Cadherin switching

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Summary

The cadherin molecules at adherens junctions have multiple isoforms. Cadherin isoform switching (cadherin switching) occurs during normal developmental processes to allow cell types to segregate from one another. Tumor cells often recapitulate this activity and the result is an aggressive tumor cell that gains the ability to leave the site of the tumor and metastasize. At present, we understand some of the mechanisms that promote cadherin switching and some of the pathways downstream of this process that influence cell behavior. Specific cadherin family members influence growth-factor-receptor signaling and Rho GTPases to promote cell motility and invasion. In addition, p120-catenin probably plays multiple roles in cadherin switching, regulating Rho GTPases and stabilizing cadherins.

Key words: Cadherin, Biology, Switching

Introduction

The term epithelial-to-mesenchymal transition (EMT) describes a process in which epithelial cells lose their characteristic polarity, disassemble cell-cell junctions and become more migratory. EMT is proposed to occur in various developmental processes and during tumor metastasis. The idea of EMTs, and their role in development and/or tumor progression, is controversial, but in some ways the controversy is a matter of semantics (Tarin et al., 2005; Thompson et al., 2005). In this Commentary, we do not go into all the studies that have added to the controversy over whether such transitions really do occur, but rather we discuss 'cadherin switching', a process in which cells shift to express different isoforms of the cadherin transmembrane proteins that underpin adherens junctions. Cadherin switching can have a profound effect on cell phenotype and behavior and is one aspect of the so-called EMT. We focus here on the role of cadherin switching during tumorigenesis, concentrating on the ramifications of amplified N-cadherin expression by tumor cells.

Epithelial cell junctions

Epithelial cell-cell junctions provide tissue integrity and promote cell polarity (Perez-Moreno et al., 2001). The junctional complex comprises tight junctions, adherens junctions and desmosomes (reviewed in Tsukita et al., 2001; Wheelock and Johnson, 2003b). The adherens junctions play a pivotal role in regulating the activity of the entire junctional complex, and the major adhesion molecules in the adherens junctions are cadherins (reviewed in Nagafuchi, 2001; Wheelock and Johnson, 2003a; Wheelock and Johnson, 2003b; Wheelock et al., 2001). In addition, members of the nectin family of cell-cell adhesion proteins localize to adherens junctions and tight junctions and promote the formation of these two structures (Sakisaka and Takai, 2004).

In some situations, including the dynamic cellular rearrangements that are integral to embryonic development, tissue integrity must be disrupted so that cells can migrate from their original position to establish new structures (Gerhart et al., 2004; Pla et al., 2001; Savagner, 2001; Shook and Keller, 2003). Likewise, when epithelial cells change their relative positions within a tissue, they become motile cells, their cell-cell junctions are disrupted and the actin cytoskeleton is reorganized (Boyer et al., 2000; Gumbiner, 2005; Keller, 2002; Savagner, 2001). It has been proposed that cancer cells use mechanisms akin to those employed during normal developmental processes to accomplish a similar goal – i.e. to invade adjacent tissues (Gotzmann et al., 2002; Thiery, 2002; Thiery, 2003). Fig. 1 shows the typical cobblestone morphology of mammary epithelial cells in culture (Fig. 1A) and the mesenchymal appearance of these cells when induced to undergo EMT by treatment with TGFβ (Fig. 1B). The actin cytoskeleton changes from a circumferential band of filaments (Fig. 1C) to stress fibers (Fig. 1D) as the cells undergo EMT.

Cadherins

Classical cadherins are the transmembrane component of the adherens junction (Fig. 2). Cadherins mediate cell-cell adhesion through their extracellular domains and connect to the actin cytoskeleton by associating with catenins through their cytosolic domain. Epithelial cells typically express E-cadherin, whereas mesenchymal cells express various cadherins, including N-cadherin, R-cadherin and cadherin-11. Endothelial cells express VE-cadherin, which is specific to these cells and is found in the junctional complex, and N-cadherin, which is not found in junctions and has an unclear function. Cadherins are important in the establishment of cell polarity and cell sorting during embryonic development (reviewed in Gumbiner, 2005; Patel, S. et al., 2003; Wheelock and Johnson, 2003a; Wheelock and Johnson, 2003b).

E-cadherin is expressed by most normal epithelial tissues and many epithelium-derived cancer cells have lost E-cadherin expression (Batlle et al., 2000; Cano et al., 2000; Comijn et al., 2001; Nieman et al., 1999; Wheelock et al., 2001). Importantly, numerous clinical studies have shown that E-cadherin is often lost in tumors in situ (Alrawi et al., 2006; Berx and Van Roy, 2001; Dunbier and Guilford, 2001; Kelleher et al., 2006; Paul et al., 1997; Wheelock et al., 2001). Mesenchymal cells, which are more motile and less polarized than epithelial cells, typically express N-cadherin. However, some cancer cells derived from epithelia inappropriately express N-cadherin, and the upregulation of N-

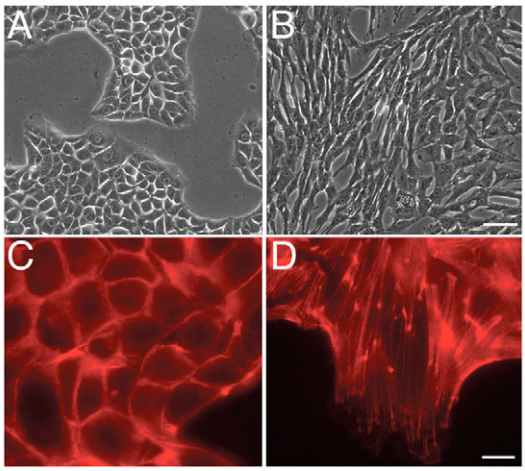

Fig. 1. Mouse mammary epithelial cells undergo EMT in response to TGFβ1. (A,B) Phase micrographs of cells from the mouse mammary epithelial cell line NMuMG in the (A) absence or (B) presence of TGFβ1 (5 ng/ml for 1 day). (C,D) Cells were stained for filamentous actin with Texas-red-labeled phalloidin. (C) Untreated cells show a peripheral band of actin filaments, typical of polarized epithelial cells. (D) TGFβ1-treated cells have prominent stress fibers. Reproduced from Maeda et al. (Maeda et al., 2005).

cadherin expression has been shown to promote motility and invasion (Hazan et al., 2000; Islam et al., 1996; Nieman et al., 1999). This loss of E-cadherin expression and gain of N-cadherin expression is reminiscent of the cadherin switching that is seen during normal embryonic development and probably underpins many of the phenotypic changes that occur in the participating cells (reviewed in Cavallaro et al., 2002; Christofori, 2003; Gerhart et al., 2004).

The term cadherin switching usually refers to a switch from expression of E-cadherin to expression of N-cadherin, but also includes situations in which E-cadherin expression levels do not change significantly but the cells turn on (or increase) expression of N-cadherin. It also includes examples in which other cadherins replace or are co-expressed with E-cadherin, including R-cadherin, cadherin 11, T-cadherin and even P-cadherin, and the expression of the 'inappropriate cadherin' might alter the behavior of the tumor cells (Derycke and Bracke, 2004; Nakajima et al., 2004; Paredes et al., 2005; Patel, I. et al., 2003; Riou et al., 2006; Stefansson et al., 2004; Taniuchi et al., 2005; Tomita et al., 2000). It has even been reported that E-cadherin can influence tumorigenesis in tissues that do not normally express this cadherin. For example, ovarian surface epithelium normally expresses N-cadherin. However, during progression to the neoplastic state, the cells show decreased N-cadherin expression and increased E-cadherin and P-cadherin expression; the E-cadherin might play a role in the initiation of the aberrant differentiation that characterizes ovarian carcinogenesis (Patel, I. et al., 2003; Wong et al., 1999; Wu et al., 2007). Table 1 presents examples of cadherin switching that have been reported during normal developmental processes and during tumorigenesis.

One role of cadherin switching is to allow a select population of cells to separate from their neighbors – for example, during processes such as gastrulation, epiblast cell ingression through the primitive streak and neural crest emigration from the neural tube (Edelman et al., 1983; Hatta and Takeichi, 1986; Takeichi, 1988; Takeichi et al., 2000). It is well known that cells expressing different cadherins segregate from one another in in vitro aggregation assays (Nose et al., 1988; Steinberg and Takeichi, 1994) and it is easy to infer that, in vivo, this ability to segregate cells allows cadherin switching to promote separation of the egressing cells from those left behind. However, it is less well understood how switching from E-cadherin to a different cadherin promotes a motile phenotype, which is also essential for processes like gastrulation and tumor invasion/metastasis. Below we discuss some of the evidence that implicates cadherin switching in the modulation of this and other aspects of cell behavior.

Transcriptional control of cadherin switching

E-cadherin expression in epithelial tumors can be downregulated by a number of mechanisms, including mutation, DNA methylation and transcriptional control. Each of these mechanisms has been implicated in various tumor types (reviewed in Peinado et al., 2007; Peinado et al., 2004). Here, we focus on transcriptional repression of E-cadherin, because this is the mechanism most likely to operate during cadherin switching, because E-cadherin transcriptional repressors have been implicated in EMT and the EMT-like changes seen in tumor cells are thought to be reversible (Thiery and Sleeman, 2006). The expression of E-cadherin is negatively regulated by a number of zinc-finger-family transcription factors, including Snail, Slug, Twist, E12 (E47, TCFE2A), SIP1 (ZEB2) and δEF1 (ZEB1), each of which has been reported to bind to the E-cadherin promoter to repress its transcription (Bolos et al., 2003; Castro Alves et al., 2007; Conacci-Sorrell et al., 2003; Grooteclaes and Frisch, 2000; Hajra et al., 2002; Huber et al., 2005; Rosivatz et al., 2002; van Grunsven et al., 2003). Although these repressors all bind to specific sequences called E-boxes within the E-cadherin promoter, the precise mechanism of repression might differ among the different repressors and among different tumor types (reviewed in Peinado et al., 2007; Peinado et al., 2004). For example, in one study, Slug not Snail expression correlated with E-cadherin repression, but in others expression of Snail or co-expression of

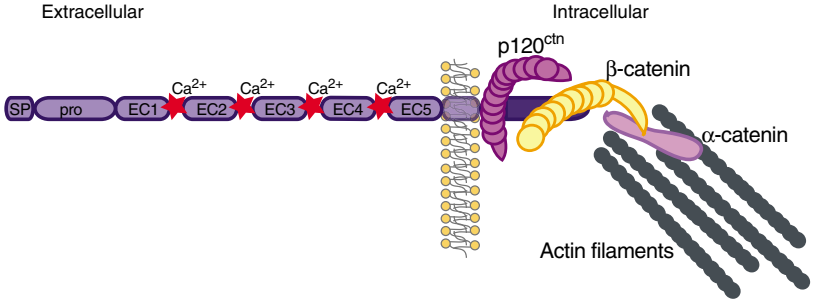

Fig. 2. Cadherin domain structure. Cadherins are single-pass transmembrane proteins that are synthesized with a signal peptide (SP) and pro-region (pro), which are removed during protein processing. The extracellular domain comprises five homologous repeats (EC1-EC5) that are bridged by calcium ions (Ca^{2+}). The cytoplasmic domain binds to p120-catenin ($p120^{ctn}$) near the plasma membrane and to β-catenin near the C-terminus. α-catenin binds to β-catenin to link the cadherin complex to the actin cytoskeleton. E-cadherin and N-cadherin domain structures are similar, as are their interactions with catenins.

Table 1. Cadherin switching during normal development and tumorigenesis

Switch	Example	Reference
Cadherin switching during normal development		
From E-cadherin to N-cadherin	Primitive streak formation	(Nakagawa and Takeichi, 1995)
	Neural plate invagination	(Hatta and Takeichi, 1986)
From N-cadherin to cadherin 6B	Generation of neural crest cells from neural plate	(Nakagawa and Takeichi, 1995)
From N-cadherin and cadherin 6B to cadherins 7	When neural crest cells become migratory	(Nakagawa and Takeichi, 1995)
From a complex mixture of cadherins to R-cadherin	Kidney morphogenesis	(Dahl et al., 2002)
From E- and P-cadherin to E-cadherin	Epidermal stratification	(Jensen et al., 1997)
Cadherin switching during tumorigenesis		
From E-cadherin to N-cadherin	Melanoma	(Li et al., 2001)
	TGFβ-induced EMT in mammary epithelial cells	(Maeda et al., 2005; Miettinen et al., 1994; Piek et al., 1999)
	Prostate cancer	(Gravdal et al., 2007; Jaggi et al., 2006; Tomita et al., 2000)
	Breast cancer	(Han et al., 1999; Hazan et al., 2000; Nieman et al., 1999)
	Pancreatic cancer	(Hotz et al., 2007; Nakajima et al., 2004)
From E-cadherin to T-cadherin	Hepatocellular carcinoma	(Riou et al., 2006)
From E-cadherin to P-cadherin	Pancreatic cancer	(Taniuchi et al., 2005)
	Gastric cancer	(Shimoyama and Hirohashi, 1991)
From E-cadherin to cadherin 11	Prostate cancer	(Bussemakers et al., 2000; Tomita et al., 2000)
	Breast cancer	(Pishvaian et al., 1999)
From E- and P-cadherin to N-cadherin	Oral squamous cell carcinoma	(Chen et al., 2004; Islam et al., 1996; Pyo et al., 2007)
From N-cadherin to E-cadherin	Ovarian cancer	(Patel, I. et al., 2003; Wong et al., 1999)

Snail with SIP1 appeared to repress E-cadherin (reviewed in Peinado et al., 2004). Thus, there are a number of ways E-cadherin transcription can be repressed.

In experimental models of EMT, such as TGFβ treatment of mammary epithelial cells, upregulation of N-cadherin has also been shown to occur at the transcriptional level (Maeda et al., 2005). However, the transcriptional regulators that influence N-cadherin expression have yet to be fully characterized. Analysis of gastrulation in the *Drosophila* embryo showed that Twist (a repressor of E-cadherin expression) can directly activate N-cadherin expression (Oda et al., 1998). In addition, overexpression of Twist has been reported in gastric cancer cells that have abnormally high N-cadherin levels (Rosivatz et al., 2002), and a recent study by Heimark and co-workers showed that Twist binds to an E-box in the first intron of the human N-cadherin gene to upregulate its expression in prostate cancer cells (Alexander et al., 2006). Other studies have not seen a correlation between the upregulation of N-cadherin and Twist expression (Maeda et al., 2005). Thus, positive regulation of the N-cadherin promoter might be as complex as negative regulation of the E-cadherin promoter, and the details remain to be established.

Post-transcriptional control of cadherin levels

Transcriptional regulation of N-cadherin expression might not be essential for a cadherin switch to occur. There is a significant body of literature documenting a role of p120-catenin in stabilizing cadherins and regulating the total level of cadherin a cell can have. p120-catenin was identified almost 20 years ago as a prominent Src substrate in transformed cells and was later shown to bind to the juxtamembrane domain of classical cadherins and prevent their degradation (Reynolds, 2007). Evidence linking p120-catenin to cadherin stability comes from a number of important studies. Reynolds and co-workers showed that the colon-cancer cell line SW48, which expresses a low level of defective p120-catenin, has very low levels of E-cadherin and impaired adhesion (Ireton et al., 2002). When they restored normal p120-catenin expression in SW48 cells, E-cadherin levels increased dramatically and the cells reverted from loosely connected colonies to the cobblestone-like colonies typical of epithelial cells. E-cadherin mRNA levels were unchanged, but the half-life of E-cadherin protein more than doubled, which suggests that p120-catenin stabilizes E-cadherin. Furthermore, Reynolds and co-workers and Kowalczyk and co-workers have independently shown that knocking down expression of p120-catenin by small interfering RNA (siRNA) results in decreased cadherin protein levels (Davis et al., 2003; Xiao et al., 2003). In the absence of p120-catenin, synthesis of cadherin is therefore not altered, but its degradation rate is increased. Fig. 3 shows the morphological change that occurs when p120-catenin is expressed in the p120-catenin-deficient pancreatic cancer cell line S2013 (Fukumoto et al., 2008).

So, what does this have to do with cadherin switching? Some years ago, forced expression of N-cadherin in epithelial cells was shown to downregulate the level of endogenous E-cadherin by accelerating its degradation (Islam et al., 1996; Nieman et al., 1999). The above studies on p120-catenin provide a hint as to why forced expression of an inappropriate cadherin promotes degradation of endogenous cadherins. In A431 cells, forced expression of R-cadherin results in degradation of endogenous E-cadherin and P-cadherin, and the degradation of endogenous cadherins is due to competition for binding to p120-catenin (Maeda et al., 2006). Thus, it is possible for cadherin switching to occur following modulation of transcription of only one cadherin. Growth factors such as TGFβ that lead to transcriptional repression of E-cadherin could also indirectly increase the expression of N-cadherin by freeing up p120-catenin to bind to N-cadherin and stabilize it at the cell surface.

Effects of inappropriate cadherins

N-cadherin promotes motility when expressed by epithelial cells (De Wever et al., 2004; Hazan et al., 2000; Nieman et al., 1999). Indeed, cells that express significant amounts of E-cadherin but only a small amount of N-cadherin still have increased motility.

This suggests that N-cadherin plays an active role in cell motility that E-cadherin cannot suppress (Hazan et al., 2000; Nieman et al., 1999). Further evidence for such a role comes from studies involving TGFβ1-induced motility of mammary epithelial cells. MCF10A cells undergo classical TGFβ1-induced morphological changes, downregulate E-cadherin and upregulate other mesenchymal proteins that are typical of TGFβ1-stimulated mammary epithelial cells. However, if short hairpin RNA (shRNA) is used to block the ability to upregulate N-cadherin expression, the cells do not show increased motility in response to TGFβ1 (Maeda et al., 2005). These studies thus convincingly show that N-cadherin promotes cell motility, implicating cadherin switching in the regulation of cell behavior.

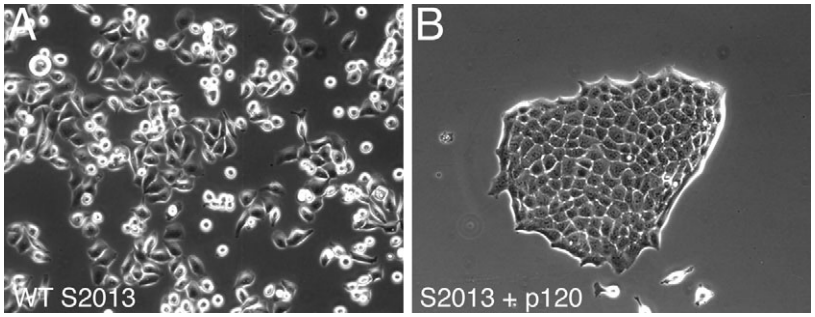

Fig. 3. p120-catenin promotes epithelial morphology in p120-deficient S2013 pancreatic cancer cells. Parental S2013 cells (wild type; WT) are scattered (A), whereas cells expressing p120-catenin form compact colonies of cobblestone-like cells (B).

Perhaps the most significant studies, however, are those showing that cadherin switching plays an important role in the behavior of tumor cells in animals. Numerous clinical studies have shown that N-cadherin and other 'inappropriate' cadherins are expressed by cells from a variety of tumors in situ (Table 1), and thus there is a correlation between cadherin switching and tumor progression in humans (Cavallaro et al., 2002). Hazan et al. showed that the non-metastatic human breast cancer cell line MCF-7 can be transformed to a metastatic cell line when transfected with N-cadherin (Hazan et al., 2000): when injected into the mammary fat pad of nude mice, N-cadherin-expressing MCF-7 cells metastasize to the liver, pancreas, salivary gland, omentum, lung, muscle and lymph nodes, whereas control MCF-7 cells do not.

Human pancreatic cancer cells upregulate N-cadherin expression in response to interactions with the extracellular matrix molecule type I collagen. Pancreatic cancer is characterized by a severe fibrotic response accompanied by extensive deposition of type I collagen. This upregulation of N-cadherin therefore has potential clinical implications. Injection of BxPC-3 human pancreatic cancer cells into the pancreas of nude mice shows that the cells indeed upregulate N-cadherin expression and they produce tumors, invade the peritoneum and induce a massive fibrotic response in the pancreas. When an shRNA directed against N-cadherin is used to abolish its expression in BxPC-3 cells, these cells instead produce tumors that are non-invasive. By contrast, expression of exogenous N-cadherin in BxPC-3 cells produces tumors that are even more aggressive than those produced by the parental cells (Shintani et al., 2006). These studies demonstrate that epithelial tumor cells that have undergone a cadherin switch show increased aggression in an orthotopic environment. Together with the numerous clinical studies showing that aggressive human tumors express N-cadherin in situ (Table 1), they emphasize the serious implications of cadherin switching in human tumorigenesis.

Does cadherin switching initiate tumorigenesis?

Is expression of inappropriate cadherins an early event in tumorigenesis or must it occur in cells that have already sustained other changes to impact tumorigenesis? Knudsen and co-workers have generated transgenic mice expressing N-cadherin under control of the mouse mammary tumor virus (MMTV) promoter (Knudsen et al., 2005). Expression of N-cadherin in the mammary glands of these mice does not induce tumors. Interestingly, even crossing these mice with mice expressing the *Neu* oncogene in the mammary gland does not produce tumors more aggressive than those in mice expressing *Neu* alone (Knudsen et al., 2005). Crossing them with mice expressing polyoma virus middle T antigen in the mammary epithelium, however, leads to increased metastasis to the lung, even though there is no difference in tumor onset (Hulit et al., 2007). These studies suggest that the effects of cadherin switching are late events in tumorigenesis and demonstrate that the influence of an inappropriate cadherin on the phenotype of the cell is context dependent. Dissecting the signaling pathways that drive cadherin-mediated changes in cell behavior is thus very important (see below).

How does cadherin switching influence cell behavior?

Growth factor receptors regulate many aspects of cell behavior, including cell motility and invasion (McKay and Morrison, 2007). A number of studies have implicated cadherins as modulators of receptor tyrosine kinase signaling. The Walsh and Doherty groups have suggested that N-cadherin facilitates dimerization of the fibroblast growth factor (FGF) receptor to initiate a growth-factor-independent signal (Doherty et al., 2000; Skaper et al., 2001). They argue that cis dimerization of N-cadherin activates an FGF-receptor-dependent signal and demonstrated that N-cadherin-mediated signaling is distinct from its adhesive activity (Utton et al., 2001; Williams et al., 2002). Earlier studies also suggested that N-cadherin influences tumor cell behavior via interactions with the FGF receptor. Our laboratory showed that downstream inhibitors of FGF-receptor signaling reduce N-cadherin-mediated invasion (Nieman et al., 1999). Extracellular domain 4 of N-cadherin is necessary and sufficient for this activity and might therefore interact directly with the FGF receptor (Kim, J. et al., 2000). Similarly, this domain of N-cadherin is required for FGF-receptor-dependent neurite extension (Utton et al., 2001; Williams et al., 2002). N-cadherin is also involved in ligand-dependent FGF-receptor signaling (Suyama et al., 2002). The extracellular domain of N-cadherin can interact directly with regions in immunoglobulin (Ig) domains 1 and 2 in the extracellular domain of FGF receptor 1. This interaction prevents receptor internalization, resulting in increased levels of receptor at the cell surface and enhanced downstream signaling (Suyama et al., 2002).

Further evidence for N-cadherin-mediated regulation of FGF-receptor signaling came from the demonstration that the N-cadherin antagonist ADH-1 reduces FGF-dependent phosphorylation of the FGF-receptor target FRS2 (Erez et al., 2004). Moreover, Cavallaro et al. have presented data indicating

that N-cadherin also interacts with FGF receptor 4. In this case, the interaction is mediated by N-CAM, another cell-cell adhesion molecule of the Ig superfamily (Cavallaro et al., 2001). Doherty and co-workers have generated further evidence for direct interactions between FGF receptors and N-cadherin, identifying the acid box that can be found in the linker region between Ig domains 1 and 2 within the receptor as the motif that mediates these interactions (Sanchez-Heras et al., 2006). Other studies, using multiple antibodies against FGF-receptor isoforms, as well as tagged versions of the receptors in N-cadherin-expressing cells, have not been able to co-immunoprecipitate N-cadherin and the FGF receptors (Kim et al., 2005), suggesting that the interactions are either transient and thus difficult to detect or cell-context dependent.

N-cadherin might interact with other receptor tyrosine kinases on tumor cells. For example, a small protein called NHERF acts as a scaffold to link N-cadherin and β-catenin to the platelet-derived growth factor (PDGF) receptor, and this complex of proteins is localized to the leading edge of migrating tumor cells, where it promotes motility (Theisen et al., 2007). Thus, when epithelial tumor cells switch from expressing E-cadherin to expressing N-cadherin, they also gain the ability to activate growth factor receptor pathways to enhance cell growth and invasion. Fig. 4 depicts some of the mechanisms by which N-cadherin might influence the phenotype of tumor cells inappropriately expressing this protein.

E-cadherin also interacts with a range of receptor tyrosine kinases, including the FGF receptor and the epidermal growth factor (EGF) receptor (Bryant et al., 2005; Perrais et al., 2007; Qian et al., 2004), and these interactions might negatively regulate growth factor receptor signaling. Lowy and co-workers showed that E-cadherin interacts with the EGF receptor and inhibits EGF-dependent signaling by restricting the mobility of the receptor (Qian et al., 2004). The inhibition is adhesion-dependent and can be blocked by adhesion-blocking antibodies. In addition, E-cadherin can inhibit ligand-dependent activation of the IGF1 receptor and the Met growth factor receptor. Gumbiner and co-workers have shown that E-cadherin-mediated inhibition of the growth-stimulatory activity of the EGF receptor is adhesion and β-catenin dependent, although this is not mediated via antagonism of the Wnt pathway (Perrais et al., 2007). E-cadherin and the FGF receptor are co-endocytosed, and high levels of E-cadherin can inhibit FGF-dependent signaling by inhibiting endocytosis and nuclear translocation of its receptor. Thus, cadherin switching can dually impact growth factor receptor signaling, by increasing the number of N-cadherin interactions with growth- and motility-stimulating receptors and decreasing the negative regulation afforded by E-cadherin.

Rho GTPases in cadherin-mediated phenotypic transformations

The Rho family of small GTPases regulate cell motility, as well as many other cellular functions (Hall, 2005; Jaffe and Hall, 2005; Raftopoulou and Hall, 2004). Rho GTPases such as Rac1, RhoA and Cdc42 exist as inactive GDP-bound forms and are converted to the active GTP-bound form by guanine nucleotide exchange factors (GEFs). GTPase-activating proteins (GAPs) activate GTPase activity to inactivate the protein, and guanine nucleotide dissociation inhibitors (GDIs) sequester the GTPase in its inactive form, which provides further negative regulation (Raftopoulou and Hall, 2004).

Cadherin-mediated cell-cell contact can activate Rac1 and/or Cdc42, depending on the experimental system, whereas RhoA activity is decreased as cells become confluent (Betson et al., 2002; Kim, S. et al., 2000; Kovacs et al., 2002; Noren et al., 2001; Yap and Kovacs, 2003). Gauthier-Rouviere and colleagues used myogenic cells to show that RhoA, but not Rac1 or Cdc42, is activated upon N-cadherin-mediated cell-cell contact (Charrasse et al., 2002). Mege and co-workers used the same system to show that inhibiting Rac1 does not influence initial N-cadherin cell-cell contact,

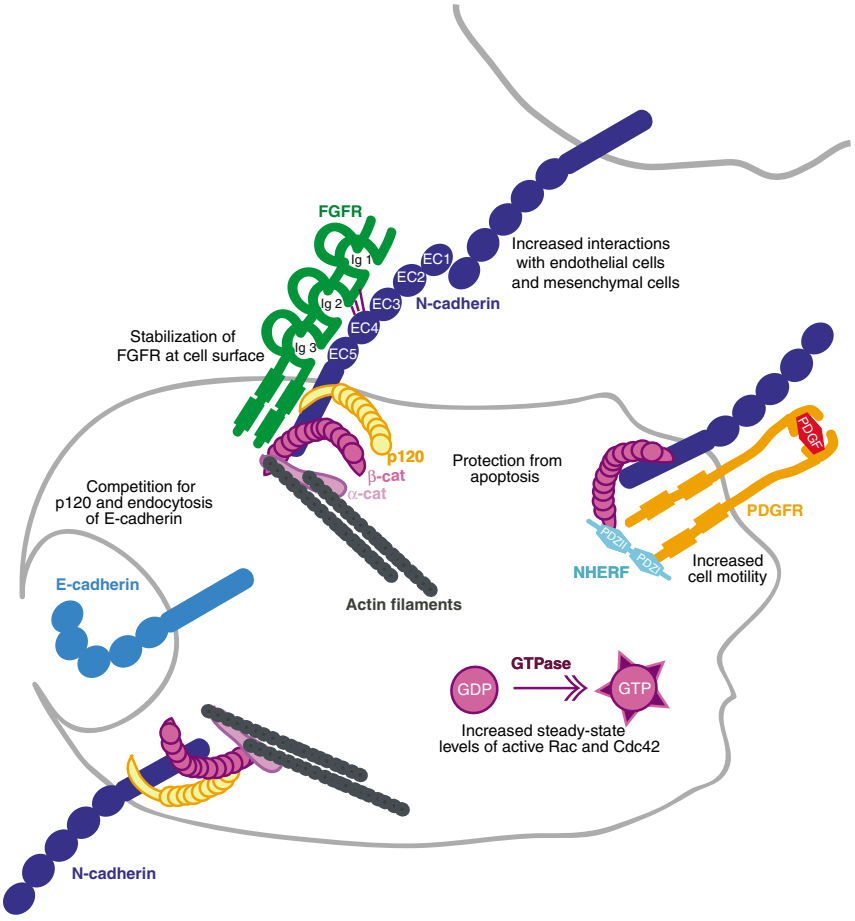

Fig. 4. Cadherin switching can influence many cellular activities. Shown are some of the changes in cellular behavior that can be induced by cadherin switching. N-cadherin interacts with the FGF receptor (FGFR) and stabilizes the receptor on the cell surface. N-cadherin increases tumor cell interactions with endothelial and mesenchymal cells. NHERF links N-cadherin to the PDGF receptor (PDGFR) via interactions with β-catenin in lamellipodia to increase cell motility. N-cadherin and R-cadherin increase steady-state levels of activated Rac and Cdc42, which promotes cell motility. N-cadherin and R-cadherin promote endocytosis of E-cadherin via competition for p120-catenin.

but that Rac1 is important for connecting the cadherin to the cytoskeleton (Lambert et al., 2002). Dejana and colleagues showed that Rac1, but not RhoA, is activated in VE-cadherin-expressing endothelial cells (Lampugnani et al., 2002), and Kouklis et al. showed that VE-cadherin that is not stably incorporated into a junction induces Cdc42 activation, leading to formation of membrane protrusions (Kouklis et al., 2003).

Inappropriate expression of R-cadherin or N-cadherin by a variety of cell types results in increased steady-state levels of active Rac1 and Cdc42. Activation of these GTPases correlates with increased cell motility and dominant-negative forms of these GTPases inhibit R-cadherin-dependent cell motility (Johnson et al., 2004; Kim et al., 2005). Taniuchi et al. showed that overexpression of P-cadherin results in increased steady-state levels of active Rac1 and Cdc42 in pancreatic cancer cells (Taniuchi et al., 2005). This was a very interesting result because, in our study, the parental cells expressed high levels of P-cadherin and yet had much lower levels of activated Rac1 and Cdc42 than did the R-cadherin transfectants (Johnson et al., 2004). Together, these studies highlight the importance of cellular context in cadherin-mediated GTPase activation and demonstrate that Rho GTPases and cadherins cooperate not only in cell adhesion but also in cell motility. In addition, they suggest that cadherin switching during tumorigenesis can influence cell behavior by activating the small GTPases that promote motility and invasion.

Interestingly, p120-catenin has another important role in this context. Cytosolic p120-catenin that is not bound to cadherin inhibits the activity of RhoA by acting as a GDI and sequestering RhoA in its inactive form (Anastasiadis, 2007; Anastasiadis et al., 2000). Taniuchi et al. showed that overexpression of P-cadherin by pancreatic cancer cells results in loss of p120-catenin from cell membranes and its accumulation in the cytosol, where it is not bound to a cadherin. They suggested that this cadherin-free p120-catenin activates Rac1 and Cdc42 by inactivating RhoA (Taniuchi et al., 2005). The reciprocal relationship between the activities of RhoA and Rac1/Cdc42 is well established (Burridge and Wennerberg, 2004), and one function of p120-catenin might be to regulate the activity of these small GTPases in the context of cadherin-mediated cell-cell adhesion and/or motility. Note that cadherin family members differentially bind p120-catenin and as a result might have different influences on RhoGTPase activity. Indeed, VE-cadherin and N-cadherin have different affinities for p120-catenin, as do R-cadherin and E-cadherin (Johnson et al., 2004; Navarro et al., 1998).

N-cadherin in tumor cell interactions with endothelial cells

In addition to modulating the invasive characteristics of tumor cells, expression of N-cadherin might also promote metastasis by facilitating interactions with the endothelium. Endothelial cells express two cadherins: VE-cadherin, which is localized in junctions and serves to organize the junctional complex in these cells, and N-cadherin, which is extrajunctional and has an unclear role (Navarro et al., 1998; Salomon et al., 1992). Endothelial cells might use N-cadherin to interact with other N-cadherin-expressing cells such as vascular smooth muscle cells and/or pericytes (Navarro et al., 1998). It is equally likely that tumor cells that express N-cadherin have an increased ability to interact with endothelial cells and that this interaction promotes metastasis by allowing the tumor cells access to the vasculature. Hazan et al. showed that MCF-7 cells transfected to express N-cadherin adhere more strongly to a monolayer of human endothelial cells than do their parental counterparts (Hazan et al., 2000). Another tumor type that switches on N-cadherin expression during tumor progression is melanoma. Antibodies that inhibit the function of N-cadherin delay transendothelial migration of melanoma cells (Sandig et al., 1997). Moreover, Herlyn and co-workers have shown that N-cadherin on melanoma cells plays a dual role promoting tumorigenesis (Li et al., 2001). Normal melanocytes interact with keratinocytes through E-cadherin, which regulates their growth. As melanocytes switch from E-cadherin expression to N-cadherin expression during melanoma progression, they are released from growth control by keratinocytes and gain the ability to interact with fibroblasts and endothelial cells, both of which express N-cadherin. Interactions with dermal fibroblasts could allow the melanoma cells to migrate through the tissue and interactions with endothelial cells could allow entry into the circulation (Li et al., 2001).

N-cadherin in cell survival

Studies show that E-cadherin-mediated cell-cell adhesion promotes cell survival in the absence of cell-substrate interactions, which might protect tumor cells from chemotherapy and from destruction while in the circulation (Kang et al., 2007; Kantak and Kramer, 1998; St Croix and Kerbel, 1997). Beyond its role stimulating cell migration, N-cadherin also promotes cell growth and survival by suppressing apoptotic signals. Granulosa cells express N-cadherin and single cells show higher levels of apoptosis than do cells in clumps. Disrupting N-cadherin function in granulosa cells using inhibitory antibodies or an inhibitory peptide induces apoptosis (Makrigiannakis et al., 1999; Peluso et al., 1996). Similarly, human hepatocellular carcinomas have been shown to express N-cadherin, and Lee and co-workers have shown that hepatocellular carcinoma cells expressing a dominant-negative N-cadherin construct that inhibits N-cadherin function are more susceptible to bile-acid-induced apoptosis than control cells (Gwak et al., 2006). When the hepatocellular carcinoma cell line HepG2 is engineered to express a mutant form of the *TIP30* tumor suppressor gene (which protects the cells from cisplatin-induced apoptosis) and subjected to RNA interference (RNAi) directed against N-cadherin expression, the cells regain sensitivity to cisplatin (Jiang et al., 2007).

As mentioned earlier, melanocytes switch from E-cadherin to N-cadherin expression when they become melanoma cells (Hsu et al., 1996). Herlyn and co-workers showed that N-cadherin-mediated adhesion protects melanoma cells from apoptosis by activating the anti-apoptotic Akt (PKB) pathway and that blocking N-cadherin function with function-perturbing antibodies induces apoptosis in these cells (Li et al., 2001). In addition, endothelial cells, which express N-cadherin and VE-cadherin, are induced to undergo apoptosis when treated with the N-cadherin antagonist ADH-1 (Erez et al., 2004). Numerous studies thus support a role for N-cadherin in the suppression of apoptosis.

Therapeutic prospects

Although we still have a lot of work to do before we fully understand how cadherin switching is achieved and how it alters the behavior of cells to promote tumor progression, we are in a position to determine whether inhibiting the activity of N-cadherin in cells that inappropriately express this protein has an impact on tumorigenesis. Blaschuk et al. reported that synthetic peptides containing the sequence His-Ala-Val (HAV), which is found in the first extracellular (EC1) domains of type I classical cadherins, can inhibit N-cadherin function (Blaschuk et al., 1990;

Williams et al., 2000). One such peptide (ADH-1) has been developed by Adherex Technologies as a potential treatment for metastatic cancers that express N-cadherin. ADH-1 has been shown to inhibit cell growth and motility in vitro, and tumor growth and invasion in vivo (Mariotti et al., 2007). In addition, Erez et al. have shown that treatment of endothelial cells, which express both N-cadherin and VE-cadherin, with ADH-1 induces apoptosis in a cell-density-dependent manner and suggest that ADH-1 might be effective at preventing tumor angiogenesis (Erez et al., 2004). It has been shown at the histological level that ADH-1 disrupts tumor angiogenesis in several mouse solid tumor models. These studies also used magnetic resonance imaging (MRI) to show that treatment with ADH-1 significantly reduces blood flow to the tumors. Importantly, the animals showed no histological sign of damage to mature vessels and no loss of blood to normal tissues (Mariotti et al., 2007). Although it is not clear why ADH-1 is selective for newly formed vessels, the data are compelling and ADH-1 has entered human clinical trials (Mariotti et al., 2007).

ADH-1 also has a direct effect on the tumor cells themselves. In a mouse orthotopic injection model for pancreatic cancer, it decreases tumor growth by increasing apoptosis in N-cadherin-expressing tumor cells (Shintani et al., 2008). Importantly, this study showed that ADH-1 effectively inhibits tumor cell invasion and metastasis.

Concluding remarks

When tumor cells switch from expressing E-cadherin to expressing N-cadherin, they show increased aggressive behavior. N-cadherin promotes aggressive behavior by a number of mechanisms, ranging from interacting with receptor tyrosine kinases at the cell surface to influencing the activation levels of RhoGTPases in the cytosol. Understanding how N-cadherin influences cell behavior will make it possible to design therapies to combat its activity and prevent tumor cell growth, invasion and metastasis. N-cadherin upregulation can be triggered by diverse extracellular signals. Future studies will be aimed at further understanding the pathways both upstream and downstream of cadherin switching in order to expand the realm of possible therapeutic interventions.

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