The regulation of cell-cell adhesion during epithelial-mesenchymal transition, motility and tumor progression

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Adherens junctions (AJs) are essential for the maintenance of epithelial homeostasis and a key factor in the regulation of cell migration and tumor progression. AJs maintain cell-cell adhesion by linking transmembrane proteins to the actin cytoskeleton. Additionally, they participate in recruitment of signaling receptors and cytoplasmic proteins to the membrane. During cellular invasion or migration, AJs are dynamically regulated and their composition modified to initiate changes in signaling pathways and cytoskeleton organization involved in cellular motility. Loss of E-cadherin, a key component of AJs, is characteristic of epithelial-mesenchymal-transition (EMT) and is associated with tumor cell invasion. We will review recent findings describing novel mechanisms involved in E-cadherin transcription regulation, endocytosis of E-cadherin and signaling associated with loss of AJs as well as reorganization of the AJ during EMT.

Introduction

Epithelial cell-cell adhesion is maintained by three basic structures connecting adjacent cells: tight junctions (zonula occludens), adherens junctions (zonula adherens) and desmosomes (macula adherens). AJs are established via calcium-dependent homophilic binding of the extracellular cadherin domains. The cytoplasmic domains of these cadherins recruit the catenins, β-catenin and p120 catenin, which in turn connect the AJs to the actin cytoskeleton. Linking catenins with the cytoskeleton may be mediated by the clustering of cadherin/catenin complexes to recruit high levels of α-catenin or by other cytoplasmic factors, such as epithelial protein lost in neoplasia (EPLIN or LIMA1).4 EPLIN, a mechanosensitive biomarker, directly binds α-catenin and is absent in many cancer cell lines.5,6 Other major components of AJs are the nectins and afadins. Unlike cadherins, nectins are capable of establishing adhesive contacts via either heterophilic or homophilic binding.7 The nectin-like protein NECL-5, better known as the poliovirus receptor (CD155), plays a role in cell motility, associating with integrin αvβ3 and platelet-derived growth factor receptor (PDGFR) at the leading edge of migrating cells.8 Other adhesion molecules have been shown to have dual function as a cell adhesion and motility promoter. In this special focus, Kiefer et al. show that L1 cell adhesion molecule (L1CAM), which plays a major role in the development of the nervous system, also functions to promote the malignancy of human tumors. While it acts as a glue between cells, L1CAM can also drive motility during neural development and supports metastasis of human cancers. Important factors that contribute to the switch in the functional mode of L1CAM are cleavage from the cell surface by membrane proximal proteolysis and the ability to change binding partners and engage in L1CAM-integrin binding. Cleavage of E-cadherin is also a mechanism by which the function of L1CAM can be disrupted.

One of the key steps in tumor progression is the loss of cell-cell adhesion. As single cells invade into the underlying stroma, they express mesenchymal markers and proteins involved in matrix degradation and motility. The induction of the EMT phenotype during cancer is reminiscent of the EMT observed during development and neural crest migration. EMT involves the suppression of E-cadherin and a switch to the expression of mesenchymal cadherins such as N-cadherin or cadherin 11.10 At the center of this process is the inhibition of E-cadherin, the major mediator of cell adhesion in AJs.11,12 The regulation of E-cadherin involves both transcriptional and post-translational mechanisms. Whether by direct gene repression or as a consequence of weaker bonds between cells, loss of AJs is concomitant to the loss of the other junction complexes during EMT.13,14 Although E-cadherin is the most studied target of EMT inducers, the transcription factor SNAIL (SNAI1) is also known to inhibit tight junction components.15,16 In this review we will consider new aspects of the regulation of AJs, particularly cadherins, focusing on their role in EMT and tumor progression.
Transcriptional Regulation and Epigenetic Silencing of E-Cadherin (CDH1)

Regulation of E-cadherin transcription during EMT is dependent upon many known transcription factors capable of binding the E-box sequence in the promoter region of CDH1. Two of these transcription factors, SNAI1 and the basic helix-loop-helix family member TWIST1, have been considered to be master regulators of EMT. Repression of E-cadherin by SNAI1/TWIST1 involves the recruitment of histone remodeling proteins to the promoter, where SNAI1 interacts with histone deacetylase (HDAC) 1 and 2. TWIST1 interacts with several other components, the Mi2/nucleosome remodeling and deacetylases (Mi2/NuRD) complex. Deacetylation of histones by HDAC prevents the opening of chromatin and the binding of the transcription machinery. SNAI1/TWIST1 can induce repression of the CDH1 promoter by phosphorylation of histone H3 at Thr45 through Akt2.

The genetic program induced in putative cancer stem cells, similarly to embryonic stem cells, can be regulated by global epigenetic changes. EMT has been shown to induce stem cell properties in tumor cells suggesting that both EMT-inducing transcription factors and epigenetic regulators do cooperate. A review by Hale et al. in this special issue highlights how cell-cell adhesion mechanisms provide molecular cues that are used by cancer stem cells. Such EMT-inducing factors have recently been found to interact with the epigenetic regulators DNA methyl transferases (DNMT), polycomb proteins and H3K9 methyl transferase (Table 1) to regulate stemness. These data have allowed for a much better understanding of how CDH1 is silenced in cancer cells.

Promoter methylation. The silencing of E-cadherin expression by hypermethylation is a common event in cancer. DNMTs target cytosine residues in CpG dinucleotides for methylation and have recently been identified in the repression of E-cadherin in normal and pathological contexts. Such colorectal cancer, gastric cancer and hepatocellular carcinomas. Multiple signaling pathways involved in EMT and tumorigenesis activate DNMTs, e.g., ras and TGF-β. DNMTs bind several histone remodeling enzymes, such as MPP8, Siruin 1 and G9a (Table 1). However, SNAI1 has been shown to be linked to DNMT1, notably in association with G9a and Suv39H1 (see also Table 2). As polycomb proteins act as a platform to recruit DNMT, the two epigenetic mechanisms could intersect.

Cooperation between Polycomb proteins and EMT-inducing transcription factors. The polycomb proteins are part of repressor complexes that inhibit gene expression through chromatin remodeling. Polycomb-mediated gene expression is essential for the maintenance of embryonic stem cells and is involved in development as well as tumor suppression. The polycomb repressive complex 2 (PRC2) recruits PRC1 after chromatin methylation at H3K27 through enhancer of Zeste Homolog 2 (EZH2), a histone H3 lysine-27-specific methyltransferase. Both, PRC1 and PRC2 have been shown to interact with SNAI1 and TWIST1 to promote EMT.

SNAI1 is stabilized through its interaction with the PRC1 component BMI-1 and interacts with Suppressor of Zeste 12 (Suz12) and EZH2 to repress CDH1 expression. Interestingly, EZH2 also participates in transforming growth factor β 1 (TGF-β1) signaling, a potent inducer of EMT. BMI-1 can also interact with TWIST1 to induce EMT.

The intricate interactions of EMT-inducing transcription factors and chromatin remodeling complexes PRC1 and PRC2 may offer novel approaches to control EMT and thus cell adhesion in cancer cells via a plethora of new drug, such as HDACs and DNMT inhibitors.

Endocytosis of AJ Components

AJs are highly dynamic structures. Remodeling of AJs and associated proteins occurs through endocytosis and recycling of the complex components. Endocytic pathways are often misregulated in cancers and a shift in the balance between recycling and degradation can result, for example, in the degradation of E-cadherin and increased cell migration. Endocytic signaling pathways enclose a vast number of mechanisms and associated proteins some of them recently described such as CD2AP. Others such as Rab, Rap and Rho GTPases, endocytosis proteins like dynamin and other associated proteins like Bar proteins etc have been extensively reviewed. Here we will here focus on some recent findings involving E-cadherin endocytosis, particularly during EMT.

E-cadherin endocytosis can be mediated by clathrin-mediated vesicles or non-clathrin pathways: caveolae- (lipid raft-mediated) endocytosis and macropinosis. The degradation or recycling of E-cadherin is dependent on its phosphorylation state. Src targets the tyrosine residues, Tyr755 and 756, at the juxtamembranous domain of E-cadherin displacing p120 catenin, thereby destabilizing AJs. E-cadherin is then degraded by the ubiquitin ligases Hakai, or MDM2 (Fig. 1). The degradation or recycling of E-cadherin is dependent on its phosphorylation state. Src targets the tyrosine residues, Tyr755 and 756, at the juxtamembranous domain of E-cadherin displacing p120 catenin, thereby destabilizing AJs.

 Src is a known oncogene and activates different signaling pathways that can ultimately inhibit E-cadherin to promote tumor progression. The proto-oncogenes epidermal growth factor receptor (EGFR) and hepatocyte growth factor receptor (HGF) target Src but also physically interact with E-cadherin.
Protein tyrosine phosphatases (PTPs) have been considered tumor suppressors as they are the functional opposites of tyrosine kinases. PTPs (PTP-μ, DEP1, PTP-LAR, PTP1B, PTB-PEST and others) activate kinases by removing inhibitory phosphates and are involved in the regulation of cell-cell junction (for a review see refs. 81 and 82). Rather than acting as pleiotropic suppressors of tyrosine kinases, PTPs recently have been described to act as specific regulators of signaling pathways. In mammary epithelial cells, loss of the PTPs PTPRG, PTPRR and PTPN23 was associated with enhanced cell motility. However only PTPN23 was been shown to directly induce E-cadherin internalization and was associated with increased invasion. Src, E-cadherin and β-catenin appear to be direct substrates of PTPN23. An increase in Src activity with the subsequent loss of PTPN23 was responsible for the expression of mesenchymal markers and the observed increased invasion in mammary cell lines (Fig. 1).

| EMT-inducing transcription factors | Epigenetic regulators | Function                      |
|-----------------------------------|-----------------------|-------------------------------|
| SNAI1                            | DNMT1                 | DNA methyl transferase        |
| SNAI1/TWIST1                     | BMI-1                 | Subunit of PRC1               |
| SNAI1                            | Suz12                 | Subunit of PRC2               |
| SNAI1                            | EZH2                  | Subunit of PRC2, H3K27 methyl transferase |
| SNAI1                            | G9a                   | H3K9 methyl transferase        |
| SNAI1                            | SuV39H1               | H3K9 methyl transferase        |
| TWIST1                           | SET8                  | H4K20 methyl transferase       |

Non-clathrin-mediated endocytic pathways. The suppression of PTPN23 is also associated with caveolin-1 mediated internalization and blocking of early endosome vesicle trafficking. Caveolin-1 plays an important role in EGFR signaling as downregulation of caveolin-1 in response to EGF results in the expression of SNAI1, EMT and loss of E-cadherin. In cancer cells, EGF can induce caveolin-1-mediated degradation of E-cadherin and tumor cell dissociation.

Reggie/flotillins are microdomain scaffolding proteins (lipid raft components) that play an important role in Wnt and Shh signaling and are involved in regulating membrane trafficking and turnover. In association with prion protein (PrP), reggies/flotillins control activation of Src kinases and the presence of receptor tyrosine kinases such as EGFR at the membrane. Reggies contribute to the internalization and turnover of E-cadherin. Together with PrP they target the recycling of...
E-cadherin to the AJs and its binding to catenins for the maintenance of cell adhesion (Fig. 2). In the context of EGFR-dependent loss of cell adhesion, reggies are necessary for EGFR endocytosis to prevent sustained activity and concurrently cell migration.

**Clathrin-mediated E-cadherin recycling.** Endocytosis of E-cadherin can be mediated by clathrin-coated vesicles upon calcium depletion. Several adaptor proteins in this pathway, notably Numb and Disabled-2 (Dab2), participate in the regulation of members of adherens junctions during EMT (Fig. 3).

Numb is often misregulated in cancers and plays many roles in the cell from endocytosis, to cell migration, cell adhesion, signaling pathways and asymmetric cell division. However, Numb’s function during tumorigenesis remains controversial: Much of the work done on Numb highlights its role as a tumor suppressor. Not only does it inhibit ubiquitination and subsequent destruction of p53, but it also has been shown to inhibit Rac1-GTP accumulation. Rac1 is initially increased by cell-cell contacts and E-cadherin but is rapidly downregulated after the establishment of adherens junctions. Indeed, Rac1-GTP accumulation is associated with loss of cell-cell adhesion and lamellipodia formation. With respect to AJs more specifically, Numb knockdown was recently shown to sensitize cells to HGF-mediated EMT.

By contrast, Numb expression at the cell membrane has been shown to promote the loss of cell-cell adhesion. It was suggested that Numb causes E-cadherin endocytosis by binding directly to p120 catenin, which itself prevents E-cadherin internalization. Sato et al. propose that phosphorylation of Numb by the atypical protein kinase aPKC prevents Numb binding to p120 and helps maintain apicobasal polarity. Overall, more work is needed to elucidate the roles of Numb in the regulation of E-cadherin endocytosis.

Dab2 is an endocytotic adaptor protein and is considered a tumor suppressor as Dab2 expression is frequently lost in different types of cancer. Interestingly, Dab2 is a central node to TGF-β1 mediated EMT-signaling. As a downstream target of TGF-β1, Dab2 gene expression is induced by Akt2, which phosphorylates hnRNP E1, thereby preventing suppression of the Dab2 promoter. However, more recent studies have found

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**Figure 2.** Reggie/Flotillin-dependent endocytosis of E-cadherin. Reggies/Flotillins regulates E-cadherin endocytosis through macropinocytosis and the recycling of E-cadherin. EGFR can phosphorylate reggie/flotillins preventing their action and thereby inducing the degradation of E-cadherin.
that the epigenetic silencing of Dab2 is responsible for the switch of TGF-β1 signaling from a tumor suppressor to an oncogenic factor. Furthermore, Dab2 participates in the endocytosis of TβRII and promotes signaling of TGFβ through canonical Smad signaling. Dab2 can decrease E-cadherin levels and upregulate the mesenchymal marker vimentin demonstrating not only a role for Dab2 in EMT, but also in the regulation of E-cadherin. In addition, a study focusing on Dab2 function in embryogenesis, identified that Dab2 mediates directional trafficking and the polarized distribution of E-cadherin. Taken together, it could be conceivable that Dab2 is affecting E-cadherin trafficking and degradation directly, but the mechanisms have yet to be identified.

The Fate of Cell-Cell Adhesion in Tumor Progression

In the absence of E-cadherin, individual tumor cells migrate by one of two modes: mesenchymal movement (directional migration with proteolysis at the leading edge) or amoeboid movement with lack of direction and of polarity. In the presence of E-cadherin, tumor cells use collective migration. Yang et al. propose that the induced mesenchymal mode of migration will be associated with local invasion but limited metastasis. Interestingly, in head-and-neck squamous cell carcinoma individual as well as collective migration can be observed. This prompts the question of whether cell-cell contacts could still be preserved after EMT.

N-cadherin adherens junctions: collective migration after EMT. Recent work demonstrates that the transition between single cell migration and collective migration in breast cancer cell lines is reversible and depends upon TGF-β1 signaling. In fact, alternating between single-cell and collective migration is conducive to a more metastatic disease phenotype. Collective cell migration requires mechanisms to maintain cell adhesion and is dependent on cell polarity.

One hallmark of EMT is the shift from E-cadherin to N-cadherin expression. The “cadherin switch” does not necessarily imply that E-cadherin will always be replaced by N-cadherin. Rather, N-cadherin expression increases as cells undergo EMT. Ectopic expression of N-cadherin increases migration of breast cancer cell lines as well as in the mouse mammary epithelial cell line NmuMG after treatment with TGF-β1. Interestingly, in this last study N-cadherin upregulation preceded morphological changes induced by EMT. The precise mechanism controlling an increase in N-cadherin expression is unknown, although previous studies have suggested it to be TWIST1-dependent.

It is important to note that although N-cadherin AJs are weaker than their E-cadherin counterparts, they may still maintain cell-cell contacts during migration. In the collective migration of neural crest cells, N-cadherin inhibits formation of protrusions at cell-cell contacts helping the cells at the leading edge to progress. The mechanism involved in the regulation of Rac1 activity to promote N-cadherin-mediated adhesion was also shown to be integrin-dependent.
N-cadherin can also play an essential role in collective migration. Cells grown in a three-dimensional environment have been shown to establish focal adhesion differently compared with cells grown in a two-dimensional environment such as on plastic, which allows for the possibility that cell-cell adhesion could also be affected. A three-dimensional environment, in which the cells were embedded in an extracellular matrix rather than grown on plastic, was required for the establishment of N-cadherin-mediated cell-cell adhesion after EMT hinting at matrix-dependent cues, such as stiffness of the matrix, which have been demonstrated to affect cell migration and invasion (see below). Shih and Yamada also described a distinct role of the cytoplasmic domain of N-cadherin in promoting migration by an unknown mechanism. However, the potential role of AJs formed by N-cadherin with respect to the migration of epithelial cells undergoing EMT remains largely unexplored.

The role of the matrix in collective invasion. Matrix rigidity and orientation promotes collective cell migration. Matrix stiffening was recently shown to sensitize cells to EGF. Moreover, as the matrix stiffens, TGF-β1 loses its anti-proliferative role to become pro-tumorigenic. This effect is not mediated by Smad signaling, but by the PI3K/Akt pathway. Matrix stiffness is important in controlling the strength of AJs in collective migration. The composition of the matrix itself is regulated by the fibroblasts, which can be activated by paracrine factors secreted from the tumor. At the same time, secretion of growth factors from the activated fibroblasts contributes to tumor progression. Carcinoma-associated fibroblasts can promote tumor invasion and generate a track in the matrix for the invading tumor cells to follow. Interestingly, lysyl oxidase-like 2 (LOXL2), a tumor marker, increases collective cell migration. LOXL2 interacts with and stabilizes Snail1 at the invasion front in actively migrating cells. In addition, LOXL2 catalyzes the cross-linking of collagen fibrils and contributes to the matrix stiffening. Proteins such as LOXL2, which participate in mechanotransduction-mediated invasion are likely the regulators of temporal and spatial assembly and disassembly of cell junctions to facilitate single vs. collective migration.

Concluding Remarks

The regulation of cell adhesion is under control of multiple signaling pathways and mechanisms that allow fine-tuning of the process. Continuous formation and disassembly is necessary during embryogenesis and development. Some of the processes involved at this stage, such as EMT, are hijacked during carcinogenesis. While the structural importance of adherens junctions has been known for a long time, the impact of the complex components on cell signaling has been identified rather recently. The central role of E-cadherin is apparent by the many layers of regulation: promoter methylation, histone methylation, transcriptional repression and post-translational modifications leading to endocytosis. As our understanding of these machineries increases, we will discover novel players and, hopefully, therapeutics that could restore cell adhesion in the clinical setting.

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111. Hocevar BA, Prunier C, Howe PH. Disabled-2 (Dab2) signaling in

109. Fazili Z, Sun W, Mittelstaedt S, Cohen C, Xu XX. 

104. Sato K, Watanabe T, Wang S, Kakeno M, Matsuzawa 

101. Pece S, Confalonieri S, R Romano P, Di Fiore PP. 

100. Wang Z, Sandiford S, Wu C, Li SS-C. Numb 

280:25920-7; PMID:15894542; http://dx.doi.org/10.1038/jcb.20031013

126. Ganz A, Lambert M, Saez A, Silberzan P, Bugain A, Mége RM, et al. Traction forces exerted through N-cadherin contacts. Biol Cell 2006; 98:721-30; PMID:16895521; http://dx.doi.org/10.1042/BC20060039

27. Theneveau E, Marchant L, Kuryyama S, Gull M, Moepps B, Parsons M, et al. Collective chemotaxis requires contact-dependent cell polarity. Dev Cell 2010; 19:39-53; PMID:20643349; http://dx.doi.org/10.1016/j.devcel.2010.06.012

16. Martin JC, Herbert B-S, Hocevar BA. Disabled-2 downregulation promotes epithelial-to-mesenchymal transition. Br J Cancer 2010; 103:1716-23; PMID:21063401; http://dx.doi.org/10.1038/bjc.6605975

https://www.landesbioscience.com Cell Adhesion & Migration 373

133. Chaudhury A, Cai KQ, Roland IH, Smith ER, Xu X-X. 

120. Rodriguez FJ, Lewis-Tuffin LJ, Anastasiadis PZ. E-cadherin 

115. Hannigan A, Smith P, Kalna G, Lo Nigro C, Orange 

119. Friedl P, Wolf K. Plasticity of cell migration: a 

20. Kadow KE, Horwitz AR. Reducing background 

17. Hao L, Ha JR, Kuzel P, Garcia E, Persad S. Cadherin 

116. Martin JC, Herbert B-S, Hocevar BA. Disabled-2 

112. Rodriguez FJ, Lewis-Tuffin LJ, Anastasiadis PZ. E- 

130. Kubow KE, Horwitz AR. Decreasing background 

118. Yang D-H, Cai KQ, Roland IH, Smith ER, Xu X-X. 

114. Hussey GS, Chaudhury A, Ray PS, Jin G, Fox PL, Howe PH. TGF-beta-mediated phosphorylation of hotRNP EI induces EMT via transcript-selective translational induction of Dab2 and ILEI. Nat Cell Biol 2010; 12:286-93; PMID:20154680

113. Chaudhury A, Hussey GS, Ray PS, Jin G, Fox PL, Howe PH. TGF-beta-mediated phosphorylation of hotRNP EI induces EMT via transcript-selective translational induction of Dab2 and ILEI. Nat Cell Biol 2010; 12:286-93; PMID:20154680

http://dx.doi.org/10.1038/onc.2011.269

2. Oncogene 2012; 31:764-75; PMID:21723656; http://dx.doi.org/10.1038/onc.2011.269

121. Giampieri S, Manning C, Hooper S, Jones L, Hill CS, Marshall JF, Harrington K, et al. Fibroblast-led collective invasion of carcinoma cells with differing 

10.1038/sj.onc.1201769

1. Wang Z, Sandiford S, Wu C, Li SS-C. Numb 

2006; 280:25920-7; PMID:15894542; http://dx.doi.org/10.1038/jcb.20031013

280:17540-8; PMID:15734730; http://dx.doi.org/10.1038/emboj.2009.190