

Regulation of Epithelial-Mesenchymal Transition by Transcriptional Factors in Cervical Carcinoma

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Abstract

Cervical carcinoma is a most prevalent cancer in women worldwide. The metastasis is one of the major issues for late-stage cervical carcinoma in patients. Epithelial-mesenchymal transition (EMT) has been implicated in cervical carcinoma progression and metastasis. During EMT, cervical carcinoma cells lose epithelial features and gain a mesenchymal phenotype. The EMT has been identified to be regulated by key transcription factors including Snail, Zeb, and Twist. In this review, we will discuss our current understanding of how these key transcription factors play important roles in EMT program of cervical carcinoma cells.

Keywords: Transcription factors; Epithelial-mesenchymal transition; Cervical carcinoma

Cervical Carcinoma

Cervical carcinoma is a most prevalent cancer and a common cause of death in women worldwide. The major types of cervical carcinoma include squamous cell carcinoma (SCC) and adenocarcinoma [1]. Squamous cell carcinoma begins in the thin and flat cells that line the cervix while adenocarcinoma begins in cervical cells that make mucus and other fluids [1]. About 90% of cervical carcinoma is squamous cell carcinoma, only 10% of cervical carcinoma is adenocarcinoma [1]. The major etiological factor of cervical carcinoma is the presence of human papillomavirus (HPV) oncogene [2]. HPV can be classified into high-risk and low-risk types. High-risk HPVs such as HPV-16, 18, and 31 are associated with more than 90% cervical carcinoma [2]. HPV is contributing to progression of cervical carcinoma through the action of HPV oncoproteins E6 and E7 which interact with tumor suppressor proteins such as p53 and pRB to interfere critical cell cycle [3]. E6 and E7 are invariably expressed in HPV-positive cervical carcinoma cells and play important roles in carcinogenesis and maintenance of the transformed phenotype [3]. Despite HPV are thought to be the major cause of cervical carcinoma, however, our data and others have shown that HPV alone is not sufficient to induce cervical carcinoma formation, suggesting that the factors other than HPV viral proteins also contribute to the progression of cervical carcinoma [4-6].

The metastasis is one of the major issues in late-stage cervical carcinoma in patients. In order to migrate, cancer cells need to activate genes for cellular proliferation, change cellular characteristics from epithelial to mesenchymal, activate anti-apoptotic signaling to initiate cell differentiation, down-regulate the receptors to help cell-cell attachment, up-regulate the cell adhesion molecules to promote cell movement, degrade cell-cell junctions, and activate proteases at the cell surface [7]. Whether or not a cancer cell successfully migrates for metastasis is related with cancer progenitor cell characteristics, environmental factors, extracellular and intracellular signaling, and epigenetic changes all influence [8].

Epithelial-mesenchymal Transition in Cervical Carcinoma

Epithelial cells have distinctive features of cell adhesion and apical-basal polarity, whereas mesenchymal cells loose cell adhesion and have a front-back cell polarity [9]. Epithelial cells can be converted to mesenchymal cells through a epithelial-mesenchymal transition (EMT) process in many cancers including cervical carcinoma, which have dramatic phenotypic changes by the loss of epithelial marker proteins such as E-cadherin and the acquisition of mesenchymal marker proteins such as vimentin [9-12]. It has been proposed that three types of EMT are involved in cancer progression. The type 1 of EMT is in the developmental processes, type 2 of EMT is in the inflammation, tissue remodeling, wound healing, and fibrosis, and type 3 of EMT is in cancer invasion and metastasis [13]. The process of EMT is reversible when mesenchymal cells gain epithelial characteristics via mesenchymal-epithelial transition (MET) process [14]. Interestingly, incomplete EMT in an epithelial cancer cell may generate a combine metastable cell which contains both epithelial and mesenchymal phenotypes and consistent with the existence of cancer cells in various tumors including cervical carcinoma [1].

The epithelial-mesenchymal transition plays an important role in metastasis of cervical carcinoma. The transfection of oncoproteins E6 and E7 in cervical carcinoma cells showed the up-regulation of mesenchymal markers SMA and vimentin and the down-regulation of epithelial marker E-cadherin during EMT [15]. Loss of E-cadherin is related to the oncoprotein E5 of human HPV, while forced expression of E-cadherin in the immortalized cell line with oncoproteins E6 and E7 can reverse the invasive phenotype [16]. The promoter DNA hypermethylation is a major contributor that regulating transcription activity of the E-cadherin gene and the hypermethylated DNA is detectable in serum of cervical carcinoma patients [17]. E-cadherin expression can be reactivated using HDAC inhibitor valproic acid (VPA), suggesting that histone modification and chromatin remodeling is involved in the regulation of E-cadherin expression in cervical carcinomas [17]. Although hypoxic has been suggested to be involved in E-cadherin

suppression in cancers, however there is no evidence to show that the oxygenation is directly related with E-cadherin expression in the squamous cell carcinoma of uterine cervix [18].

Loss of E-cadherin during EMT

E-cadherin is expressed primarily in epithelial cells as a single-span transmembrane glycoprotein of five repeats and one cytoplasmic domain [19]. E-cadherin mediates cell-cell adhesion via interacting with a number of proteins including α -catenin, β -catenin, and p120 catenin which link E-cadherin to the actin cytoskeleton in its cytoplasmic domain [20]. The extracellular domain of E-cadherin contains characteristic repeats that regulate homophilic and heterophilic interactions [21]. The evidences suggest that the combination of *cis*-dimerization of two cadherin molecules on the same cell surface and *trans*-interactions between cadherin dimers on opposing cell surfaces which maximizes homophilic adhesion [22-25].

Loss of E-cadherin is a common feature of EMT in epithelial cancers including cervical carcinoma, which has been found to increase cancer cell invasion and metastasis [7]. E-cadherin is a tumor suppressor of many tumors and its down-regulation provokes the development of malignant epithelial cancers. Several important transcription factors have been shown to associate with E-cadherin during EMT. As a member of the Snail family of transcriptional repressors, Slug is capable of repressing E-cadherin expression to trigger EMT, suggesting that it may play a role as an invasion promoter. The evidence suggests that both Snail and its family member Slug are capable of repressing E-cadherin in epithelial cells via the E-box elements in the proximal E-cadherin promoter [26]. Behrens et al. [27] have demonstrated that epithelial cells assume invasive characteristics due to loss of E-cadherin-mediated cell adhesion. Burdsal et al. [28] have shown that blocking E-cadherin is sufficient to trigger EMT in mammalian cell systems. Therefore, loss of E-cadherin is frequently associated with strong invasive tendencies and can be considered as a classical marker of poor prognosis of cervical carcinoma.

Regulation of EMT by Transcription Factors in Cervical Carcinoma

Many transcription factors have been reported to associate with the regulation of EMT. These transcription factors include the Snail family of zinc-finger transcription factors such as Snail1 (Snai1), Snail2 (Slug), and Snail3 (Smuc); the two-handed zinc-finger factors of d-crystallin/E2 box factor family proteins zinc-finger E-box-binding homeobox (Zeb)1 and Smad-interacting protein Zeb2; and the basic helix-loop-helix factors Twist1 and Twist 2 [17,29,30]. These transcription factors recognize the DNA sequences of E-box in the promoter region of E-cadherin and recruit cofactors and histone deacetylases resulted in repressing E-cadherin expression [31]. In addition, these transcription factors act as molecular switches response to the signaling pathways and regulate the EMT program [32].

Snail, Slug and Smuc

The Snail family proteins include Snail (Snai1), Slug (Snai2), and Smuc (Snai3) which are zinc finger transcriptional regulators [33]. The Snail family proteins encode transcription factors of the zinc-finger type and share a highly conserved carboxy-terminal region and a divergent amino-terminal region [34]. The zinc-finger type includes the cysteines and histidines (C2H2) type and function as sequence-specific DNA-binding motifs [35]. The amino-terminal part of the zinc-finger type can bind to a major groove of the DNA [36]. In addition, the zinc-finger type includes two beta-strands followed by alpha-helix [36]. The two conserved C2H2 coordinate the zinc ion [37]. It has been shown that the consensus binding site of Snail-related genes contains a core of six bases, CAGGTG [38]. This motif is identical to the core binding site of basic helix-loop-helix (bHLH)

transcription factors [39], suggesting that Snail proteins might compete with them for the same binding sequences.

Snail has been shown to convert normal epithelial cells into mesenchymal cells through the direct repression of E-cadherin expression [40]. More importantly, Snail knockout mice die at gastrulation stages and show defects in EMT [41]. Mutant embryos retain all characteristic of epithelial cells with apical-basal polarity, microvilli and Adherens Junctions [42]. This study indicates that Snail acting as a repressor of E-cadherin expression and loss of Snail proteins in epithelial cells resulted in failing to undergo EMT. Snail and Slug are considered major transcription factors that regulate EMT in various cancers including cervical carcinoma [9,43,44]. Several studies have shown that Snail family proteins play important role in induction of EMT in cervical carcinoma [1,6] (Figure 1). The Snail inhibits the expression of claudins, occludin, and thrombomodulin in cervical carcinoma cells [1,45,46]. Snail and Smuc have been reported to associate with lymph node metastasis [47]. It was shown that Snail and Slug bind to the E-cadherin promoter, up-regulate mesenchymal makers such as Vimentin, and ultimately promote EMT [48]. The up-regulation and nuclear accumulation of Snail are correlated with EMT in cervical carcinoma [47]. These data suggest that Snail family proteins play important role during EMT in cervical carcinoma.

Zeb1 and Zeb2

Zeb family proteins including Zeb1 and Zeb2 are sequence-specific DNA-binding transcription factors [49]. Several studies have shown that Zeb1 and Zeb2 can regulate E-cadherin expression in multiple human cancers through binding the E-boxes of E-cadherin [17,50,51]. Both Zeb1 and Zeb2 contain the helix-loop-helix motif that bind to the bipartite E-boxes of E-cadherin promoter region [17]. Polycomb protein Pc2 is required for E-cadherin repression mediated by small ubiquitin-like modifier (SUMO) conjugated lysine residues Lys391 and Lys866 in Zeb proteins [17,52]. In addition, Zeb proteins control the microRNA expression by interfering in microRNA promoter activity to form a reciprocal feedback loop in EMT [53]. Dysregulation of both Zeb1/2 and E-cadherin can be found in a lot of tumorigenic processes such as the stem-like cell character, development of mesenchymal phenotype,

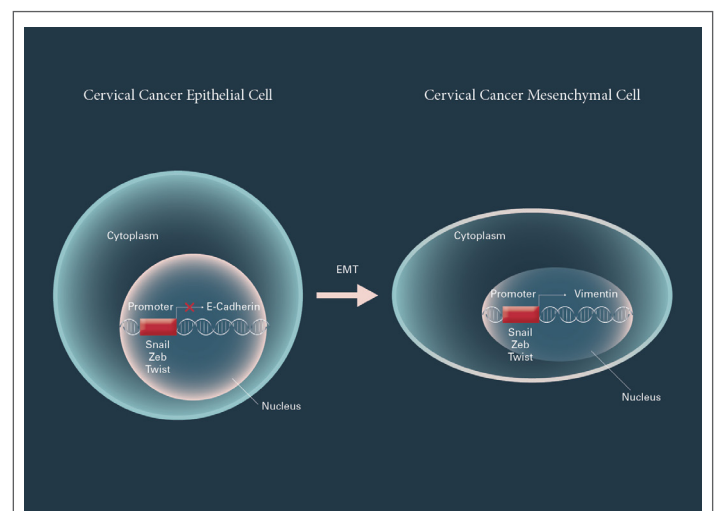


Figure 1: Transcription factors regulate EMT in cervical carcinoma cells. Cervical cancer epithelial cell can be converted to cervical cancer mesenchymal cell by regulation of Snail, Zeb, and Twist family proteins in nucleus, respectively during an epithelial-mesenchymal transition (EMT) process, which has dramatic phenotypic changes by the loss of epithelial marker proteins such as E-cadherin and the acquisition of mesenchymal marker proteins such as vimentin.

aggressiveness in EMT, resistance to therapeutic agents, adaptive stages under hypoxic microenvironment, and cancer progression [17,54-55].

Normally, mesenchymal cells highly express Zeb1, whereas epithelial cells lack Zeb1 expression [56]. Zeb1 can induce EMT through suppressing E-cadherin and other genes to participate in epithelial cell polarity, when Zeb1 is inappropriately expressed in cervical carcinomas [57]. Nuclear Zeb1 expression is detected in most of invasive cervical carcinomas [58]. In addition, Nuclear Zeb1 expression is associated with high grades in cervical carcinoma [35]. Although hypoxia has been suggested to be involved in E-cadherin suppression in solid tumors, however the oxygenation status has no direct correlation with E-cadherin level in the cervical carcinoma [18]. Clinically, Zeb1 expression has been found in more than 95% cervical carcinoma and the expression level of Zeb1 was significantly associated with International Federation of Gynecology and Obstetrics stages and regional lymph node metastasis [17]. At present, whether Zeb1 and Zeb2 are involved in the cervical carcinomas remained to be determined.

Twist1/2

Twist is a transcription factor protein that belongs to the family of basic-helix-loop-helix proteins (bHLH) [20]. Twist includes a conserved domain with two α -helices separated by an inter-helical loop [59]. Twist can form dimers by its helices and binds to the DNA sequences 5'-CANNTG-3' called E-boxes [60]. In vertebrate animals, Twist encodes two similar genes, Twist1 and Twist2 which are 90% identical. The C-terminal sequence of E-box in Twist is associated with the anti-osteogenic function. Twist1 has a glycine-abundant region in the N-terminal of E-box, whereas Twist2 does not have such region. Both Twist1 and Twist2 are associated with the differentiation of muscle, cartilage and osteogenic cells [61]. Twist is mainly found in neural crest cells in vertebrates [62]. The absence of Twist2 function in mice is associated with cachexia [60].

Twist family proteins have been reported to contribute in tumor metastasis by promoting EMT [63]. Twist2 protein regulates E-cadherin expression by down-regulating E-cadherin promoter activity [64]. Twist1 is a master regulator and a primary cause of EMT in cervical carcinoma [22,35]. The expression of Twist1 is associated with chemotherapy and radiotherapy resistance while the inactivation of Twist1 by RNA interference induces cell apoptosis in cervical carcinoma cells [65]. In addition, the overexpression of Twist1 leads to a poor prognosis and the knockdown of Twist1 induces down-regulation of MDR1/P-gp (multi-drug resistance protein) expression, inhibiting its efflux activity, and sensitizing cervical cancer cells to cisplatin treatment in cervical carcinomas [66]. Twist2 expression in cervical squamous cell carcinoma patients is a predictor for metastatic potential and Twist2 increases the rate of migration and invasion more than Twist1 [67]. Twist plays a role in the regulation of EMT in cervical cells through maintaining the CD44 expression and stem cell-like properties associated with EMT [68]. The expression of Twist is critical for EMT induction by increasing the expression of CD44, enhancing tumor sphere formation, and promoting ALDH1 activity during cervical carcinoma development [8]. Twist induces the activation of β -catenin pathway and Wnt3 signaling in Twist-overexpressing cells [68]. The aberrant expression of Twist1 and Twist2 in cervical carcinoma cells is associated with activation of AKT pathway resulted in phosphorylation and suppression of GSK-3 β [40]. These data suggested that both Twist1 and Twist2 play important role through regulation of EMT during cervical carcinoma development.

Conclusion

Various transcription factors have been reported to associate with the regulation of EMT in cancer. In this review, we discussed how some of the transcriptional factors such as Snail, Zeb, and Twist proteins play important

roles in EMT during cervical carcinoma development. Metastasis is the major cause of death in cervical carcinoma and EMT plays a key role in metastasis of cervical carcinoma by down-regulation of epithelial marker E-cadherin and up-regulation of mesenchymal marker vimentin, resulted in increasing cancer cell survival, migration, invasion, metastasis, and recurrence. Interestingly, many studies have shown that activation of EMT transcriptional factors is associated with oncogenic transformation which make them more aggressive and promote the development of metastatic properties. As molecular switches, these activated EMT transcriptional factors can respond to complex signaling pathways and regulate the EMT program. In addition, these activated EMT factors can recognize the E-box DNA sequences in the promoter region of E-cadherin, recruit cofactors and histone deacetylases to repress its expression. Therefore, these activated EMT transcriptional factors have been implicated in the cancer stem cell property, cancer recurrence, resistance of radio therapeutic and chemotherapeutic drugs, and immune suppression. Studies in cell lines and xenograft mice models have identified that the function of activated EMT transcriptional factors in cancer is not only as important diagnostic and prognostic biomarkers, but also as potential therapeutic targets. Taken together, a better understanding the role of transcriptional factors in promoting EMT and cancer stem cells in cervical carcinoma will lead to develop more new prognostic biomarkers and therapeutic targets for cervical cancer invasion and metastasis.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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