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Adherens junction turnover: regulating adhesion through cadherin endocytosis, degradation, and recycling

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Abstract

Adherens junctions are important mediators of intercellular adhesion, but they are not static structures. They are regularly formed, broken, and rearranged in a variety of situations, requiring changes in the amount of cadherins, the main adhesion molecule in adherens junctions, present at the cell surface. Thus, endocytosis, degradation, and recycling of cadherins are crucial for dynamic regulation of adherens junctions and control of intercellular adhesion. In this chapter, we review the involvement of cadherin endocytosis in development and disease. We discuss the various endocytic pathways available to cadherins, the adaptors involved, and the sorting of internalized cadherin for recycling or lysosomal degradation. In addition, we review the regulatory pathways controlling cadherin endocytosis and degradation, including regulation of cadherin endocytosis by catenins, cadherin ubiquitination, and growth factor receptor signaling pathways. Lastly, we discuss the proteolytic cleavage of cadherins at the plasma membrane.

Introduction

Cell contacts are not static structures. They are regularly formed, broken, and rearranged both during normal physiological processes and in disease states. In order to allow for dynamic changes in cell contact strength, adherens junctions must themselves be plastic. A key mechanism for modulating adhesion strength is the adjustment of the amount of cadherin, the main adhesion molecule in adherens junctions, present at the plasma membrane (unless otherwise noted, we use ‘cadherin’ to mean classical cadherins, the cadherin subfamily which forms adherens junctions). Cadherin levels are determined by the balance between endocytosis and degradation, which remove cadherin from the plasma membrane, and synthesis and recycling, which increase the amount of cadherin available. Transcriptional regulation of cadherins also plays an important role in development and disease (Peinado et al., 2004). However, because the metabolic half-life of cadherins is long, approximately five to ten hours in cultured cells (McCrea and Gumbiner, 1991; Shore and Nelson, 1991), transcriptional regulation cannot account for more rapid changes in adhesion strength. As we discuss in this chapter, endocytosis, degradation, and recycling of cadherins are crucial for dynamic regulation of adherens junctions and control of intercellular adhesion.

Cadherins are named for their calcium-dependent adhesion. Depletion of extracellular calcium disrupts adherens junctions (Kartenbeck et al., 1982), and it was this process that provided the first evidence that cadherin turnover might play a role in the dynamic control of cell adhesion. Classic electron microscopy and immunofluorescence studies demonstrated that, subsequent to calcium depletion, cadherins are removed from cell junctions by endocytosis (Kartenbeck et al., 1991; Matthey and Garrod, 1986). Cadherin endocytosis plays a role in physiological processes as well. For example, cells undergoing mitosis often appear to adopt a rounded morphology, suggesting that they have become detached from their neighbors. Cadherin endocytosis was found to accompany mitosis-related cell rounding, decreasing the junctional pool of cadherin to allow for decreased adhesion, even as the total amount of cadherin expression remained constant (Bauer et al., 1998). More recent work suggests that cadherin endocytosis is a particularly important mechanism for the disassembly of cadherin-based adhesive contacts (Trojanovsky et al., 2006). The significance of cadherin internalization to the dynamic regulation of cell-cell adhesion is now well established. Cadherin endocytosis has been observed in a large variety of developmental and disease processes, and in recent years, tremendous progress has been made toward understanding the molecular mechanisms involved in cadherin internalization and degradation.

In this chapter, we review the evidence for the involvement of cadherin endocytosis during development and its misregulation in disease. We also discuss the rapidly accumulating body of work detailing the trafficking pathways involved in cadherin endocytosis. Both clathrin-dependent and clathrin-independent pathways have been implicated, and several endocytic adaptors which interact with cadherins have been identified. In addition, we consider the process of sorting internalized cadherin for recycling or degradation and how the regulation of cadherin recycling may be used to control adherens junction turnover. Regulation of cadherin endocytosis by catenins is also important, and we review the effects of catenins on cadherin internalization. p120-catenin in particular has gained prominence as a “set-point” for cadherin levels, but α - and β -catenins may have important roles as well. We also review the evidence for cadherin ubiquitination as a signal for adherens junction turnover and the ubiquitin ligases which have been found to target cadherins and affect cadherin trafficking. In order to further consider the regulation of cadherin internalization, we discuss the many growth factor signaling pathways that affect cadherin trafficking. Interestingly, in some cases the connection is bidirectional, with growth factor signaling altering cadherin trafficking and cadherins modulating growth factor receptor signaling. Finally, we briefly discuss another important mechanism for adherens junction turnover, the proteolytic degradation of cadherins at the plasma membrane.

Cadherin endocytosis in development and disease

Perhaps the best examples of the importance of cadherin endocytosis and the dynamic regulation of adherens junctions come from tissue patterning and development. Initially, cadherins were observed to control tissue patterning by facilitating cell sorting based on the type of cadherin expressed (Nose et al., 1988). However, Steinberg and Takeichi also demonstrated that varying the expression level of a single cadherin could also be used as a mechanism for cell sorting (Steinberg and Takeichi, 1994). Thus, the prominent role of

cadherin endocytosis in development should come as no surprise. For example, during epithelial-mesenchymal transitions, cells decrease the expression level of cadherins through a process involving cadherin internalization (Miller and McClay, 1997). Cadherin internalization has also been reported during gastrulation in a variety of organisms (Oda et al., 1998; Ogata et al., 2007), where it may be controlled by Wnt signaling (Ulrich et al., 2005). Other developmental processes where cadherin internalization is important include nervous system development, where both the Rab-5-dependent endocytosis and Rab-11-mediated recycling of N-cadherin are required for neuronal patterning (Kawauchi et al., 2010). Two lines of investigation also demonstrate the importance of cadherin endocytosis for developmental processes involving planar cell polarity. First, convergent extension in *Xenopus* embryos typically involves the coordinated down-regulation of C-cadherin in response to mesoderm-inducing signals (Brieher and Gumbiner, 1994; Zhong et al., 1999). Inhibiting dynamin in *Xenopus* embryos blocks C-cadherin endocytosis, disrupting convergent extension (Jarrett et al., 2002). Second, in *Drosophila*, planar-polarized endocytosis of DE-cadherin mediates cell intercalation necessary for germ band extension, and blocking cadherin endocytosis prevents this critical developmental process (Levayer et al., 2011). Thus, cadherin internalization plays a key role in a variety of developmental processes.

Of course, processes which play important roles in development often contribute to disease when they are activated inappropriately. Cadherin internalization is no exception, and loss of cell adhesion is a key requirement for cancer metastasis. Loss of adhesion in many types of cancer is often attributed to decreased E-cadherin expression (Hirohashi, 1998). While this is most often due to decreased synthesis, there is some evidence that increased cadherin endocytosis may also play a role. One recent study found that a non-junctional, presumably internalized, E-cadherin expression pattern was associated with poor survival in nasopharyngeal cancer (Xie et al., 2010). Another found Src-dependent E-cadherin internalization with shear stress in an oropharyngeal cancer cell line (Lawler et al., 2009). Increased E-cadherin internalization has also been found in a mouse model of UV-irradiation-induced squamous cell carcinoma (Brouxhon et al., 2007). As discussed below, there is also considerable evidence for the involvement of cancer-associated signaling molecules, such as receptor tyrosine kinases and v-Src, in cadherin internalization.

Cadherin endocytosis may play a role in other disease processes as well. For example, internalization of E-cadherin by pancreatic acinar cells was found to be increased in an experimental model of acute pancreatitis (Lerch et al., 1997). Acute pancreatitis is classically associated with significant pancreatic edema, and increased cadherin endocytosis leading to loss of epithelial integrity is an attractive pathophysiological mechanism. Another disease process in which cadherin endocytosis has been implicated is the autoimmune blistering disease pemphigus vulgaris. Auto-antibodies from pemphigus patients cause increased internalization of the desmosomal cadherin desmoglein 3, which may contribute to loss of epithelial integrity and blister formation (Calkins et al., 2006; Delva et al., 2008). Intriguingly, cadherin endocytosis may also be involved in infectious processes. The bacterium *Listeria monocytogenes* appears to hijack a constitutive cadherin endocytic pathway in order to gain entry to cells, a key contributor to the pathogen's virulence (Veiga

and Cossart, 2005). The potential involvement of cadherin endocytosis in such a variety of diseases makes it a tempting target for new therapies, though it remains to be seen whether aberrant cadherin internalization in disease can be inhibited without affecting cadherin endocytosis necessary for normal biological processes. Turning these discoveries into a new generation of anti-cancer drugs will certainly require a better understanding of the molecular mechanisms and regulation of adherens junction turnover.

Cadherin trafficking pathways

Understanding the pathways cadherins use to move in and out of adherens junctions has been a major research focus over the past decade (Chiasson and Kowalczyk, 2008). This work has significantly increased our understanding of how cadherins are internalized and how they are selected for degradation or for recycling back to the plasma membrane. Trafficking pathways essentially control the rate of cadherin turnover; the higher the rate of cadherin endocytosis and the higher the proportion of endocytosed cadherin selected for degradation rather than recycling, the lower the amount of cadherin that will be available to form adherens junctions. We review the clathrin-dependent endocytosis of cadherins and the adaptor proteins involved, as well as several clathrin-independent endocytic pathways and pathways involved in the recycling of internalized cadherin (Figure 1).

Clathrin-mediated endocytosis—Cadherin internalization occurs through several distinct endocytic pathways. Of them, most work has focused on clathrin-mediated endocytosis, which is also the endocytic pathway understood in the greatest detail (Bonifacino and Traub, 2003). Proteins are targeted for clathrin-mediated endocytosis by the binding of adaptor protein complexes. Once bound, adaptor proteins recruit other components of the endocytic machinery and cluster into clathrin-coated pits. Clathrin-coated pits containing proteins targeted for endocytosis then undergo dynamin-mediated scission from the plasma membrane, budding off to form endocytic vesicles. Internalized proteins can be sorted for recycling back to the plasma membrane or sorted to the lysosome for degradation.

Cadherin was first recognized to undergo clathrin-mediated endocytosis by Le and colleagues, who observed constitutive clathrin-mediated endocytosis and recycling of E-cadherin in MDCK cells (Le et al., 1999). We also found that endocytosis of VE-cadherin in endothelial cells occurs through a clathrin-mediated pathway ultimately resulting in degradation of the cadherin by the lysosome (Xiao et al., 2003b). Furthermore, clathrin-mediated endocytosis appears to be responsible for two types of growth factor-induced cadherin internalization, FGF-mediated internalization of E-cadherin (Bryant et al., 2005) and VEGF-mediated internalization of VE-cadherin (Gavard and Gutkind, 2006). Interestingly, clathrin-mediated endocytosis of E-cadherin may be related to the cadherin's adhesive state. Izumi and colleagues isolated adherens junction-containing membrane from rat liver and, using a reconstitution system, observed budding of E-cadherin into clathrin-coated vesicles with electron microscopy and biochemical fractionation. Adding antibody against the extracellular domain of E-cadherin, which blocks *trans* interactions, to the reconstitution system increased the amount of cadherin which entered clathrin-coated vesicles, while adding E-cadherin extracellular domain fragments decreased recruitment of

cadherin to clathrin-coated vesicles. They also found that *trans* interaction-mediated inhibition of cadherin endocytosis involved activation of the small G-proteins Rac and Cdc42, as well as the actin-binding protein IQGAP1 (Izumi et al., 2004). In addition, exposing an intestinal epithelial cell line to low-calcium conditions, which disrupts cadherin *trans* interactions, results in the clathrin mediated endocytosis of E-cadherin, along with other adherens junction and tight junction components, into a unique syntaxin-4-positive compartment (Ivanov et al., 2004). Thus, clathrin-mediated endocytosis appears to modulate cadherin function in a variety of biological contexts.

Endocytic adaptors—Clathrin-mediated endocytosis depends on adaptor proteins to recognize proteins targeted for internalization and to recruit other components of the endocytic machinery. Identifying clathrin-mediated endocytosis as a pathway for cadherin internalization raises the question of what endocytic adaptors might recognize cadherins. One likely candidate is the adaptor protein complex AP-2, which commonly recognizes cargo proteins with a tyrosine- or dileucine-based motif (Traub, 2003). E-cadherin contains a putative dileucine-based AP-2 binding motif in its cytoplasmic tail, and mutating those residues disrupts the normal basolateral localization of E-cadherin (Miranda et al., 2001) and prevents E-cadherin clathrin-mediated endocytosis (Miyashita and Ozawa, 2007b). This motif is also present in many other classical cadherins, including N- and P-cadherins. It is not, however, present in VE-cadherin or in *Drosophila* DE-cadherin. Nonetheless, the VE-cadherin cytoplasmic tail is sufficient to mediate clathrin-dependent endocytosis when attached to an unrelated transmembrane protein, strongly suggesting that cadherins may contain other endocytic adaptor binding sequences as well (Xiao et al., 2005). In recent years, more direct evidence for the involvement of AP-2 in the clathrin-mediated endocytosis of cadherins has begun to accumulate. We found that internalization of VE-cadherin is clathrin-, dynamin-, and AP-2-dependent and that AP-2 both co-localizes with VE-cadherin and co-immunoprecipitates with the VE-cadherin cytoplasmic tail (Chiasson et al., 2009). An AP-2 subunit was also found to co-immunoprecipitate with the E-cadherin cytoplasmic tail (Sato et al., 2011). Interestingly, Levayer and colleagues also found that AP-2-and clathrin-mediated endocytosis of DE-cadherin is crucial for the establishment of planar cell polarity in germ band extension. Polarized distributions of Dia and Myosin-II induce planar DE-cadherin clustering in junctions perpendicular to the developing long axis of the germ band. DE-cadherin clustering recruits AP-2 and clathrin to these junctions, leading to the preferential endocytosis of DE-cadherin from perpendicular junctions and the relative accumulation of DE-cadherin in junctions parallel to the germ band axis (Levayer et al., 2011).

However, the question of what endocytic adaptors are important for cadherin endocytosis remains incompletely resolved. It is not yet clear that AP-2 interacts directly with cadherins. It is also possible that other endocytic adaptors may be involved depending on the biological context. Mice null for Dab-2, another adaptor protein associated with clathrin-mediated endocytosis, support this possibility. They exhibit loss of apical-basal polarized distribution of E-cadherin, as well as the LDL receptor-related protein megalin, in the developing endoderm (Yang et al., 2007). Several reports also suggest a role for the endocytic adaptor Numb in cadherin internalization. In radial glial cells, Numb co-immunoprecipitates with

cadherins, and Numb depletion disrupts adherens junctions (Rasin et al., 2007). Numb also binds to E-cadherin in epithelial cell lines and mediates endocytosis of cadherins specifically from the apical surface, contributing to the lateral localization of cadherins in adherens junctions (Lau and McGlade, 2011; Wang et al., 2009). This polarization is due to localized phosphorylation and inactivation of Numb at lateral membranes by the PAR polarity complex member aPKC (Sato et al., 2011). Consequently, the role of adaptor proteins in cadherin endocytosis remains an exciting area for future discovery.

Clathrin-independent endocytic pathways—Cadherin turnover has also been associated with clathrin-independent endocytic pathways, though considerably less work has been done in this area compared to clathrin-mediated cadherin endocytosis. Studies have suggested that cadherin endocytosis may occur through both caveolin-mediated and macropinocytosis-like pathways. Akhtar and colleagues found that a dominant-active form of the small GTPase Rac1 could disrupt cell-cell adhesion in keratinocytes. This was associated with the endocytosis of E-cadherin through a pathway that appeared to be distinct from the uptake of transferrin, which is clathrin-mediated, and through structures that co-localized with caveolin (Akhtar and Hotchin, 2001). Further evidence for caveolin-mediated cadherin endocytosis was provided by Lu and colleagues, who demonstrated that EGF signaling could disrupt cell-cell adhesion by triggering the caveolin-mediated internalization of E-cadherin, a mechanism which may be relevant to epithelial-to-mesenchymal transition in cancers (Lu et al., 2003). In contrast, Bryant and colleagues characterized the EGF-induced internalization of E-cadherin in a breast carcinoma cell line, in which E-cadherin was internalized along with the cadherin-binding proteins p120 and β -catenin, as Rac1-modulated macropinocytosis, rather than caveolin-mediated (Bryant et al., 2007). It is not clear if the EGF-related mechanisms described by Lu and Bryant are in fact different and, if they are, how they can be reconciled. However, Paterson and colleagues have observed E-cadherin endocytosis that is both clathrin- and caveolin-independent, but dynamin-dependent. This pathway, which they identify as similar to macropinocytosis, appears to affect cadherin that is not engaged in *trans* interactions in an adherens junction (Paterson et al., 2003). Lastly, the desmosomal cadherin desmoglein 3 undergoes lipid-raft-mediated endocytosis, though it is unclear if this pathway is available to classical cadherins as well (Delva et al., 2008). Though some of the specific details of the clathrin-independent pathways remain unclear, it appears that both clathrin-dependent and clathrin-independent endocytic pathways play a role in cadherin turnover.

Recycling pathways—Not all molecules that enter an endocytic pathway face immediate degradation in the lysosome. Some are sorted and recycled back to the plasma membrane. Recycling pathways are particularly important for cadherins, and the choice between degradation and recycling can help fine-tune the amount of cadherin present at adherens junctions and the strength of cell-cell adhesion. The first suggestion of the importance of a recycling pathway to cadherin trafficking came from the discovery that E-cadherin does not travel directly from the Golgi complex to the cell surface, but transits first through Rab11-positive recycling endosomes (Lock and Stow, 2005). Interestingly, while expressing dominant-negative Rab11 blocked delivery of wild type E-cadherin to the plasma membrane, an E-cadherin mutant lacking the dileucine motif important for clathrin-mediated

endocytosis traffics to the plasma membrane without impediment, though it is mislocalized to the apical surface (Lock and Stow, 2005; Miranda et al., 2001). In contrast, Drosophila DE-cadherin traffics through Rab11-positive endosomes and inhibiting Rab-11 disrupts the integrity of the embryonic ectoderm, even though DE-cadherin lacks the dileucine motif (Roeth et al., 2009). In addition to acting as way stations for newly synthesized cadherin on its way to the plasma membrane, Rab11-positive recycling endosomes can also sort internalized cadherin for recycling back to the cell surface. In fact, Classen and colleagues found that Rab11 recycling of cadherin mediates the rearrangements in cell-cell contacts seen in the hexagonal packing of Drosophila wing disk cells (Classen et al., 2005). Desclozeaux and colleagues also found that cadherin recycling is necessary for maintaining adherens junctions and epithelial polarity and that disrupting the recycling endosome with dominant-negative Rab11 prevented MDCK cells from forming cysts when grown in three-dimensional culture (Desclozeaux et al., 2008).

Additional work has begun to illuminate the molecular mechanisms responsible for cadherin recycling. In particular, components of the exocyst complex appear to be critical. Sec5, sec6, and sec15 are all required for DE-cadherin trafficking from recycling endosomes to the plasma membrane (Langevin et al., 2005). Depletion of the scaffolding protein PALS1 also causes the mislocalization of the exocyst complex and disrupts recycling of E-cadherin (Wang et al., 2007). Recently, Guichard and colleagues identified Rab11- and exocyst complex-mediated recycling of cadherins as a target of the pathogen *Bacillus anthracis*, highlighting its pathophysiological importance. *B. anthracis*, the causative agent of anthrax, produces two different toxins, lethal factor and edema factor, which both inhibit the exocyst complex through independent mechanisms. This results in the loss of cadherin from adherens junctions, potentially contributing to the toxin-mediated epithelial and vascular disruption which occurs with *B. anthracis* infection (Guichard et al., 2010). In addition to the exocyst complex, another potential mediator of cadherin recycling is the adaptor protein complex AP-1B, which usually mediates recycling of basolaterally targeted proteins. Ling and colleagues found that AP-1B interacts with E-cadherin through phosphatidylinositol-4-phosphate 5-kinase type I γ (PIPKI γ), which binds directly to the E-cadherin cytoplasmic tail near the β -catenin binding site (Ling et al., 2007). Interestingly, an E-cadherin mutation at the PIPKI γ binding site is associated with familial diffuse gastric cancer (Yabuta et al., 2002).

Our understanding of cadherin recycling remains incomplete. Though many of the important components of the cadherin recycling pathway have been identified, the list is likely to grow further. Furthermore, although we review below some evidence that ubiquitination may trigger the selection of cadherin for degradation rather than recycling (Palacios et al., 2005), the regulation of the cadherin recycling pathways remains, for now, only partially elucidated.

Regulation of cadherin endocytosis by catenins

Given the importance of cadherin endocytosis for the proper maintenance and dynamic regulation of cell-cell adhesion, identifying the regulatory mechanisms controlling cadherin internalization and recycling has become a significant research focus. Much attention has

been paid to the catenins, the cytoplasmic binding partners of cadherins, which stabilize adherens junctions and link them to the actin cytoskeleton (Delva and Kowalczyk, 2009). These include α -catenin, β -catenin, and p120-catenin. β -catenin binds to the C-terminal catenin-binding domain of cadherins and, along with α -catenin, helps link the cadherin to the actin cytoskeleton. p120-catenin binds to the juxtamembrane domain, N-terminal to the β -catenin binding site, and stabilizes cadherin at the adherens junction. All three catenins contribute to the regulation of adherens junctions.

p120-catenin—p120-catenin (p120) plays a key role as an inhibitor of cadherin turnover and as a “set-point” for cadherin expression levels (Figure 2). A member of the armadillo family of proteins, p120 binds to the juxtamembrane domain of cadherins (Reynolds, 2007). Ireton and colleagues discovered that epithelial morphology in a colon carcinoma cell line lacking p120 could be restored with exogenous p120 expression. Furthermore, p120 rescue of epithelial morphology required p120 binding to E-cadherin. The mechanism of this activity involved increased E-cadherin protein levels and half-life without changes to E-cadherin mRNA levels (Ireton et al., 2002). Those results, which strongly suggested that p120 binding to cadherin is necessary to prevent rapid cadherin turnover, were confirmed by studies directly demonstrating that loss of p120 results in cadherin endocytosis (Davis et al., 2003; Xiao et al., 2003a). Importantly, p120 acts not only as an inhibitor of cadherin endocytosis, but as a “set-point” for cadherin expression (Figure 2A). Expressing cadherin mutants which compete for p120 binding results in the endocytosis of endogenous cadherin, while cadherin mutants which cannot bind to p120 lack this activity (Xiao et al., 2003a; Xiao et al., 2005). This raises the interesting possibility that p120 might serve as a master regulator of cadherin levels in cells. For example, increased expression of one cadherin might, through competition for p120 binding, cause increased turnover and down-regulation of other cadherins in the cell. Exactly this dynamic has been reported to occur in two studies of cells expressing multiple cadherin types. In A431 cells, exogenously expressing R-cadherin caused the endocytosis and down-regulation of endogenous E- and P-cadherins (Maeda et al., 2006). Similarly, in endothelial cells, which express both VE- and N-cadherins, but which rely primarily on VE-cadherin to form adherens junctions, altering expression levels of one cadherin inversely affects protein levels of the other cadherin (Ferrerri et al., 2008).

Multiple mechanisms have been proposed to explain how p120 regulates cadherin turnover. Cadherin internalization mediated by p120 loss is clathrin-dependent, as discussed above in more detail (Xiao et al., 2005). Clathrin-dependent endocytosis requires an adaptor protein to bind to cargo and recruit other components of the endocytic machinery. p120 binding to the cadherin cytoplasmic domain could potentially mask the binding site of such an endocytic adaptor. Alternatively, p120 could regulate cadherin turnover by locally modifying actin dynamics through its well-described role as an inhibitor of the small GTPase RhoA (Anastasiadis, 2007). For example, cells exogenously expressing high levels of p120 display increased actin branching and the formation of long dendritic spines (Anastasiadis et al., 2000; Noren et al., 2000; Reynolds et al., 1996). It has become increasingly clear however, that p120 binding to cadherins, not p120 inhibition of RhoA, is the mechanism of p120-mediated cadherin stabilization (Figure 2B). First, our lab and others

have shown that p120 binding to cadherin is an absolute requirement for p120-mediated cadherin stabilization (Ireton et al., 2002; Miyashita and Ozawa, 2007b; Xiao et al., 2005). We also demonstrated that inhibition of RhoA signaling is insufficient to block cadherin endocytosis and that cadherin can also be stabilized by a p120 mutant unable to inhibit Rho (Chiasson et al., 2009). Neither of these observations support a role for RhoA in p120 regulation of cadherin endocytosis. Lastly, our observation that p120 prevents VE-cadherin from clustering into AP-2- and clathrin-enriched membrane domains directly supports the hypothesis that p120 masks an endocytic adaptor binding site on the cadherin cytoplasmic tail (Chiasson et al., 2009). This model received additional support from the recently published crystal structure of a portion of the E-cadherin cytoplasmic domain in complex with p120. The E-cadherin-p120 interface contains both static and dynamic binding regions, an interaction which could support binding competition or regulated exchange with an endocytic adaptor protein (Ishiyama et al., 2010).

Numerous studies of animal models have underscored the physiological importance of p120 to adherens junction regulation, at least in mammals. p120 binding to cadherin is apparently dispensable in *Drosophila* and *C. elegans* (Myster et al., 2003; Pacquelet et al., 2003; Pettitt et al., 2003). However, p120 binding is critical for adherens junction stability in mice. Numerous tissue-specific p120-null mouse models have been developed, and all of them display disrupted cadherin-mediated cell adhesion (summarized in Table 1). The reasons for the different requirements for p120 in mammals and invertebrates remains unknown. Though, as outlined above, cadherin trafficking pathways in *Drosophila* appear similar to those in mammalian systems, there may be significant differences in their regulation. Interestingly, the p120 sub-family of catenins is considerably larger in vertebrates than in invertebrates, with additional members including p0071, δ -catenin/NPRAP, ARVCF, and the plakophilins (Hatzfeld, 2005). These observations suggest that vertebrate tissue patterning requires additional levels of control over cadherin trafficking, with both the expanded role of vertebrate p120 and the expanded size of the vertebrate p120 sub-family serving as points of regulation not present in simpler organisms.

β -catenin and α -catenin—Another cytoplasmic binding partner of cadherins is β -catenin, which binds to the C-terminal portion of the cadherin cytoplasmic tail, termed the catenin-binding domain. β -catenin plays an important role in adherens junction structure, contributing to the link between cadherins and the actin cytoskeleton (Hartsock and Nelson, 2008). β -catenin binding to cadherins is clearly important for its ability to recruit α -catenin, which, through a mechanism that is not fully understood, links cadherins to actin (Yamada et al., 2005). In fact, this may be the primary role of β -catenin in adherens junctions, since mutant cadherin which cannot bind to β -catenin but is fused to α -catenin forms junctions that are apparently normal (Nagafuchi et al., 1994; Pacquelet and Rorth, 2005). Further support for the hypothesis that β -catenin stabilizes adherens junctions through the recruitment of α -catenin comes from a knock-in mouse model recently created by Schulte and colleagues with a mutant VE-cadherin which does not bind to β -catenin but is fused to α -catenin replacing the wild-type VE-cadherin gene. The mutant mice are viable, though they are not born at mendelian frequencies, and are resistant to inflammatory stimuli that

trigger increased vascular permeability in wild-type mice, suggesting supra-physiological stabilization of their endothelial adherens junctions (Schulte et al., 2011).

Though β -catenin clearly has an important role in adherens junction regulation, its role in cadherin trafficking is far from clear. One report does suggest that β -catenin is required for proper cadherin localization and that disrupting β -catenin binding to cadherins results in cadherin accumulation in intracellular compartments (Chen et al., 1999). However, other studies have yielded conflicting results, though several studies have found at least circumstantial evidence for a β -catenin role in cadherin trafficking. First, Dupre-Crochet and colleagues found that casein kinase 1 (CK1) inhibition stabilizes adherens junctions, while CK1 over-expression disrupts adherens junctions. CK1 phosphorylates E-cadherin, primarily on a serine residue within the catenin binding domain. They also found that a phosphomimetic mutation at that site weakens β -catenin binding to E-cadherin and increases E-cadherin internalization (Dupre-Crochet et al., 2007). Second, Tai and colleagues report that in cultured hippocampal neurons, NMDA inhibits N-cadherin turnover and causes β -catenin to accumulate in dendritic spines. Both effects are related to β -catenin phosphorylation (Tai et al., 2007). Lastly, Sharma and colleagues report that β -catenin is internalized by macropinocytosis in cultured fibroblasts, and that internalized β -catenin co-localizes with N-cadherin. This process appears to be mediated by IQGAP1 binding to β -catenin (Sharma and Henderson, 2007). These three accounts are somewhat contradictory. The first two suggest that β -catenin binding to cadherin inhibits its endocytosis, while the last one suggests that β -catenin binding has a role in mediating cadherin endocytosis. Complicating things further, Miyashita and Ozawa report that, while β -catenin binding to E-cadherin may affect E-cadherin localization, the mechanism is unrelated to cadherin turnover. They find that an E-cadherin mutant which cannot bind to β -catenin is mislocalized to an intracellular compartment. However, this mislocalization occurs even with the co-expression of dominant-negative dynamin, which blocks all dynamin-mediated endocytosis. Interestingly, mislocalization of the non- β -catenin-binding mutant cadherin is dependent on the dileucine motif important for clathrin-mediated internalization of E-cadherin; mutant cadherin which cannot bind β -catenin and lacks the dileucine motif traffics to the plasma membrane and does not accumulate intracellularly (Miyashita and Ozawa, 2007a). Given the conflicting evidence, more work is needed to understand how β - and α -catenin-mediated cytoskeletal linkages might affect cadherin endocytosis, as well as any other effects that β -catenin binding to cadherins might have on cadherin trafficking.

Regulation of cadherin endocytosis and degradation by ubiquitination

Cadherin ubiquitination also plays an important role in regulating cadherin turnover. Proteins are selected for ubiquitination through interaction with E3 ubiquitin ligase proteins which recruit E2 ubiquitin conjugating enzymes charged with ubiquitin and catalyze the transfer of ubiquitin to the target molecule, usually on lysine residues. Ubiquitin molecules can be attached singly or linked together to form a poly-ubiquitin chain. While poly-ubiquitination is usually associated with targeting intracellular proteins for degradation by the 26S proteasome, mono-ubiquitination can also trigger the endocytosis and lysosomal degradation of membrane proteins (Clague and Urbe, 2010). Because of its association with endocytosis and degradation, cadherin ubiquitination has been an attractive candidate

process for regulating cadherin turnover. Additionally, as a posttranslational modification, cadherin ubiquitination could potentially be influenced by a variety of signaling pathways, ensuring ample control points for the modulation of cadherin endocytosis and degradation. Circumstantial support for a role for ubiquitination in cadherin turnover comes from studies showing that proteasome inhibitors such as MG-132 can block cadherin endocytosis, though the mechanism of this effect remains unclear (Xiao et al., 2003b). In fact, a significant body of work has now developed to establish the importance of ubiquitination in cadherin turnover.

The first ubiquitin ligase identified to target cadherin was Hakai, a c-Cbl-like protein with phosphotyrosine-binding, RING finger, and proline-rich domains characterized by Fujita and colleagues. Hakai associates with and ubiquitinates E-cadherin, causing its internalization. Interestingly, this function is dependent on Src-mediated phosphorylation of E-cadherin at two specific tyrosine residues in the juxtamembrane domain (Fujita et al., 2002). This both explains the previously reported ability of v-Src to transform cultured epithelial cells to a fibroblastic phenotype (Behrens et al., 1993) and provides a potential explanation for the ability of p120 to inhibit cadherin internalization, since p120 binding could mask or prevent the phosphorylation of the E-cadherin tyrosine residues required for Hakai binding. However, these tyrosine residues are not conserved in all classical cadherins. P-cadherin contains only one of the two tyrosine residues, and N- and VE-cadherins lack both of them. Hakai-mediated down-regulation of cadherins therefore may not play a role at all adherens junctions.

Further work by Palacios and colleagues has clarified the mechanism of Hakai-induced E-cadherin turnover. Hakai-mediated ubiquitination of E-cadherin may not directly trigger E-cadherin internalization, since an E-cadherin mutant that cannot interact with Hakai can still be internalized. However, Hakai-mediated ubiquitination of E-cadherin changes the destination of E-cadherin once it has been internalized, redirecting it from a recycling pathway to degradation in the lysosome (Palacios et al., 2005). This redirection requires Hrs, a ubiquitin-interacting protein with a role in shuttling mono-ubiquitinated cargo to the lysosome (Palacios et al., 2005; Toyoshima et al., 2007). Studies have also linked Hakai to developmental and disease processes. Hakai is essential for the maintenance of epithelial integrity in *Drosophila*, though its interaction with DE-cadherin is considerably different than the interaction of mammalian Hakai with E-cadherin. *Drosophila* Hakai can interact with DE-cadherin based on the extracellular and transmembrane portions of the cadherin without the intracellular portion (Kaido et al., 2009). Because Hakai is a cytoplasmic protein, it is not clear how this interaction can occur without the assistance of another protein. Hakai has also been linked to disease in some human colorectal carcinomas, where elevated Slit-Robo signaling induces an epithelial to mesenchymal transformation by recruiting Hakai to ubiquitinate E-cadherin, causing its down-regulation. Elevated Slit-Robo expression is also associated with increased risk of metastasis and decreased survival (Zhou et al., 2011). Though the function of Hakai may be limited to only a subset of adherens junctions, it clearly plays an important role.

Hakai is not the only ubiquitin ligase that has been connected to adherens junction turnover. The ubiquitin ligase MDM2 also ubiquitinates and causes the degradation of E-cadherin, and

in human breast carcinoma specimens, increased MDM2 expression was associated with decreased E-cadherin protein levels (Yang et al., 2006). A third ubiquitin ligase, the viral protein K5, has also been shown to target VE-cadherin (Mansouri et al., 2008). K5 is expressed by human herpesvirus-8 (HHV-8), which causes the angioproliferative neoplasm Kaposi sarcoma. K5 is thought to play a role in the virus's ability to evade the host immune response by ubiquitinating and causing the internalization of immune recognition components such as the class I major histocompatibility complex. The increased vascular permeability associated with Kaposi sarcoma may be due to a similar mechanism inducing the endocytosis and down-regulation of VE-cadherin (Qian et al., 2008). Because K5 is a member of the membrane-associated RING-CH (MARCH) family of ubiquitin ligases, which includes several human proteins expressed in a variety of tissues (Nathan and Lehner, 2009), it is possible that HHV-8 may be appropriating a more generally important cellular mechanism for cadherin regulation involving endogenous MARCH proteins.

Growth factor signaling and cadherin endocytosis

Cell-cell junctions are fundamental links between a cell and its environment. It is not a surprise then, that adherens junctions are not regulated only by intracellular processes, but also by intercellular cues. A variety of growth factor signaling pathways have been tied to the dynamic regulation of cadherin endocytosis, including hepatocyte growth factor (HGF), epithelial growth factor (EGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and transforming growth factor β (TGF β). Many of these pathways affect cadherin trafficking and catenin binding, which are discussed above in more detail.

The first growth factor receptor associated with cadherin endocytosis was the HGF receptor, c-Met (Figure 3A). HGF is also called scatter factor for its ability to stimulate epithelial cell motility. Treatment of cultured cells with HGF or a small molecule HGF receptor agonist causes the co-endocytosis of the HGF receptor and associated E-cadherin (Kamei et al., 1999). This effect requires the activation of the small GTPase Arf6 (Palacios et al., 2001). Additionally, HGF signaling causes Numb, an endocytic adaptor which may play a role in establishing the lateral localization of cadherins by facilitating their specific endocytosis from the apical surface, to decouple from E-cadherin and associate with aPKC and Par6 instead, disrupting cell polarity (Wang et al., 2009). Thus, HGF appears to cause both the general down-regulation of cadherin and the disruption of adherens junction polarity. Both effects are consistent with the ability of HGF to induce a fibroblast-like phenotype. However, the cause of HGF-mediated cell scattering remains in dispute, since, in MDCK cells, HGF enhances integrin-mediated interactions with the extracellular matrix which pull the cells apart, but does not appear to disrupt E-cadherin mediated adhesion (de Rooij et al., 2005). More work will be needed to understand the functional importance and precise mechanism of HGF-mediated cadherin endocytosis.

EGF signaling has also been tied to cadherin endocytosis (Figure 3B). The effect of EGF receptor signaling is notable because it causes cadherin internalization through a clathrin-independent pathway. As discussed above, however, beyond clathrin independence, there is disagreement over which endocytic pathway is involved. Lu and colleagues reported that EGF receptor over-expression caused E-cadherin internalization through a caveolin-

mediated pathway (Lu et al., 2003). In contrast, Bryant and colleagues reported that EGF induced E-cadherin internalization through macropinocytosis (Bryant et al., 2007). More work will need to be done to sort out these conflicting findings. Interestingly, the relationship between the EGF receptor and cadherins appears to be bidirectional. Certain mutations in the extracellular domain of E-cadherin are associated with decreased formation of E-cadherin-EGF receptor complexes, resulting in increased EGF receptor signaling in both cultured cells and human gastric carcinoma samples (Bremm et al., 2008). This finding suggests that while EGF signaling can cause E-cadherin endocytosis, E-cadherin can inhibit EGF signaling. Clearly, adherens junctions are not simply acted upon by signaling pathways, but are active participants in them as well.

A third growth factor associated with cadherin internalization is VEGF, an important growth factor in vasculogenesis and angiogenesis, which increases vascular permeability by disrupting endothelial cell-cell junctions (Figure 3C). Gavard and Gutkind demonstrated that VEGF signaling causes the Src-mediated phosphorylation of VE-cadherin, resulting in the recruitment of β -arrestin and the subsequent clathrin-mediated endocytosis of VE-cadherin (Gavard and Gutkind, 2006). The pathway is interrupted by angiopoietin-1, which strengthens vascular integrity and decreases permeability. Angiopoietin-1 inhibits Src activation by the VEGF receptor, counteracting VEGF-mediated cadherin internalization (Gavard et al., 2008). As with E-cadherin and the EGF receptor, the relationship between VE-cadherin and the VEGF receptor is bidirectional. In cell culture, confluent endothelial cells are resistant to the effects of VEGF, an effect which requires both VE-cadherin and β -catenin (Grazia Lampugnani et al., 2003). VE-cadherin association with the VEGF receptor prevents VEGF receptor internalization in response to VEGF binding. When internalized in response to VEGF binding, the VEGF receptor is not degraded. Rather, it enters an endosomal signaling compartment where it activates the MAP kinase pathway. Thus, by preventing VEGF receptor endocytosis, VE-cadherin can inhibit VEGF signaling (Lampugnani et al., 2006).

A similar two-way interaction also occurs between cadherins and the FGF receptor (Figure 3D). FGF activation of the FGF receptor induces macropinocytosis of E-cadherin (Bryant et al., 2007; Bryant et al., 2005). Conversely, increased expression of E- or N-cadherin inhibits internalization of the FGF receptor (Bryant et al., 2005; Suyama et al., 2002). In contrast to the VEGF receptor, however, internalization of ligand-bound FGF receptor serves to shut off FGF signaling, primarily through subsequent degradation of the receptor. Thus, FGF signaling down-regulates cadherins and cadherins support FGF signaling, essentially forming a negative-feedback loop. Lastly, cadherin trafficking can be affected by TGF β signaling. TGF β and Raf-1 synergistically induce E-cadherin endocytosis and epithelial to mesenchymal transition in mammary epithelial cells (Janda et al., 2006). Interestingly, TGF β - and Raf-1-induced cadherin internalization is associated with cadherin ubiquitination.

The large variety of growth factor signaling pathways affecting cadherin endocytosis clearly indicates the importance of the dynamic and coordinated regulation of cadherin internalization and intercellular adhesion. More work is needed, however, to understand how these disparate pathways are interrelated in different biological contexts. The potential for

two-way communication between growth factor receptors and adherens junctions is particularly intriguing, and the full potential of these mechanisms has yet to be explored.

Cadherin shedding

In this chapter, we have focused mainly on down-regulation of adherens junctions through the removal of cadherin from the cell surface. However, this is not the only mechanism available for reducing the amount of cadherin available to form adhesive contacts. In some situations, cadherins may be proteolytically cleaved while they remain at the plasma membrane. This process, often termed cadherin “shedding,” can lead to the release of cadherin extracellular domains from the cell or fragments of the cadherin cytoplasmic tail into the cytoplasm, with potential effects beyond loss of adhesion.

Released fragments of cadherin extracellular domains were first identified as factors that inhibited cell adhesion in conditioned medium from a breast cancer cell line (Damsky et al., 1983; Wheelock et al., 1987). Inducing E-cadherin shedding in cell culture can also promote cell invasion into a collagen substrate (Noe et al., 2001). Consequently, there has been considerable excitement for the possible involvement of cadherin shedding in the loss of intercellular adhesion in cancer and the use of cadherin extracellular domain fragments as tumor biomarkers. However, results from observational studies have been mixed (reviewed in De Wever et al., 2007). While serum levels of E-cadherin extracellular domains are elevated approximately three-fold in patients with several types of cancer, there is no correlation with disease progression. It is also possible that increased cadherin shedding detected in these studies is related to general inflammatory processes rather than to the tumor specifically (Pittard et al., 1996). In addition to possible roles in cancer and inflammation, cadherin shedding appears to be involved in several developmental processes. N-cadherin is cleaved during chick retinal development, where, counter-intuitively, the truncated product promotes cell adhesion and neurite development (Paradies and Grunwald, 1993). N-cadherin shedding has also been reported in neural crest delamination and in adult neurons (Marambaud et al., 2003; Shoval et al., 2007). Lastly, in response to Eph/ephrin signaling, E-cadherin shedding plays a role in cell sorting (Solanas et al., 2011). Given the variety of processes in which it has been implicated, cadherin shedding appears to have an important role in development. However, more work will need to be done to understand the role cadherin shedding in more detail and in additional developmental processes.

Many of the proteases responsible for cadherin shedding have been identified. Members of the “a disintegrin and metalloprotease” (ADAM) family, and ADAM10 in particular, appear to be an important generators of free E-cadherin and N-cadherin extracellular domain fragments (Maretzky et al., 2005; Reiss et al., 2005). Interestingly, EGFR-mediated down-regulation of desmosomal cadherins appears to occur, at least in part, through ADAM proteases, a result suggesting how cadherin shedding might be connected to signaling pathways (Klessner et al., 2009). A variety of other proteases have also been implicated in cadherin shedding, including matrix metalloproteinases and kallikreins (Klucky et al., 2007; McGuire et al., 2003; Noe et al., 2001). Still other proteases, including caspases and presenilin, can cleave cadherins intracellularly, releasing a soluble cadherin fragment into the cytoplasm (Marambaud et al., 2002). Interestingly, these intracellular fragments can

traffic to the nucleus, potentially affecting a variety of transcription factors (Ferber et al., 2008). The relationship of intracellular cadherin proteolysis to extracellular cadherin shedding is not yet understood, but, in addition to modulating intercellular adhesion, these mechanisms have the potential to integrate adherens junctions with cell signaling networks.

Summary and future perspectives

Cadherin endocytosis and degradation play crucial roles in the dynamic control of intercellular adhesion. By adjusting the rate of cadherin internalization, cells are able to quickly modify the strength of their adherens junctions, rearranging their relationship with their environment. This process is absolutely critical during development, and, as we have seen, cadherin endocytosis and degradation have been linked to a growing number of developmental processes in a variety of species. A particularly exciting area of current research focuses on planar-cell-polarized endocytosis of cadherin as a mechanism for the establishment of planar polarization of an epithelial layer. The role of cadherin endocytosis during development may turn out to be more complicated – and more important – than simply allowing cells to switch between epithelial and mesenchymal phenotypes. The misregulation of cadherin endocytosis also appears to be increasingly important in disease processes, and, consequently, as a possible therapeutic target. However, our understanding remains incomplete, and devising a new generation of anti-cancer drugs targeting cadherin endocytosis will require further work.

In addition to contributing to our understanding of the role of cadherin internalization in development and disease, recent work has also advanced our understanding of the molecular mechanisms underlying cadherin endocytosis. In particular, we have learned a great deal about clathrin-mediated cadherin endocytosis and its contribution to adherens junction dynamics. However, more needs to be done in order to characterize the clathrin-independent endocytic pathways that cadherins can enter, as well as to better understand which pathways are active in different biological contexts. Furthermore, while several endocytic adaptors have been associated with adherens junction turnover, the nature of the interactions between these adaptors and cadherins remains largely unknown. In order to unwind the pathways regulating cadherin endocytosis, it will be necessary to more precisely identify the cadherin domains which drive their removal from the cell membrane. Do cadherin endocytic signals overlap with the p120 binding site, allowing p120 to compete with endocytic adaptors for cadherin binding, thus stabilizing cadherins at the cell membrane, as has been proposed? Furthermore, how does cadherin shedding relate to cadherin internalization? Answers to these questions must await further investigation.

In addition to better understanding the molecular mechanisms of cadherin endocytosis, another important focus of future research will be the signaling pathways that allow for its dynamic regulation. One possibility is raised by studies supporting the role of α - and β -catenins in cadherin regulation. Since α - and β -catenins link cadherins to the actin cytoskeleton, might this link play some role in cadherin trafficking? For now, the evidence is unclear. A second possibility is that cadherin ubiquitination may be used as signal to promote cadherin endocytosis. Several ubiquitin ligases have been found to mediate the ubiquitination and down-regulation of cadherins. However, based on what is known so far,

the scope of each of the pathways identified remains limited to specific biological contexts. Further research will be needed to determine whether cadherin ubiquitination is a broadly applicable mechanism that regulates cell-cell adhesion. Finally, the many growth factor signaling pathways implicated in cadherin endocytosis suggest several opportunities to link intercellular contacts to intercellular signaling. The possibility that this relationship might be bidirectional, allowing growth factors to affect cadherin endocytosis and cadherins to affect growth factor signaling pathways, is particularly exciting. Still, it will take more work to integrate the disparate pathways that have been identified.

Though our understanding of cadherin internalization and degradation and the mechanisms that regulate them is far from complete, much has been learned in the decades since cadherin endocytosis was first observed in response to calcium depletion. Cadherin endocytosis is now recognized as an important factor in the dynamic control of intercellular adhesion. It remains an active area of research, with the promise to further our understanding of the ever-changing adhesive interactions between cells and the implications of adherens junction dynamics for development and disease.

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References

- Akhtar N, Hotchin NA. RAC1 regulates adherens junctions through endocytosis of E-cadherin. *Mol Biol Cell*. 2001; 12:847–862. [PubMed: 11294891]
- Anastasiadis PZ. p120-ctn: A nexus for contextual signaling via Rho GTPases. *Biochim Biophys Acta*. 2007; 1773:34–46. [PubMed: 17028013]
- Anastasiadis PZ, Moon SY, Thoreson MA, Mariner DJ, Crawford HC, Zheng Y, Reynolds AB. Inhibition of RhoA by p120 catenin. *Nat Cell Biol*. 2000; 2:637–644. [PubMed: 10980705]
- Bauer A, Lickert H, Kemler R, Stappert J. Modification of the E-cadherin-catenin complex in mitotic Madin-Darby canine kidney epithelial cells. *J Biol Chem*. 1998; 273:28314–28321. [PubMed: 9774455]
- Behrens J, Vakaet L, Friis R, Winterhager E, Van Roy F, Mareel MM, Birchmeier W. Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-cadherin/beta-catenin complex in cells transformed with a temperature-sensitive v-SRC gene. *J Cell Biol*. 1993; 120:757–766. [PubMed: 8425900]
- Bonifacino JS, Traub LM. Signals for Sorting of Transmembrane Proteins to Endosomes and Lysosomes. *Annual Review of Biochemistry*. 2003; 72:395–447.
- Bremm A, Walch A, Fuchs M, Mages J, Duyster J, Keller G, Hermannstadter C, Becker KF, Rauser S, Langer R, von Weyhern CH, Hofler H, Lubber B. Enhanced activation of epidermal growth factor receptor caused by tumor-derived E-cadherin mutations. *Cancer Res*. 2008; 68:707–714. [PubMed: 18245470]
- Brieher WM, Gumbiner BM. Regulation of C-cadherin function during activin induced morphogenesis of *Xenopus* animal caps. *J Cell Biol*. 1994; 126:519–527. [PubMed: 8034750]
- Brouxhon S, Kyrkanides S, O'Banion MK, Johnson R, Pearce DA, Centola GM, Miller JN, McGrath KH, Erdle B, Scott G, Schneider S, VanBuskirk J, Pentland AP. Sequential down-regulation of E-cadherin with squamous cell carcinoma progression: loss of E-cadherin via a prostaglandin E2-EP2 dependent posttranslational mechanism. *Cancer Res*. 2007; 67:7654–7664. [PubMed: 17699770]

- Bryant DM, Kerr MC, Hammond LA, Joseph SR, Mostov KE, Teasdale RD, Stow JL. EGF induces macropinocytosis and SNX1-modulated recycling of E-cadherin. *J Cell Sci.* 2007; 120:1818–1828. [PubMed: 17502486]
- Bryant DM, Wylie FG, Stow JL. Regulation of endocytosis, nuclear translocation, and signaling of fibroblast growth factor receptor 1 by E-cadherin. *Mol Biol Cell.* 2005; 16:14–23. [PubMed: 15509650]
- Calkins CC, Setzer SV, Jennings JM, Summers S, Tsunoda K, Amagai M, Kowalczyk AP. Desmoglein endocytosis and desmosome disassembly are coordinated responses to pemphigus autoantibodies. *J Biol Chem.* 2006; 281:7623–7634. [PubMed: 16377623]
- Chen YT, Stewart DB, Nelson WJ. Coupling assembly of the E-cadherin/beta-catenin complex to efficient endoplasmic reticulum exit and basal-lateral membrane targeting of E-cadherin in polarized MDCK cells. *J Cell Biol.* 1999; 144:687–699. [PubMed: 10037790]
- Chiasson, CM.; Kowalczyk, AP. *Cell Junctions.* Wiley-VCH Verlag GmbH & Co.; KGaA: 2008. *Cadherin Trafficking and Junction Dynamics.*; p. 251-270.
- Chiasson CM, Wittich KB, Vincent PA, Faundez V, Kowalczyk AP. p120-catenin inhibits VE-cadherin internalization through a Rho-independent mechanism. *Mol Biol Cell.* 2009; 20:1970–1980. [PubMed: 19211843]
- Clague MJ, Urbe S. Ubiquitin: same molecule, different degradation pathways. *Cell.* 2010; 143:682–685. [PubMed: 21111229]
- Classen AK, Anderson KI, Marois E, Eaton S. Hexagonal packing of *Drosophila* wing epithelial cells by the planar cell polarity pathway. *Dev Cell.* 2005; 9:805–817. [PubMed: 16326392]
- Damsky CH, Richa J, Solter D, Knudsen K, Buck CA. Identification and purification of a cell surface glycoprotein mediating intercellular adhesion in embryonic and adult tissue. *Cell.* 1983; 34:455–466. [PubMed: 6352050]
- Davis MA, Ireton RC, Reynolds AB. A core function for p120-catenin in cadherin turnover. *J Cell Biol.* 2003; 163:525–534. [PubMed: 14610055]
- Davis MA, Reynolds AB. Blocked acinar development, E-cadherin reduction, and intraepithelial neoplasia upon ablation of p120-catenin in the mouse salivary gland. *Dev Cell.* 2006; 10:21–31. [PubMed: 16399075]
- de Rooij J, Kerstens A, Danuser G, Schwartz MA, Waterman-Storer CM. Integrin-dependent actomyosin contraction regulates epithelial cell scattering. *J Cell Biol.* 2005; 171:153–164. [PubMed: 16216928]
- De Wever O, Derycke L, Hendrix A, De Meerleer G, Godeau F, Depypere H, Bracke M. Soluble cadherins as cancer biomarkers. *Clin Exp Metastasis.* 2007; 24:685–697. [PubMed: 17952616]
- Delva E, Jennings JM, Calkins CC, Kottke MD, Faundez V, Kowalczyk AP. Pemphigus vulgaris IgG-induced desmoglein-3 endocytosis and desmosomal disassembly are mediated by a clathrin- and dynamin-independent mechanism. *J Biol Chem.* 2008; 283:18303–18313. [PubMed: 18434319]
- Delva E, Kowalczyk AP. Regulation of cadherin trafficking. *Traffic.* 2009; 10:259–267. [PubMed: 19055694]
- Desclozeaux M, Venturato J, Wylie FG, Kay JG, Joseph SR, Le HT, Stow JL. Active Rab11 and functional recycling endosome are required for E-cadherin trafficking and lumen formation during epithelial morphogenesis. *Am J Physiol Cell Physiol.* 2008; 295:C545–556. [PubMed: 18579802]
- Dupre-Crochet S, Figueroa A, Hogan C, Ferber EC, Bialucha CU, Adams J, Richardson EC, Fujita Y. Casein kinase 1 is a novel negative regulator of E-cadherin-based cell-cell contacts. *Mol Cell Biol.* 2007; 27:3804–3816. [PubMed: 17353278]
- Elia LP, Yamamoto M, Zang K, Reichardt LF. p120 catenin regulates dendritic spine and synapse development through Rho-family GTPases and cadherins. *Neuron.* 2006; 51:43–56. [PubMed: 16815331]
- Ferber EC, Kajita M, Wadlow A, Tobiansky L, Niessen C, Ariga H, Daniel J, Fujita Y. A role for the cleaved cytoplasmic domain of E-cadherin in the nucleus. *J Biol Chem.* 2008; 283:12691–12700. [PubMed: 18356166]
- Ferreri DM, Minnear FL, Yin T, Kowalczyk AP, Vincent PA. N-cadherin levels in endothelial cells are regulated by monolayer maturity and p120 availability. *Cell Commun Adhes.* 2008; 15:333–349. [PubMed: 18979298]

- Fujita Y, Krause G, Scheffner M, Zechner D, Leddy HE, Behrens J, Sommer T, Birchmeier W. Hakai, a c-Cbl-like protein, ubiquitinates and induces endocytosis of the E-cadherin complex. *Nat Cell Biol.* 2002; 4:222–231. [PubMed: 11836526]
- Gavard J, Gutkind JS. VEGF controls endothelial-cell permeability by promoting the [beta]-arrestin-dependent endocytosis of VE-cadherin. *Nat Cell Biol.* 2006; 8:1223–1234. [PubMed: 17060906]
- Gavard J, Patel V, Gutkind JS. Angiopoietin-1 Prevents VEGF-Induced Endothelial Permeability by Sequestering Src through mDia. *Developmental Cell.* 2008; 14:25–36. [PubMed: 18194650]
- Grazia Lampugnani M, Zanetti A, Corada M, Takahashi T, Balconi G, Breviario F, Orsenigo F, Cattelino A, Kemler R, Daniel TO, Dejana E. Contact inhibition of VEGF-induced proliferation requires vascular endothelial cadherin, beta-catenin, and the phosphatase DEP-1/CD148. *J Cell Biol.* 2003; 161:793–804. [PubMed: 12771128]
- Guichard A, McGillivray SM, Cruz-Moreno B, van Sorge NM, Nizet V, Bier E. Anthrax toxins cooperatively inhibit endocytic recycling by the Rab11/Sec15 exocyst. *Nature.* 2010; 467:854–858. [PubMed: 20944747]
- Hartsock A, Nelson WJ. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim Biophys Acta.* 2008; 1778:660–669. [PubMed: 17854762]
- Hatzfeld M. The p120 family of cell adhesion molecules. *Eur J Cell Biol.* 2005; 84:205–214. [PubMed: 15819401]
- Hirohashi S. Inactivation of the E-cadherin-mediated cell adhesion system in human cancers. *Am J Pathol.* 1998; 153:333–339. [PubMed: 9708792]
- Ireton RC, Davis MA, van Hengel J, Mariner DJ, Barnes K, Thoreson MA, Anastasiadis PZ, Matrisian L, Bundy LM, Sealy L, Gilbert B, van Roy F, Reynolds AB. A novel role for p120 catenin in E-cadherin function. *J Cell Biol.* 2002; 159:465–476. [PubMed: 12427869]
- Ishiyama N, Lee SH, Liu S, Li GY, Smith MJ, Reichardt LF, Ikura M. Dynamic and static interactions between p120 catenin and E-cadherin regulate the stability of cell-cell adhesion. *Cell.* 2010; 141:117–128. [PubMed: 20371349]
- Ivanov AI, Nusrat A, Parkos CA. Endocytosis of epithelial apical junctional proteins by a clathrin-mediated pathway into a unique storage compartment. *Mol Biol Cell.* 2004; 15:176–188. [PubMed: 14528017]
- Izumi G, Sakisaka T, Baba T, Tanaka S, Morimoto K, Takai Y. Endocytosis of E-cadherin regulated by Rac and Cdc42 small G proteins through IQGAP1 and actin filaments. *J Cell Biol.* 2004; 166:237–248. [PubMed: 15263019]
- Janda E, Nevolo M, Lehmann K, Downward J, Beug H, Grieco M. Raf plus TGFbeta-dependent EMT is initiated by endocytosis and lysosomal degradation of E-cadherin. *Oncogene.* 2006; 25:7117–7130. [PubMed: 16751808]
- Jarrett O, Stow JL, Yap AS, Key B. Dynamin-dependent endocytosis is necessary for convergent-extension movements in *Xenopus* animal cap explants. *Int J Dev Biol.* 2002; 46:467–473. [PubMed: 12141433]
- Kaido M, Wada H, Shindo M, Hayashi S. Essential requirement for RING finger E3 ubiquitin ligase Hakai in early embryonic development of *Drosophila*. *Genes Cells.* 2009; 14:1067–1077. [PubMed: 19682089]
- Kamei T, Matozaki T, Sakisaka T, Kodama A, Yokoyama S, Peng YF, Nakano K, Takaishi K, Takai Y. Coendocytosis of cadherin and c-Met coupled to disruption of cell-cell adhesion in MDCK cells--regulation by Rho, Rac and Rab small G proteins. *Oncogene.* 1999; 18:6776–6784. [PubMed: 10597286]
- Kartenbeck J, Schmelz M, Franke WW, Geiger B. Endocytosis of junctional cadherins in bovine kidney epithelial (MDBK) cells cultured in low Ca²⁺ ion medium. *J Cell Biol.* 1991; 113:881–892. [PubMed: 2026652]
- Kartenbeck J, Schmid E, Franke WW, Geiger B. Different modes of internalization of proteins associated with adherens junctions and desmosomes: experimental separation of lateral contacts induces endocytosis of desmosomal plaque material. *EMBO J.* 1982; 1:725–732. [PubMed: 6821357]

- Kawauchi T, Sekine K, Shikanai M, Chihama K, Tomita K, Kubo K, Nakajima K, Nabeshima Y, Hoshino M. Rab GTPases-dependent endocytic pathways regulate neuronal migration and maturation through N-cadherin trafficking. *Neuron*. 2010; 67:588–602. [PubMed: 20797536]
- Klessner JL, Desai BV, Amargo EV, Getsios S, Green KJ. EGFR and ADAMs cooperate to regulate shedding and endocytic trafficking of the desmosomal cadherin desmoglein 2. *Mol Biol Cell*. 2009; 20:328–337. [PubMed: 18987342]
- Klucky B, Mueller R, Vogt I, Teurich S, Hartenstein B, Breuhahn K, Flechtenmacher C, Angel P, Hess J. Kallikrein 6 induces E-cadherin shedding and promotes cell proliferation, migration, and invasion. *Cancer Res*. 2007; 67:8198–8206. [PubMed: 17804733]
- Lampugnani MG, Orsenigo F, Gagliani MC, Tacchetti C, Dejana E. Vascular endothelial cadherin controls VEGFR-2 internalization and signaling from intracellular compartments. *J Cell Biol*. 2006; 174:593–604. [PubMed: 16893970]
- Langevin J, Morgan MJ, Sibarita JB, Aresta S, Murthy M, Schwarz T, Camonis J, Bellaiche Y. Drosophila exocyst components Sec5, Sec6, and Sec15 regulate DE Cadherin trafficking from recycling endosomes to the plasma membrane. *Dev Cell*. 2005; 9:365–376. [PubMed: 16224820]
- Lau KM, McGlade CJ. Numb is a negative regulator of HGF dependent cell scattering and Rac1 activation. *Exp Cell Res*. 2011; 317:539–551. [PubMed: 21147098]
- Lawler K, O'Sullivan G, Long A, Kenny D. Shear stress induces internalization of E-cadherin and invasiveness in metastatic oesophageal cancer cells by a Src-dependent pathway. *Cancer Sci*. 2009; 100:1082–1087. [PubMed: 19432901]
- Le TL, Yap AS, Stow JL. Recycling of E-cadherin: a potential mechanism for regulating cadherin dynamics. *J Cell Biol*. 1999; 146:219–232. [PubMed: 10402472]
- Leher MM, Lutz MP, Weidenbach H, Muller-Pillasch F, Gress TM, Leser J, Adler G. Dissociation and reassembly of adherens junctions during experimental acute pancreatitis. *Gastroenterology*. 1997; 113:1355–1366. [PubMed: 9322531]
- Levayer R, Pelissier-Monier A, Lecuit T. Spatial regulation of Dia and Myosin-II by RhoGEF2 controls initiation of E-cadherin endocytosis during epithelial morphogenesis. *Nat Cell Biol*. 2011; 13:529–540. [PubMed: 21516109]
- Ling K, Bairstow SF, Carbonara C, Turbin DA, Huntsman DG, Anderson RA. Type I gamma phosphatidylinositol phosphate kinase modulates adherens junction and E-cadherin trafficking via a direct interaction with mu 1B adaptin. *J Cell Biol*. 2007; 176:343–353. [PubMed: 17261850]
- Lock JG, Stow JL. Rab11 in recycling endosomes regulates the sorting and basolateral transport of E-cadherin. *Mol Biol Cell*. 2005; 16:1744–1755. [PubMed: 15689490]
- Lu Z, Ghosh S, Wang Z, Hunter T. Downregulation of caveolin-1 function by EGF leads to the loss of E-cadherin, increased transcriptional activity of beta-catenin, and enhanced tumor cell invasion. *Cancer Cell*. 2003; 4:499–515. [PubMed: 14706341]
- Maeda M, Johnson E, Mandal SH, Lawson KR, Keim SA, Svoboda RA, Caplan S, Wahl JK 3rd, Wheelock MJ, Johnson KR. Expression of inappropriate cadherins by epithelial tumor cells promotes endocytosis and degradation of E-cadherin via competition for p120(ctn). *Oncogene*. 2006; 25:4595–4604. [PubMed: 16786001]
- Mansouri M, Rose PP, Moses AV, Fruh K. Remodeling of endothelial adherens junctions by Kaposi's sarcoma-associated herpesvirus. *J Virol*. 2008; 82:9615–9628. [PubMed: 18667499]
- Marambaud P, Shioi J, Serban G, Georgakopoulos A, Sarner S, Nagy V, Baki L, Wen P, Efthimiopoulos S, Shao Z, Wisniewski T, Robakis NK. A presenilin-1/gamma-secretase cleavage releases the E-cadherin intracellular domain and regulates disassembly of adherens junctions. *EMBO J*. 2002; 21:1948–1956. [PubMed: 11953314]
- Marambaud P, Wen PH, Dutt A, Shioi J, Takashima A, Siman R, Robakis NK. A CBP binding transcriptional repressor produced by the PS1/epsilon-cleavage of N-cadherin is inhibited by PS1 FAD mutations. *Cell*. 2003; 114:635–645. [PubMed: 13678586]
- Marciano DK, Brakeman PR, Lee CZ, Spivak N, Eastburn DJ, Bryant DM, Beaudoin GM 3rd, Hofmann I, Mostov KE, Reichardt LF. p120 catenin is required for normal renal tubulogenesis and glomerulogenesis. *Development*. 2011; 138:2099–2109. [PubMed: 21521738]
- Maretzky T, Reiss K, Ludwig A, Buchholz J, Scholz F, Proksch E, de Strooper B, Hartmann D, Saftig P. ADAM10 mediates E-cadherin shedding and regulates epithelial cell-cell adhesion, migration,

- and beta-catenin translocation. *Proc Natl Acad Sci U S A*. 2005; 102:9182–9187. [PubMed: 15958533]
- Mattey DL, Garrod DR. Splitting and internalization of the desmosomes of cultured kidney epithelial cells by reduction in calcium concentration. *J Cell Sci*. 1986; 85:113–124. [PubMed: 3793787]
- McCrea PD, Gumbiner BM. Purification of a 92-kDa cytoplasmic protein tightly associated with the cell-cell adhesion molecule E-cadherin (uvomorulin). Characterization and extractability of the protein complex from the cell cytostructure. *J Biol Chem*. 1991; 266:4514–4520. [PubMed: 1999432]
- McGuire JK, Li Q, Parks WC. Matrilysin (matrix metalloproteinase-7) mediates E-cadherin ectodomain shedding in injured lung epithelium. *Am J Pathol*. 2003; 162:1831–1843. [PubMed: 12759241]
- Miller JR, McClay DR. Characterization of the role of cadherin in regulating cell adhesion during sea urchin development. *Dev Biol*. 1997; 192:323–339. [PubMed: 9441671]
- Miranda KC, Khromykh T, Christy P, Le TL, Gottardi CJ, Yap AS, Stow JL, Teasdale RD. A dileucine motif targets E-cadherin to the basolateral cell surface in Madin-Darby canine kidney and LLC-PK1 epithelial cells. *J Biol Chem*. 2001; 276:22565–22572. [PubMed: 11312273]
- Miyashita Y, Ozawa M. A dileucine motif in its cytoplasmic domain directs beta-catenin-uncoupled E-cadherin to the lysosome. *J Cell Sci*. 2007a; 120:4395–4406. [PubMed: 18057030]
- Miyashita Y, Ozawa M. Increased internalization of p120-uncoupled E-cadherin and a requirement for a dileucine motif in the cytoplasmic domain for endocytosis of the protein. *J Biol Chem*. 2007b; 282:11540–11548. [PubMed: 17298950]
- Myster SH, Cavallo R, Anderson CT, Fox DT, Peifer M. Drosophila p120catenin plays a supporting role in cell adhesion but is not an essential adherens junction component. *J Cell Biol*. 2003; 160:433–449. [PubMed: 12551951]
- Nagafuchi A, Ishihara S, Tsukita S. The roles of catenins in the cadherin-mediated cell adhesion: functional analysis of E-cadherin-alpha catenin fusion molecules. *J Cell Biol*. 1994; 127:235–245. [PubMed: 7929566]
- Nathan JA, Lehner PJ. The trafficking and regulation of membrane receptors by the RING-CH ubiquitin E3 ligases. *Experimental Cell Research*. 2009; 315:1593–1600. [PubMed: 19013150]
- Noe V, Fingleton B, Jacobs K, Crawford HC, Vermeulen S, Steelant W, Bruyneel E, Matrisian LM, Mareel M. Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1. *J Cell Sci*. 2001; 114:111–118. [PubMed: 11112695]
- Noren NK, Liu BP, Burrridge K, Kreft B. p120 catenin regulates the actin cytoskeleton via Rho family GTPases. *J Cell Biol*. 2000; 150:567–580. [PubMed: 10931868]
- Nose A, Nagafuchi A, Takeichi M. Expressed recombinant cadherins mediate cell sorting in model systems. *Cell*. 1988; 54:993–1001. [PubMed: 3416359]
- Oas RG, Xiao K, Summers S, Wittich KB, Chiasson CM, Martin WD, Grossniklaus HE, Vincent PA, Reynolds AB, Kowalczyk AP. p120-Catenin is required for mouse vascular development. *Circ Res*. 2010; 106:941–951. [PubMed: 20110533]
- Oda H, Tsukita S, Takeichi M. Dynamic behavior of the cadherin-based cell-cell adhesion system during Drosophila gastrulation. *Dev Biol*. 1998; 203:435–450. [PubMed: 9808792]
- Ogata S, Morokuma J, Hayata T, Kolle G, Niehrs C, Ueno N, Cho KW. TGF-beta signaling-mediated morphogenesis: modulation of cell adhesion via cadherin endocytosis. *Genes Dev*. 2007; 21:1817–1831. [PubMed: 17639085]
- Pacquelet A, Lin L, Rorth P. Binding site for p120/delta-catenin is not required for Drosophila E-cadherin function in vivo. *J Cell Biol*. 2003; 160:313–319. [PubMed: 12551956]
- Pacquelet A, Rorth P. Regulatory mechanisms required for DE-cadherin function in cell migration and other types of adhesion. *J Cell Biol*. 2005; 170:803–812. [PubMed: 16129787]
- Palacios F, Price L, Schweitzer J, Collard JG, D'Souza-Schorey C. An essential role for ARF6-regulated membrane traffic in adherens junction turnover and epithelial cell migration. *EMBO J*. 2001; 20:4973–4986. [PubMed: 11532961]
- Palacios F, Tushir JS, Fujita Y, D'Souza-Schorey C. Lysosomal targeting of E-cadherin: a unique mechanism for the down-regulation of cell-cell adhesion during epithelial to mesenchymal transitions. *Mol Cell Biol*. 2005; 25:389–402. [PubMed: 15601859]

- Paradies NE, Grunwald GB. Purification and characterization of NCAD90, a soluble endogenous form of N-cadherin, which is generated by proteolysis during retinal development and retains adhesive and neurite-promoting function. *J Neurosci Res.* 1993; 36:33–45. [PubMed: 8230319]
- Paterson AD, Parton RG, Ferguson C, Stow JL, Yap AS. Characterization of E-cadherin endocytosis in isolated MCF-7 and chinese hamster ovary cells: the initial fate of unbound E-cadherin. *J Biol Chem.* 2003; 278:21050–21057. [PubMed: 12657640]
- Peinado H, Portillo F, Cano A. Transcriptional regulation of cadherins during development and carcinogenesis. *Int J Dev Biol.* 2004; 48:365–375. [PubMed: 15349812]
- Perez-Moreno M, Davis MA, Wong E, Pasolli HA, Reynolds AB, Fuchs E. p120-catenin mediates inflammatory responses in the skin. *Cell.* 2006; 124:631–644. [PubMed: 16469707]
- Pettitt J, Cox EA, Broadbent ID, Flett A, Hardin J. The *Caenorhabditis elegans* p120 catenin homologue, JAC-1, modulates cadherin-catenin function during epidermal morphogenesis. *J Cell Biol.* 2003; 162:15–22. [PubMed: 12847081]
- Pittard AJ, Banks RE, Galley HF, Webster NR. Soluble E-cadherin concentrations in patients with systemic inflammatory response syndrome and multiorgan dysfunction syndrome. *Br J Anaesth.* 1996; 76:629–631. [PubMed: 8688260]
- Qian L-W, Greene W, Ye F, Gao S-J. Kaposi's Sarcoma-Associated Herpesvirus Disrupts Adherens Junctions and Increases Endothelial Permeability by Inducing Degradation of VE-Cadherin. *J Virol.* 2008; 82:11902–11912. [PubMed: 18815301]
- Rasin MR, Gazula VR, Breunig JJ, Kwan KY, Johnson MB, Liu-Chen S, Li HS, Jan LY, Jan YN, Rakic P, Sestan N. Numb and Numbl are required for maintenance of cadherin-based adhesion and polarity of neural progenitors. *Nat Neurosci.* 2007; 10:819–827. [PubMed: 17589506]
- Reiss K, Maretzky T, Ludwig A, Tousseyn T, de Strooper B, Hartmann D, Saftig P. ADAM10 cleavage of N-cadherin and regulation of cell-cell adhesion and beta-catenin nuclear signalling. *EMBO J.* 2005; 24:742–752. [PubMed: 15692570]
- Reynolds AB. p120-catenin: Past and present. *Biochim Biophys Acta.* 2007; 1773:2–7. [PubMed: 17175391]
- Reynolds AB, Daniel JM, Mo YY, Wu J, Zhang Z. The novel catenin p120cas binds classical cadherins and induces an unusual morphological phenotype in NIH3T3 fibroblasts. *Exp Cell Res.* 1996; 225:328–337. [PubMed: 8660921]
- Roeth JF, Sawyer JK, Wilner DA, Peifer M. Rab11 helps maintain apical crumbs and adherens junctions in the *Drosophila* embryonic ectoderm. *PLoS ONE.* 2009; 4:e7634. [PubMed: 19862327]
- Sato K, Watanabe T, Wang S, Kakeno M, Matsuzawa K, Matsui T, Yokoi K, Murase K, Sugiyama I, Ozawa M, Kaibuchi K. Numb controls E-cadherin endocytosis through p120 catenin with aPKC. *Mol Biol Cell.* 2011
- Schulte D, Kuppers V, Dartsch N, Broermann A, Li H, Zarbock A, Kamenyeva O, Kiefer F, Khandoga A, Massberg S, Vestweber D. Stabilizing the VE-cadherin-catenin complex blocks leukocyte extravasation and vascular permeability. *EMBO J.* 2011
- Sharma M, Henderson BR. IQ-domain GTPase-activating protein 1 regulates beta-catenin at membrane ruffles and its role in macropinocytosis of N-cadherin and adenomatous polyposis coli. *J Biol Chem.* 2007; 282:8545–8556. [PubMed: 17255093]
- Shore EM, Nelson WJ. Biosynthesis of the cell adhesion molecule uvomorulin (E-cadherin) in Madin-Darby canine kidney epithelial cells. *J Biol Chem.* 1991; 266:19672–19680. [PubMed: 1918074]
- Shoval I, Ludwig A, Kalcheim C. Antagonistic roles of full-length N-cadherin and its soluble BMP cleavage product in neural crest delamination. *Development.* 2007; 134:491–501. [PubMed: 17185320]
- Smalley-Freed WG, Efimov A, Burnett PE, Short SP, Davis MA, Gumucio DL, Washington MK, Coffey RJ, Reynolds AB. p120-catenin is essential for maintenance of barrier function and intestinal homeostasis in mice. *J Clin Invest.* 2010; 120:1824–1835. [PubMed: 20484816]
- Solanas G, Cortina C, Sevillano M, Batlle E. Cleavage of E-cadherin by ADAM10 mediates epithelial cell sorting downstream of EphB signalling. *Nat Cell Biol.* 2011; 13:1100–1107. [PubMed: 21804545]

- Stairs DB, Bayne LJ, Rhoades B, Vega ME, Waldron TJ, Kalabis J, Klein-Szanto A, Lee JS, Katz JP, Diehl JA, Reynolds AB, Vonderheide RH, Rustgi AK. Deletion of p120-catenin results in a tumor microenvironment with inflammation and cancer that establishes it as a tumor suppressor gene. *Cancer Cell*. 2011; 19:470–483. [PubMed: 21481789]
- Steinberg MS, Takeichi M. Experimental specification of cell sorting, tissue spreading, and specific spatial patterning by quantitative differences in cadherin expression. *Proc Natl Acad Sci U S A*. 1994; 91:206–209. [PubMed: 8278366]
- Suyama K, Shapiro I, Guttman M, Hazan RB. A signaling pathway leading to metastasis is controlled by N-cadherin and the FGF receptor. *Cancer Cell*. 2002; 2:301–314. [PubMed: 12398894]
- Tai CY, Mysore SP, Chiu C, Schuman EM. Activity-regulated N-cadherin endocytosis. *Neuron*. 2007; 54:771–785. [PubMed: 17553425]
- Toyoshima M, Tanaka N, Aoki J, Tanaka Y, Murata K, Kyuuma M, Kobayashi H, Ishii N, Yaegashi N, Sugamura K. Inhibition of tumor growth and metastasis by depletion of vesicular sorting protein Hrs: its regulatory role on E-cadherin and beta-catenin. *Cancer Res*. 2007; 67:5162–5171. [PubMed: 17545595]
- Traub LM. Sorting it out: AP-2 and alternate clathrin adaptors in endocytic cargo selection. *J Cell Biol*. 2003; 163:203–208. [PubMed: 14581447]
- Troyanovsky RB, Sokolov EP, Troyanovsky SM. Endocytosis of cadherin from intracellular junctions is the driving force for cadherin adhesive dimer disassembly. *Mol Biol Cell*. 2006; 17:3484–3493. [PubMed: 16760429]
- Ulrich F, Krieg M, Schotz EM, Link V, Castanon I, Schnabel V, Taubenberger A, Mueller D, Puech PH, Heisenberg CP. Wnt11 functions in gastrulation by controlling cell cohesion through Rab5c and E-cadherin. *Dev Cell*. 2005; 9:555–564. [PubMed: 16198297]
- Veiga E, Cossart P. Listeria hijacks the clathrin-dependent endocytic machinery to invade mammalian cells. *Nat Cell Biol*. 2005; 7:894–900. [PubMed: 16113677]
- Wang Q, Chen XW, Margolis B. PALS1 regulates E-cadherin trafficking in mammalian epithelial cells. *Mol Biol Cell*. 2007; 18:874–885. [PubMed: 17182851]
- Wang Z, Sandiford S, Wu C, Li SS. Numb regulates cell-cell adhesion and polarity in response to tyrosine kinase signalling. *EMBO J*. 2009; 28:2360–2373. [PubMed: 19609305]
- Wheelock MJ, Buck CA, Bechtol KB, Damsky CH. Soluble 80-kd fragment of cell-CAM 120/80 disrupts cell-cell adhesion. *J Cell Biochem*. 1987; 34:187–202. [PubMed: 3611200]
- Xiao K, Allison DF, Buckley KM, Kottke MD, Vincent PA, Faundez V, Kowalczyk AP. Cellular levels of p120 catenin function as a set point for cadherin expression levels in microvascular endothelial cells. *J Cell Biol*. 2003a; 163:535–545. [PubMed: 14610056]
- Xiao K, Allison DF, Kottke MD, Summers S, Sorescu GP, Faundez V, Kowalczyk AP. Mechanisms of VE-cadherin processing and degradation in microvascular endothelial cells. *J Biol Chem*. 2003b; 278:19199–19208. [PubMed: 12626512]
- Xiao K, Garner J, Buckley KM, Vincent PA, Chiasson CM, Dejana E, Faundez V, Kowalczyk AP. p120-Catenin regulates clathrin-dependent endocytosis of VE-cadherin. *Mol Biol Cell*. 2005; 16:5141–5151. [PubMed: 16120645]
- Xie LQ, Bian LJ, Li Z, Li Y, Li ZX, Li B. Altered expression of E-cadherin by hepatocyte growth factor and effect on the prognosis of nasopharyngeal carcinoma. *Ann Surg Oncol*. 2010; 17:1927–1936. [PubMed: 20131016]
- Yabuta T, Shinmura K, Tani M, Yamaguchi S, Yoshimura K, Katai H, Nakajima T, Mochiki E, Tsujinaka T, Takami M, Hirose K, Yamaguchi A, Takenoshita S, Yokota J. E-cadherin gene variants in gastric cancer families whose probands are diagnosed with diffuse gastric cancer. *Int J Cancer*. 2002; 101:434–441. [PubMed: 12216071]
- Yamada S, Pokutta S, Drees F, Weis WI, Nelson WJ. Deconstructing the cadherin-catenin-actin complex. *Cell*. 2005; 123:889–901. [PubMed: 16325582]
- Yang DH, Cai KQ, Roland IH, Smith ER, Xu XX. Disabled-2 is an epithelial surface positioning gene. *J Biol Chem*. 2007; 282:13114–13122. [PubMed: 17339320]
- Yang JY, Zong CS, Xia W, Wei Y, Ali-Seyed M, Li Z, Broglio K, Berry DA, Hung MC. MDM2 promotes cell motility and invasiveness by regulating E-cadherin degradation. *Mol Cell Biol*. 2006; 26:7269–7282. [PubMed: 16980628]

- Zhong Y, Brieher WM, Gumbiner BM. Analysis of C-cadherin regulation during tissue morphogenesis with an activating antibody. *J Cell Biol.* 1999; 144:351–359. [PubMed: 9922460]
- Zhou WJ, Geng ZH, Chi S, Zhang W, Niu XF, Lan SJ, Ma L, Yang X, Wang LJ, Ding YQ, Geng JG. Slit-Robo signaling induces malignant transformation through Hakai-mediated E-cadherin degradation during colorectal epithelial cell carcinogenesis. *Cell Res.* 2011; 21:609–626. [PubMed: 21283129]

Figure 1. Cadherin trafficking pathways

Cadherin internalization can occur through either clathrin-mediated, caveolin-mediated, or macropinocytosis-like pathways. Internalized cadherin is then sorted either for lysosomal degradation or recycling back to the plasma membrane.

Figure 2. p120-catenin regulates cadherin endocytosis

A. p120 acts as a “set-point” for cadherin levels. Increased expression of a second cadherin type competes for p120 binding, causing the internalization of the first cadherin type. This activity allows p120 to serve as a master regulator of cadherin expression in cells (Ferreri et al., 2008; Maeda et al., 2006; Xiao et al., 2003a; Xiao et al., 2005). B. p120 binds to cadherins and masks an endocytic adaptor binding site. When p120 dissociates from the cadherin, the adaptor binding site is exposed, allowing the endocytic adaptor to bind to the cadherin, triggering cadherin endocytosis (Chiasson et al., 2009; Ishiyama et al., 2010).

Figure 3. Growth factor signaling pathways influence cadherin endocytosis

A. HGF activation of c-Met causes co-endocytosis of the receptor with E-cadherin (Kamei et al., 1999; Palacios et al., 2001). B. EGFR over-expression induces E-cadherin endocytosis and E-cadherin binding to the receptor inhibits EGFR signaling (Bremm et al., 2008; Bryant et al., 2007; Lu et al., 2003). C. VEGFR activation triggers the phosphorylation of VE-cadherin through a Src, Vav2, Rac, and PAK signaling cascade. Phosphorylated VE-cadherin recruits β -arrestin and triggers clathrin-mediated endocytosis of the cadherin (Gavard and Gutkind, 2006; Gavard et al., 2008). Conversely, VE-cadherin inhibits the internalization of VEGFR into signaling compartments upon ligand binding (Lampugnani et al., 2006). D. FGFR activation induces E-cadherin endocytosis, and cadherins inhibit the endocytosis and degradation of FGFR, forming a negative feedback loop (Bryant et al., 2007; Bryant et al., 2005; Suyama et al., 2002).

Table 1

Tissue-specific p120-null mouse models display phenotypes characteristic of decreased cadherin levels and impaired intercellular adhesion.

Tissue / Cell Type	Phenotype	Reference
Salivary gland	E-cadherin levels reduced; acinar development blocked	(Davis and Reynolds, 2006)
Skin	Reduced levels of cadherins and other adherens junction proteins; chronic inflammation due to NF κ B activation	(Perez-Moreno et al., 2006)
Hippocampal neurons	Decreased cadherin levels; fewer synapses	(Elia et al., 2006)
Endothelium	VE-cadherin and N-cadherin levels reduced; vascular patterning defects and hemorrhaging	(Oas et al., 2010)
Intestinal epithelium	Down-regulation of adherens junction proteins; compromised barrier function	(Smalley-Freed et al., 2010)
Oropharyngeal epithelium	Decreased E-cadherin expression; development of invasive squamous cell carcinoma	(Stairs et al., 2011)
Kidney	Decreased cadherin levels; impaired tubule morphogenesis; development of cystic kidney disease	(Marciano et al., 2011)