Cadherins: Cellular adhesive molecules serving as signaling mediators

Mark Yulis[1], Dennis H.M. Kusters[1], Asma Nusrat[1#]

[1]Department of Pathology, The University of Michigan, Ann Arbor, Michigan 48109, USA

[#]Corresponding Author

Asma Nusrat

Department of Pathology

The University of Michigan

109 Zina Pitcher Place, Room 4057

Ann Arbor, Michigan 48109.

E-mail: anusrat@umich.edu

Mark Yulis received his BS in Biochemistry at Muhlenberg College in Pennsylvania and his PhD at Emory University. His studies examine mechanisms by which inflammatory mediators promote desmoglein 2 cadherin cleavage to influence epithelial apoptosis. **Dennis Kusters** received his PhD in Biochemistry at the University of Maastricht in the Netherlands. For his postdoctoral training at the University of Michigan he is investigating the role of desmosomal cadherins in controlling intestinal epithelial homeostasis. **Asma Nusrat's** research group investigates mechanisms by which intercellular junction proteins control epithelial homeostasis and barrier function, pathobiology of chronic mucosal inflammation and mechanisms of wound repair.

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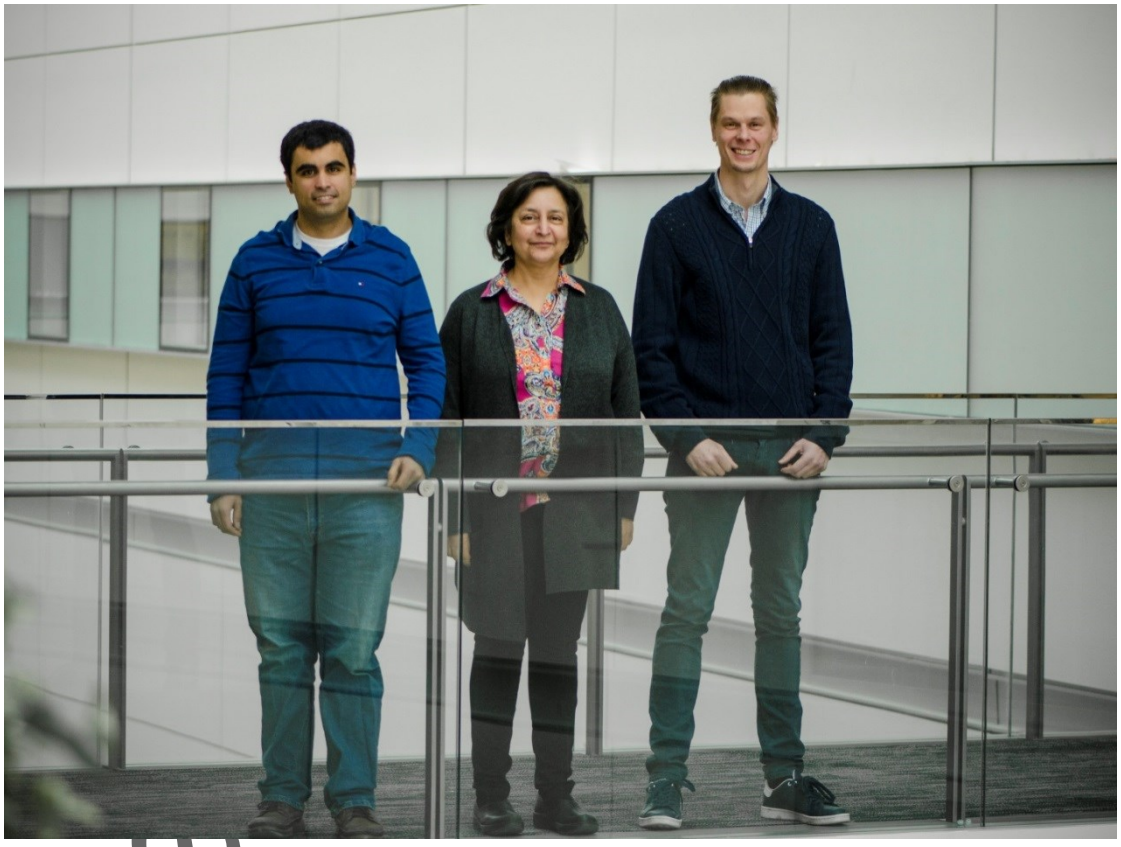

Abstract

The single pass, transmembrane proteins of the cadherin family have been appreciated as important proteins that regulate intercellular adhesion. In addition to this critical function, cadherins contribute to important signaling events that control cellular homeostasis. Many examples exist of classical, desmosomal, and atypical cadherins participating in the regulation of signaling events that control homeostatic functions in cells. Much of the work on cadherin mediated signaling focuses on classical cadherins or on specific disease states such as pemphigus vulgaris. Cadherin mediated signaling has been shown to play critical roles during development, in proliferation, apoptosis, disease pathobiology, and beyond. It is becoming increasingly clear that cadherins operate through a range of molecular mechanisms. The diversity of pathways and cellular functions regulated by cadherins suggests that we have

only scratched the surface in terms of the roles that these versatile proteins play in signaling and cellular function.

Introduction

Cadherins serve as calcium dependent transmembrane adhesion proteins and are subdivided into classical-, proto-, desmosomal, and atypical cadherins (Harris & Tepass, 2010). The classical and desmosomal cadherins have an ectodomain composed of five extracellular cadherin repeats, a single transmembrane region, and a cytoplasmic domain that interacts with either β-catenin (classical cadherins) or plakoglobin (Pg) (desmosomal and classical cadherins) (Shapiro & Weis, 2009). In addition to the classical adhesive function of cadherins, they contribute to a number of other cellular processes including cellular signaling (Kovacs, 2003; Wheelock & Johnson, 2003; Heuberger & Birchmeier, 2010). Dynamic changes in cell adhesion are needed to establish and re-establish cell-cell contacts in multiple contexts, including developmental cell movements, tissue renewal, and wound repair (Harris & Tepass, 2010; Pandya *et al.*, 2017). These processes require cells to integrate and process a variety of signals from the external- as well as the internal milieu (**Table 1**). In many cases, the precise mechanism by which the cadherins participate in a signaling pathway to influence cellular outcome is not fully understood, even though their involvement in the pathway is clearly demonstrated. One of the most well characterized signaling functions of cadherins is by acting as scaffolds to either promote or inhibit certain signaling pathways, which most often depend on the presence of the cadherin in the junction or its adhesion state. Through these and other mechanisms, cadherins participate in regulation of cellular homeostatic events that encompass proliferation, differentiation and apoptosis. For the purposes of this review, we define "homeostatic pathways" as pathways that regulate proliferation, cell death, or the

differentiation of cells. We are also including mechanisms that that may be required for cells to maintain a homeostatic state (i.e. glucose metabolism) as "non-homeostatic" though they can play a role in homeostasis. In this review, we will discuss the emergence of distinct classes of cadherin mediated signaling mechanisms. Mechanisms that are directly dependent upon changes in the adhesion state or junctional residence of cadherins are discussed first. We will refer to these as adhesion or junctional residence dependent cadherin signaling mechanisms. Thereafter, mechanisms that are not themselves dependent upon a change in adhesion are discussed.

A) Cadherin mediated regulation of proliferation

Cadherins have been implicated in the regulation of cell proliferation through interaction with a wide range of signaling mediators. One example, and arguably the most classic paradigm of cadherin mediated signaling, is by modulating β-catenin signaling (Harris & Tepass, 2010; Clevers & Nusse, 2012). The classical cadherins E-cadherin, N-cadherin, and VE-cadherin can prevent nuclear localization of β-catenin by direct interaction, thereby inhibiting TCF/LEF mediated stimulation of proliferation (Solanas *et al.*, 2008; Kourtidis *et al.*, 2017). In addition to sequestration through direct binding, E-cadherin can suppress β-catenin signaling through other mechanisms. For example, re-expression of E-cadherin in SW480 colon carcinoma cells leads to increased β-catenin phosphorylation and co-localization at the plasma membrane with APC, thus promoting β-catenin degradation (Maher *et al.*, 2009). Furthermore, the authors show that neither phosphorylated β-catenin nor members of the destruction complex co-fractionate with E-cadherin suggesting that regulation of β-catenin stability by E-cadherin can be independent of direct binding (Maher *et al.*, 2009). However, another study found that re-expression of E-cadherin in this same cell line results in repression of fibronectin and LEF1 gene expression through prevention of β-

catenin nuclear localization via direct binding (Solanas *et al.*, 2008). These data suggest that E-cadherin can regulate the function of β-catenin through multiple functionally distinct mechanisms within the same cell type. Additionally, depending on the circumstances and the mechanism used, E-cadherin can either promote or repress β-catenin signaling and therefore proliferation. Such differences highlight the intricacies and complexities of cadherin mediated signaling. Binding of E-cadherin to β-catenin also affects other signaling pathways in addition to TCF/LEF mediated transcriptional regulation. E-cadherin engagement directly regulates the Hippo signaling pathway in a β-catenin dependent manner through prevention of YAP nuclear localization, thereby inhibiting cell proliferation (Kim *et al.*, 2011a). Howard *et al.* noted that when comparing two common model epithelial cell lines, MDCK and HEK293, MDCK cells are more classically "epithelial" and have well developed tight and adherens junctions, whereas the HEK293 cells lack such properties. Exposure of MDCK cells to hepatocyte growth factor (HGF) results in transition from the epithelial phenotype to one resembling HEK293s. While treatment of standard MDCK cells with Wnt3a does not noticeably affect β-catenin signaling, pretreatment with HGF (or treatment of HEK293 cells) increases β-catenin signaling compared to standard MDCK cells. E-cadherin knock-down in the HGF pretreated MDCK cells or in the HEK293 cells abolishes these signaling effects (Howard *et al.*, 2011). These findings suggest that when epithelial cells transition to a more mesenchymal phenotype, the ability of Wnt ligands to activate β-catenin signaling is enhanced and this effect is dependent on E-cadherin. In light of the results discussed above, the outcome of E-cadherin on β-catenin signaling is dependent on whether or not the cells exhibit an epithelial- or a mesenchymal phenotype. During development several populations of cells undergo an epithelial to mesenchymal transition (EMT), including epiblast cells in the primitive streak(Yoshikawa *et al.*, 1997; Chapman & Papaioannou, 1998). As part of this process, these cells switch from predominantly expressing E-cadherin to expressing N-

cadherin with increased Wnt/β-catenin signaling that has been linked to differentiation of these migrated cells into the mesoderm. To test the functional relevance of their *in vitro* findings, the authors analyzed the levels of the Wnt/ β-catenin target gene *Tbx6* (known to be important for the differentiation of migrated epiblasts into mesoderm) in N-cadherin knock-out mouse embryos(Yoshikawa *et al.*, 1997; Chapman & Papaioannou, 1998). Reduced *Tbx6* expression was observed in these knock-out mice compared to controls, suggesting that cadherin mediated increase of Wnt/β-catenin signaling may be required for critical steps in embryonic development (Howard *et al.*, 2011). Together, these data suggest that through modulation of β-catenin activity, E-cadherin can participate in a variety of signal transduction pathways that influences cellular outcomes.

In addition to regulation of β-catenin, E-cadherin influences proliferation through other pathways as well. For example, engagement of E-cadherin either through direct cell-cell contact or through application of E-cadherin Fc coated beads in the absence of cell spreading enhances the number of cells in proliferative S-phase compared to controls. This increase in proliferation was found to be dependent upon the activation of Rac1 and was lost upon E-cadherin knock-down or treatment with the E-cadherin adhesion inhibitory antibody HECD1 (Liu *et al.*, 2006). Such observations suggest that the E-cadherin mediated regulation of proliferation is controlled by signaling events that are directly dependent on its adhesive role in adherens junctions and not due to the steric blockage of cell spreading. Knock-down of the adherens junction associated protein PLEKHA7 in Caco2 model intestinal epithelial cells results in increased cadherin-11, phopsho-p130CAS, cyclin D1, SNAI1, and c-Myc protein levels. Additional knock-down of E-cadherin or preventing the increase in cadherin-11 via shRNA reverses these changes (Kourtidis *et al.*, 2015). Although the authors focus more on the mechanism through which PLEKHA7 itself participates in this signaling pathway, it is clear that the effects of PLEKHA7 loss are dependent on the presence of cadherins.

Besides E-cadherin, other cadherins regulate cell proliferation through a variety of mechanisms. VE-cadherin inhibits cell growth by binding to a variety of growth factor receptors such as TGF-βR (Rudini *et al.*, 2008) and VEGF-RII (Grazia Lampugnani *et al.*, 2003). In neural precursor cells located within the ventricular zone, N-cadherin knock-down results in reduced β-catenin activity and Akt phosphorylation, as opposed to the increased β-catenin activity seen after knock-down of E-cadherin and VE-cadherin. The authors found that Akt prevented premature maturation of these neural progenitors (Zhang *et al.*, 2013). Some unconventional or atypical cadherins can also modulate proliferative signaling. For example, cadherin-17 knock-down has been shown to result in decreased proliferation through reduced Erk phosphorylation (Lin *et al.*, 2014). Even though these studies reporting detailed mechanisms by which cadherin family members other than E-cadherin regulate proliferation are limited, it is evident that these proteins participate in these biological responses.

The role of desmosomal cadherins in proliferative signaling is complex and depends on the cell-type and/or cadherin involved. In intestinal epithelial cells, Dsc-2 knock-down results in increased proliferation through EGFR/β-catenin signaling (Kolegraff *et al.*, 2011), while Dsg-2 knock-down reduces EGFR phosphorylation and inhibits proliferation (Kamekura *et al.*, 2014). In line with these results, expression of Dsg-2 in the suprabasal layer of the epidermis in mice leads to epidermal hyperplasia. In western blots comparing transgenic and wild-type mouse skin, changes in the phosphorylation of several signaling mediators known to regulate proliferation were observed. These include, MAPK, Akt, PDK-1, PTEN (inhibitory modification) and GSK-3β as well as increased PTEN, c-Myc, and NF-κB protein levels (Brennan *et al.*, 2007). Exposure of intestinal epithelial cells to either IL-1β or TNF-α results in extracellular cleavage of Dsg-2 by the matrix metalloproteases MMP9 and ADAM10. The resulting Dsg-2 cleaved fragment reduces intercellular adhesion and

promotes proliferation through activation of Her2/3 signaling (**Figure 1A**) (Kamekura *et al.*, 2015). Analogous to Dsg-2 ectodomain cleavage, ADAM15 promotes extracellular cleavage of E-cadherin and the resulting fragment promotes Her2/3 heterodimerization and activation in breast cancer cell lines (Najy *et al.*, 2008). The authors suggest that proliferation mediated by activation HER2/3 signaling could be associated with the oncogenic properties of soluble E-cadherin. Since these extracellular fragments affect both proliferation and adhesion, we have included this signaling mechanism as being adhesion dependent, despite the requirement for cadherin cleavage. Re-expression of Dsc-3 in Dsc-3-deficient lung cancer cell lines results in decreased basal levels of phosphorylated Erk1/2 and reduced proliferation (Cui *et al.*, 2012). Thus the loss of Dsc-3 in lung cancer cells appears to activate pro-proliferative oncogenic Erk1/2 activity. These findings highlight a role of desmosomal cadherins that, analogous to classical cadherins, may control proliferation and invasion of tumor cells.

B) Cadherin mediated regulation of apoptosis

In addition to controlling proliferation, cadherins act as important mediators of apoptotic signaling. Much of the work into E-cadherin mediated regulation of apoptosis has been done in the context its role in cancer development and progression. E-cadherin homophilic engagement in HC11 mouse breast cancer cells enhances STAT3 phosphorylation and disrupting this binding reduces pSTAT3 levels through activation of Rac1 leading to increased apoptosis (**Figure 2A**) (Arulanandam *et al.*, 2009). This increased apoptosis and reduced STAT3 activation highlights disrupting E-cadherin engagement in breast cancer as a potentially attractive therapeutic strategy. Taken together with the findings of Liu *et al.*, E-cadherin adhesion dependent activation of Rac1 appears to both enhance proliferation and reduce apoptosis, suggesting that this is one of the E-cadherin dependent mechanisms that are known to be suppressed in cancer. Expression of E-cadherin in lung, colorectal and pancreatic cancer lines enhances apoptosis and caspase-8 activation through

direct interaction with the TNF-α Related Apoptosis Inducing Ligand (TRAIL) receptors, death receptors 4 and 5 (DR4/5) (Lu *et al.*, 2014). Apoptosis sensitization was enhanced by E-cadherin adhesion and promoted formation of the Death Inducing Signaling Complex (DISC) in response to TRAIL treatment (**Figure 2B**) (Lu *et al.*, 2014). This is an example of cadherins acting as scaffolds for transmembrane signaling proteins in addition to functioning as scaffolds for cytoplasmic/intracellular signaling proteins. E-cadherin adhesion deficient mutant proteins were used to show that E-cadherin mediated adhesion participates in the stimulation of notch signaling, resulting in increased expression of the anti-apoptotic protein Bcl-2 (Ferreira *et al.*, 2012). These data provide examples showing that the adhesion state of E-cadherin, in addition to ensuring cell-cell cohesion, acts as a signal that modulates a variety of downstream signaling events to influence cellular homeostasis. Using mouse models expressing different chimeric cadherins containing portions of N-cadherin and E-cadherin it was shown that the extracellular domain of E-cadherin interacts with the Igf-1 receptor, which promotes activity of this receptor and survival of trophectoderm cells during blastocyst formation (Bedzhov *et al.*, 2012). Through its robust regulation of both cell proliferation and cell death, E-cadherin functions to control key cellular outcomes that influence cellular homeostasis.

The role of desmosomal cadherin mediated adhesion or junctional residence in apoptotic signaling is less well understood, but a few such mechanisms have been described. For example, expression of Dsg-2 in suprabasal cells of the murine epidermis increases NF-κB and Bcl-X_L protein levels. Keratinocytes derived from these mice display greater survival compared to cells derived from WT mice. These effects are enhanced by EGF treatment and are abolished by inhibition of NF-κB (Brennan *et al.*, 2007). As with proliferation, other classical cadherins in addition to E-cadherin participate in the regulation of apoptosis. In the absence of VE-cadherin, endothelial cells express N-cadherin through enhanced β-catenin

signaling as well as display increased apoptosis, proliferation, and migration and re-expression of VE-cadherin in these cells reverses these effects (Giampietro *et al.*, 2012). The authors found that altering the balance of normal VE-cadherin to N-cadherin expression levels leads to vascular elongation and increased angiogenesis (Giampietro *et al.*, 2012). Following a series of co-expression experiments the authors suggest that VE-cadherin and N-cadherin play similar but divergent roles in the regulation of proliferation, apoptosis, and migration of endothelial cells.

C) Cadherin mediated control of cellular differentiation

In addition to being important regulators of proliferation and apoptosis, cadherins also regulate cell differentiation in various contexts. Loss of E-cadherin in *in vitro* cultured murine embryonic stem cells decreases Klf4 and Nanog expression as well as pSTAT3 levels and interaction of STAT3 with the Nanog promoter (Hawkins *et al.*, 2012). In another study using embryonic stem cells, treatment with E-cadherin adhesion blocking antibodies reduced expression of several transcriptional regulators as well as Tet1 and Esrrb (Segal & Ward, 2017). These reports highlight the importance of E-cadherin in the maintenance of the embryonic stem cell transcriptional program and in the ability of embryonic stem cells to maintain their pluripotency and stem cell properties. Overexpression of cadherin-11 in *Xenopus* embryos, inhibits cranial neural crest migration and is phenocopied by ADAM13 inhibition. The authors found that ADAM13 cleaves cadherin-11 generating an extracellular fragment and treatment with this cadherin-11 extracellular fragment alleviates the effects of cadherin-11 overexpression and ADAM13 inhibition on cranial neural crest migration through binding of cadherin-11 (McCusker *et al.*, 2009). This is another example of the effects of cadherin extracellular fragments in modulation of cadherin mediated adhesion via competitive binding. Such observations highlight the importance of cadherin extracellular

fragments functioning as signaling molecules that influence cadherin mediated adhesion. Generation of these fragments can be exquisitely regulated in order to facilitate specific functional outcomes like increased proliferation or migration of cells.

Much of the research describing roles of cadherins in the regulation of differentiation are centered on the intricately regulated expression patterns of desmosomal cadherins, which play an essential role in ensuring appropriate epidermal differentiation. This topic has been extensively discussed in previous reviews and will therefore not be detailed here (Green & Simpson, 2007; Nekrasova & Green, 2013). Exogenously expressed Dsg-1 co-immunoprecipitates (Co-IPs) Erbin in normal human epidermal keratinocytes (NHEKs) (Harmon *et al.*, 2013). Induction of differentiation in NHEKs leads to increased expression of many differentiation specific genes including loicrin and involucrin. However, after induction of differentiation in Erbin knock-down cells, these changes are all diminished, resulting in a lack of appropriate cellular differentiation. In differentiated wild-type NHEK cells pErk is predominantly confined to the basal layer, whereas after downregulation of Erbin, pErk was detected within the suprabasal cells of the epidermis. Dsg-1 expression facilitates co-immunoprecipitation of Ras-Raf scaffold protein Shoc2 with Erbin and diminishes Erbin's ability to Co-IP K-Ras (Harmon *et al.*, 2013). It is important to note that expression of WT Dsg-1 reduces pErk1/2 levels by two thirds, whereas expression of Dsg-1 lacking the Erbin binding site decreases pErk1/2 levels by half. These data suggest that while the ability of Dsg-1 to bind Erbin plays an important role in its regulation of Erk1/2 phosphorylation, this signaling function is not exclusively mediated by Erbin binding (Harmon *et al.*, 2013). These data suggest that desmogleins can modulate binding properties of other important signaling scaffold proteins, in addition to acting as a scaffold themselves. Abnormal differentiation of the suprabasal layers was observed in differentiated epidermal raft cultures derived from Dsg-1 knock-down NHEKs. Furthermore, inducing differentiation in Dsg-1 knock-down

keratinocytes increases pEGFR and pErk1/2 compared to control cells. Moreover, Erk1/2 inhibition was found to reverse the effects of Dsg-1 knock-down on suprabasal epidermal differentiation (Getsios *et al.*, 2009). Using NHEKs cultured in organotypic raft cultures, this same group showed that knock-down of the guanine nucleotide exchange factor Bcr or blockade of MAL/SRF-induced transcriptional regulation leads to reduced expression of multiple epidermal differentiation associated proteins. Re-expression of Dsg-1 in these cells rescues the loss of these differentiation markers (Dubash *et al.*, 2013). These findings indicate that Dsg-1 dependent regulation of Erk1/2 phosphorylation, GEF Bcr, and MAL/SRF-induced transcription are critical events in epidermal differentiation and their loss results in impairment of this vital process. Exposure of NHEKs to UVB prior to the induction of differentiation caused a dose dependent decrease in Dsg-1, Dsc-1, and multiple keratins as well as an increased pErk which is associated with delayed/impaired epidermal differentiation. Through overexpression and knockdown studies the authors show an influence of Dsg-1 in protection from the adverse effects UVB exposure on epidermal differentiation (Johnson *et al.*, 2014). These example provide evidence for the importance of desmosomal cadherins in controlling epidermal differentiation which is vital for the defense properties of the skin.

D) Other cadherin signaling events

The role of cadherins in signaling extends beyond the basic homeostatic mechanisms of life, death, and differentiation. In fact, recent research into cadherin mediated signaling has begun to focus more on the roles that cadherins play in other signaling pathways. Since research into these aspects of cadherin signaling is in its infancy, this section will primarily focus on some isolated examples from various systems ranging from regulation of Schwann cell mediated myelination (Basak *et al.*, 2015) to insulin production by pancreatic β-cells

(Parnaud *et al.*, 2015). The rate and extent of myelination in the peripheral nervous system of E-cadherin knock-out mice is significantly reduced compared to wild-type animals which would result in impaired peripheral nervous system function. (Basak *et al.*, 2015). E-cadherin together with neuregulin 1 increases phosphorylation of HER2, Akt, and Erk to facilitate myelination (**Figure 2C**) (Basak *et al.*, 2015). Since very little is known about mechanisms that govern Schwann cell myelination, the finding that E-cadherin and neuregulin 1 work together to enhance this process is of great interest. Pernaud *et al*. observed that primary human pancreatic β-cells subjected to high glucose display a noticeable increase in insulin production when plated on E-cadherin Fc coated glass compared to controls. This effect is blocked by treatment with the E-cadherin adhesion blocking antibody DECMA-1 (Parnaud *et al.*, 2015). These findings suggest that engagement of E-cadherin plays a role in insulin production in β-cells thereby controlling overall glucose metabolism. Furthermore, a recent study by Park *et al*. has also supported a role of E-cadherin in controlling glucose metabolism. Re-expression of E-cadherin in gastric cancer epithelial cells enhances oxidative phosphorylation and expression of glucose transporters. Glucose metabolism in cancer cells shifts from predominantly oxidative phosphorylation to anaerobic glycolysis during EMT, suggesting that loss of E-cadherin may play a role in this metabolic shift. These effects are reduced following NF-κB knock-down, suggesting that NF-κB participates in promoting oxidative phosphorylation upon E-cadherin re-expression in cancer cells (Park *et al.*, 2017). Using MCF10A human mammary epithelial cells, Bays *et al*. showed that application of extracellular force to endogenous E-cadherin through the use of E-cadherin Fc coated magnetic beads stimulates AMPK activation as well as increased phosphorylation of MLC, vinculin, and CrkL. This was associated with reinforcement of the actin cytoskeleton and enhanced barrier function. Additionally, application of force to E-cadherin increases glucose uptake and cellular ATP levels. These effects are inhibited by knock-down of E-cadherin,

AMPK, and Lkb1 as well as E-cadherin adhesion blocking antibody treatment (Bays *et al.*, 2017). Together, these results suggest that, in addition to its roles in adhesion and regulation of epithelial barrier properties, E-cadherin contributes to nutrient signaling that has not been fully explored. Additionally, the work of Bays *et al.* highlights the diversity of cadherin mediated signaling mechanisms that are dependent on its mechanotransduction properties. Loss of E-cadherin is known to play a role in the epithelial to mesenchymal transition (EMT) and the subsequent increased invasiveness and dissemination of metastatic cancer cells. Knock-down of E-cadherin in A549 non-small cell lung carcinoma (NSCLC) cells increases invasiveness as well as enhances expression of multiple MMPs and transcriptional regulators. These effects of E-cadherin knockdown were reversed by MEK inhibition (Bae *et al.*, 2013). Such findings suggest that the contribution of E-cadherin loss to epithelial - mesenchymal transition (EMT) and tumor invasion are at least partly mediated through activation of MEK/Erk signaling.

Analogous to E-cadherin, other classical cadherins contribute to diverse signaling event. For example, extracellular cleavage of N-cadherin on microglial cells by ADAM10 and MMP9 and treatment of cultured primary microglial cells with the resulting protein fragment increases nuclear NF-κB translocation as well as release of TNF-α, MMP9, and MCP-1 indicating increased microglial activity (Conant *et al.*, 2017). In light of the many examples of N-cadherin participating in signaling mechanisms in epithelial, endothelial, and neuronal cells, it will be of great interest to further explore and define the overall signaling repertoire of this protein and its control of cellular functions.

There have been recent examples of cadherin mediated signaling that involve their residence in multi-protein complexes other than their traditional junctions. For example, Labernadie *et al*. showed that Cancer Associated Fibroblasts (CAFs) exert forces on A431 human epidermal carcinoma cells in *in vitro* co-culture. Co-cultured CAFs form heterotypic

N-cadherin/E-cadherin junctions with the A431 cells. These junctions can withstand mechanical forces and are involved in mechanotransdution cross-talk between cancer cells and CAFs (Labernadie *et al.*, 2017). Such cadherin mediated mechanotransduction influences CAF polarization and directional migration of cancer cells in three-dimensional cultures led by CAF movement. E-cadherin was required for the transduction of force between these two cell types. Thus, classical cadherins can participate in heterotypic junctions that can withstand force and these junctions can have an impact on cellular outcomes (Labernadie *et al.*, 2017). In endothelial cells, it was shown that the transmembrane domain (TMD) of Notch1 forms a mechanosensory complex containing the transmembrane tyrosine phosphatase leukocyte common-antigen related (LAR), GEF Trio, and Rac1, which participate in the activation of Rac1 and functions to regulate the formation of adherens junctions and endothelial permeability. This complex is targeted to adherens junctions through binding of the Notch1 TMD with VE-cadherin (Polacheck *et al.*, 2017).

Tsang *et al*. examined the effects of Dsg-3 on junctional and cytoskeletal components and observed that knock-down of Dsg-3 in HaCaT cells results in decreased expression of adherens junction, desmosome, and actin cytoskeleton associated proteins that was linked to impaired development and function of intercellular junctions and cell polarization. In addition, Dsg-3 knock-down cells displayed decreased β-catenin tyrosine phosphorylation as well as several other distinct effects including diminished re-integration of junctional E-cadherin and β-catenin following calcium switch supporting its role in the assembly of intercellular junctions in HaCaT cells. This loss of Dsg3 impaired the formation of adherens junctions and E-cadherin dependent Src activation (Tsang *et al.*, 2012a; Tsang *et al.*, 2012c). On the converse overexpression of Dsg-3 in HaCaT and A431 cells increases the number of actin protrusions and the rate of actin turnover as well as activation of several Rho family small GTPases thereby influencing cellular polarization and tissue remodeling. When

combined with Rac1 inhibition, Dsg-3 overexpression increases A431 cell migration (Tsang *et al.*, 2012a). The authors also described interaction between a non-junctional pool of Triton X-100 soluble Dsg-3 and E-cadherin in a calcium, p120, and plakoglobin dependent manner (Tsang *et al.*, 2012c). Such observations suggest that Dsg-3 (and potentially others desmogleins) play a much larger role in overall junctional and cytoskeletal regulation and the subsequent control of cellular polarization and tissue remodeling than previously appreciated.

E) Cadherin mediated signaling in the context of disease

Cadherin mediated signaling mechanisms have been shown to play an important role in multiple disease states and pathologies. One of the most well studied examples is in the context of cancer. A number of previous reports have addressed cadherin dependent regulation of cancer pathogenesis and progression that involves control of cellular proliferation, apoptosis, invasiveness, metabolism, and metastasis in the context of this disease (Conacci-Sorrell *et al.*, 2002; Cavallaro & Christofori, 2004; Jeanes *et al.*, 2008; Onder *et al.*, 2008; Solanas *et al.*, 2008; Maher *et al.*, 2009; Bae *et al.*, 2013; Lu *et al.*, 2014; Park *et al.*, 2017). Although E-cadherin is a classic example of cadherin mediated signaling in cancer, N-cadherin, VE-cadherin, desmosomal cadherins, and other cadherins have also been shown to participate in oncogenic signaling in cancer pathogenesis. Since we have mentioned examples of cadherin signaling in the context of oncogenesis above, and this topic has been detailed in previous reviews, we focus on cadherin contribution to other disease mechanisms in this section.

We will in large part address the role of cadherin signaling in the autoimmune skin diseases pemphigus vulgaris (PV) and pemphigus foliaceus (PF). Several signaling mechanisms have been implicated in the pathobiology of pemphigus. Pemphigus vulgaris and pemphigus foliaceus have been reported to be mediated by autoantibodies that target Dsg-3

and Dsg-1 respectively (Mao *et al.*, 2011; Schulze *et al.*, 2012). Studies using multiple systems have shown that the use of monoclonal antibodies targeting desmogleins can recapitulate pemphigus pathology (Schulze *et al.*, 2012; Bektas *et al.*, 2013). However, the contribution of desmogleins to these signaling events remains incompletely understood (Chernyavsky *et al.*, 2007). We will highlight some key examples of signaling caused by pemphigus autoantibodies because this field of study has been previously reviewed more comprehensively (Sharma *et al.*, 2007; Schmidt & Waschke, 2009; Spindler & Waschke, 2014). Following 48 hours of treatment with PV autoantibodies, mouse keratinocytes display reduction in nuclear localization of plakoglobin (Pg). Plakoglobin is a member of the armadillo family of proteins and is the most closely related protein to the famous signaling mediator β-catenin. Plakoglobin is a critical component of the desmosomal plaque and directly binds to the intracellular tails of desmogleins and desmocollins. After prolonged PV autoantibody treatment, keratinocytes display noticeably increased proliferation, c-Myc expression, and c-Myc nuclear localization, which was shown to be reversed after plakoglobin knock-out. Analogous increased proliferation is also seen in human PV patient skin samples (Williamson *et al.*, 2006). NHEKs treated with PV autoantibodies display internalization and depletion of Dsg-3, which are prevented by p38MAPK inhibition (Jolly *et al.*, 2010). It was later shown that in response to patient derived PV autoantibody treatment p38MAPK phosphorylation occurs prior to the activation of EGFR resulting in blister formation. The PV autoantibody treatment causes an increase in pErk1/2, which is enhanced by inhibition of either p38MAPK or EGFR phosphorylation (Bektas *et al.*, 2013). These observations suggest that some of the changes in signaling mediators observed upon PV autoantibody treatment can be antagonistic in nature. For example, the PV autoantibody induced increases in p38MAPK and EGFR dampening the increase in pErk1/2 caused by the same treatment. PV autoantibody or AK23 (a monoclonal antibody against mouse Dsg-3 that

causes PV phenotypes) treatment causes endocytosis of EGFR, which is prevented by p38MAPK inhibition resulting in reduced blister formation. Exposure of NHEKs with PV autoantibodies leads to Dsg-3 internalization and overall loss of the protein. These effects are reduced by pre-treatment with inhibitors of either p38MAPK or EGFR or knock-down of EGFR. The functional outcome of EGFR inhibition in these models was prevention of the loss of Dsg-3 mediated adhesion resulting in decreased blister formation (Bektas *et al.*, 2013).

Dysregulation of apoptosis is also known to be important in PV pathobiology. A mouse model often used to study these diseases is referred to as a passive transfer mouse, in which IgG or serum derived from PV or PF patients is injected into the blood stream of embryonic mice after which the mice develop symptoms and clinical manifestations of the parent disease. PV passive transfer mice show higher Bax and lower Bcl-2 protein levels, as well as increased caspase-3 and -9 activity. In addition, the PV transfer treated mice have enhanced phosphorylation of m-TOR and Src as well as increased blister formation and apoptosis, all of which are abolished or reduced by FAK inhibition (Gil *et al.*, 2012). Increased apoptosis was observed in the epidermis of PF passive transfer mice which led to the development of experimental PF. These effects were reversed using pan-caspase or caspase-3 selective inhibitors (Li *et al.*, 2009). In biopsy samples derived from the skin surrounding the injection site of PF passive transfer mice, Lee *et al*. observed an increase in phospho-p38MAPK in two peaks following treatment. The authors also described apoptosis execution beginning 30 hours post treatment and increasing in severity over time, which is prevented by p38MAPK inhibition. Only inhibition of the first p38MAPK peak but not the second prevented blister formation in these mice (Lee *et al.*, 2009). These data suggest that this first burst of p38MAPK activation mediates blister formation in PF. In the basal cells of PV passive transfer mice, Pretel *et al*. describe increased phosphorylated Her family growth factor receptors and downstream signaling molecules including Src and m-TOR, which were

all inhibited by EGFR inhibitor treatment. The authors also found increased betacellulin, TGF-α, and EGF in these cells. Pre-treatment with an EGFR inhibitor, a Src kinase family inhibitor, rapamycin, or pan-caspase inhibitors all prevent the apoptosis and other clinical manifestations normally seen in PV passive transfer mice (Pretel *et al.*, 2009). These data suggest that the increased apoptosis seen in response to pemphigus autoantibody treatment is necessary for the development of clinical symptoms. Passive transfer mice have proven useful in exploring non-apoptotic aspects of pemphigus related signaling as well. Using passive transfer of AK23 (monoclonal mouse anti-Dsg-3 antibody), Schulze *et al.* observed increased activation or expression of several signaling mediators that promote proliferation. They also observed decreased expression of total and phospho p38MAPK, Dsg-1/2, Dsg-3 and EGFR. These AK23 passive transfer mice developed typical blistering and other symptoms associated with clinical PV (Schulze *et al.*, 2012). *Ex vivo* treatment of human skin tissue with PV or PF autoantibodies results in blister formation that is repressed by enhanced RhoA signaling. In HaCat cells, inhibition of p38MAPK or activation of Rho family members prevents the disruption of several desmosomal proteins and E-cadherin normally seen in response to PV autoantibody treatment. As discussed above, disruption of these proteins is important to the development of blistering and other PV or PF symptoms. Treatment of HaCaT cells with PV or PF autoantibodies also results in reduced active RhoA, which is prevented by p38MAPK inhibition (Waschke *et al.*, 2006). These data suggest that inhibition of RhoA occurs following p38MAPK activation and is an important step in pemphigus pathobiology through preventing RhoA activity from inhibiting the disruption of desmosomal proteins and E-cadherin. Using a panel of PV monoclonal antibodies, only those that cause a loss of intercellular adhesion activate p38MAPK. The authors also found that inhibition of p38MAPK prevents Dsg-3 internalization and depletion in response to the PV mAbs (Mao *et al.*, 2011). These data suggest that the disruption of desmoglein mediated

adhesion through internalization and loss of the desmogleins themselves may be a critical factor in pathogenicity of these autoantibodies.

Cadherin mediated signaling has been shown to participate in other disease states besides cancer and pemphigus, but these are far fewer. For example, Dsg-1 mRNA and protein expression is reduced in eosinophilic esophagitis (EoE), that has been associated with reduced barrier integrity and adhesion (Sherrill *et al.*, 2014a). These effects are recapitulated by Dsg-1 knock-down of cultured esophageal epithelial cells. Dsg-1 knock-down promotes pro-inflammatory transcriptional responses as analyzed by genome-wide by RNAseq (Sherrill *et al.*, 2014a). Both Dsg-1 knock-down in cultured esophageal epithelial cells and in human EoE patient samples display noticeably increased mRNA expression of the integrin ligand protein periostin compared to controls (Sherrill *et al.*, 2014a). Periostin is known to be highly expressed in EoE (Dellon *et al.*, 2016; Politi *et al.*, 2017). This data suggests that increased periostin expression after loss of Dsg-1 may contribute to the pathobiology of EoE. Samples from human patients with striate palmoplantar keratoderma (SPPK) arising from mutations in Dsg-1 display increased Erk activity and reduced Dsg-1, loricrin, and keratin-10 expression. These samples also display increased interaction of Shoc2 with K-ras and reduced interaction of this protein with Erbin (Harmon *et al.*, 2013). Combined with the pemphigus examples above, desmogleins play a key role in the pathobiology of a number of disorders that include skin diseases and cancer.

F) Cadherin intracellular fragment signaling

The findings we have described thus far in this review highlight the diversity and importance of cadherin mediated signaling. However, most of these mechanisms are either dependent on or in some way influenced by changes in cadherin mediated adhesion in intercellular junctions. In the next section we will highlight an emerging class of cadherin

mediated signaling mechanism that do not appear to be influenced by changes in cadherin adhesion. Most of these mechanisms are based on intracellular cadherin cleavage and the generation of a functional protein fragment. For example, E-cadherin can be cleaved intracellularly by gamma secretase resulting in generation of the E-cadherin C-Terminal Fragment 2 (CTF2) (Marambaud *et al.*, 2002). Expression of CTF2 results in its nuclear localization and increased proliferation, which are both enhanced by co-expression with p120. E-cadherin CTF2 promotes Kaiso mediated transcriptional regulation and is able to Co-IP both with Kaiso and p120 (**Figure 1B**) (Ferber *et al.*, 2008). In line with the E-Cadherin CTF2, expression of N-cadherin c-terminal fragment 2 (CTF2) leads to N-Cadherin CTF2 nuclear localization and increased expression of β-catenin and cyclin D1 as well as increased emigration of neural crest cells from the developing neural tube in quail embryos (Shoval *et al.*, 2007). In response to UV induced apoptosis in keratinocytes, desmoglein-1 is cleaved intracellularly by caspases-3 and -7 and knock-down of desmoglein-1 protects cells from UV induced apoptosis (Dusek *et al.*, 2006). We have recently shown that in response to TNF-α and IFN-γ as well as TRAIL treatment, Dsg-2 undergoes intracellular cleavage that is mediated by caspase-8. The resultant Dsg-2 intracellular fragment (Dsg-2 ICF) sensitizes intestinal epithelial cells to apoptosis through increased expression of the anti-apoptotic Bcl-2 family members Bcl-X_L and MCl1 (Yulis *et al.*, 2018). Taken together, these findings highlight the importance of cadherin intracellular fragments serving as key signaling mediators.

G) Cadherin signaling in cells lacking intercellular adhesion

There are also a few examples of cadherin mediated signaling that remain intact in cells lacking intercellular adhesion. In immortalized untransformed human mammary epithelial cells E-cadherin knock-down results in increased N-cadherin and vimentin

expression as well as reduced expression of cytokeratin 18, indicative of an epithelial to mesenchymal transition. However, this was not seen after expression of adhesion blocking dominant negative E-cadherin, indicating that the E-cadherin dependent signaling mechanisms preventing cells from undergoing EMT in this system are independent of E-cadherin's intercellular adhesion properties (Onder *et al.*, 2008). In addition, E-cadherin knock-down results in increased motility, invasiveness, and reduced sensitivity to apoptosis, all of which are rescued by expression of the adhesion blocking E-cadherin dominant negative protein (Onder *et al.*, 2008). While organoids generated from primary mammary epithelial cells derived from mice with inducible E-cadherin knock-out displayed a normal epithelial phenotype upon E-cadherin knock-out, the cells displayed a more classically mesenchymal phenotype. In spite of this phenotype however, few cells disseminate out as single cells and therefore do exhibit classic invasive properties. The transcription factor Twist1 has been suggested to play an active role in promoting EMT through regulation of E-cadherin and other mediators. Primary mammary epithelial cell organoids derived from mice with overexpression of Twist1 exhibit single cell dissemination, which was inhibited upon E-cadherin knock-down (Shamir *et al.*, 2014). These data support a role of E-cadherin – Twist signaling in the development of metastatic potential cells. Thus, E-cadherin signaling modulates cancer phenotypes through signaling mechanisms that remain intact even in the absence of intercellular adhesion. Similarly, in *Xenopus* embryos knock-down of cadherin-11 inhibits cranial neural crest cell migration and this effect is rescued by expression of cadherin-11 lacking the extracellular domain. However, expression of cadherin-11 lacking the β-catenin binding domain or the transmembrane domain does not rescue this loss of cranial neural crest cell migration. These effects are also reversed by supplementation with recombinant Rho family small GTPases or the Rho GEF Trio (Kashef *et al.*, 2009). Although research into cadherin signaling in cells lacking intercellular adhesion is not completely

understood, these examples open the door to a new frontier of possibilities regarding the breadth of cadherin mediated signaling.

Conclusion

The cadherin family of transmembrane proteins serve as important intercellular adhesion molecules. Over the years, appreciation of their function as critical signaling mediators has been steadily growing. Classical, desmosomal, atypical, and unconventional cadherins all participate in many signaling cascades that influence proliferation, apoptosis, and differentiation in a number of tissues and cell types as well as in disease. In more recent years, the critical roles that cadherins play in the regulation of non-homeostatic cellular functions have emerged. Dysregulation of cadherin mediated signaling results in disease states encompassing epithelial barrier defects, inflammation, and neoplasia. While great pioneering research has been performed, we have only just begun to understand the extent to which cadherins participate in signaling events. Due to their importance in intercellular adhesion, most of the cadherin mediated signaling described thus far is directly influenced by either the junctional residence or the adhesive state of the cadherin itself. Further study of these mechanisms will continue to expand our understanding of the repertoire of cadherin functions.

Additional Information:

Competing Interests:

The authors declare no conflict of interest.

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Figure Legends:

Graphical Abstract) Modalities of cadherin mediated signaling: Cadherins have been shown to participate in many diverse signaling pathways and mechanisms. Not only is the range of functional outcomes that cadherins regulate wide, the number of different types of mechanisms that cadherins participate in to mediate their signaling is also impressive.

A) Many times, cadherins themselves act as scaffolds for important signaling events and the ability of the cadherin to participate in these signaling mechanisms is often dependent upon their participation in cadherin mediated adhesion.

B) Other mechanisms requiring cadherin mediated adhesion include cadherin protein association with other transmembrane signaling proteins and receptors.

C+D) There are several reports of cadherin mediated signaling that requires the formation of stable cadherin protein fragments via regulated proteolytic cleavage. Most of these mechanisms require cadherin extracellular fragment generation and often times both stimulate receptor signaling pathways and interfere with cadherin mediated adhesion **(C)**. There are, however, multiple studies describing cadherin intracellular protein fragment

generation (**D**). These mechanisms often entail binding of the intracellular fragment to a cytoplasmic signaling partner, nuclear re-localization, and regulation of gene transcription.

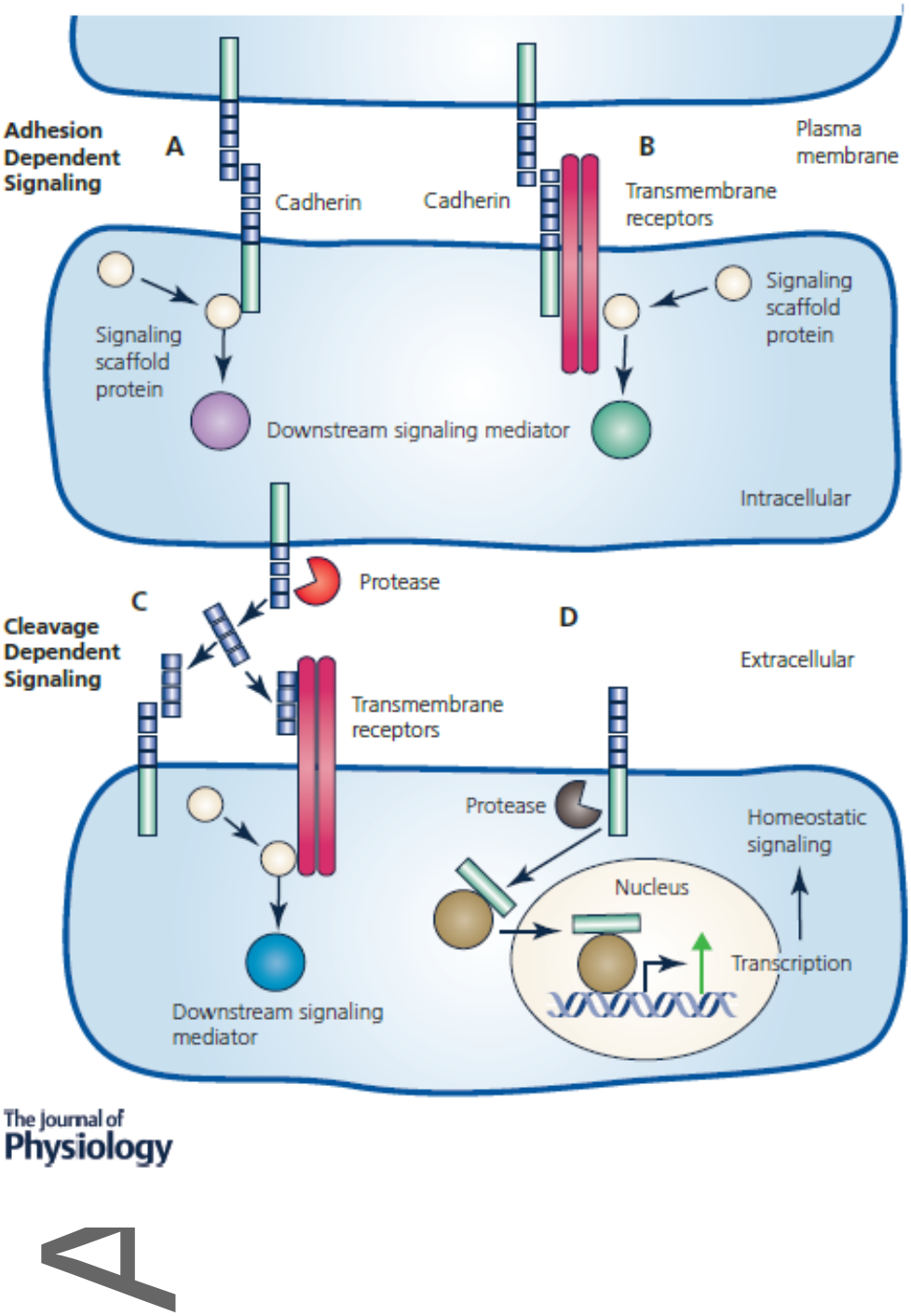

Figure 1) Examples of cleavage dependent cadherin mediated signaling. A growing number of reports have shown that cadherin protein fragments generated through regulated cleavage play important signaling roles.

A) Many of these mechanisms are dependent upon cadherin extracellular fragment generation. These usually influence signaling of transmembrane receptors and/or cadherin mediated adhesion in a paracrine and/or autocrine manner. The extracellular fragment of Dsg2 has been shown to participate in both of these activities interfering with Dsg2 extracellular adhesive binding and increasing proliferation through stimulation of the Her2 and Her3 receptors (Kamekura *et al.*, 2015).
B) Cadherin intracellular fragments also mediate important signaling events. An example of this is the enhancement of E-cadherin C-Terminal Fragment 2 (CTF2) nuclear localization through binding to p120 and increased Kaiso mediated nuclear transcription (Ferber *et al.*, 2008).

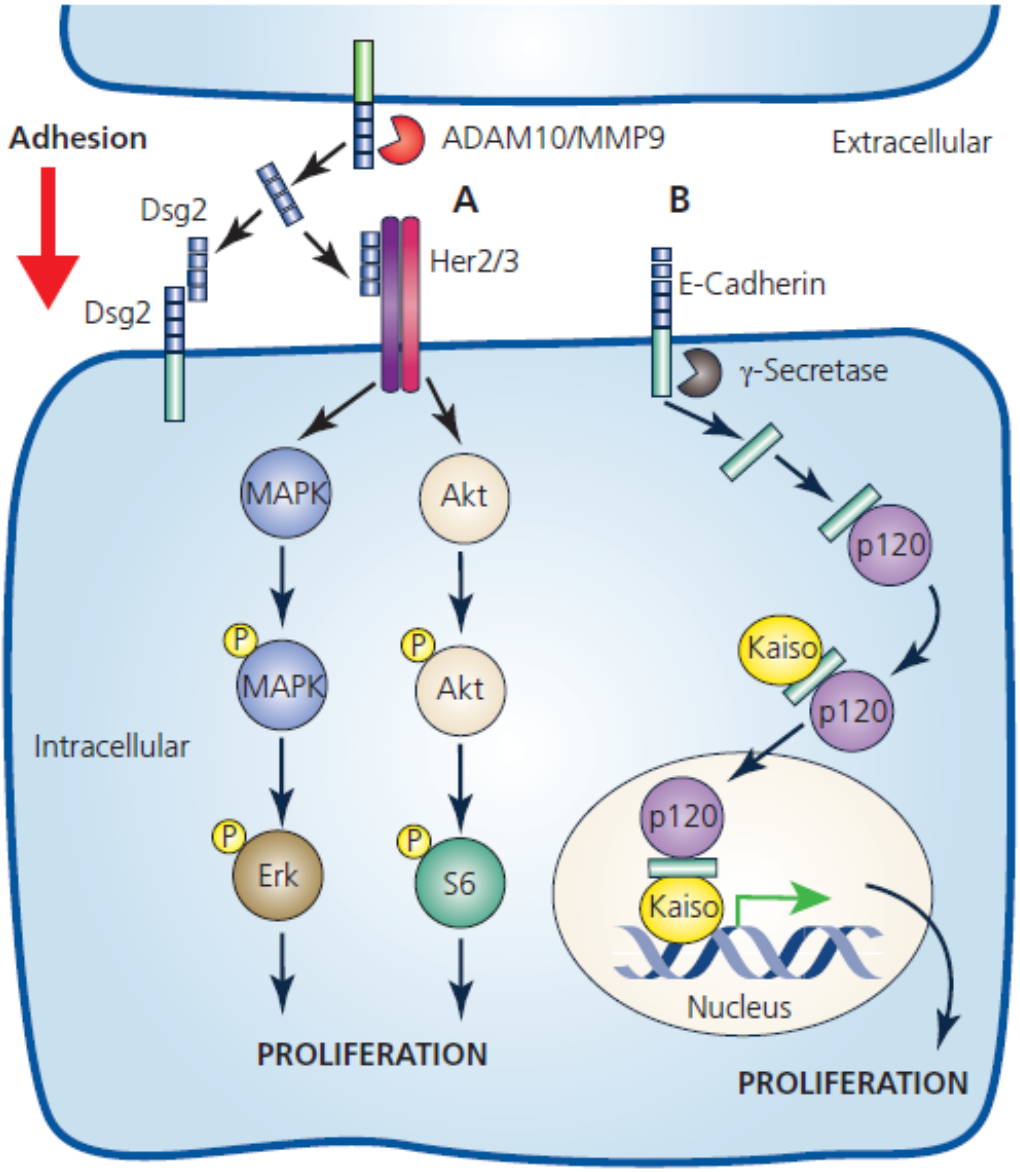

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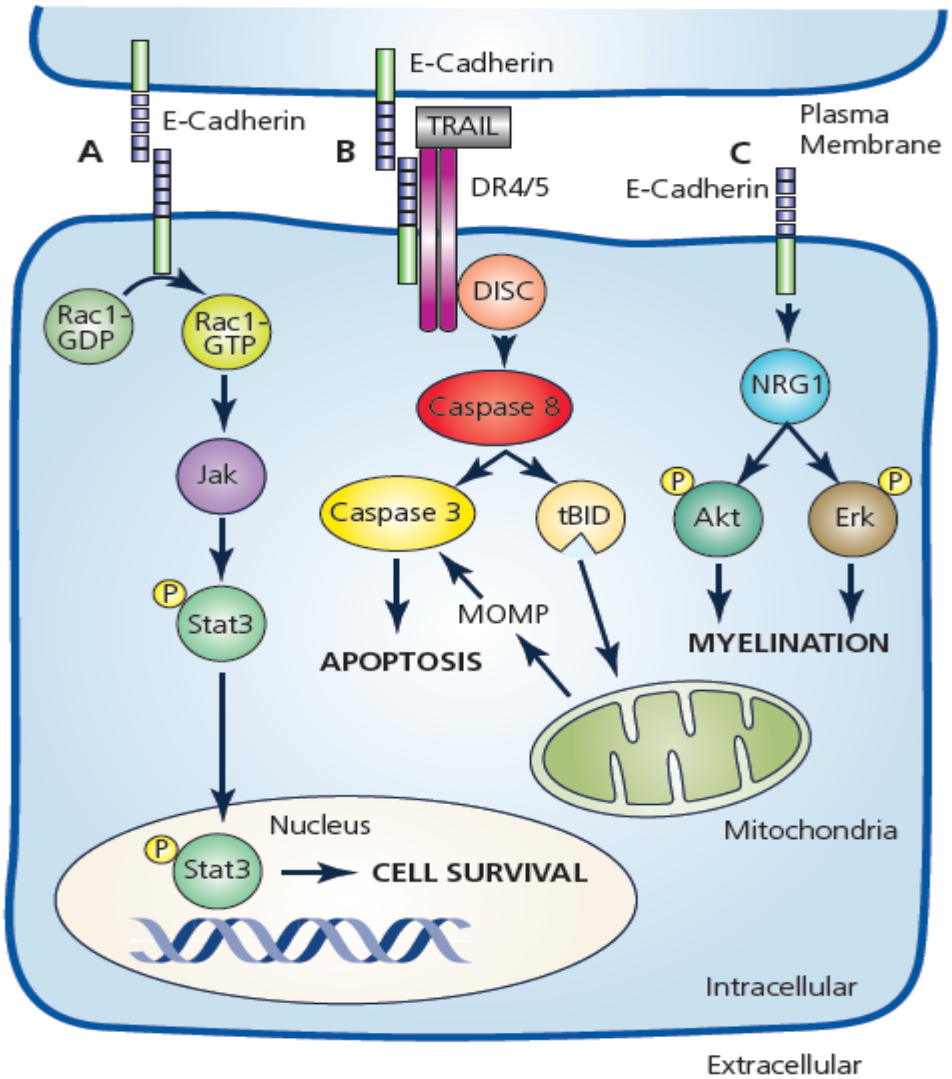
Figure 2) Examples of cadherin mediated signaling dependent on junctional residence and adhesive state. Cadherin cytoplasmic tails can act as scaffolds for intracellular signaling molecules thereby promoting distinct signaling pathways under various conditions. These scaffolding functions are often dependent upon the following:
A+B) The adhesive state of the cadherin or **C)** the presence of the cadherin within a stable junction. Examples of these types of mechanisms include activation of Rac1 and Stat3 signaling through E-cadherin adhesive binding leading to repression of apoptosis (Arulanandam *et al.*, 2009) or reduced peripheral nervous system myelination in the absence of E-cadherin (Basak *et al.*, 2015). There have even been a few reports of cadherin adhesive binding leading to the cadherin acting as a scaffold for and enhancing the activity of transmembrane signaling receptors such as the receptors for TNF-α Related Apoptosis Inducing Ligand (TRAIL), Death Receptors 4 and 5 (DR4 and 5) leading to enhanced Death Inducing Signaling Complex (DISC) formation and apoptosis sensitivity (Lu *et al.*, 2014).



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Functional Output	Cadherin (/fragment)	Associated Proteins	Effectors	Reference
Proliferation	E-Cadherin	β-catenin	TCF/LEF; Fibronectin	(Solanas *et al.*, 2008)
			TCF/LEF; β-catenin	(Maher *et al.*, 2009)
		β-catenin	Hippo; YAP	(Kim *et al.*, 2011b)
		β-catenin*	TCF/LEF†; Wnt3a‡Wnt3A	(Howard *et al.*, 2011)
	(NTF#)	HER2/3	ErBb	(Najy *et al.*, 2008)
	(CTF2#)	p120; Kaiso	Kaiso†	(Ferber *et al.*, 2008)
		EGFR	EGF‡; Stat5; ERK	(Perrais *et al.*, 2007)
		p120	Rac1	(Liu *et al.*, 2006)
	E-cadherin/ cadherin-11	p120; PLEKHA7	p120; Src; p130 CAS; c-Myc; FAK; SNAI1;	(Kourtidis *et al.*, 2015)
	N-Cadherin	β-catenin	Akt	(Zhang *et al.*, 2013)
		β-catenin*	TCF/LEF†; Wnt3a‡; TBX6	(Zhang *et al.*, 2013)
	(CTF2#)		Cyclin D1; β-catenin	(Shoval *et al.*, 2007)
	VE-Cadherin	TGF-βR; ALK5; ALK1	PAI-1, Id1; TGF-β‡; Smad1/5, 2, 3	(Rudini *et al.*, 2008)
		VEGF-RII; β-catenin	p44/42 MAPK VEGF‡; EGF‡	(Grazia Lampugnani *et al.*, 2003)

[Multiple processes affected]	VE-Cadherin / N-Cadherin	β-catenin	PI3K; FoxO1; Akt; SOD[‡]; β-catenin; Caspase-3; FGF-2; MMP3; PDGFRA; ANGPT2; FRS-2α	(Giampietro *et al.*, 2012)
	Cadherin-17		Ras; Raf; MEK; Erk; Integrin β1, β4, β5; p21; p53;	(Lin *et al.*, 2014)
	Dsg-2		EGFR; Dsc-2; Histone H3; Src; Erk1/2	(Kamekura *et al.*, 2014)
[Multiple processes affected]			MAPK; Akt; PDK-1; GSK-3β; PTEN; c-Myc; NFκB; MEK1/2; p90RSK; Raf; Stat3; Bcl-X_L	(Brennan *et al.*, 2007)
	(ECF[#])	HER2/3	S6; mTOR; MAPK; Akt; Erk1/2	(Kamekura *et al.*, 2015)
	Dsc-2		EGFR; CD44; Akt; β-catenin[†]	(Kolegraff *et al.*, 2011)
	Dsc-3		Erk1/2	(Cui *et al.*, 2012)
Apoptosis	E-Cadherin	DR4/5	DISC *[complex formation]*	(Lu *et al.*, 2014)
			Stat3; Rac1; Cdc42	(Arulanandam *et al.*, 2009)
			Notch; Bcl-2	(Ferreira *et al.*, 2012)
		Igf-1r	Igf-1[‡]	(Bedzhov *et al.*, 2012)
[Multiple			Twist; β-catenin;	(Onder *et al.*, 2008)

processes affected]			Cytokeratin-8; N-cadherin; vimentin; fibronectin	
[Multiple processes affected]	VE-Cadherin / N-Cadherin	β-catenin	PI3K; FoxO1; Akt; SOD‡; β-catenin; Caspase-3; FGF-2; MMP3; PDGFRA; ANGPT2; FRS-2α	(Giampietro *et al.*, 2012)
	Dsg-1		Caspase-3	(Dusek *et al.*, 2006)
[Multiple processes affected]			MAPK; Akt; PDK-1; GSK-3β; PTEN; c-Myc; NFκB; MEK1/2; p90RSK; Raf; Stat3; Bcl-X_L	(Brennan *et al.*, 2007)
Differentiation	E-Cadherin		Klf4; Nanog; Stat3; Erk1/2; Akt; N-cadherin	(Hawkins *et al.*, 2012)
			Stat3; Tet1; Esrrb; Tbx3; Nanog; Klf4; Nr0B1; Nr5a2; Erk1/2	(Segal & Ward, 2017)
	Dsg-1	Erbin; Shoc2	Erk1/2; K-Ras; loricrin; keratin-10	(Harmon *et al.*, 2013)
			EGFR; Erk1/2; Lamin A/C; keratin-10; desmocollin-1; fillagrin; loricrin; c-Raf; Mek1/2; EphA2; ErbB2	(Getsios *et al.*, 2009)
		Bcr◊	desmocollin-1; MAL; SRF; keratin-10; keratin-1; loricrin	(Dubash *et al.*, 2013)
	Dsg-1/Dsc-1		keratin1; keratin 10; p63;	(Johnson *et al.*,

			desmocollin-1	2014)
	Cadherin-11 [extracellular fragment[#] *{fragment is unnamed}*]	ADAM13; ADAM9	?	(McCusker *et al.*, 2009)
"Non-homeostatic signaling" / disease	E-Cadherin	Neurgulin1[◊]; HER2; ErbB2	Akt; Erk; P0	(Basak *et al.*, 2015)
		ROCK[▪]; Rac1[▪]; FAK[▪]	?	(Parnaud *et al.*, 2015)
[Multiple processes affected]		β-catenin	NFκB; Wnt; c-Myc; IκBα; GLUT1; GLUT3; GLUT4	(Park *et al.*, 2017)
		Lkb1; AMPK[*]	MLC; Vinculin; CrkL; Abl; RhoA	(Bays *et al.*, 2017)
			EGFR; Erk; ZEB1; N-cadherin; CDH1; Twist; Slug; ZEB1; MMP2; p53; GSK3β	(Bae *et al.*, 2013)
[Multiple processes affected]			Twist; β-catenin; Cytokeratin-8; N-cadherin; vimentin; fibronectin	(Onder *et al.*, 2008)
		Twist1[◊]	N-cadherin; αE-catenin; β-catenin	(Shamir *et al.*, 2014)
	E-cadherin/ N-cadherin		Afadin; Nectin-2; Nectin-3	(Labernadie *et al.*, 2017)

	N-cadherin [extracellular fragment[#] *{fragment is unnamed}*]	ADAM10; MMP7	NFκB; Iba-1; TNF-α; MMP9; MCP-1	(Conant *et al.*, 2017)
	VE-cadherin	Notch-1; LAR; Rac-1; TRIO	?	(Polacheck *et al.*, 2017)
	Dsg-1		periostin	(Sherrill *et al.*, 2014b)
	Dsg-3	E-Cadherin; Actin; Rac1; RhoA; Cad42	Src; p120; β-catenin; MLC	(Tsang *et al.*, 2012d)
		β-catenin; E-cadherin; P-cadherin; desmoglein-2; plakoglobin[*]; p120[*]	desmolgien-1/2; desmocollin-2; plakoglobin; PKP2; desmoplakin; PKP1; Src;	(Tsang *et al.*, 2012b)
	Cadherin-11	β-catenin	GEF-Trio; Rho; Rac; Cdc42	(Kashef *et al.*, 2009)

*Interaction is inferred but not directly demonstrated
†Inferred through effects on promoter based reporter activity assay
‡The cadherin influences the outcome of this mediator's signaling, but is not activating or repressing this mediator directly
#Cadherin cleavage fragment shown to mediate this signaling
◊Although no interaction is shown or inferred, the cadherin directly modulates the signaling of this protein
▪Inhibition of this protein enhances effects of cadherin mediated signaling, although no direct binding was detected or inferred