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Epithelial-Mesenchymal Transition Pathways in Breast Cancer

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Abstract

Epithelial-Mesenchymal Transition (EMT) is one of the key molecular mechanisms contributing to metastatic progression. This process is involved in the local invasion and migration of various cancers, including breast cancer. Changes in the EMT regulatory pathways during tumorigenesis lead to the loss of cellular adhesions, changes in the polarity of the cell and cytoskeleton, detachment of the cell, migration, intravasation, survival in the vascular system, extravasation, and, finally, metastasis. EMT is largely mediated by a core set of EMT-activating transcription factors. Many pathways are involved in the EMT regulation; the primary mediators include TGF, Notch, and Wnt. Several in vitro studies on normal and malignant mammary epithelial cells, as well in vivo studies on mouse models with breast cancer, have shown the role of EMT in this cancer. Investigation of the EMT regulatory pathways can help in cancer surveillance and treatment and make potential direct targets for new-combination anticancer drugs and personalized medicine.

INTRODUCTION

In breast cancer, as in similar to most solid tumors, the majority of breast cancer-related deaths are due to metastasis rather than the primary lesion (1). The reported incidence of breast cancer is increasing (2). This fact has led to increasing efforts to improve the efficacy of the treatments and patient management, as well as identifying the genetic and phenotypic characteristics of the metastatic breast cancer cells. Metastasis is the most difficult feature of the cancerous cell to simulate. Therefore, studies investigating this phenomenon are usually in vitro because the process is a sequence of events that are dependent on the different characteristics of the tumor cells and their interactions with the tissue microenvironment (3). Epithelial-Mesenchymal Transition (EMT) is one of the key molecular mechanisms contributing to metastasis. This process is involved in the local invasion and migration of various cancers, including breast cancer. Through this process, epithelial cells lose their adherent and tight junctions that keep them attached to the neighboring cells and components and gain the mesenchymal properties, including fibroblastoid morphology, characteristic gene expression changes, and increased motility, enabling them to break through the basal membrane and migrate over a long distance (4-5). The concept of EMT was developed in embryology. However, it has recently been extended to tumor progression and metastasis. Changes in the EMT regulatory pathways during tumorigenesis lead to the loss of cellular adhesions, changes in the polarity of the cell and cytoskeleton, detachment of the cell, migration, intravasation, survival in the vascular system, extravasation, and, finally, metastasis (6). Regarding the morphology, the EMT is classically characterized by a de-differentiation from an epithelial phenotype to a mesenchymal phenotype and is marked by decreasing the E-cadherin expression while increasing the expressions of N-cadherin, vimentin, and cellular proteases. The EMT is also believed to be a critical step in the transition of the pre-invasive cancers to the invasive forms, such as in DCIS, PIN, etc., as well as from the organ-confined disease to the metastatic disease (7). EMT is largely mediated by a core set of EMT-activating transcription factors (EMT-TFs), including Snail (or SNAI1), SLUG (or SNAI2), Twist-Related Protein 1 (TWIST1), Zinc-Finger E-box-Binding Homeobox 1 (ZEB1), and ZEB2. These factors activate the classic EMT program, resulting in the disjunction of cellular adhesions, loss of epithelial cell polarity, and gaining a mesenchymal, motile phenotype (8). Many pathways are involved in the

EMT regulation; the primary mediators include TGF, Notch, and Wnt. These pathways are also affected by the tumor microenvironment, such as hypoxia and the differential expression of microRNAs (miRNAs) (9). Several studies have shown the role of EMT in breast cancer, including in vitro studies on normal and malignant mammary epithelial cells and in vivo studies on mouse models with breast cancer (10-11). When breast cancer cells undergo EMT, they gain a basal-like phenotype, suggesting that EMT occurs within a specific genetic context in breast tumors (12). Regarding the heterogeneity of this cancer in histology, clinical presentations, and response to therapy, as well as the problem of metastasis as the main cause of breast cancer-related death, a deeper understanding of the mechanisms underlying the EMT in breast tumors may improve the therapeutics. In this review, we evaluated the signaling pathways related to this process and their effective factors.

TGF-B SIGNALING PATHWAY AND EMT

Several studies have shown an extensive range of potential roles for TGF-β in metastasis. Cancer cells need to lose their cellular polarity and acquire mesenchymal characteristics for local invasion and metastasis (13). Therefore, EMT is one of the important steps for metastasis (14). TGF-β signaling can downregulate claudins, occludins, and ZO1, leading to tight junction degradation (15). Snail1/2, HMGA2, and ZeB1/2 suppress the expression of E-cadherins through pathways mediated by TGF-β (16). In addition, TGF-β alters the PAR6 complex structure in a non-genomic manner [44]. A study by Ozdamar et al. (17) demonstrated that TGF-β stimulation induced by TbR-II-mediated phosphorylation of PAR6, is led to the degradation of RhoA, a key molecule that stabilizes the cell junctions.. Moreover, oncogene Ras is involved in the TGF-β-induced EMT in a variety of cancers, including breast cancer (18). Also, Araki et al. (19) reported that destabilization of p53 by Mdm2 was a key step in the EMT of breast cancer cells. Mdm2 expression mediated by TGF-β was associated with the progression of breast cancer to late-stage metastasis. TGF-β signaling plays an instrumental role in activating this transcriptional network by inducing the expression of several pleiotropically acting transcription factors, also known as “master regulators” of EMT. These factors include the Snail protein, which belongs to the Snail family of proteins (20); SLUG (21); ZEB1/deltaEF1 (22) and ZEB2/SIP1, the two-handed zinc finger factors (23); Twist, the basic helix-loop-helix (bHLH) protein (24) that can be upregulated by Wnt; EGFR; and STAT3 signaling (25-27). Other EMT transcription factors include HMGA2 (28) and Ets1 (29), acting as upstream regulators in this network by upregulating the expression of Snail and ZEB family members, respectively. HMGA2 and Ets1 can also be induced by the TGFβ-Smad pathway. On the other hand, FOXC2 is a factor whose functions downstream the Snail and Twist to promote EMT (30). In addition recent studies indicated that overactive TGFβ-Smad2 signaling further contributed to establishing an EMT phenotype by maintaining the epigenetic silencing of key epithelial marker genes, such as E-cadherin, claudin-4, kallikrein-10, and cingulin. This appears to be mediated via Smad2-dependent regulation of DNA methyltransferase 1 (DNMT1) binding activity and DNA methylation of the corresponding gene promoter regions (31). Therefore, EMT induction is one of the main mechanisms by which TGFβ promotes cell migration, invasion, and metastasis. Studies have shown that stimulation of TGF-β in cultures of carcinoma-derived cell lines can lead to the activation of this reversible process (32). Acquisition of an EMT phenotype results in cells with diminished adhesive capacity that are highly migratory and invasive due to increased secretion of extracellular proteases. Therefore, EMT enhances intravasation of carcinoma in situ cells through the basement membrane in the circulation while facilitating the extravasation at the distal tissues and formation of micrometastases in distal organs (33). In summary, it is becoming increasingly clear that TGF-β signaling controls a complex network of interconnected pathways to regulate EMT and, therefore, the metastatic properties of cancer cells.

WNT SIGNALING PATHWAY AND EMT

Wnt signaling is a highly conserved signaling pathway with a critical role in regulating embryonic and organ development, as well as cancer progression. Genome-wide sequencing and gene expression profile analyses have demonstrated that Wnt signaling is greatly involved in breast cancer cell proliferation and metastasis (34). Wnt proteins, a group of secreted glycoproteins, mostly bind with Frizzled (FZD) receptors and Low-density-lipoprotein-receptor-Related Proteins 5/6 (LRP5/6), initiating signaling pathways dependent or independent from β-catenin (35). The Wnt pathway plays a critical role in cellular proliferation and oncogenesis. β-catenin is a downstream signaling molecule activated by canonical Wnt signaling with a dual role in EMT. When bound to cadherin complexes in adherens junctions, β-catenin acts as a bridge to enhance cell-to-cell adhesion. Moreover, it is a transcription co-factor with DNA-binding proteins of the T-Cell Factor (TCF)/Lymphoid Enhancer Factor (LEF) family (36). Thus, β-catenin is considered an ideal target for studying the molecular basis of EMT and malignancy development. 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) (37). 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 complexes (LRP5 or LRP6) to inactivate GSK-3β in the destruction complex, leading to β-catenin stabilization. Also, β-catenin accumulates and translocates into the nucleus, where it forms a complex with TCF/LEF transcription factors, regulating the transcription of Wnt target genes. In this pathway, GSK-3β is a nodal protein that negatively regulates the stability and activity of β-catenin, as well as mediating the phosphorylation of Snail (38). The first phosphorylation motif regulates its β-Trcp-mediated ubiquitination, whereas the second phosphorylation motif controls its subcellular localization. Phosphorylation of Snail by GSK-3β facilitates its proteasomal degradation (39). Conversely, inhibition of GSK-3β leads to Snail accumulation, E-cadherin down-regulation, and EMT development. Wnt ligands can activate EGFR signaling through Fzd, whereas EGFR can activate β-catenin, a downstream effector of the Wnt pathway, via the RTK-PI3K/Akt pathway. Furthermore, EGFR has been shown to form a complex with β-catenin, increasing the invasion and metastasis of cancer cells.

NOTCH SIGNALING PATHWAY AND EMT

Notch signaling is a primitive signaling pathway with various roles in the normal origin of each multicellular organism and its development. Therefore, any aberration in the pathway will inevitably lead to fatal outcomes, such as cancer. It has been shown that Notch is an oncogene in breast cancer, as overexpression of Notch1IC, Notch3IC, or Notch4IC is sufficient for the transformation of normal breast epithelial cells into cancerous cells (40-42). Overexpression of Notch1 and/or Jagged1 predicts the poorest prognosis for women with breast cancer. Early studies have shown that normal breast tissue has a high expression of the negative regulator of Notch, Numb, while the expression is lost in breast tumors. Additionally, Notch signaling activates the genes required for EMT, a prerequisite for metastasis from the primary site (breast) to distant organs, such as lymph nodes, bone, brain, lung, or liver (43). Data suggest that positive regulation of SLUG through Jagged1-mediated activation of NotchIC results in the repression of E-cadherin, thus allowing EMT in breast cancer cells. Jagged1 overexpression alone is associated with increased bone metastasis, while the disruption of the Notch pathway by using the γ-Secretase Inhibitor (GSI) MRK-003 reversed the bone metastasis mediated by Jagged-1 in mice (44). Increased expression of Notch targets, HEY2 and HES1, down-regulation of E-cadherin and β-catenin, and increased migration and invasion of breast cancer cells cultured in low-oxygen conditions demonstrated that the hypoxia-induced activation of Notch promotes EMT in breast cancer cells (45). Importantly, EMT gives rise to a cancerous, stem-cell-like phenotype in immortalized Human Mammary Epithelial Cells (HMECs). Studies have shown that HMECs with only the overexpression of SNAIL or TWIST successfully undergo EMT, and most, if not all, transitioned cells have CD44 overexpression and CD24 underexpression, which are markers for breast cancer stem cells. Furthermore, BCSCs have cell fate plasticity and can change from epithelial phenotypes to mesenchymal phenotypes.

CONCLUSION

In cancer, It seems that EMT is a key pathological process characterized by abnormal metabolic reprogramming of cancer cells toward an invasive and pro-metastatic phenotype. The signals responsible for the EMT activation can derive directly from alterations in the cancer cell metabolism. Subsequently, tumor cells undergoing EMT modify the surrounding environment, which actively helps the tumor invade and metastasize. TGF-β, Wnt, and Notch signaling pathways, participating actively in the tumor spread, have been proposed as possible tools for cancer surveillance and treatment. On the one hand, these are biomarkers for disease regression or relapse, while on the other hand, they are possible direct targets for new-combination anticancer drugs and personalized medicine.

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