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Review Article

Epithelial–mesenchymal transition-related factors in solid tumor and hematological malignancy

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Abstract

The epithelial–mesenchymal transition (EMT) process plays pivotal roles in regulatory mechanisms of embryogenesis and wound healing physiologically, and organ fibrosis, cancer progression, and metastasis pathologically. EMT is classified as primary, secondary, and tertiary during embryonic development. EMT contributes to repair of tissue injury and fibrogenesis by re-epithelialization and regeneration of fibroblasts, respectively. The hallmarks of EMT include loss of contact inhibition, remodeling of extracellular matrix, and reorganization of cytoskeleton, along with expression of mesenchymal markers and reduction of epithelial markers. Cancer cells acquire stemness, migration and invasive capability, evade apoptosis, and initiate metastasis to distant organs. Several EMT regulators including Snail, Zeb1, Zeb2, and Twist in solid tumor and Sox4, distal-less homeobox gene 4 (*DLX4*), Prdm14, Bmi1, and the forkhead box family in hematological malignancy are reviewed with regard to their signaling pathways, regulatory mechanisms, and clinical interactions.

Keywords: epithelial–mesenchymal transition; hematological malignancy; solid tumor; transcription factor

1. Introduction

The epithelial–mesenchymal transition (EMT) is the process of conversion of cells from a differentiated epithelial state into a dedifferentiated migratory mesenchymal phenotype, which is crucial for regulatory mechanisms in embryogenesis, cancer metastasis, organ fibrosis, and wound healing.[1]

2. Physiologic EMT

During embryogenesis, EMT is classified as primary, secondary, and tertiary.[2] In the process of primary EMT, the first EMT incident in the embryo occurs during gastrulation when cells from the epiblast move to a primitive streak in the midline and undergo EMT to form the mesoderm and the ectoderm.[3] Another primary EMT process occurs during the generation of neural crest cells, when epithelial cells become neural crest cells and migrate to form neural tubes, which develops into the central nervous system in mammals.[4]

During embryogenesis, transient epithelial structures including the notochord, somites, somatopleures, and splanchnopleures also undergo secondary EMT to generate mesenchymal cells, which subsequently differentiate into specific cell types, such as those of connective tissue, hematopoietic stem cells, endocardium, muscle, and neural arches. The mesenchyme of liver and islets of Langerhans also develop through secondary EMT from the liver diverticulum and pancreatic bud, respectively.[5,6] The physiologic event involving tertiary EMT is the formation of mesenchymal cardiac jelly from endothelial cells located in the

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atrioventricular canal and the outflow tract in the heart, which becomes the endocardial cushion.[7]

In addition to embryogenesis, EMT also participates in the physiologic repair of tissue injury, that is, the re-epithelialization of wounds. Keratinocytes assembling around the border of a wound will undergo phenotypic conversion to an intermediate "metastable" phenotype and then acquire mesenchymal characteristics including loss of cell–cell adherence and polarity, and gain in migratory activity.[8] The ovarian epithelial cells also utilize EMT to assume a fibroblast-like phenotype in the postovulatory phase and reside in the ovary mesenchyme, which promotes tissue repair in response to ovulation.[9]

3. Pathologic EMT

During renal fibrogenesis, interstitial fibroblast can originate from progenitors of tissue fibroblasts (bone marrow derived) migrating in the circulation to repopulate in peripheral organs; however, they also can originate *de novo* from the epithelium through the EMT process to generate a much larger number of fibroblasts.[10] It is not a unique process for renal fibrogenesis, and hepatocytes, lens epithelium, endothelium, and cardiomyocytes can contribute to tissue fibrosis in a similar manner. In a murine animal model, hepatocytes derived from cirrhotic liver demonstrated features of EMT including mitogen-activated protein kinase-dependent increased expression of vimentin and type I collagen, suggesting the involvement of EMT in the mechanism of hepatocellular carcinoma genesis.[11] Cardiac fibrosis was associated with the recruitment of fibroblasts from endothelial cells undergoing EMT.[12] For patients receiving peritoneal dialysis, mesothelial cells go through EMT to transform into cells with epithelioid morphology, resulting in peritoneal fibrosis, and eventually, dysfunction of the peritoneal membrane.[13]

4. EMT in solid tumors

Accumulated evidence suggests that EMT plays pivotal roles in cancer progression and metastasis, but the effects of EMT on human tumors remain inconclusive.[14,15] Cancer cells acquire their stem cell-like property, that is, the capability of metastatic colonization and resistance to treatment, through the process of EMT to promote deposition of extracellular matrix.[2]

In the invasive front of tumor of colon carcinoma, migratory tumor cells at the edge will display morphological features of EMT, including loss of E-cadherin and basement membrane.[16] Similar phenomena were also observed in papillary thyroid carcinoma, in which EMT was associated with tumor invasion and nodal metastasis.[17]

Several hallmark processes are crucial in EMT, including loss of E-cadherin and polarity but increase of N-cadherin and vimentin. Upon initiation of EMT, loss of E-cadherin promotes invasion during carcinoma progression and E-cadherin is repressed by EMT-related factors either directly or indirectly. Snail, Zeb, E47, and KLF8 directly bind to the promoter of *CDH1*, which encodes E-cadherin and down-regulates the expression of E-cadherin,[18,19] whereas Twist, goosecoid, E2-2A, E2-2B, and FoxC2 indirectly transcriptionally inhibit E-cadherin.[20,21]

5. Direct regulators of EMT

5.1. Snail

Snail is of enormous significance in physiologic EMT, such as in gastrulation and formation of neural crest. Snail1 is one of the repressive transcription factors directly binding to the promoter of *CDH1*. In breast carcinoma, the expression of Snail1 was associated with repression of E-cadherin and lymph node metastasis.[22]

Snail1 interacts with Suz12 and Ezh2 and recruits polycomb complex 2 to repress CDH1.[23] Snail1 binds to the E2-box [C/A(CAGGTG)] on the promoter with its C-terminal domain or interacts with histone deacetylases with its SNAG sequence in the N-terminal domain.[24,25] The translation of Snail1 messenger RNA (mRNA) can be activated by Y box binding protein 1 in breast carcinoma,[26] and its nuclear localization is promoted by LIV1, which is a downstream signaling target of signal transducer and activator of transcription 3.[27] Many post-translational modifications have been found, such as the p21-activated kinase 1 regulating the level of subcellular localization by phosphorylation of Snail[28] and glycogen synthesis kinase 3β (GSK3β)-mediated phosphorylation facilitating the ubiquitin-dependent degradation of Snail.[29] By contrast, *Lox2* counteracts GSK3β and stabilizes Snail.[30] The cooperative corepressors may be required for Snail to function; for example, these corepressors are required for the SMAD protein to bind to Snail to form a repressive complex inhibiting transforming growth factor-β (TGF-β)-induced EMT.[31]

5.2. Zeb1

The expression of Zeb1 can be induced by Snail1,[32] but its function is independent of Snail because it is associated with repression of *CDH1* in the absence of Snail in colon carcinoma, which implies that the inducers of EMT are dependent on the cellular context.[33]

5.3. Twist

Twist belongs to the category of basic helix–loop–helix factor transcription factors. Besides being a master regulator of embryogenesis, Twist also induces EMT and metastasis and is associated with poor survival in invasive breast ductal carcinoma, endometrial cancer, hepatocellular cancer, and melanomas.[34–37] Downstream targets of Twist include platelet-derived growth factor receptor-α, Akt2, Snail1, and Snail2. Twist is upregulated by nuclear factor-kB (NF-kB), hypoxia-inducible factor 1-α, and SRC-1/PEA3.[38–42] Twist represses E-cadherin and upregulates N-cadherin and vimentin, which are the hallmarks of EMT.

Twist is associated with nodal metastasis in breast cancer.[43] In phyllodes tumor, methylation of the promoters of Twist1 was noted in approximately 10.7% of tumor samples, which correlated with more high-grade malignancy.[44] In breast carcinoma, Twist is overexpressed in all high-grade tumors and represses the expression of estrogen receptor (ER) by recruiting DNA methyltransferase to the ER promoter and by interacting with histone deacetyltransferase 1.[45] Twist1 and Bmi1 are mutually essential for promoting EMT and tumor-initiating capability in head and neck squamous cell carcinoma, which implies that chromatin remodeling was crucial in the mechanisms of Twist-induced EMT.[46] Twist1 also binds to the Twist box in the C-terminus of p53 to counteract the post-translational modifications of p53 and facilitates its degradation mediated by mouse double minute 2 homolog protein (MDM2).[47] Overexpression of Twist transforms breast cancer cells into cancer stem cell phenotypes with characteristics of high CD44 expression, no or little CD24 expression, and increased aldehyde dehydrogenase 1 activity independent of the mechanisms of EMT.[48] By contrast, activation of β-catenin and Akt pathways is required for overexpressing Twist to maintain the EMT-associated cancer stem cells of breast carcinoma and cervical carcinoma.[49]

Twist overexpression contributes to cisplatin and anthracycline resistance in bladder cancer cells,[50] and development of multidrug resistance might be explained by increase of Twist expression and adenosine triphosphate-binding cassette transporters.[51]

6. EMT in hematological malignancy

Although leukemia or lymphoma cells of hematological malignancy are embryonically developed from the mesoderm, the so-called EMT, or mesenchymal–epithelial transition (MET), was rarely reported in previous studies. However, because EMT-related factors are associated with invasiveness, migration capability, stemness, and drug resistance, EMT is a crucial mechanism for cancer cells during disease progression. Even for normal physiologic development of blood cells, EMT-related factors may also play pivotal roles. For example, extracellular signal-regulated kinase-mediated phosphorylation of E2A, which is also directly related to EMT, controls its degradation in response to Notch signaling during lymphocyte differentiation.[52] *E2A* gene products (E12 and E47) are also targets for G_1 cyclin-dependent kinases regulating B-cell growth and survival during development.[53] Twist overexpression is associated with the resistance to imatinib in chronic myeloid leukemia cases; in addition, Twist expression is repressed by tyrosine kinase inhibitors but is upregulated when the resistance develops.[54]

7. Factors related to cell cycle control or DNA repair

7.1. Sox4

Sex-determining region Y-related high-mobility-group-box transcription factors belong to a large family composed of eight groups from A to H in vertebrates.[55] Sox4 regulates embryogenesis and mesenchymal differentiation.[56] Knockout of Sox4 leads to the arrest of B-cell development at the pro-B-cell stage and impaired TGF-β-mediated T-helper type 2 cell differentiation.[57,58] In adults, Sox4 expression is distributed in the tissues of pancreatic islets cells, gonads, thymus, and hematopoietic stem cells.[59,60] Sox4 has been identified as the most common integration site for retrovirus in leukemia and lymphomas, suggesting that Sox4 plays roles in inducing hematologic malignancy such as mouse myeloid leukemia and splenic marginal zone lymphoma.[61,62] The expression of Sox4 correlates with the cyclic adenosine monophosphate response element-binding protein expression in patients with acute myeloid leukemia (AML), both of which coexist to promote proliferation of hematopoietic progenitors.[63] Both Sox4 and PBX1 are well-known important hematopoietic stem cell regulators.[64] However, the detailed mechanisms involving Sox4 in generation of leukemic cells remain unresolved. In addition, Sox4 also binds to the promoter of CD56 in myeloma cells and is associated with lytic bone lesions and worse survival.[64] Sox4 is also involved in EMT in hepatocellular carcinoma or mammary epithelial cells and in increased stemness, inducing migration capability, and metastasis and invasiveness, which might be regulated by TGF-β.[65] Sox4 also acts cooperatively with Oct4 to bind to the Sox2 promoter, contributing to the stemness of glioma-initiating cells and disease progression.[66] However, besides its role in EMT, Sox4 has also been identified as a tumor suppressor because it interplays with p53 in the DNA repair pathway involving activation of ataxia telangiectasia mutated/ataxia telangiectasia and Rad3-related kinases in medulloblastoma.[67]

7.2. Bmi1

B-cell-specific Moloney murine leukemia virus integration site 1 (Bmi1) is a member of polycomb repressive complex 1, which plays a pivotal role in self-renewal of cancer stem cells, which explains tumor recurrence and resistance to chemotherapeutic drugs in melanoma, neuroblastoma, and oropharyngeal squamous cell carcinoma.[68] Bmi1 is located on 10p11.23 and universally expressed in all human tissues over the body.[69] Increased expression of Bmi1 is specifically found in hematopoietic and neural stem cells because it inhibits senescence and maintains immortality of cells by activating telomerase.[68,70]

It is co-expressed with other cancer stem cell markers in lymphoma.[71] Bmi1 promotes the proliferation of leukemia stem cells and normal hematopoietic stem cells[72] by abolishing the p16INK4A/RB and p53/MDM2 pathways to prevent cell arrest.[73] Bmi1 acts synergistically with Twist to induce EMT in head and neck squamous carcinoma, and therefore, increased expression of Bmi1 is correlated with worst prognosis.[46] In addition to EMT and stemness, Bmi1 is also associated with chemoresistance. In studies of ovarian carcinoma, prostate cancer, and pancreatic cancer, Bmi1 increased resistance to chemotherapeutic drugs including cisplatin, paclitaxel, docetaxel, and gemcitabine, respectively, by

reducing intracellular glutathione levels, which were believed to be related to reactive oxygen species.[74] Bmi1 also activates NF-kB, rendering glioma cells antiapoptic and resistant to chemotherapy and radiotherapy.[75] In nasopharyngeal carcinoma, upregulation of Bmi1 stabilized Snail and repressed *PTEN*, i.e. phosphatase and tensin homolog, which was identified as a tumor suppressor in a large body of cancers, whereas the abrogating PTEN reversed EMT-associated invasion and migration.[76] Bmi also promoted proliferation of keratinocyte by upregulating the expression of cyclin-dependent kinase 2 and 4, and cyclin D1, whereas it inhibited the activity of caspase and poly-adenosine diphosphate-ribose polymerase cleavage.[77] Bmi1 was involved in the differentiation of helper T cells (Th2) through stabilization of GATA-binding protein 3.[78]

7.3. Forkhead box

Forkhead box (Fox) proteins are among a large family of transcription factors regulating cell growth, differentiation, and embryogenesis of the mesoderm.[79] The Fox family shares a conserved Fox domain also known as "Winged-helix domain," which is involved in DNA binding, and another extra-Fox protein–protein interaction domain, which is involved in interactions with other transcription factors or DNA repair complexes.[80] As the Fox family contains more than 30 factors, it is beyond the scope of this review to detail all the factors; instead, we only focus on factors with regard to EMT and hematologic malignancy.

FOXM1 oncogene, located at chromosome 12p13.33, was found to be amplified in 42% of non-Hodgkin's lymphoma, including diffuse large B-cell lymphoma, follicular lymphoma, and B-cell chronic lymphocytic leukemia, and associated with increased expression of Myc.[81] Unphosphorylated *FOXM1* located in the cytoplasm at the G_1/S phase will be transported to the nucleus after phosphorylation by MEK1 at the G_2/M phase, and abolishment of MEK1 will lead to repression of *FOXM1* and delayed cell cycle in the G_2/M phase.[82,83] In pancreatic cancer overexpressing *FOXM1*, activation of EMT regulators including Zeb1, Zeb2, and Snail2 was noted along with downregulation of microRNAs (miRNAs; let-7a, let-7b, let-7c, miR-200b, and miR-200c).[84] *FOXM1* regulates cell cycle control through centromere protein A, centromere protein B, cell division cycle 25B, aurora B kinase, survivin, Skp2, and Cks1 and angiogenesis through matrix metalloproteinases and vascular endothelial growth factor.[83,85,86] Chromosomal translocations leading to fusion of Foxp1 and immunoglobulin heavy-chain locus [t(3;14)] or amplifications of Foxp1 were frequently noted in mucosa-associated lymphoid tissue (MALT) lymphoma and diffuse large B-cell lymphoma but these are not responsible for the overexpression of Foxp1.[87,88] However, high expression of Foxp1 was associated with nongerminal center, activated B-cell-type diffuse large B-cell lymphoma as well as with extremely poor prognosis.[89] Similarly, high expression of Foxp1 and stages were both significant prognostic factors for gastric MALT lymphoma.[90] Foxp1 is essential for B lymphocyte differentiation in both transition from pro-B to pre-B cell stage and control of variable-joining recombination of genes encoding immunoglobulin heavy chain.[91] High expression of Foxc2 was associated with basal-like breast cancer and several EMT-related factors including Snail, Twist, goosecoid, Zeb1, Zeb2, and Ets-1 enhance the ability of metastasis.[92,93]

8. Factors related to epigenetic modulation or pluripotency

8.1. Prdm14

Prdm14 is a transcription factor of the PR domain-containing large family, which is suggested to have histone methyltransferase activity.[94] Prdm14 is normally expressed specifically in pluripotent cells and it functions as repressors of differentiation in embryonic stem cells to maintain the renewal of stem cells.[95] Prdm14 can cooperatively act with Oct4, Sox2, and Klf to regulate the reprogramming of human fibroblasts and the expression of *POU5F1*, which is a gene of pluripotency.[96] Prdm14 is also expressed in 25% of lymphoid leukemia, which can block B-cell differentiation at the pro-B-cell stage. In common lymphoid progenitors transduced with Prdm14, genes associated with pluripotency, EMT, tumor formation, Wnt/Ras signaling, and early B-cell commitment were upregulated.[97]

8.2. Distal-less homeobox gene 4 (DLX4)

Distal-less homeobox gene 4, *DLX4*, putatively termed as "beta protein 1" is a member of the *DLX4* family, and performs the functions of repressing the expression of beta-globin in early erythroid cells and controlling bone morphogenesis and skeletal patterning.[98] However, aberrantly high expression of *DLX4* mRNA was found in 63% of AML blasts and in 32% of T-cell acute lymphoblastic leukemia but not in B-lineage acute leukemia. Besides its expression in leukemia blasts, expression of *DLX4* is noted only in CD34-negative cells in normal bone marrow.[99] *DLX4* can induce EMT and promote migration and invasiveness of breast cancer cells by upregulating Twist.[100]

9. Future perspectives

EMT-related factors are crucial for normal physiologic development and pathologic cancerous progression. Whereas carcinoma cells utilize the reprogramming process of EMT to acquire their stemness, migratory, invasive, and metastatic abilities, cancers of mesenchymal origin such as leukemia and lymphoma seem to derive their chemoresistance or their ability to evade key driver signaling pathways through other functions of EMT-related factors (Figure 1). On the other side, MET is another subject of investigation field, which seems not just a reversal of EMT but an entirely divergent process.

Because these regulators of EMT program are transcription factors, cancer therapeutics targeting them are very difficult to achieve. Although some *in vivo* and *vitro* studies utilized RNA interference specific to the EMT regulators to intervene in the

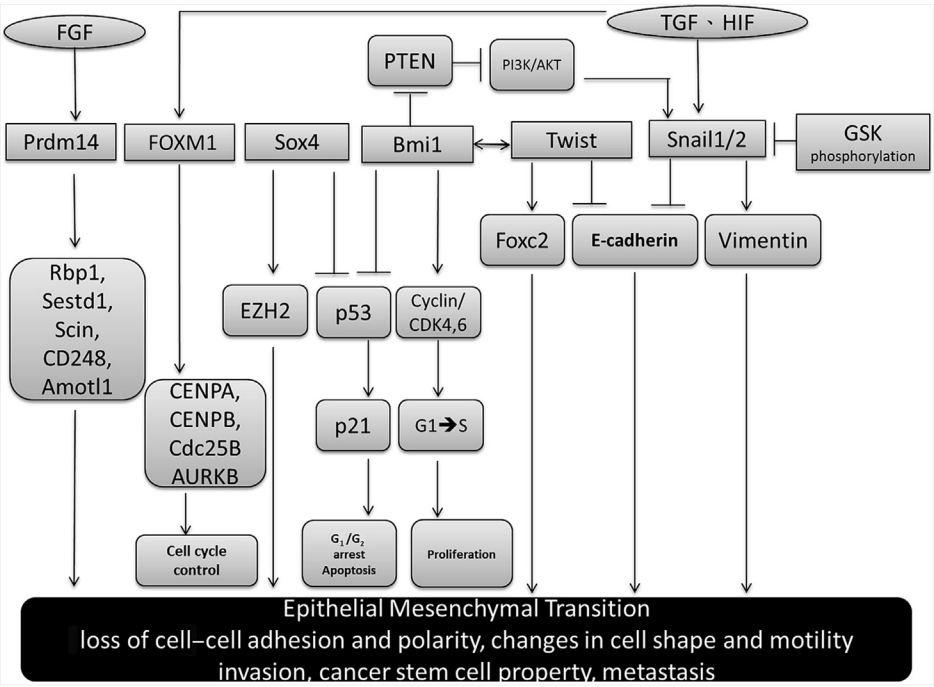

Figure 1. Connecting signaling pathways to epithelial–mesenchymal transition (EMT)-related transcriptions factors. Snail and Twist inhibit epithelial marker (E-cadherin) but promote mesenchymal marker (vimentin), both of which are hallmarks of EMT. Snail is induced by transforming growth factor-β (TGF-β) directly or by Bmi1 through the phosphatase and tensin homolog/phosphoinositide 3-kinase (PI3K)/Akt pathway, but is inhibited by glycogen synthase kinase-3β (GSK3β)-mediated phosphorylation. In addition to direct promotion of EMT, Twist acts synergistically with Bmi1 or enhances EMT thorough FoxC2. Bmi1 also regulates cell cycle control through cyclin and cyclin-dependent kinase 4 and 6 (CDK-4,6) as well as through DNA repair mechanisms, such as p53 and p21. The regulation of cell cycle control by *FOXM1* is associated with centromere protein A (CENPA), centromere protein B (CENPB), cell division cycle 25B (CDC25B), and aurora kinases B (AURKB). Overexpression of Prdm14 is associated with EMT and retinol binding protein 1 (Rbp1), SEC14 and spectrin domain 1 (Sestd1), scinderin (Scin), CD248, and angiomotin like 1 (Amotl1). Fox = forkhead box; HIF = hypoxia-inducible factor.

EMT process, future development of delivery systems, vectors conferring more stability and specificity of miRNA, which is tolerated more by immune surveillance of hosts, are warranted. In addition to inhibiting positive regulators of the EMT program, augmentation of negative transcription factors might be an alternative to revert it, such as *KLF17* or *DEAR1* in breast cancer. Other strategies to target TGF-β, the Hedgehog/Snail pathway, Notch, or Wnt/β-catenin pathways by antibody or small-molecule tyrosine kinase inhibitors are currently under development. Alternative approaches are developing drugs targeting cancer stem cells or circulating tumor cells featuring upregulation of EMT factors, such as salinomycin, although this concept remains preliminary currently.

Many EMT regulators have been reported to be prognostically significant in numerous studies. Nonetheless, their role as predictive factors are lacking, as cancer therapeutics targeting them are still in trial. Detecting the dedifferentiated circulating tumor cells with overexpression of Twist, Bmi1, and other markers of EMT provides some hope to predict risk of distant metastasis in early breast cancer.

EMT involves an extremely large network of transcription factors, such as Snail, Twist, Zeb1, Zeb2, Bmi1, Sox4, *DLX4*, Prdm14, Bmi1, and Fox family, in a cellular context-dependent manner. It is fundamental to delineate the mechanisms of EMT to understand cancer progression and metastasis and, more importantly, to develop drugs targeting these pathways. Appreciating these factors provides a rationale and standpoint for targeted therapeutic agents and helps to prognostically stratify patients into different subgroups that share different survivals and possibly individualized treatments.

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