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REVIEW

Epithelial to mesenchymal transition inducing transcription factors and metastatic cancer

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Abstract The epithelial to mesenchymal transition (EMT) is an important step for the developmental process. Recent evidences support that EMT allows the tumor cells to acquire invasive properties and to develop metastatic growth characteristics. Some of the transcription factors, which are actively involved in EMT process, have a significant role in the EMT–metastasis linkage. A number of studies have reported that EMT-inducing transcription factors (EMT-TFs), such as Twist, Snail, Slug, and Zeb, are directly or indirectly involved in cancer cell metastasis through a different signaling cascades, including the Akt, signal transducer and activator of transcription 3 (STAT3), mitogen-activated protein kinase (MAPK) and Wnt pathways, with the ultimate consequence of the downregulation of E-cadherin and upregulation of metastatic proteins, such as N-cadherin, vimentin, matrix metalloproteinase (MMP)-2, etc. This review summarizes the update information on the association of EMT-TFs with cancer metastasis and the possible cancer therapeutics via targeting the EMT-TFs.

Keywords Epithelial to mesenchymal transition · Metastasis · Twist · Snail · Slug · Zeb · Cancer therapeutics

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Introduction

The epithelial to mesenchymal transition (EMT) is a biological process by which polarized epithelial cells loss the adherent and tight cell–cell junction, enhance the migratory capacity, elevate the resistance to apoptosis, greatly increase the production of extracellular matrix (ECM) components and gain the invasive properties to become mesenchymal cells. EMT and its reverse process, mesenchymal to epithelial transition (MET), are essential for embryonic development and several developmental processes, such as gastrulation, neural tube formation, mesoderm formation, wound healing, and organ fibrosis [1, 2]. The concept of EMT was first recognized in the field of embryology but has recently been reported to play a vital role in cancer progression and metastasis [3, 4].

Metastasis is a multistep process combining local invasion, intravasation, transport, extravasation, and colonization, by which cancer cells are spread from the site of primary tumors through the circulation to form secondary or metastatic tumors at a distant site or another nonadjacent organ [5]. As metastasis remains one of the most enigmatic aspects of the disease and causes most cancer deaths, it is one of the major threats in modern life [6]. EMT allows the tumor cells to acquire invasive properties and to develop metastatic growth characteristics. These events are facilitated by the reduction of cell–cell adhesion molecule E-cadherin; upregulation of more plastic mesenchymal proteins such as vimentin, N-cadherin, and smooth muscle actin; and deregulation of the Wnt pathway, and these break through the basement membrane [2]. Many EMT-inducing transcription factors (EMT-TFs), such as Twist1, Snail1, Snail2 (also named "Slug"), Zeb1, and Zeb2, can repress E-cadherin directly or indirectly [4, 7, 8]. These master EMT-TFs along with other factors facilitate EMT by repressing E-cadherin and other junctional proteins [2]. Association of EMT-TFs with

cancer progression has become an interesting field of cancer research, especially in pathogenic and therapeutic aspects.

Role of EMT-TFs in metastatic cancers

Several EMT-TFs factors act as master molecular switches that recognize the E-box DNA sequences in the promoter region of E-cadherin, recruit transcriptional cofactors and histone deacetylases, regulate the EMT process by responding to the known signaling pathways, and thereby repress its expression [9].

Twist

Twist is a member of the basic helix-loop-helix (bHLH) transcription factor family which is one of the important EMT-TFs [10]. Twist (also called Twist1) and Twist-related protein (Twist2) share extensive homology [11], while Twist1 is essential for the neural tube formation and thought to be involved in regulating the differentiation of osteogenic and chondrogenic cells from mesenchymal precursors during skeletal development and remodeling [12]. On the other hand, Twist2 maintains cells in a preosteoblast phenotype by inhibiting osteoblast maturation during osteoblast development through the similar mechanisms, but not identical to those utilized by Twist1 [13]. An aberrant Twist1 expression or its gene methylation is frequently found in metastatic carcinomas. In 2004, Yang et al. [14] first identified Twist1 as a pro-metastatic factor in murine isogenic breast cancer cell lines, and Sahlin et al. [15] indicated *TWIST1* as a breast cancer susceptibility gene. The overexpression of Twist1 is also associated with the development and progression of prostate cancer, gynecological cancer like epithelial ovarian cancer, urological cancers like urothelial and bladder cancer, hepatocellular carcinoma (HCC), gastric cancer, colorectal carcinoma, and thyroid cancer [4].

In addition to overexpression, epigenetic changes (hypermethylation) in *TWIST1* promoter have been known to be associated with different types of cancer [16–18]. Methylation of CpG islands in promoter regions of *TWIST1* abolishes proper TATA-binding protein binding to the promoter, which permits cells to disassemble cell adherence, reduce DNA repairing capacity, and avoid apoptosis [19]. Hypermethylated *TWIST1* provides a valuable tool for the diagnosis of cancer, but the exact mechanism remains still unknown as there is no direct correlation between Twist1 promoter methylation and *TWIST1* protein or RNA expression [16].

Twist1 plays an essential role in cancer metastasis through different signaling pathways, including Akt (a serine/threonine-specific protein kinase), signal transducer and activator of transcription 3 (STAT3), mitogen-activated protein kinase (MAPK), Ras, transforming growth factor beta (TGFβ), and Wnt signaling. In addition to the classical oncogenic signaling pathways, some other signaling molecules like the increased activity of nuclear factor kappa B (NF-κB), neurotrophic receptor tyrosine kinase B (TrkB), and steroid receptor co-activator (SRC)-3 protein also induce cancer initiation and progression via Twist1 activation. Activated Twist1 has a significant role in tumor invasion and metastatic cancer development, as Twist1 downregulates E-cadherin and upregulates N-cadherin expression, which are the hallmarks of EMT [4, 9]. Moreover, Twist1 plays critical roles in some physiological process related to metastasis, such as angiogenesis, extravasation, invadopodia, vasculogenic mimicry, and chromosomal instability. Twist1 interacts with Mi2/nucleosome remodeling and deacetylase components and forms the protein complex Mi2/NuRD, which plays an essential role in cancer cell migration and invasion [20]. Experimental works showed that Twist1 is involved in tumor invasion and metastasis also by upregulating the expression of matrix metalloproteinases (MMPs) and downregulating the expression of TIMP, a naturally occurring specific inhibitor of MMPs [21, 22]. In addition, Twist1 is responsible for the loss of estrogen receptor (ER) activity by regulating ER function and thus is involved in the generation of hormone-resistant, ER-negative (ER−) breast cancer [23].

Recent evidences showed that the overexpression of Twist1 in cancer cells can promote the generation of a cancer stem cell (CSC) phenotype which possess self-renewal properties and are resistant to chemo/radiation therapy [4, 9, 24, 25]. In EMT-associated cancer stem cell traits, Twist1 directly activates the polycomb protein, Bmi1, which is frequently overexpressed in various types of human cancers and can confer drug resistance [24]. Twist1 also plays a critical role through the activation of β-catenin and Akt pathways which is thought to be associated with the generation of CSCs [25].

Snail

The *Drosophila* embryonic protein, Snail, is a zinc finger containing transcription factor which is required for proper development, such as mesoderm and neural crest formation and central nervous system (CNS) development of vertebrate and invertebrate embryos. The Snail superfamily includes Snail1 and Snail2 (also called Slug), and all the family members encode transcriptional repressors and share a similar organization with a highly conserved carboxy-terminal domain that binds to the E-box hexa-nucleotide DNA motif (5′-CACCTG-3′) in the human E-cadherin promoter [2, 26]. The expression of Snail/Slug is closely associated with cancer metastasis as it is a critical regulator of multiple signaling molecules such as epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), TGFβ, bone morphogenetic proteins (BMPs), Wnt, Notch, tumor

necrosis factor alpha (TNF-α), and cytokines. These signaling molecules from tumor microenvironment have been shown to activate Snail/Slug in different cellular contexts. The activated Snail/Slug prominently induces EMT by downregulating E-cadherin and promoting cell migration, invasion, and tumor progression [26, 27]. It was reported that the overexpression of Snail is correlated with deacetylation of histones H3 and H4 at the E-cadherin promoter [28]. Snail can deacetylate histones H3 and H4 by the recruitment of a co-repressor complex containing histone deacetylase 1 (HDAC1) and HDAC2 (HDAC1/2) and Sin3A through the SNAG domain, leading to the repression of E-cadherin expression [28].

The overexpression of Snail1 has been found to be correlated with breast cancer, ovarian cancer, gastric cancer, hepatocellular carcinoma, colon cancer, and synovial sarcomas [27]. Snail and Slug are also critical for a cancer cell to acquire CSC-like properties toward resisting radio/chemotherapy-mediated cellular stress. They indirectly increase the activation of a self-renewal program through loss of binding to specific gene promoters including NANOG, HDAC1, TCF4, Krueppel-like factor 4 (KLF4), HDAC3, and GPC3 and further induce the expression of other stem cell markers including Oct4, Bmi1, and nestin as well as increase the number of cells with CD44+CD117+ (represent as ovarian CSC markers) [29].

Zeb

The Zeb family (Zeb1 and Zeb2) is a group of zinc finger/homeodomain transcription factors, which play a pivotal role in the formation of neural crest cells and the derivative structures during normal development of the vertebrate embryo [2, 30]. Both of the Zeb family members contain two widely separated zinc finger clusters (ZFCs), located toward the N-terminal (Nt-ZFC) and C-terminal (Ct-ZFC) ends of the protein and interacting with CACC T(G) E-box-like DNA sequences located in the target gene promoters [31]. Zeb proteins induce EMT by downregulating E-cadherin and upregulating a number of other mesenchymal markers, vimentin, fibronectin, N-cadherin, and MMPs, facilitating cell migration, invasion, and eventual metastasis to distant organs [2]. The expression of Zeb proteins is activated by several signaling molecules such as growth and steroid hormones, hypoxia-inducible factor-1 alpha (HIF-1α) in hypoxic conditions, FGF, insulin growth factor 1, platelet-derived growth factor (PDGF) receptor, Ras-ERK2-Fra1, NF-κB, and Janus kinase (JAK)/STAT3 and classical signaling pathways including TGFβ/Smad, Wnt, and Notch [9]. The overexpression of Zeb proteins is responsible for the increased aggressiveness and higher metastatic capacity in a wide range of primary human carcinomas including ovarian, breast, endometrium, lung, prostate, colon, gallbladder, pancreatic, and bladder cancer [32].

The epigenetic alterations, such as promoter hypermethylation and histone modifications of *ZEB1*, contribute to cancer metastasis. Although the functional mechanism of epigenetic alterations is not clear, some studies reported the prevalent hypermethylation and silencing of *ZEB1*, whose protein product suppresses E-cadherin expression, leading to the progression of different types of cancers [33–35]. It was also reported that Zeb maintains CSC-like properties and promotes tumorigenicity. Zeb represses the expression of stemness-inhibiting microRNAs (miRNAs), including miR-200, miR-183, and miR-203, which in turn increase stem cells markers (including Bmi1 and KLF4) and thereby act as a promoter of cancer stem cells [36].

A summary of EMT-TF expression patterns in different cancers has been presented in Table 1 [7, 17, 18, 29, 33–77].

EMT-TFs in cancer therapeutics

Metastatic cancers are among the major threats in human health. Despite the tremendous efforts in basic and clinical research, the pathogenic mechanism by which tumor cells escape the local environment and colonize distant organs is unclear [4, 78, 79]. The understanding of the mechanistic role of the EMT markers that have been associated with metastases and their regulation is thought to be essential to develop the potential treatment strategies of metastatic cancers.

Recently, Twist1 or Twist1-mediated signaling pathways have been indicated as the promising target in cancer therapeutics. Twist1 is thought to be inactivated by RNA interference (RNAi) by promoting cellular senescence and growth arrest which may control Twist1-mediated tumor metastasis. In a study, Zhuo et al. [80] utilized RNAi technology to knockdown Twist1 expression in A549 cell and reported that Twist1 depletion significantly sensitized A549 cell to cisplatin via MAPK/mitochondrial pathway. Their study suggested that Twist1 depletion might be a promising approach to treat cisplatin-resistant lung cancer [80]. Li et al. [81] reported that adriamycin treatment induces apoptosis in cancer cells, and their study indicated that Twist1 RNAi raises apoptosis rate and improves the efficacy in adriamycin-based chemotherapies for breast cancer. Knockdown of Twist1 by RNAi in anaplastic thyroid carcinoma cells reduces cell migration and local invasion and increases apoptosis [46]. Several chemotherapeutic agents are also able to downregulate Twist1 and Twist1-associated molecules to control tumor formation and cancer metastasis. The natural product curcumin was found to downregulate Twist1 and reduce malignant glioma growth by inhibiting the JAK1,2/STAT3 signaling pathway [82]. The cruciferous vegetable component sulforaphane (SFN) in

Table 1 Epithelial to mesenchymal transition inducing transcription factor expression patterns in different cancers

EMT-TFs	Overexpressed in	Hypermethylated in
Twist1	Breast cancer [7], epithelial ovarian cancer [37], hepatocellular carcinoma [38], gastric carcinoma [39], esophageal squamous cell carcinoma [40], nasopharyngeal carcinoma [41], prostate cancer [42], bladder cancer [43], brain cancers [44], head and neck cancer [45], thyroid cancer [46], giant tumor of bone [47]	Bone and brain cancer [17], colorectal cancer [18], breast cancer [48], uterine cervix cancer [49], ovarian cancer [50], bladder cancer [51], gastric cancer [52], lung cancer [53], tonsillar squamous cell carcinomas [54]
Snail1	Breast cancer [55], colorectal cancer [56], oral cancer [57], gastric cancer [58], ovarian cancer [59], colon cancer [60], skin cancer [61], cell renal cell carcinoma [62], prostate cancer [63]	No clear report of Snail hypermethylation in cancer
Snail2/Slug	Ovarian cancer [29], gastric cancer [64], lung cancer [65], colorectal cancer [66], glioma [67], pancreatic cancers [68], breast cancer [69], prostate cancer [70], hepatocellular carcinoma [71]	No clear report of Slug hypermethylation in cancer
Zeb	Pancreatic cancer [36], endometrial cancer [72], colorectal cancer [73], lung cancer [74], prostate cancer [75], gallbladder cancer [76], breast cancer [69], bladder cancer [77]	Hepatocellular carcinoma [33], breast cancer [34], pancreatic cancer [35]

combination with well-known polyphenol and flavonoid quercetin can eliminate cancer stem cell characteristics of pancreatic CSCs by downregulating Twist1 and other EMT proteins [83]. Quercetin reduces the migration ability of head and neck cancer-derived sphere cells partially by decreasing the productions of Twist1, N-cadherin, and vimentin [84]. The orchid component moscatilin inhibits migration and metastasis of human breast cancer MDA-MB-231 cells through inhibition of Akt and Twist signaling pathway [85]. Recently, Twist1 and its target protein N-cadherin were found to be downregulated by thymoquinone treatment in HeLa and MDA-MB-435 cells, while thymoquinone showed antimetastatic activity in these cancer cell lines [86].

Snail is another attractive target for the drug development of cancer therapeutics. Blocking the Snail activity has shown great potential to prevent cell migration, invasion, and cancer metastasis [26]. Shaoyao decoction (SYD), a traditional Chinese medicine prescription formulated by Liu Wan-Su is commonly used in treating ulcerative colitis [87]. SYD inhibits colorectal cancer (CRC) cell proliferation and induces CRC cell apoptosis by downregulating Snail and other EMT markers like N-cadherin, fibronectin, and vimentin. SYD might be an alternative therapy for colitis-associated CRC (caCRC) as it ameliorates caCRC by suppressing inflammation and inhibiting EMT [87]. Grape seed pro-anthocyanidins (GSPs), an efficient antioxidant and anticarcinogenic agent, ameliorates the radiation-induced lung injury through suppressing the TGFβ1/Smad3/Snail signaling pathway [88]. In a study, Lv et al. suggested that hydrogen sulfide (H_2S) has a novel cancer therapeutic effects on breast cancer cells, as it reduces the expression of Snail and phospho-p38 (a signaling protein associated with apoptosis) through the activation of CSE/H_2S pathway [89]. Forkhead box Q1 (FoxQ1), a member of the forkhead transcription factor family, is an important therapeutic target for pancreatic cancer treatment. Knocking down of FoxQ1 by small interfering RNA (siRNA) results in the inhibition of tumor formation and metastasis in pancreatic cancer stem-like cells via the reduction of Snail expression [90]. There is evidence that miRNA-148a suppresses the nuclear accumulation of Snail and metastasis of hepatoma cells by targeting Met/Snail signaling-like activated phosphorylation of Akt-Ser473 and inhibits the phosphorylation of GSK-3β-Ser9 [91]. Downregulating the expression of Snail was also found as a potential therapeutic approach for the treatment of metastasis and invasion of cervical carcinomas [92].

Snail2/Slug might be another target for cancer therapy. Slug expression was found to be significantly reduced in breast cancers after neoadjuvant chemotherapy [93]. Receptor tyrosine kinase (RTK) is one of the promising targets in

molecularly targeted therapy for cancer. Axl is one of the most frequently activated RTK in liver cancer cell lines. Knocking down of Axl by RNAi significantly suppressed Slug expression and reduced the invasiveness of HCC cell lines [94]. Slug inhibition was also found as a target for the inhibition of metastatic capacity of prostate cancer by the combination of mTOR/Erk/HSP90 inhibitor (combination of rapamycin, CI-1040, and 17-AAG) treatment [95]. Among the miRNAs involved in breast cancer, miR-221 plays a crucial role, which actually downregulates the oncosuppressor p27Kip1 and upregulates Slug. By using antisense miRNA (antagomir) molecules, targeting miR-221 induces the downregulation of Slug and the upregulation of p27Kip1 [96]. Knockdown of Slug by using short hairpin RNA (shRNA) inhibits the proliferation and invasion of HCT116 colorectal cancer cells and lung cancer growth and metastasis [97, 98].

Both members of the Zeb family of transcription factors have the capacity to control cellular processes, and they could open up new possibilities for the treatment of advanced carcinomas [30]. Zeb1 was found to be highly expressed in lung adenocarcinoma A549 and H1299 cell lines. Knockdown of Zeb1 expression by lentivirus-delivered siRNA introduced a novel therapeutic target for the treatment of lung cancer, as it could decrease lung adenocarcinoma cell proliferation by delaying S-phase entry, induce cell apoptosis, and inhibit tumor formation in A549 and H1299 cell lines [99]. Inactivation of the retinoblastoma protein (RB) is the key determinant of breast cancer phenotype. Arima et al. [100] developed a screening program and thereby identified the cyclin-dependent kinase inhibitor (CDK4/6 inhibitor PD0332991) which reduces cell invasiveness and proliferation by down-regulating Zeb1 expression and blocking RB phosphorylation. Shen et al. [101] transfected Zeb1 siRNA into the osteosarcoma MG-63 cells and found that the transfected MG-63 cells with Zeb1 siRNA result in reduced expression of Zeb1 and decrease the invasion ability of MG-63 cells, which might provide the information for the targeted therapy for osteosarcoma.

Thus, targeting EMT-TFs has a significant role in cancer therapeutics. Specified research in this area can aid more success in target-specific drug development for cancer treatments.

Conclusion

EMT is a key step in tumor progression and metastasis. Recently, EMT-TFs have been identified as molecular markers in some human metastatic cancers. Success in targeting EMT-TFs by small RNA technology or chemotherapeutic approach may show a new hope to control metastatic cancer. Study on the upstream or downstream molecules of these TFs in different signaling pathways might be useful in finding vital targets of cancer treatment. The better understanding of action of EMT-TFs on CSCs as well as metastasis will make target-specific cancer therapy easier. Since EMT-inducing signals are diverse and often context-dependent, the knowledge about the sequential changes in activity and expression pattern in EMT-TFs during EMT may provide the information on the mechanism of the progression of metastasis. More investigations are necessary for better understanding of the roles of individual EMT-TFs that may provide an insight into tumor formation and progression as well as provide a basis for innovative therapeutic approaches and diagnostic markers for metastatic cancers.

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Conflicts of interest None

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