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Mini Review

Molecular mechanisms controlling E-cadherin expression in breast cancer

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ABSTRACT

Disruption of cell–cell adhesion, which is essential for the maintenance of epithelial plasticity and is mediated by a class of proteins called cadherins, is an initial event in the progression of cancer. Cadherins are Ca^{2+}-dependent transmembrane proteins that are associated with actin via other cytoplasmic proteins. Disruption of cell–cell adhesion during cancer progression is an important event during cancer initiation and metastasis. E-cadherin, one of the most widely studied tumor suppressors in breast cancer, belongs to a family of calcium-dependent cell adhesion molecules. Various signaling molecules and transcription factors regulate the expression of E-cadherin. Loss of E-cadherin has been reported to induce epithelial–mesenchymal transition in several cancers. This review highlights recent advances in defining the mechanisms that regulate E-cadherin expression in breast cancer.

Introduction

Epithelial cancers account for 90% of cancer-related mortality and among them, breast cancer is the leading cause of death, with annual mortality of 45,000 in 2008. One basic characteristic of cancer cells is that they adhere poorly to each other. Cell–cell adhesion is mediated by a variety of membrane proteins such as classical cadherins (E and N, P, R, VE, etc.), claudins, occludins, nectins, and desmosomal cadherins. The classical cadherins, the most extensively studied class of cadherins, are named based on the tissue from which they were first isolated. For example, E-, N-, and R-cadherins were derived, respectively, from epithelial, neural, and retinal tissues. Classical cadherins are required to initiate cell–cell contacts; other adhesion protein complexes subsequently assemble and maintain the structural continuum of the epithelium [1].

E-cadherin, a single-span transmembrane glycoprotein of five repeats and cytoplasmic domain, is expressed primarily in epithelial cells. Its extracellular region has a Ca^{2+}-dependent homophilic adhesion function and the cytoplasmic domain interacts with catenins (Fig. 1) [2,3]. The critical importance of E-cadherin to normal development and tissue function is demonstrated by the lethality of E-cadherin gene knockout in mice in the very early stage of embryogenesis [4]. E-cadherin is a tumor suppressor protein that is used as a prognostic marker for breast cancer [5]. One important event in the progression of cancer is switching of the expression of cadherin from E-cadherin to N-cadherin [6]. By various mechanisms, expression of N-cadherin promotes aggressive behavior of tumor cells, ranging from interacting with receptor tyrosine kinases at the cell surface to influencing the activation levels of Rho-GTPases in the cytosol [7].

N-cadherin is over-expressed in most invasive and metastatic human breast cancer cell lines and tumors [6,8]. N-cadherin induces metastasis due to its ability to bind and potentiate signaling by the FGF receptor [9]. N-cadherin is emerging as a potential therapeutic target. A peptide based on the N-cadherin antagonist (ADH-1) has been developed and is currently undergoing clinical trials [10]. ADH-1 has been shown to inhibit cell growth and motility *in vitro*, as well as tumor growth and invasion *in vivo* [10,11].

E-cadherin has gained special interest because its function is frequently perturbed in metastatic cancer cells. E-cadherin is linked to the cytoskeleton through association with several cytoplasmic catenins [1]. The intracellular juxtamembrane part of E-cadherin binds to p120 catenin, an armadillo repeat protein capable of modulating E-cadherin clustering [12]. The distal segment of E-cadherin's cytoplasmic domain can interact with β-catenin or plakoglobin, armadillo repeat proteins that in turn bind to α-catenin. The carboxyl end of α-catenin binds directly to F-actin. Through a direct mechanism, α-catenin can then link the membrane-bound cadherin–catenin complex to the actin cytoskeleton (Fig. 2) [13].

Studies using cell–cell adhesion mediated by cadherin–catenin complex demonstrate the role of Rho GTPases, Rac1, and RhoA in reorganization of the actin cytoskeleton [14]. Frequent down-regulation of E-cadherin during the progression of cancer correlates with aggressive behavior of tumors and a poor prognosis [15]. In experimental systems, expression of E-cadherin has been shown to reduce the progression and invasiveness of tumors, as well as

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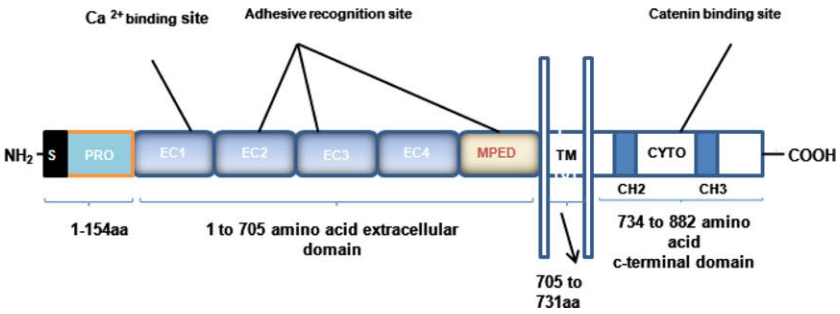

Fig. 1. Schematic representation of different domains of E-cadherin. E-cadherin is transmembrane glycoprotein with four extracellular domains (EC domain) with several Ca^{2+} binding and adhesive recognition site, one membrane proximal extracellular domain (MPED), transmembrane domain and cytoplasmic domain. Human E-cadherin is synthesized as pro-peptide of 1036 amino acids. Functional human E-cadherin is 882 amino acids protein with first 154 amino acids are involved in signaling and processing to the membrane.

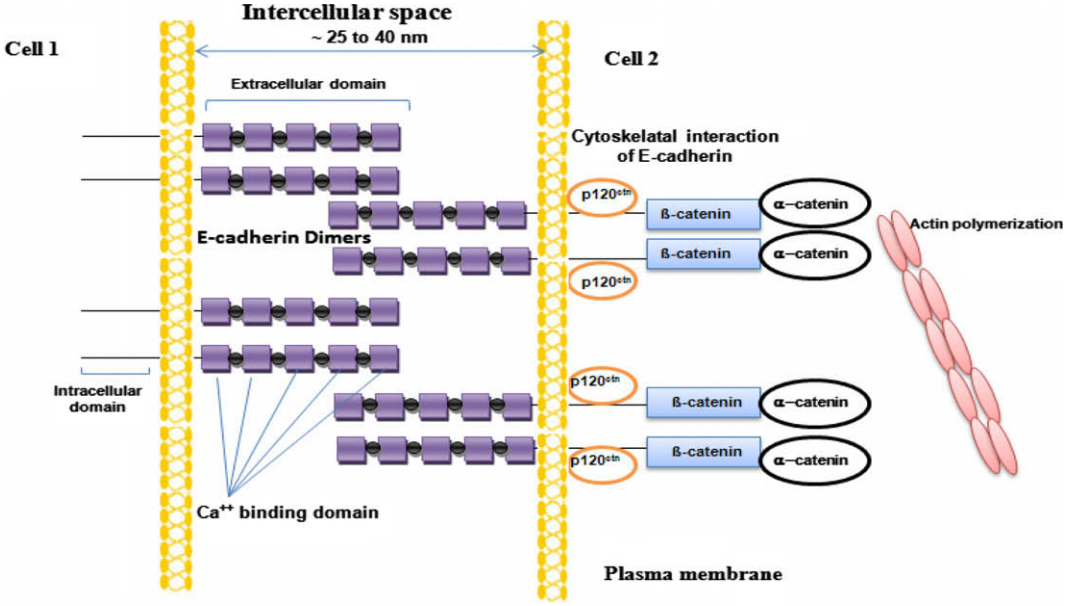

Fig. 2. Schematic illustration of E-cadherin in adherens junction formation. E-cadherin forms homodimer in the extracellular domain in a Ca^{2+}-dependent manner, while cytoplasmic domain binds with catenin and in turn regulates actin reorganization.

the formation of metastases [16]. Loss of E-cadherin during tumor progression leads to accumulation of β-catenin in the cytoplasm, which leads to stimulation of the β-catenin pathway. Based on these results, it was hypothesized that E-cadherin serves as an invasion or metastasis suppressor and that perturbation of its function occurs rather late in tumor progression [17]. E-cadherin also has an essential function in normal physiologic processes such as development, cell polarity, and tissue morphology [18], as well as in pathologic states such as epithelial–mesenchymal transition (EMT), a process whereby tumor cells lose their epithelial markers and migrate to distal organs.

Genetic and epigenetic control of E-cadherin expression

Both reversible and irreversible loss of E-cadherin is important in the progression of cancer. Human E-cadherin (CDH1) is located on chromosome 16q22.1, which has been reported to confer loss of heterozygosity in sporadic breast cancer [19]. Also, promoter hypermethylation is known to result in transcriptional down-regulation of many genes, including the E-cadherin gene [20]. This finding demonstrates that the E-cadherin promoter can be partially methylated. In general, the methylation profile of the E-cadherin promoter fragment contains unmethylated or partially methylated CpG islands. Alternation of E-cadherin expression by transcriptional silencing has also been reported to cause reduced expression of E-cadherin [21]. This transcriptional silencing is mediated by a class of zinc finger binding proteins that target the promoter region of E-cadherin and suppress its expression. It has been reported that zinc-finger transcription factors such as Snail/Slug are over-expressed in advanced carcinomas [22]. Interestingly, these transcription factors are important in the embryonic development during the epithelial–mesenchymal transition, when E-cadherin expression is lost. Experimental knockdown of E-cadherin in epithelial breast cancer cell lines has confirmed the role of E-cadherin as a tumor suppressor protein [23]. Over-expression of E-cadherin in metastatic breast cancer cells inhibits breast cancer invasion and metastases *in vitro* and *in vivo* [24].

Signaling control of E-cadherin expression

Several signaling molecules have been reported to control E-cadherin expression. Kleinberg et al. [25] have found that the epithelial–mesenchymal transition and E-cadherin expression are influenced by several growth factors that are responsive to signaling, as well as by a variety of polypeptide growth pathways. Loss of E-cadherin has wide-ranging transcriptional and functional conse-

quences for human breast epithelial cells. Loss of E-cadherin is sufficient to confer metastatic ability on breast cancer cells that are otherwise essentially nonmetastatic. Using a dominant-negative E-cadherin mutant, Onder and colleagues [23] have determined that the acquisition of metastatic ability by cancer cells after E-cadherin loss is not attributable solely to disruption of intercellular adhesion contacts. Instead, it is the additional loss of E-cadherin protein that activates an epithelial–mesenchymal transition, attained by increased cellular motility, invasiveness, and resistance to apoptosis. This study also suggests that point mutations in the extracellular domain that preserve the cytoplasmic tail of E-cadherin are not likely to result in an epithelial–mesenchymal transition (EMT) or to afford functional traits that allow completion of the later steps of metastasis. In contrast, complete elimination of E-cadherin expression via truncation mutation or locus repression or loss results in the activation of malignancy-associated traits [26]. Thus, loss of E-cadherin, in combination with certain additional oncogenic lesions, results in the acquisition of multiple functional traits that contribute to the completion of several rate-limiting steps in the invasion-metastasis cascade.

Interestingly, Lester et al. [27] showed that breast cancer cells cultured in 1.0% O_2 (hypoxic condition) induce epithelial–mesenchymal transition (EMT). Hypoxia induces translocation of Snail to the nucleus, at which time E-cadherin is lost from plasma membranes. Hypoxia-induced E-cadherin loss increases expression of the urokinase-type plasminogen activator receptor (uPAR), which in turn activates cell signaling factors such as Akt and Rac1 downstream of uPAR. Hypoxia also induces phosphorylation of glycogen synthase kinase-3beta and increases Snail 1 expression.

p120 catenin binds to the juxtamembrane domain [12,28] and is critical for cadherin stability and turnover. p120 insufficiency in SW48 breast cells appears to be directly responsible for a significant reduction in the amount of E-cadherin, as well as associated defects in epithelial morphology. Accumulating evidence suggests that GSK-3beta participates in modulating the activity of Snail1 [29]. Snail1 contains several consensus sites for GSK-3beta. Phosphorylation of Snail by GSK-3beta facilitates its proteasomal degradation [30]. Conversely, inhibition of GSK-3beta leads to Snail 1 accumulation, E-cadherin down-regulation, and the development of EMT in cultured epithelial cells. Several signaling pathways implicated in the progression of EMT, including the Wnt and phosphoinositide 3-kinase pathways, use GSK-3beta to mediate their responses. In these pathways, regulation of other transcriptional effectors like β-catenin by GSK-3beta works in concert with changes in Snail 1 to orchestrate E-cadherin regulation (Fig. 3) [18].

Furthermore, Soto et al. [31] have determined that p120 has an unexpected dual role in the transformed growth of breast tumor cells. In the presence of E-cadherin expression, p120 stabilizes E-cadherin complexes and promotes their tumor-suppressive function, potently inhibiting Ras activation. Upon E-cadherin loss during tumor progression, the negative regulation of Ras is relieved; under these conditions, endogenous p120 induces transformed cell growth by activating Rac1 and the subsequent phosphorylation of Raf and MEK. These events result in constitutive, anchorage-independent activation of the Ras–MAPK–ERK signaling pathway. p21-activated kinase 1 (PAK1), a major signaling molecule downstream of growth factors and the small GTPases, is required for the optimum transcription repression activity of Snail 1 [32]. The underlying mechanism of Pak1 regulation of Snail1 activity involves Pak1 phosphorylation of Snail on serine 246 and its accumulation in the nucleus to exert its repressor functions [32].

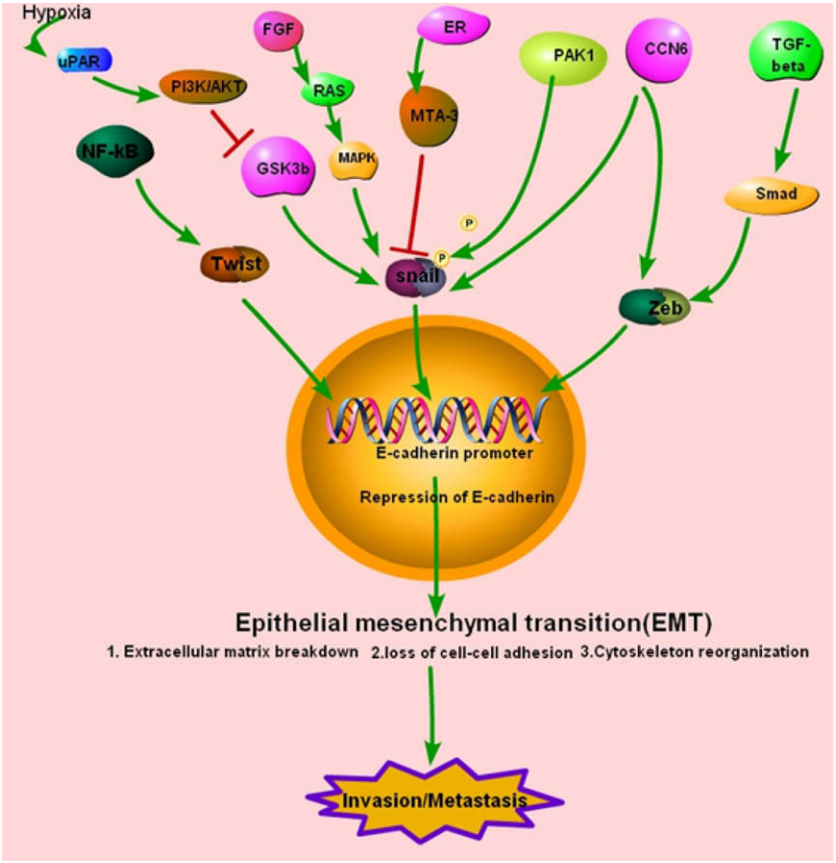

Fig. 3. Role of various signaling pathways in modulating the expression of E-cadherin. Different E-cadherin signaling pathways regulates the key process of epithelial–mesenchymal transition and the progression of breast cancer.

In addition, several tumor suppressor proteins have also been shown to regulate the expression of E-cadherin in breast cancer. Retinoblastoma (Rb), a classical tumor suppressor, has an important role in progression of the cell cycle. Loss or reduction of Rb expression is seen most commonly in high-grade breast adenocarcinomas, suggesting that a relationship may exist between loss of Rb function and a less-differentiated state, increased proliferation, and high metastatic potential. A interesting study by Arima et al., [33] suggests that Rb has a critical role in the expression of E-cadherin in breast cancer. Ectopic expression and knockdown of Rb resulted in either increased or reduced expression of E-cadherin. Other EMT-related transcriptional factors, including Slug and Zeb-1, were also induced by Rb depletion. Also these authors confirmed that Rb binds to an E-cadherin promoter sequence in association with the transcription factor activator protein-2α. Then, using breast cancer specimens, they observed a concurrent down-regulation of Rb and E-cadherin expression in mesenchymal-like invasive cancers. They concluded that inactivation of retinoblastoma contributes to tumor progression not only due to loss of cell proliferation control, but also conversion to an invasive phenotype and the loss of E-cadherin, which is a novel tumor suppressor of Rb.

Transcriptional control of E-cadherin expression

Detailed analysis has shown that E-cadherin in primary breast tumors and cell lines of distal histotypes does not have significant mutations in the E-cadherin gene, despite the fact that these tumors often show reduced expression of E-cadherin gene and protein expression. Detailed analysis of E-cadherin promoter has identified E-box elements that are responsible for the transcriptional repression of E-cadherin in non-E-cadherin-expressing mesenchymal cells [34]. It has been found that a zinc-finger transcription factor, Snail 1, directly binds to E-boxes of E-cadherin promoter and represses its expression [35]. Further studies have demonstrated the function of additional zinc-finger transcription factors in repressing E-cadherin transcription, thereby causing the disruption of cell–cell adhesion that occurs during EMT; these transcription factors include Slug, a close relative of Snail [22], and two members of the ZEB family of transcription factors, ZEB1 (δEF1) [36] and ZEB2 (SIP1) [37].

More recent studies have found that ZEB1, Snail 1, and Slug are capable of repressing the transcription of several polarity factors, including *Crumbs3* and *Lgl2* [38,39], indicating their function in suppressing critical components of epithelial cell traits. In addition to zinc-finger transcription factors, which bind to E-cadherin promoter with high affinity, the bHLH factor Twist has been found to be capable of repressing E-cadherin expression and thereby inducing epithelial–mesenchymal transition in human mammary epithelial cells [40]. One unique aspect of Twist is that it does not seem to directly repress the transcription of E-cadherin. Like Twist, two additional embryonic transcription factors, FOXC2 [41] and Goosecoid [42], have been shown to induce EMTs in certain epithelial cells, although they seem to lack the ability to directly bind to the E-cadherin promoter.

Several studies have indicated that activity of the NF-kB transcription factor family is required for maintenance of an invasive phenotype in cancers induced either by Ras or carcinogen treatment or in sporadic breast cancer cells. Wirth and coworkers identified NF-kB as a central mediator of EMT in a mouse model of breast cancer progression [43]. Specifically, inhibition of NF-kB in Ras transformed epithelial cells (EpRas cells) induces expression of the master regulators Snail 1, Slug, ZEB1, ZEB2 and Twist, which repress the expression of E-cadherin (Fig. 4). All the above described studies reveal that E-cadherin expression is regulated by several different proteins thorough different signaling cascades.

miRNA and E-cadherin expression in breast cancer

miRNAs are short noncoding RNAs of 20–22 nucleotides (nt) that control gene expression at the post-transcriptional level by pairing their seed sequences (2–8 nt at the 5′ end) to complementary sequences typically located in the 3′ untranslated region (UTR) of target mRNAs. This pairing results in degradation of the target mRNAs and/or inhibition of the translation process. miRNAs regulate diverse cellular processes. Some miRNAs have been shown to function as either tumor suppressors or oncogenes. The mechanism whereby members of the Zeb family of transcription factors are expressed in the regulation of E-cadherin is poorly understood. Initial studies showed down-regulation of ZEB1 and ZEB2 factors by over-expression of, respectively, miR-200c and miR-200b miRNAs and the upregulation of E-cadherin, the main target of the ZEB1 and ZEB2 repressors [44,45]. This finding is concomitant with the alteration of cell morphology in breast carcinoma cells [44]. Another link between E-cadherin and miRNAs has been emphasized [46] with the demonstration that the EMT regulator Twist

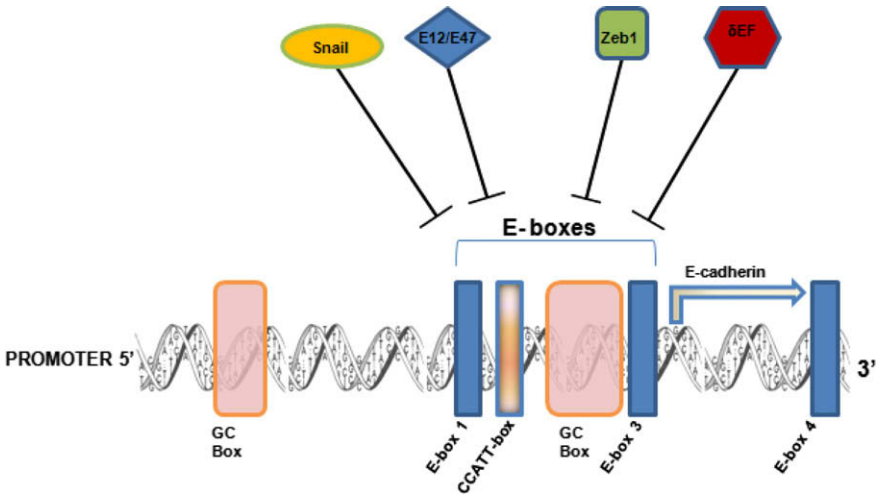

Fig. 4. Zinc-finger transcription factors target the proximal region of E-cadherin promoter in regulating its expression. Zinc-finger transcription factors are over-expressed in metastatic breast cancer cells, and they regulate the expression of E-cadherin through binding with the E-box of the E-cadherin promoter.

is a positive, direct activator of miR-10b, an miRNA that is over-expressed in human breast carcinoma.

Conclusions

Inactivation of E-cadherins is important in the progression of sporadic breast cancer. E-cadherin is completely and irreversibly lost in infiltrative lobular breast cancer, which suggests its function as a tumor suppressor. In the last few years, several cytoplasmic proteins have been identified that regulate the expression of E-cadherin. A recent study has identified a novel role of tumor suppressor in the regulation of E-cadherin [33]. Using various tissue culture breast cancer cells as models, several cytoplasmic proteins have been identified as regulators of E-cadherin. miRNA studies suggest the critical function of the miRNA 200 family in the regulation of E-cadherin and its potential use as a therapeutic approach to restore its expression.

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