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Review

Epithelial-mesenchymal transition in breast cancer progression and metastasis

Yifan Wang[1,2] and Binhua P. Zhou[1,2]

Abstract

Breast cancer is the most common cancer in women, and approximately 90% of breast cancer deaths are caused by local invasion and distant metastasis of tumor cells. Epithelial-mesenchymal transition (EMT) is a vital process for large-scale cell movement during morphogenesis at the time of embryonic development. Tumor cells usurp this developmental program to execute the multi-step process of tumorigenesis and metastasis. Several transcription factors and signals are involved in these events. In this review, we summarize recent advances in breast cancer researches that have provided new insights in the molecular mechanisms underlying EMT regulation during breast cancer progression and metastasis. We especially focus on the molecular pathways that control EMT.

Key words Breast cancer, epithelial-mesenchymal transition, metastasis, signaling pathway, Snail

Epithelial-mesenchymal transition (EMT) is a process through which epithelial cells lose the adherent and tight junctions that keep them in contact with their neighbors and gain mesenchymal properties, including fibroblastoid morphology, characteristic gene expression changes, and increased potential for motility, enabling them to break through the basal membrane and migrate over a long distance[1,2]. The concept of EMT was developed in the field of embryology but has recently been extended to tumor progression and metastasis.

EMT is classified into three types based on the biological context under which it occurs[2-4]. Type 1 EMT describes the transition events that allow epithelial cells to become motile mesenchymal cells during implantation, embryo formation, gastrulation, and neural crest migration. These primary mesenchymal cells act as progenitors and generate secondary epithelia in mesodermal and endodermal organs via mesenchymal-epithelial transition (MET). Type 2 EMT is associated with wound healing, tissue regeneration, and organ fibrosis. In this process, tissue fibroblasts are generated from epithelial or endothelial cells during injury and chronic inflammation. Type 3 EMT, which occurs in epithelial cancer cells, is the process through which cancer cells at the invasive front of primary tumors undergo a phenotypic conversion to invade and metastasize through the circulation and generate a metastatic lesion at distant tissues or organs by MET. Although these three types of EMT represent considerably different biological processes, some genetic elements and mechanisms of regulation may be similar and well-conserved. The similarity of genetic controls and biochemical mechanisms underlying the acquisition of the invasive phenotype and the subsequent systemic spread of cancer cells highlights that tumor cells usurp the developmental pathways for their metastatic dissemination. The role of EMT in breast cancer has been demonstrated via numerous *in vitro* studies in normal and malignant mammary epithelial cells and via *in vivo* studies using mouse models of breast cancers[5,6]. Breast tumors undergo EMT and show a basal-like phenotype, suggesting that EMT occurs within a specific genetic context in breast tumors [7]. Because breast cancer is a heterogeneous disease in terms of tumor histology, clinical presentation, and response to therapy

Authors' Affiliations: [1]Department of Molecular and Cellular Biochemistry, [2]Markey Cancer Center, University of Kentucky College of Medicine, Lexington, KY 40506, USA.

Corresponding Author: Binhua P. Zhou, [1]Department of Molecular and Cellular Biochemistry, [2]Markey Cancer Center, University of Kentucky College of Medicine, Lexington, KY 40506, USA. Tel: +1-859-323-4474; Fax: +1-859-257-6030; Email: peter.zhou@uky.edu.

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and because breast cancer-related deaths are primarily due to metastatic progression, a deeper understanding of the mechanisms that underlie the EMT program in breast tumors will lead to the development of better therapeutic strategies.

EMT Regulators

A hallmark of EMT is losing expression of E-cadherin, a key cell-cell adhesion molecule. As a caretaker of the epithelial phenotype, E-cadherin helps to assemble epithelial cell sheets and maintain the quiescence of the cells within these sheets[8]. The vast majority of known signaling pathways have been implicated in the regulation of EMT. Several transcription factors, including the Snail/Slug family, Twist, δEF1/ZEB1, SIP1/ZEB2, and E12/E47[9-11], respond to these signals and function as master molecular switches of the EMT program. These transcription factors recognize E-Box DNA sequences located near the transcription initiation site of E-cadherin, where they recruit co-factors and histone deacetylases[12]. Snail was the first discovered transcription repressor of E-cadherin, and is one of the most important transcription factors involved in EMT. First described in Drosophila as a repressor of the transcription of shotgun (an E-cadherin homolog) and thereby control embryogenesis, Snail was later found to play a fundamental role during EMT in mammalian cells[9,13,14]. In addition to its E-cadherin repression function, Snail also down-regulates the expression of other epithelial molecules, including claudins, occludins, and mucin-1, and induces the expression of genes associated with a mesenchymal and invasive phenotype[15]. Three Snail family proteins have been identified in vertebrates: Snail1 (Snail), Snail2 (Slug), and Snail3 (Smuc). Snail was observed to be highly expressed in both epithelial and endothelial cells of invasive breast cancer but undetectable in normal breast[16,17]. Snail has also been linked to tumor grade, metastasis, recurrence, and poor prognosis[18-20]. In addition, Snail family proteins collaborate with other transcription factors to orchestrate concerted regulation of EMT. Recent research shows that expression of Slug and Twist is highly correlated in human breast tumors[21]. Snail and Twist cooperate in inducing ZEB1 expression during EMT[22]. Moreover, current findings show that microRNAs (miRNAs) are also master regulators of EMT. MicroRNAs are single-stranded, small, 20–22 nucleotide long, non-coding RNAs that modulate gene expression at the post-transcriptional level[23,24]. miRNAs have been implicated in regulating diverse cellular pathways and are commonly dysregulated in human cancers. Several reports suggest that some miRNAs, such as *miRNA-200*, directly target *ZEB1* and *ZEB2* mRNAs by up-regulating E-cadherin in cancer cell lines, thereby suppressing cell motility[25]. Here, we discuss the major signaling pathways that regulate EMT during breast cancer progression and metastasis, with a specific focus on the role of Snail in this complex signaling network (Figure 1).

Microenvironmental Signaling Pathways Leading to EMT Induction

TGF-β/Smad pathway

Transforming growth factor-β (TGF-β) signaling, implicated as the primary inducer of EMT, plays a dual role in cancers. During early stages of tumor growth, TGF-β seems to act as a tumor suppressor by inducing growth arrest and apoptosis. During tumor progression, the growth inhibitory response to TGF-β is decreased, whereas the EMT response is retained or even increased[26,27]. TGF-β and its receptors, together with other receptor tyrosine kinases (RTKs), regulate transcription by Smad-dependent and -independent TGF-β receptor signaling pathways. TGF-β binds to type I and type II serine-threonine kinase receptors, termed TβRI and TβRII, respectively[28]. After ligand binding, TβRII transphosphorylates TβRI, which activates the receptor-regulated Smad2 and Smad3. Activated Smad2/3 forms complexes with Smad4, a DNA-binding partner common to all receptor-regulated Smads, and translocates into the nucleus. Smad complexes interact with various transcription factors and transcription co-activators to regulate target gene transcription. Overexpression of Smad2 and Smad3 results in increased EMT in a mammary epithelial model[29]. Knockout of Smad3 blocks TGF-β–induced EMT in primary tubular epithelial cells, and reducing the functions of Smad2 and Smad3 decreased the metastatic potential of breast cancer cell lines in a xenograft model[30]. Interestingly, Smad3 and Smad4 interact and form a complex with Snail. This complex then targets the promoters of the Coxsackie-adenovirus receptor (CAR), a tight-junction protein, and E-cadherin in breast epithelial cells during TGF-β–induced EMT[31]. TGF-β–activated Smads and Ras-activated mutant *p53* can intercept *p63*, which is responsible for protecting normal epithelial stem cells from apoptosis and coordinating their differentiation, to form a ternary complex in which the p63 transcriptional functions are antagonized, unleashing TGF-β–driven metastasis. In this context, Smads are not operating as transcription factors, but as adapters, bridging together mutant *p53* and *p63*[32]. In addition, TGF-β signaling can occur via Smad-independent pathways, including the activation of phosphatidylinositol 3-kinase (PI3K), Akt,

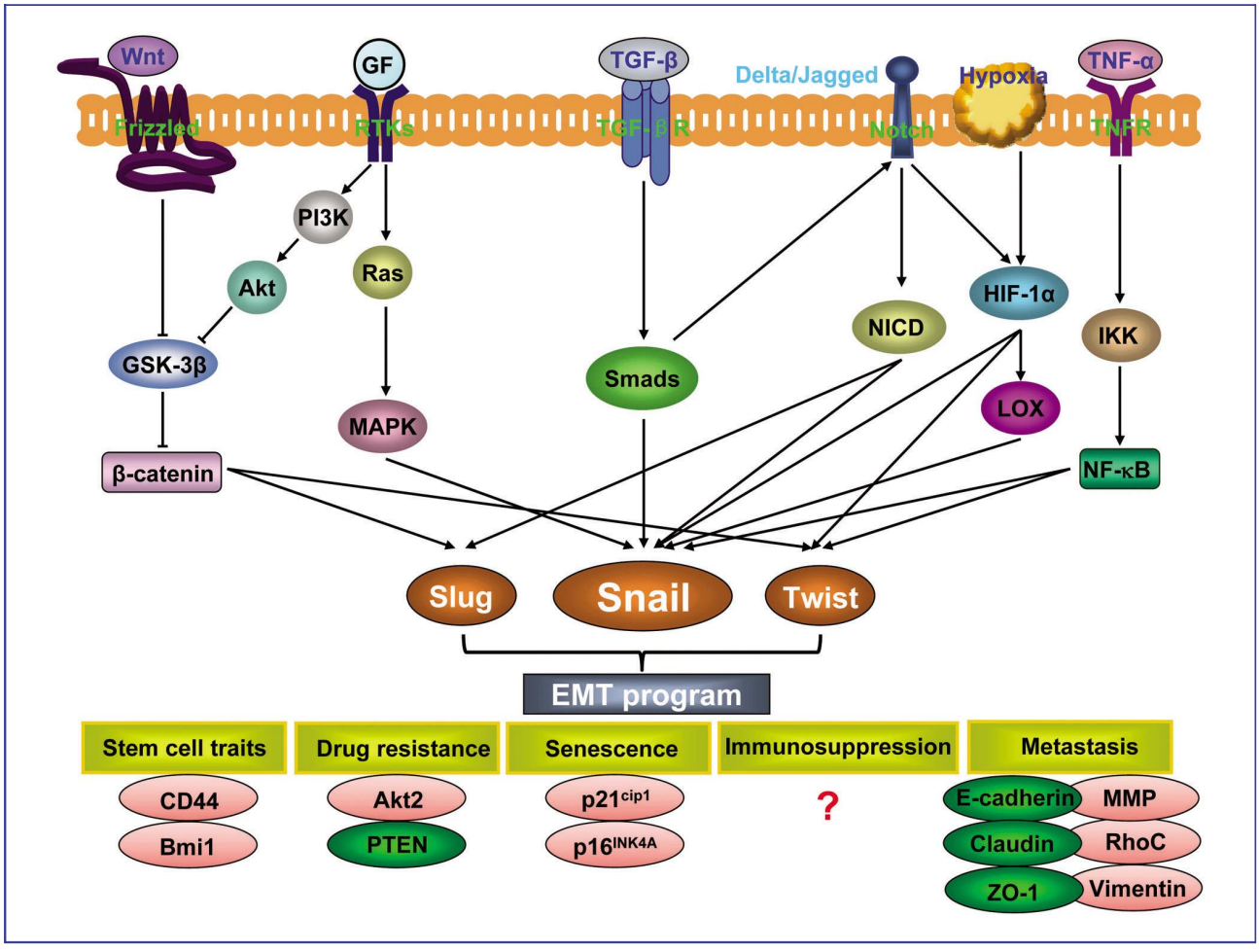

Figure 1. **An overview of signaling networks in controlling epithelial-mesenchymal transition (EMT) and metastasis.** Activation of tumor growth factor-β (TGF-β), Wnt, Notch, receptor tyrosine kinases (RTKs), and tumor necrosis factor-α (TNF-α) signaling pathways leads to the activation of several EMT transcription factors, such as Snail, Slug, and Twist, thus resulting in the induction of EMT. EMT bestows tumor cells with stem cell-like characteristics, resistance to immunosuppression and senescence, and survivability against chemotherapy and endocrine therapy during metastasis.

mitogen-activated protein kinase (MAPK), and small GTPases of the Rho family. Both Smad-dependent and -independent pathways overlap and function together to regulate the transcription of EMT master regulators, including Snail, Slug, and Twist[33]. Moreover, TGF-β can collaborate with other signaling pathways, including Notch, Wnt/β-catenin, nuclear factor (NF)–κB, and RTKs, to induce complete EMT and maintain the mesenchymal phenotype of invasive/metastatic tumor cells [34-36]. The platelet-derived growth factor (PDGF)/PDGF receptor autocrine loop, which is essential for the acquisition of a complete EMT phenotype [37], can be induced during TGF-β–driven EMT. Annexin A1 (AnxA1), an inducible endogenous inhibitor of NF-κB, promotes metastasis by enhancing TGF-β/Smad signaling and actin reorganization, which facilitates an EMT-like switch, thereby allowing efficient cell migration and invasion of metastatic breast cancer cells[38,39].

Wnt signaling pathway

The Wnt pathway plays a critical role in cell proliferation and oncogenesis. One of the downstream signaling molecules activated by canonical Wnt signaling is β-catenin. β-catenin has a dual role in EMT, acting not only as a bridge to enhance cell-cell adhesion when bound to cadherin complexes in adherens junctions, but also as a transcription co-factor with DNA-binding proteins of the T-cell factor (TCF)/lymphoid enhancer factor (LEF) family. Thus, β-catenin is considered an ideal target for studying the molecular basis of both EMT and malignant cancer formation. In the absence of Wnt, cytoplasmic β-catenin is phosphorylated by a destruction complex consisting of axin, adenomatous polyposis coli

(APC), glycogen synthase kinase-3β (GSK-3β), and casein kinase I (CKI). Phosphorylated β-catenin is recognized by the E3 ubiquitin ligase β-Trcp, which targets β-catenin for proteosomal degradation. In the presence of Wnt ligands, Wnt binds to 7-transmembrane domain receptor Frizzled (Fzd) and the lipoprotein receptor-related protein (LRP5 or LRP6) complexes to inactivate GSK-3β in the destruction complex, thus stabilizing β-catenin. β-catenin accumulates and translocates into the nucleus, where it forms a complex with TCF/LEF transcription factors and regulates the transcription of Wnt target genes [40]. In this pathway, GSK-3β is a nodal protein, which not only negatively regulates the stability and activity of β-catenin but also mediates phosphorylation of Snail [41]. We have reported that GSK-3β binds to and phosphorylates Snail at two consensus motifs to dually regulate its function. The first phosphorylation motif regulates its ubiquitination mediated by β-Trcp, whereas the second phosphorylation motif controls its subcellular localization[42]. Phosphorylation of Snail by GSK-3β facilitates its proteasomal degradation. Conversely, inhibition of GSK-3β leads to Snail accumulation, E-cadherin down-regulation, and EMT development. Interestingly, the target phosphorylation site for GSK-3β is conserved in β-catenin and Snail proteins. In human breast cancer cells, canonical Wnt signaling activates the EMT program by inducing the expression of intracellular protein Axin2 to stabilize Snail. Axin2 mediates this effect by acting as a nucleocytoplasmic chaperone for GSK-3β and forming a Wnt-Axin2-GSK-3β cascade [43]. Therefore, Wnt can stabilize the levels of Snail and β-catenin to induce EMT and cancer metastasis by blocking the activity of GSK-3β. Moreover, Snail can functionally interact with β-catenin to enhance the activation of Wnt signaling, thus establishing a positive feedback loop for Wnt-dependent transcription [44]. In addition to the canonical Wnt/β-catenin pathway, Wnt signaling activates the extracellular signal-regulated kinase-1/2 (ERK1/2) pathway in mouse mammary epithelial cells via epidermal growth factor receptor (EGFR) transactivation. Wnt and EGFR signaling pathways crosstalk and transactivate each other in cancer. Wnt ligands can activate EGFR signaling through Fzd, whereas EGFR can activate β-catenin, a downstream effector of Wnt pathway, via the RTK-PI3K/Akt pathway. Furthermore, EGFR has been shown to form a complex with β-catenin and increase the invasion and metastasis of cancer cells [45].

Notch signaling pathway

The Notch signaling pathway not only maintains a balance between cell proliferation, differentiation, and apoptosis, but also plays an important role in determining cell fate and maintaining the progenitor cell population. Four Notch receptors (Notch1–4) and five ligands (Jagged1, 2 and Delta-like1, 3, 4) have been identified. Notch signaling is normally initiated by ligand-receptor binding between adjacent cells, followed by intramembrane cleavage of Notch receptor by γ-secretase and release of Notch intracellular domain (NICD), which then translocates to the nucleus to generate a transcription factor complex with transcriptional regulators CSL (RBP-Jk), Mastermind-like 1 (MAML1), and histone acetyltransferase p300/CBP. This complex activates canonical Notch target genes Myc, cell cycle regulator p21, Hairy/Enhancer of split (HES), and the HES-related repressor (HERP, HRT, and HEY) families [46]. Notch signaling is insufficient and must be coordinated with other signals to promote EMT. Early evidence showed that Notch activity is required for TGF-β-induced EMT during cardiac development [47]. Further studies revealed that TGF-β increases Notch activity through Smad3, which up-regulates both Jagged1 and HEY1. Elevated Jagged1 and Notch promote Slug expression, thereby suppressing E-cadherin. HEY is considered a potential marker of human breast cancers that exhibit activation of the Jagged1-Notch-Slug signaling axis. This Slug-induced EMT is also accompanied by activation of β-catenin and resistance to anoikis, which also contributes to breast cancer metastasis [48]. Moreover, crosstalk occurs between the Wnt and Notch pathways. Ectopic expression of Wnt1 is sufficient to transform primary human mammary epithelial cells, resulting in tumorigenic conversion. The Wnt1-transformed cells have enhanced expression of the Delta-like1, 3, and 4 ligands. Increased Notch signaling is also needed for the tumorigenic phenotype [49]. In addition, activation of Notch signaling is also required for hypoxia-induced EMT. Hypoxia has received considerable attention as an inducer of tumor metastasis. Notch serves as a critical intermediate in conveying the hypoxic response into EMT. In this process, Notch signaling controls Snail expression by two distinct but synergistic mechanisms, including direct transcriptional activation of Snail and an indirect mechanism operating via lysyl oxidase (LOX). Notch increases LOX expression by recruiting hypoxia-inducible factor 1α (HIF-1α) to LOX promoter, which stabilizes the Snail protein, resulting in up-regulation of EMT and migration and invasion of cancer cells [46,50]. Recent research shows that hypoxia-induced Jagged2 promotes breast cancer metastasis and self-renewal of cancer stem-like cells[51].

TNF-α/NF-κB signaling pathway

Tumor necrosis factor-α (TNF-α) is a pivotal pro-inflammatory cytokine involved in inflammation, immunity, cellular homeostasis, and tumor progression.

As a tumor-promoting factor, TNF-α is linked in many cancers to all steps of tumorigenesis, including transformation, proliferation, angiogenesis, invasion, and metastasis [15]. TNF-α signals through two distinct cell surface receptors, TNFR1 and TNFR2. TNFR1-associated death domain protein (TRADD) recruits TNF receptor-associated factor (TRAF2) and receptor-interacting protein (RIP). TRAF2 in turn recruits IκB kinase (IKK) complex, a multicomponent protein kinase complex, which is then activated in a RIP-dependent manner. The IKK complex phosphorylates inhibitory protein IκB, which normally binds to NF-κB and inhibits its translocation. After phosphorylation, IκB proteins undergo rapid ubiquitination and proteasome-mediated degradation, releasing NF-κB. The free NF-κB translocates to the nucleus and mediates the transcription of a vast array of proteins involved in tumor progression, such as proliferation, migration, and apoptosis[52]. Unequivocal evidence shows that NF-κB activation is associated with the induction of many transcription factors involved in EMT, such as Snail, Slug, Twist, and ZEB1/ZEB2 [53]. We have found that in the classical TNF-α/NF-κB activation pathway, Snail can be stabilized by TNF-α through the activation of NF-κB, which prevents Snail phosphorylation by GSK-3β and subsequent degradation [54]. NF-κB also binds to Snail promoter, leading to increased Snail transcription [55]. Knockdown of Snail expression not only inhibits TNF-α–induced cancer cell migration and invasion *in vitro* but also suppresses lipopolysaccharide-mediated metastasis *in vivo* [54]. Recently, Raf kinase inhibitor protein (RKIP), a metastatic suppressor, was shown to inhibit NF-κB activity, and conversely, Snail was shown to repress the expression of RKIP. Therefore, there is a circuitry between RKIP, NF-κB, and Snail in which overexpression of Snail in tumors inhibits RKIP and induces EMT [56-58]. In addition, NF-κB can mediate important aspects of TGF-β signaling essential for inducing and maintaining EMT in RAS-transformed mammary epithelial cells, facilitating an invasive/metastatic tumor phenotype[59]. NF-κB is also responsible for the activation of mesenchymal marker vimentin and matrix metalloproteinases (MMPs), such as MMP2 and MMP9 [60,61]. In addition to the TNF-α/NF-κB signaling pathway, NF-κB activation may result from different pathways triggered by a variety of cytokines, growth factors, and tyrosine kinases[52].

Receptor tyrosine kinase signaling pathway

In addition to the signaling pathways implicated in EMT mentioned above, numerous other RTKs have also been found to contribute to EMT and tumor cell invasion[62]. Growth factors, such as hepatocyte growth factor (HGF), epidermal growth factor (EGF), or fibroblast growth factors (FGF), transduce signals via activation of RTKs and their central downstream effector Ras[63,64]. HGF that signals via the RTK c-Met and ERK/MAPK cascade was among the first observed to play a role in reshaping epithelial differentiation towards a scattering phenotype characterized by robust down-regulation of E-cadherin and with critical links to tumor metastasis[65]. The HGF pathway has also been linked to the regulation of the transcription factor Snail, a major inducer of EMT [66]. Most frequently, the constitutive activation of RTKs and their downstream signaling effectors such as MAPK or PI3K endow epithelial cells with an increased rate of proliferation and are crucial events in establishing hyperplastic/premalignant lesions. Signaling via either MAPK or PI3K has been reported to be necessary and sufficient to regulate EMT in collaboration with TGF-β[67]. Ras-activated MAPK can promote EMT and metastasis of breast tumor cells via increasing Twist1 serine 68 phoshorylation and stabilization [68]. PI3K plays critical roles in the establishment of EMT and provides crosstalk between growth factor signaling, integrin receptors, and small GTPases of the Rho family that control cytoskeletal organization[69,70]. Furthermore, many studies indicate that Wnt and EGFR signaling crosstalk via RTKs pathways. Although the critical role of RTKs signaling in EMT has been established, research on the regulation of epithelial cell polarity shows that oncogenic pathways whose signaling involves RTKs may not be sufficient to elicit EMT. Despite having the ability to destroy polarity and tight junction assembly, these oncogenic pathways fail to induce a mesenchymal, migratory phenotype [71]. This is compatible with the overwhelming evidence that EMT in various carcinomas involves an intricate interplay of multiple signaling pathways, including TGF-β, Wnt, Notch, PDGF, and downstream β-catenin, NF-κB, and ERK/MAPK pathways[72].

Roles of EMT in Cancer Progression and Metastasis

EMT and stem cells

Stem cells have long been investigated for their central role in organ development. However, current studies suggest that cells with stem/progenitor characteristics also play critical roles in tumor formation and progression [73,74]. Although the concept of cancer stem cells (CSCs, also known as tumor-initiating cells, TICs) has been long proposed, recent advances in the identification of CSCs from several human cancers have provided more convincing evidence for this concept. CSCs were first identified in human acute myeloid leukemia malignancies and have been subsequently

described in solid tumors, including tumors of the breast, brain, colon, and others[75-77]. In human breast tumors, a small subpopulation of cancer cells with the $CD44^{high}/CD24^{low}$ antigenic phenotype is present. These cells are highly enriched for CSCs compared to the majority of carcinoma cells within the same tumors that have the $CD44^{low}/CD24^{high}$ phenotype. Recent observations connect EMT to CSCs, suggesting that the EMT process may facilitate the generation of cancer cells with the mesenchymal traits needed for dissemination as well as the self-renewal properties needed for initiating secondary tumors[78]. The induction of EMT in immortalized human mammary epithelial cells (HMLE) by ectopic expression of Snail or Twist or exposure to TGF-β results in an increased ability to form tumorspheres and yield cells with a $CD44^{high}/CD24^{low}$ stem cell signature. Such cells adopt a mesenchymal phenotype are greatly enriched in TICs, and are akin to breast CSCs[79]. This evidence suggests that there may be a direct link between EMT and the gain of CSC-like properties, which may be prerequisites for cancer cell metastasis. A major driving force for these processes is the TGF-β signaling pathway[80]. The ZEB/miR-200 feedback loop is speculated to account for such an EMT-CSC link at the molecular level. This feedback loop, in particular, is a driving force for cancer progression towards metastasis by controlling the state of CSCs[81]. Our recent research shows that activation of β-catenin and Akt pathways by Twist are critical for the maintenance of EMT-associated, CSC-like features[82].

EMT and drug resistance

Although advancement in early detection technologies and cancer therapies has greatly improved the survival of cancer patients, the majority of patients will die because of relapse and drug resistance. More than 45% to 50% of patients diagnosed with breast cancer will develop refractory or resistant disease[78]. The existence of a subpopulation of tumor cells with stem cell-like characteristics, such as very slow replication and resistance to standard cancer therapy, poses a new concept to explain the phenomena of drug resistance and tumor recurrence[82,83]. For instance, in human breast tumors, neoadjuvant chemotherapeutic treatment of patients with locally advanced breast cancer results in the enrichment of $CD44^{+}/CD24^{-/low}$ cells and increased efficiency of mammosphere formation[84]. The subpopulation of CSCs with a $CD44^{high}/CD24^{low}$ cell surface marker profile was more resistant to cancer therapies (chemotherapy, hormone therapy, and radiotherapy) than the major population of more differentiated breast cancer cells[85]. Considering the relationship between CSCs and EMT, indirect resistance of CSCs to therapies suggests that the EMT program contributes to drug resistance. In addition, direct evidence also exists for the connection between the EMT phenotype and therapeutic resistance. For example, in the breast cancer cell line MCF7, EMT induced by EGFR signaling has been linked to tamoxinfen resistance and increased invasiveness[86]. In addition, basal subtype breast cancer, a mesenchymal-like cancer, is highly resistant to chemotherapies and is associated with higher mortality rates[78]. The mechanism by which the EMT program mediates drug resistance is not well understood. However, Twist may play a role in this process. Increased expression of Twist was found in highly invasive breast cancer cell lines, and the elevated Twist was reported to up-regulate the transcription of AKT-2 to promote cell survival and resistance to paclitaxel[87]. Furthermore, aberrant expression of Snail and/or Slug in breast cancer cells has also been related with invasive growth potential. Snail or Slug expression promotes cellular resistance to programmed cell death in MCF7 cells elicited by the DNA-damaging chemotherapeutic agent doxorubicin and promotes acquisition of invasive growth properties[88]. The elucidation of a molecular mechanism underlying the contribution of CSCs and EMT to drug resistance is crucial for finding useful therapeutic strategies to overcome this problem. CSCs are often resistant to common drugs *in vivo* and *in vitro* when compared with the majority of the cancer cell population, which raises the question of whether traditional therapy only "debulks" tumors, leaving CSCs to repopulate the original tumor, thus resulting in disease recurrence. Therefore, more effective therapies will be required to selectively target this crucial cell population.

EMT and immunosuppression

Recent research shows that immunosuppression also plays an important role in cancer metastasis. This work suggests that tumor progression and metastasis evolves via an immunosuppressive network, which is mediated by several tumor-derived soluble factors (TDSFs) such as TGF-β, interleukin-10 (IL-10), and vascular endothelial growth factor (VEGF), and extends from the primary tumor site to secondary lymphoid organs and peripheral vessels. TDSFs act as strong chemoattractants to recruit immature myeloid cells (iMCs), including immature dendritic cells and macrophages, from bone marrow to the tumor site in accordance with tumor progression, resulting in the inhibition of dendritic cell maturation and T-cell activation in a tumor-specific immune response[89]. Snail-induced EMT is involved in this network and has been demonstrated to accelerate cancer metastasis, using not only an enhanced invasive ability but also induction of immunosuppression through immunosuppressive cytokines, regulatory T cells, impaired dendritic cells, and cytotoxic T lymphocyte

resistance. Therefore, therapies directed to interfere with EMT might be both anti-invasive and able to restore immunocompetence in cancer patients[90].

Conclusions and Perspective

EMT is a complex, stepwise phenomenon that occurs during embryonic development and tumor progression and involves major reprogramming of gene expression that leads to alterations in cell fate and behavior. A recent link between EMT and CSCs has sparked considerable interest, as it is widely accepted that only a minor population of tumor cells can initiate and support tumor development and that the highly aggressive tumor cells share many characteristics of embryonic progenitor cells. The finding that EMT confers tumor cells with traits of CSCs provides a plausible molecular mechanism for tumor metastasis and recurrence. Several challenges remain to fully address the fundamental mechanism and the regulation of EMT. Many embryonic transcription factors and pathways that regulate EMT have been found to be activated in breast tumor models and to promote EMT in the context of tumor progression by forming a complex signaling network. Within this signaling network, EMT is regulated through signal integration, crosstalk, and feedback control. Despite recent advances, much remains unknown about the EMT program in cancer progression and metastasis because cancer is a complex and multi-step process, and EMT represents only part of the process of tumor invasion and metastasis. It is often hard to identify whether a particular molecule or pathway under investigation is specific to the EMT program or is operating in parallel with other programs, such as cell survival and proliferation. Currently, it is clear that EMT is not only triggered from the program inside tumor cells but also appears dependent on the signals from the tumor microenvironment, including extracellular matrix, blood vasculature, inflammatory cells, and fibroblasts, as specific cancers indisputably prefer specific sites for metastasis. A number of studies on the role of EMT in cancer were performed in cultured tumor cell lines. However, considering the tissue-specific interaction occurring during tumor progression and metastasis, new 3-dimensional or organ culture systems or *in vivo* investigation will be required to identify the molecular steps that control EMT. Furthermore, EMT and MET are transient and dynamic events, particularly at the tumor invasive front, and a powerful imaging system that can capture these processes will be needed to reveal the mystery of EMT. Unlike the classical EMT known to occur in embryonic development, which represents a relatively permanent change in cell identity and fate, EMT markers and phenotype are often not apparent in distant metastases. The mechanisms underlying the expression of these EMT markers are not well established. Therefore, we need to develop better markers and detection methods as a prerequisite to causally link EMT with distinct steps of metastasis in the future. Large-scale analysis of transcripts by sequencing or microarray can be applied to uncover new candidates for EMT. In summary, dissecting the gene expression patterns at the invasive front of tumors and uncovering the complex signaling networks that orchestrate the EMT process in human cancers will help us to better understand breast cancer progression and metastasis and delineate more effective therapeutic strategies for future metastasis prevention.

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