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Focus On

Regulation of E-cadherin

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Abstract Numerous studies suggest that loss of E-cadherin is necessary to induce Epithelial–mesenchymal transition (EMT) and metastasis. Snail is a major contributor to EMTs. The Snail family of zinc-finger transcription factors interact with the E-cadherin promoter to repress transcription during EMT. The present article reviews the regulation of E-cadherin and discusses recent novel insights into the molecular basis in the process of EMT.

Keywords: E-cadherin; Snail; Epithelial–mesenchymal transition

E-cadherin (uvomorrulin), the founding member of the cadherin superfamily of calcium-dependent, transmembrane glycoproteins, plays a critical role in establishing adherens-type junctions. E-cadherin contains a prototypic extracellular domain that mediates homophilic protein–protein interactions in a zipper-like fashion. The intracellular domains of cadherins interact with several proteins collectively known as catenins. The resulting molecular complex binds to α-catenin and assembles other peripheral cytoplasmic proteins to connect E-cadherin to the actin cytoskeleton. E-cadherin plays an essential role in normal physiologic processes such as development, cell polarity, and tissue morphology [1], and in pathologic states such as epithelial–mesenchymal transition (EMT), a process usually accompanied by tumor dedifferentiation, infiltration, and metastasis. Alterations in E-cadherin expression or functions are common during carcinogenesis. In general, aberrant spatial–temporal expression or repression of E-cadherin is accompanied during EMT, an essential component of cancer progression to more aggressive phenotypes. In contrast, restoration of E-cadherin expression enhances intercellular adhesion, inhibits tumorigenicity, and suppresses the invasiveness of epithelial tumor cells [2,3]. Due to the loss of cell-to-cell junctions during EMT, E-cadherin loss leads to an increased pool of the cytoplasmic β-catenin as well as its transcription activity, resulting in the stimulation of β-catenin-responsive growth-regulatory genes [2]. Here we will briefly summarize the regulation of E-cadherin expression and discuss novel insights by which upstream regulators of E-cadherin might control the process of EMT, with a particular emphasis in breast cancer cells.

Genetic control of E-cadherin expression

The E-cadherin encoding gene, *CDH1*, maps to a region on chromosome 16q22.1, a region frequently associated with the loss of heterozygosity in sporadic breast cancers [4]. Since somatic mutations inactivating the *CDH1* gene are found in over 50% of diffuse-type gastric and infiltrative lobular breast cancers [5], E-cadherin has been proposed to have a causal role in some human cancers. However, the finding that E-cadherin mutations are rare in ductal breast cancers [4] suggests the potential involvement of epigenetic modifications in controlling the functions of E-cadherin.

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Epigenetic control of E-cadherin inactivation

Epigenetic mechanisms such as hypermethylation of the E-cadherin promoter [6,7], Histone H3 deacetylation (HDAC) in the context of CpG-methylation-mediated gene silencing [8], and transcriptional silencing have all been linked to the inactivation of E-cadherin expression. Analysis of somatic cell hybrids of E-cadherin-positive and -negative breast cancer cells suggests that the loss of E-cadherin expression in some breast cancers may be linked with a dominant repression transacting pathway [9], presumably due to dysregulation of transcription factors, support the notion of direct transcriptional silencing of E-cadherin as a major regulatory mechanism in human cancers.

Transcriptional controls of E-cadherin

The transcription of E-cadherin is controlled by both positive- and negative-regulatory elements located in its 5V promoter region [3,10–12]. The V promoter region also contains three E-box motifs, and protein-binding motifs with a core consensus sequence of CANNTG [3,13–17], which have both negative- and positive-regulatory functions. The E-box motifs function as negative regulators of E-cadherin in mesenchymal and in transformed cells by binding to Snail [3,13–17] or Zeb [13]. In addition, the E-box motifs could also impart a positive-regulatory function in epithelial cells [12] due to interactions with bHLH transcription factors [14].

The demonstration that the Snail family of zinc-finger transcriptional repressors control E-cadherin expression in epithelial cells has opened a new avenue of research in the field EMT. Examples of zinc-finger transcription repressors of E-cadherin include: Snail, Slug, ZEB1 and SIP1 (ZEB2) [17], and Twist [18]. Two widely studied repressors of E-cadherin expression and consequently, of EMT, are Snail and Slug [3,14,15], with ectopic expression of Snail or Slug in epithelial cells downregulating E-cadherin and promoting fibroblastic, tumorigenic and invasive characteristics [3,14,15]. Snail also activates the transcription of vimentin and fibronectin, which are *bona fide* markers of mesenchymal differentiation. Accordingly, carcinoma cells with low or no E-cadherin contain high levels of Snail-1 [3,14,19,20]. In addition to cancer, Snail is also critical during developmental processes, as Snail-1 knockout mice were unable to undergo gastrulation and neural-crest EMT [16], and is embryonic lethal due to its inability to downregulate E-cadherin and undergo EMT [17].

Slug was first identified as another repressor of E-cadherin in chicken, where it was shown to be critical in the induction of EMT during embryonic development [21]. However, this function is not conserved in all vertebrates, as its expression does not correlate with the loss of E-cadherin expression or EMT during mouse embryogenesis [3,22], and unlike Snail, Slug-null mice are viable and fertile [22]. Interestingly, the status of Slug expression correlates well with the loss of E-cadherin in human breast carcinomas [15]. There is also evidence to suggest that Slug might participate in EMT via downregulation of the components of the desmosome adhesion complex [23], in addition to downregulation of E-cadherin in some epithelial cell lines in a specific cellular contexts.

Transcriptional repressor activity of Snail has been linked with the C-terminus zinc fingers which mediate the sequence specific DNA binding to the E-box consensus sequence of CANNTG [3,14,19,23–28]. It is hypothesized that the repressor function of the Snail proteins is partially mediated via its competition with the bHLH transcription factors to bind the E-box motif [16,26]. Although the repressor activity depends greatly on the zinc-finger region, at least two other regions in the N-terminus are important for repressor function. The SNAG (Snail/Gfi) domain is conserved in the N-terminal region of all vertebrate Snail genes, and shown to be important for the repressor function of the Snail proteins in mammalian cells. Several vertebrate Snail family members also contain a partial CtBP interaction domain (CID) consensus sequence. The transcriptional repressor role of the Snail family of proteins is therefore mediated by its ability to compete for regulatory elements in the E-cadherin promoter, the SNAG domain, its interaction with CtBP, or a combination of the all three routes.

Cooperation of transcriptional repression with epigenetic modification

One widely accepted mechanism by which Snail represses the E-cadherin promoter includes the recruitment of repressor complexes involving Sin3A, or HDACs [9]. In addition, E-cadherin transcription is shown to be silenced by a CtBP corepressor complex containing different methylase and HDAC activities, as well as EF1 and SIP1 [19]. The complexity of repression of E-cadherin is further evident by the recent finding that the Snail gene is directly inhibited, in an HDAC-dependent manner, by MTA3, an estrogen-dependent component of the large transcriptional corepressor complex Mi-2/NuRD [29–31]. In addition, corepressor function of Snail is also compromised by its subcellular relocalization to the cytoplasm [31,32]. Overall, these findings indicate that Snail regulation of E-cadherin might be regulated by the dynamic interplay among multiple coregulators in a temporal and spatial manner.

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Signaling control of E-cadherin expression

The process of EMT as well as E-cadherin expression could be influenced by a variety of polypeptide growth factors and growth-factor-responsive signaling pathways. However, mechanisms by which signaling pathways regulates the expression of E-cadherin are poorly understood, but widely believed to involve modulation of the phosphorylation and/or the steady-state level of Snail [23]. For example, GSK-3β-mediated phosphorylation of Snail (at motif 2) negatively controls the stability of Snail protein, and thus, could lead to re-expression of E-cadherin in cells [32]. In contrast, stimulation of p21-activated kinase 1, a major signaling nodule downstream of growth factors and the Small GTPases, is required for an optimum transcription repression activity of Snail [33]. The underlying mechanism of Pak1 regulation of Snail activity involves Pak1 phosphorylation of Snail on serine 246 and its accumulation in the nucleus to exert its repressor functions [33]. Therefore, the corepressor functions of Snail and consequently, the process of EMT, could be modulated in both a negative and a positive manner depending on the nature of the signaling kinase activated or post-translational modification of Snail.

Future direction

Regulation of E-cadherin plays a crucial role in EMT and tumor progression. A hierarchy of different mechanisms at multiple levels, including genetic, epigenetic, and transcriptional regulations, may finally define the E-cadherin activity in a dynamic, as well as a cell and tissue specific manner. Since most of our current understanding of EMT in cancer cells is derived from tissue-culture model systems, it will be important to start combining these approaches with whole animal models as well as with human tumor specimens, to gain a comprehensive view of upstream regulators of E-cadherin that may be important in tumor invasion.

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