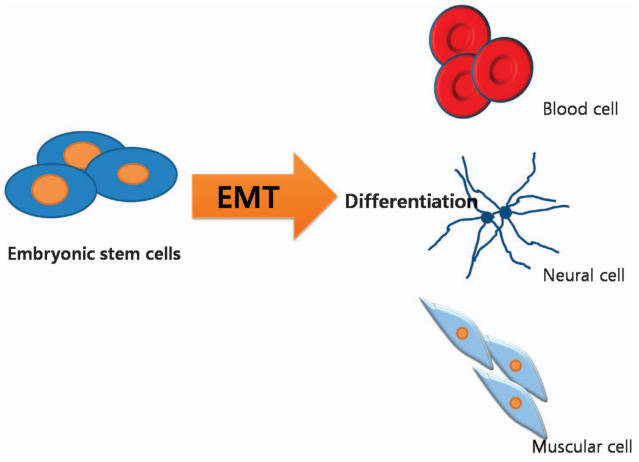

Figure 2 Potential effect of epithelial–mesenchymal transition (EMT) on the differentiation of embryonic stem cells (ESCs). Transcription factors, that is, the snail family, and regulatory factors, that is, microRNA, that regulate downstream signaling pathways associated with the EMT response might affect EMT in ESCs. ESCs (embryo blastocyst; undifferentiated) differentiate into many cell types in the body, suggesting that EMT might be involved in the differentiation of ESCs.

cytoskeleton rearrangement.[53] The 5T4 antigen is expressed on the cell surface, generating a mesenchymal phenotype. The expression of the 5T4 oncofetal antigen is upregulated in colorectal, gastric and ovarian carcinomas.[53–56] Overexpression of 5T4 antigen on mouse ESCs reduces cell–cell contact, downregulates E-cadherin expression and alters the actin cytoskeleton in epithelial cells.[53,57,58] The lack of 5T4 antigen is involved in increase of the restoration of cell–cell contact, and the upregulation of E-cadherin production prevents the development of a mesenchymal morphology.[53] This effect might reflect the stabilization of the cortical actin cytoskeleton rearrangement through E-cadherin, which blocks 5T4 antigen localization. Human ESC colonies, cultured under feeder-free conditions, undergo differentiation and develop a mesenchymal cell-like phenotype.[52] The mesenchymal-like cells express more mesenchymal markers compared with a single upper layer of columnar (zone 1) cells.[52] In another study,[59] EMT occurred not only in the embryoid body formed from human ESCs or human induced pluripotent stem cells but also in cultures of human pluripotent stem cells. Thus, it is reasonable to expect that human pluripotent stem cells resemble cells obtained from the epiblast. Thus, the occurrence of EMT in human pluripotent stem cells and embryoid bodies reflects the EMT observed during gastrulation in human development. In addition, the relationship between protein tyrosine kinase 7 (ptk7) and EMT was also examined in this study. The ptk7 has been considered an important factor for embryonic development in mice, particularly during gastrulation and neural tube closure.[59,60] Although ptk7-positive cells lose pluripotent marker and E-cadherin expression and acquire mesenchymal marker expression, ptk7 did not affect pluripotency or lineage marker changes.[59] In mouse ESCs, Ell3, a testis-specific RNA polymerase 2 elongation factor, promotes lineage differentiation through the upregulation of EMT-associated gene expression and the downregulation of pro-apoptotic gene expression. The local

expression of Ell3 has been observed in undifferentiated cells, and this expression affects embryoid body formation.[61] However, Ell3 does not influence the expression of self-renewal markers, suggesting that ZEB1 might have an important role in differentiation through Ell3. Thus, EMT in ESCs might induce differentiation through several pathways.

MicroRNA acts as a regulator of EMT in many cell types. For example, snail promotes EMT through the repression of microRNA 200 family members and induces mesoderm differentiation during the epiblast stem cell stage, suggesting that microRNA 200 family members might suppress EMT and the differentiation of ESCs at the epiblast stem cell stage.[62] Another study reported that the mesenchymal–epithelial transition might be important in reprogramming fibroblasts. OCT4, SOX2, Klf4 and c-Myc are imported into fibroblasts, and these proteins induce the rapid downregulation of microRNA-155 and microRNA-10b, associated with EMT, and the upregulation of microRNA-205 and -429 during the development of induced pluripotent stem cells.[63] Therefore, it is reasonable to assume that EMT might be associated with the differentiation of ESCs, suggesting that certain factors, that is, the microRNA family, which regulate EMT at various pluripotent stages, might exist. Naïve cells are thought to be more undifferentiated than primed cells; if this is correct, then these regulating factors may have an influence on pluripotent stages of naïve and primed cells.

CONCLUSION

EMT is important for embryonic development, and this process affects metastasis and the invasion of various cancers. Several molecules, including TGF-β and other growth factors, induce EMT. These factors bind to their respective receptors and might also interact with each other. As shown in Figure 1, various signaling pathways (for example, the TGF-β, Wnt/glycogen synthase kinase-3β, Notch and RTK signaling pathways) might be critical for the induction of EMT. ESCs are pluripotent, suggesting that these cells can differentiate into many cell types (Figure 2). Although the exact mechanism(s) underlying EMT activity has not been clarified in these cells, ESCs might be controlled through EMT under various circumstances during differentiation. Transcriptional activators of EMT, such as snail and ZEB, have also been implicated in the differentiation of ESCs, and regulating factors, such as the microRNA family, specifically promoted or inhibited EMT at the pluripotent cell stage. Thus, ESCs remain at either the pluripotent stage or the more differentiated stage. EMT and regulating factors regulate the differentiation of ESCs. Because ESCs, rather than epiblast stem cells, are more undifferentiated, these cells undergo EMT differentiation, suggesting that this process contributes to naïve and primed cell types. Thus, further studies should examine the mechanism(s) underlying EMT in ESCs required to generate transgenic organisms and induced pluripotent stem cells in order to develop the treatments for diseases using stem cells.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Next-Generation BioGreen 21 Program (no. PJ009599), Rural Development Administration, Republic of Korea.

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