REVIEW ARTICLE

Role of epigenetic mechanisms in epithelial-to-mesenchymal transition of breast cancer cells

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The epithelial-to-mesenchymal transition (EMT) is a crucial process during normal development that allows dynamic and reversible shifts between epithelial and mesenchymal cell states. Cancer cells take advantage of the complex, interrelated cellular networks that regulate EMT to promote their migratory and invasive capabilities. During the past few years, evidence has accumulated that indicates that genetic mutations and changes to epigenetic mechanisms are key drivers of EMT in cancer cells. Recent studies have begun to shed light on the epigenetic reprogramming in cancer cells that enables them to switch from a noninvasive form to an invasive, metastatic form. The authors review the current knowledge of alterations of epigenetic machinery, including DNA methylation, histone modifications, nucleosome remodeling and expression of microRNAs, associated with EMT and tumor progression of breast cancer cells. Last, existing and upcoming drug therapies targeting epigenetic regulators and their potential benefit for developing novel treatment strategies are discussed. (Translational Research 2014;■:1–17)

Abbreviations: 5-aza-CdR = 5-aza-2′-deoxycytidine; AML = acute myeloid leukemia; ANRIL = antisense non-coding RNA in the INK4 locus; CBP = CREB-binding protein; CDKN2A = Cyclin-Dependent Kinase Inhibitor 2A; CoREST = co-repressor for element-1-silencing transcription factor complex; CtBP = C-terminal binding protein; CSC = cancer stem cell; DNMT = DNA methyltransferase; EMT = epithelial-to-mesenchymal transition; ER-α = estrogen receptor α; EZH2 = enhancer of zeste homolog 2; HDAC = histone deacetylase; HMT = histone methyltransferase; HOTAIR = HOX transcript antisense RNA; HtrA = high-temperature requirement factor A; Id1 = inhibitor of DNA-binding 1; Id3 = inhibitor of DNA binding 3; IGF = insulin-like growth factor; IRS1 = insulin receptor substrate-1; JARID1B = Jumonji/ARID Domain-Containing Protein 1B; lncRNA = long noncoding RNA; LSD1 = lysine-specific demethylase 1; MBD = methyl-CpG binding-domain protein; MeCP2 = methyl CpG binding protein 2; MET = mesenchymal-to-epithelial transition; miRNA = microRNA; mRNA = messenger RNA; MTA = metastasis tumor antigen; ncRNA = noncoding RNA; NuRD = Nucleosome Remodeling and Deacetylase; PRC2 = polycomb repressive complex 2; Snail = snail family zinc finger 1; Snail2/Slug = snail family zinc finger 2; SUZ12 = suppressor of zeste 12 homolog; TGF-β = transforming growth factor β; TIC = tumor initiating cell; TP53 = tumor protein p53; Twist = twist basic helix-loop-helix transcription factor 1; ZEB1/2 = zinc finger E-box binding homeobox 1/2

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Excluding skin cancers, breast cancer is the malignancy with the greatest incidence and is the second leading cause of cancer deaths among women in the United States.[1] More than 90% of cancer deaths from solid tumors are the result of metastases, not the primary tumors from which these malignancies arise.[2] During the metastatic process, the epithelial cells of a primary tumor acquire migratory and invasive skills that allow them (1) to detach from the primary tumor site and invade the surrounding tissue; (2) to intravasate the lymphatic or blood system, where they are disseminated to distant sites of the body; and (3) to extravasate and colonize at a secondary site.[2-4] Widespread cell-biologic reorganization is necessary for a tumor cell of epithelial origin to detach from the primary tumor and infiltrate surrounding tissues. These tumor cells have to diminish cell-cell contacts, remodel cell-matrix adhesions, and gain migratory and invasive abilities, all of which are mesenchymal properties. Dynamic, reversible transitions between an epithelial and mesenchymal phenotype can be commonly observed during normal developmental processes, including gastrulation, neural crest formation, and heart morphogenesis. The reversible switches between epithelium and mesenchyme are known as epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET), respectively.[5] During EMT, polarized, nonmotile epithelial cells downregulate their cell-cell adhesion molecules (eg, E-cadherin, β-catenin), detach from their cell collective, and convert to nonpolarized, motile, fibroblastic cells.[6,7] In the adult, EMT-like events can be observed during wound healing[8] and in pathologic states as fibrosis[9] and tumor metastasis.[7] Thus, comparable with events in embryonic development, EMT as well as MET are considered to play important roles in tumorigenesis. Although EMT has been demonstrated to promote tumor invasion and metastatic dissemination, the reversed process, MET, is emphasized after dissemination of tumor cells and seems to contribute to the colonization and formation of distant metastases.[10-13] A common spectrum of EMT-MET-typical changes in morphology and gene expression patterns is associated with these processes. In terms of cancer, EMT does not necessarily describe a complete switch from a differentiated epithelial state to a dedifferentiated mesenchymal state. In human carcinomas, multiple intermediate phenotypic stages between the 2 end points of epithelium and mesenchyme can be observed, each of which can be characterized by a different set of active epithelial and mesenchymal marker proteins and functional signaling traits.[14] Because various extracellular ligands can contribute to the induction of an EMT program, the signals from the nearby microenvironment of a tumor determine which individual EMT transcription factors, such as twist basic helix-loop-helix transcription factor 1 (Twist), snail family zinc finger 1 (Snail), snail family zinc finger 2 (Snail2/Slug), zinc finger E-box binding homeobox 1 (ZEB1), forkhead box C2 (FoxC2), become expressed and activated functionally.[14,15] The complexity of the signaling pathways upstream and downstream of the induction of EMT or MET explains the diversity of phenotypic effects that can be observed depending on the microenvironmental context of each individual type of cancer. Recent studies have demonstrated that EMT can induce tumor cells to take on stem cell-like traits. This small minority of neoplastic cells has the ability to seed new tumors—the reason why they are also termed tumor-initiating cells (TICs).[16] The stem cell-like characteristics conferred to epithelial cells by EMT endow the tumor cells with exactly the skill set needed for dissemination and formation of metastases (ie, the ability to proliferate and the potential of self-renewal).[6] For example, it was shown in mammary epithelial cells, that expression of *TWIST1* or *SNAI1* or treatment with transforming growth factor β (TGF-β) leads to a greater number of cells with cancer stem cell (CSC)-like properties. The stem cell features of these cells were demonstrated by their gene expression profiles, cell surface antigen patterns ($CD44^{high}/CD24^{low}$), potential to form mammospheres in Matrigel cultures, and ductal outgrowths in mouse xenograft assays.[17,18] Lending additional support to the hypothesis that EMT is associated with the formation of cells with CSC character, it was shown that breast cancer cells with a $CD44^{high}/CD24^{low}$ signature express significantly greater levels of cancer progression and metastasis-associated genes, such as *SNAI1*, *TWIST1*, or *FOXC2*, which promote the aggressiveness and metastatic potential of these cells.[19,20] Moreover, EMT and the acquisition of CSC traits have been associated with the basallike subtype of human breast cancer, a form of breast tumor with a poor prognosis because of its elevated potential to form distant metastases. Several studies showed that basallike breast cancer cells are enriched in $CD44^{high}/CD24^{low}$ CSCs and display high levels of EMT transcription factors.[15,21-24]

More interesting and important, there is increasing evidence that the process of MET is as crucial to aspects of tumor metastasis as EMT. Although EMT is a driver of tumor dissemination, switching to an epithelial phenotype by undergoing MET is described as an important facilitator enhancing metastatic colonization.[25-27] Clinical and experimental studies examining breast cancer metastases showed that the colonizing cells display epithelial and TIC-like characteristics, indicating that successful metastatic growth is supported by MET.[13,26-28] In breast cancer cells, the MET process can be promoted by the loss or inhibition of well-