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Cross talk between focal adhesion kinase and cadherins: Role in regulating endothelial barrier function

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Abstract

A layer of endothelial cells attached to their underlying matrices by complex transmembrane structures termed focal adhesion (FA) proteins maintains the barrier property of microvascular endothelium. FAs sense the physical properties of the extracellular matrix (ECM) and organize the cytoskeleton accordingly. The close association of ahderens junction (A) protein, cadherin, with the cytoskeleton is known to be essential in coordinating the appropriate mechanical properties to cell-cell contacts. Recently, it has become clear that a crosstalk exists between focal adhesion kinase (FAK) and cadherin that regulates signaling at intercellular endothelial junctions. This review discusses recent advances in our understanding of the dynamic regulation of the molecular connections between FAK and the cadherin complex and cadherin-catenins-actin interaction-dependent changes as well as the role of small GTPases in endothelial barrier regulation. This review also discusses how a signaling network regulates a range of cellular processes important for barrier function and diseases.

Keywords

Barrier function; focal adhesion (FA); Focal adhesion kinase(FAK); Adherens junction (AJ); cadherin

INRODUCTION

Cell adhesion relies upon specialized transmembrane adhesion proteins, the cell adhesion molecules, through which cell-cell and cell-matrix interactions are mediated. These families of molecules act through receptor-ligand interactions that usually extend from the intracellular space to the extracellular space where they bind to other cell membranes (cell-cell) or to the cell-matrix. Adhesion sites that defined structural contact between cells and the ECM were initially described in studies using interference-reflection microscopy and electron microscopy (Abercrombie and Dunn, 1975; Abercrombie et al., 1971; Izzard and Lochner, 1976; Izzard and Lochner, 1980). These studies revealed that matrix adhesion occurs at many specialized, elongated small regions along the ventral plasma membrane tightly connected with the substrate. Moreover, these sites termed FAs, are associated with

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actin microfilaments at their cytoplasmic aspects and play an important role in the regulation of actin cytoskeleton organization, the adhesive interaction between integrins and their extracellular ligand, and the regulation of endothelial barrier integrity. FAK has emerged as a mediator of crosstalk between integrin-mediated FAs and intercellular adherens junction (AJs). FAK plays a central role in initiating and integrating various signaling pathways that ultimately affect barrier function. Evidence points to the importance of FAK activation in the regulation of microvascular barrier function (Holinstat et al., 2006; Quadri et al., 2003; Quadri and Bhattacharya, 2007). On one hand, FAK activation is essential in the maintenance of endothelial barrier properties, and inhibition of FAK activity leads to leaky microvessels (Holinstat et al., 2006; Quadri et al., 2003; Quadri and Bhattacharya, 2007). Conversely, FA assembly and activation serve as important signaling events in increasing endothelial permeability under stimulatory conditions, such as in the presence of angiogenic factors (Eliceiri et al., 2002; Zachary, 2003) and inflammatory mediators (Uehata et al., 1997).

Adhesion between cells is mediated by junctional proteins that constitute the intercellular junctional complex, which has an important role in defining the physiological function of a cell. Cadherins are plasma membrane proteins associated with AJs that make important contributions to barrier function, embryogenesis, and tissue homeostasis (Gumbiner, 2005; Halbleib and Nelson, 2006; Nishimura and Takeichi, 2009). AJs are characterized ultrastructurally as plasma membrane associated organelles comprised of opposing dense plaques at cell-cell contacts. The extracellular domains of cadherins are involved in homotypic interactions required for the formation of AJs; the cytoplasmic domain associates with catenins that link AJs to the actin cytoskeleton for junctional stabilization (Hirokawa and Heuser, 1981; Miyaguchi, 2000). The close association of the cadherin molecules with the cytoskeleton is known to be essential in coordinating the appropriate mechanical properties to cell-cell contacts. How adhesive interactions between cells generate and maintain the endothelial barrier remains one of the most challenging questions in understanding the basis of endothelial barrier function. AJs and the cadherin-catenin complex are therefore the subjects of intense research. Recent work has greatly advanced our understanding of the molecular organization of AJs and how cadherin-catenin complexes engage actin.

We reviewed the molecular structure and function of FAK (Sunita Bhattacharya, 2005) and cadherins (Parthasarathi, 2009). The review addresses FAK and VE/E-cadherin signaling in endothelial barrier regulation. This review also addresses cadherin-actin based adhesion, specifically the association between intracellular VE/E-cadherin molecules and the actin cytoskeleton. In addition to their adhesive function, cell adhesion molecules modulate signal transduction pathways by interacting with receptor tyrosine kinases and Rho-family GTPases for example (Braga, 2002; Noren et al., 2003; Yap and Kovacs, 2003). Hence, changes in the expression of cell adhesion molecules affect not only the adhesive properties, but also the signal transduction status of a cell. Conversely, signaling pathways modulate the function of cell adhesion molecules, altering the interactions between cells and their environment. This leads to changes in cell-cell and cell-matrix interactions, hence, microvascular endothelial barrier regulation. The combined application of new approaches, such as live cell imaging with molecular manipulation by DNA, protein transfection, and gene silencing will continue to provide excellent tools for FA and AJ regulation studies. Quantitative confocal and two photon microscopy methods that allow for simultaneous measurements of AJ protein dynamics and permeability in the intact microvessel provide a unique direction of future studies.

FAK LOCALIZATION AND FUNCTION

FAK is a major player in mediating signaling initiated at sites of cell-matrix attachment and at activated growth factor receptors, such as those for platelet-derived growth factor (PDGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF) (Chen and Chen, 2006; Garces et al., 2006; Sieg et al., 2000). FAK is most commonly found at the cell membrane, in FAs, in smaller focal contacts, or in nascent spreading adhesions (de Hoog et al., 2004; Serrels et al., 2007). In epithelial cell-cell junctions the FAK NH2-terminal domain targeted to the nuclei and intercellular junction (Stewart et al., 2002), raises the possibility that the generation of NH2-terminal FAK fragments by post-translational processing may provide a novel mechanism for modulating cell junction. Different FAK domains may be postulated to play distinct cell type specific roles. FAK NH2-terminal fragments are generated during apoptosis (Lobo and Zachary, 2000). FAK resides in the nucleus, implying that it may ‘travel’ between subcellular locations. The role of nuclear localization of the FAK NH2-terminal domain is not known.

Protein interactions with the FAK carboxy-terminal, the focal adhesion targeting sequence (Hildebrand et al., 1993), are thought to determine its subcellular localization. FAK localization to focal adhesions is mediated primarily by the COOH-terminal focal adhesion targeting (FAT, residues 840–1052) domain (Hildebrand et al., 1993). The COOH-terminal domain of FAK is expressed in some tissues as an alternative transcript encoding a 41–43kDa protein called FRNK (for FAK-related non-kinase) (Schaller et al., 1993), and this domain antagonises FAK signaling by competing for binding to focal contacts (Taylor et al., 2001). Evidence shows (Holinstat et al., 2006) that inhibition of FAK by adenoviral expression of FRNK (a dominant negative FAK construct) in monolayer prevented p190RhoGAP phosphorylation, increased RhoA activity, induced actin stress fiber formation, and produced an irreversible increase in endothelial permeability in response to thrombin. Expression of FRNK in lung microvessel endothelia increased vascular permeability. RhoA is known to increase endothelial monolayer permeability by disrupting adherens junctions and reorganizing the cell-ECM attachment sites (Carbajal et al., 2000).

FAK activity is necessary for barrier enhancement (Holinstat et al., 2006; Quadri et al., 2003; Quadri and Bhattacharya, 2007) and controls diverse cellular processes, as well as biological properties associated with barrier function (Mehta et al., 2002; Quadri et al., 2003) and disease, such as vascular development, cardiomyocyte-induced hypertrophy, fibrosis, and epithelial cancer (Chishti et al., 1998; Lim et al., 2008; Luo and Guan, 2010; van Nimwegen and van de Water, 2007; Zhao and Guan, 2009). This range of functions is evidence that FAK performs fundamentally important roles in cells, the details of which continue to be uncovered. Indeed, at the cell cortex, FAK regulates integrin-dependent cell-matrix interactions, promoting dynamic actin and adhesion changes at the membrane and signaling to proliferation and survival pathways. Although FAK associated with cadherin, but does not have any binding site for cell-cell junctional protein for example cadherin / catenin, hence FAK does not interact directly with junctional protein. FAK transmit signals to junctional protein through intermediate molecules. It is not yet clear how the combined scaffold and kinase functions of FAK integrate signaling outputs that coordinate cell adhesion and barrier regulation.

FAK AND ACTIN SIGNALING

FAK influences adhesion by its direct or indirect effects on actin and adhesion regulators, such as the RhoGTPases (Noren et al., 2003). Neural Wiskott–Aldrich syndrome protein (n-WASP), which is an effector for the RhoGTPase CDC42 (Wu et al., 2004) is a binding

partner and substrate of FAK. WASP transduces extracellular signals into reorganization of the cytoskeleton and regulates actin-related protein (Arp2/3). Phosphorylation of n-WASP on Tyr256 affects its nuclear localization and promotes cell migration (Wu et al., 2004). In addition to n-WASP, the FAK-FERM domain (N-terminal) also binds directly to the Arp3, which induces an activating conformational change in the Arp2/3 complex (Fig. 1). This promotes nucleation by bringing an actin monomer to Arp2/3 via WASP-homology 2 domain, enhancing actin polymerization (Serrels et al., 2007), and stabilizing the newly formed actin (Winder, 2003). FAK is needed for proper assembly of nascent integrin adhesions. Arp3 is located at the tip; of nascent adhesion structures as they form (Serrels et al., 2007). The FAK FERM–Arp3 interaction is an example that directly links integrin signaling with actin polymerization machinery in the vicinity of nascent adhesions (Fig. 1).

Receptor for activated kinase C1 (RACK1) is found in nascent integrin adhesions but not in mature focal adhesion structures (de Hoog et al., 2004; Serrels et al., 2007). RACK1 binds to the FERM domain of FAK, at different FERM sequences to ARP3 (Fig. 1), and this complex is also located at nascent adhesions (Serrels et al., 2010). The FAK FERM–RACK1 interaction enables directional responses, and this may contribute to FAK's role in an invasive cancer phenotype (Lahlou et al., 2007; Luo and Guan, 2010; McLean et al., 2004). Although this interaction between two molecular scaffolds (FAK and RACK1) is important physiologically, it is not clear whether RACK1 binding to the FERM domain activates FAK kinase activity (Serrels et al., 2010). Key effecter substrates of FAK in different functions have not widely been identified. The relative importance of the adaptor and kinase functions in all of FAK's biological activities and endothelial barrier regulation remains unknown.

CADHERIN ORGANIZATION AND FUNCTION

The mechanisms of cellular signaling and adhesion are thought to be closely connected, such that adhesion components have double (or more) functions and interconnect in a signaling structural network (Pece and Gutkind, 2000). AJs function as clusters during zonula adherens assembly and dynamic cell–cell interactions. Cadherins are the principal components of AJs and clusters at sites of cell–cell contact (Parthasarathi, 2009). The cadherin family consists of classical cadherins, which are the main mediators of calcium-dependent cell–cell adhesion, and non-classical cadherins, which include desmosomal cadherins and the recently discovered large subfamily of protocadherins, which are implicated in neuronal plasticity. Classical cadherins are a family of single-span, transmembrane-domain glycoproteins that function specifically as cell–cell adhesion molecules. The classical cadherins are further subdivided into types I and II on the basis of sequence homology. These are three major cadherins found: vascular endothelial (VE), epithelial cadherin (E), and neuronal cadherins (N) (Corada et al., 1999; Liaw et al., 1990) in the vascular endothelium. VE-cadherin (also cadherin-5) is located at intercellular junctions of all endothelial types, and its expression has been confirmed both *in vitro* and *in vivo* (Dejana et al., 1999; Liaw et al., 1990). In the intact pulmonary vasculature, large vessels primarily express VE-cadherin (Gao et al., 2000; Parker et al., 2006; Safdar et al., 2003). Evidence show strong VE-cadherin expression in arteries, arterioles, and capillaries but almost no expression in veins and venules, suggesting vessel type-specific expression of VE-cadherin in regular human lung tissue, independent of age or sex (Herwig et al., 2008). Rat pulmonary microvessels express E-cadherin (Godzich et al., 2006; Ofori-Acquah et al., 2008; Parker et al., 2006; Quadri et al., 2003). VE-cadherin belongs to the type II sub group; only 23% of its sequence is identical with the classical cadherins, E-, and N-cadherins from the type I sub group (Breier et al., 1996). N-cadherin is not clustered at cell–cell junctions, but distributed diffusely in the cell membrane (Salomon et al., 1992). A morphological and functional endothelial heterogeneity has been proven for micro- and macrovascular

endothelial cells of different organs, different species, and different compartments of the same organ (Cines et al., 1998; Volk and Kox, 2000).

Crystal structure studies show that cadherins contain a N-terminal extracellular region, a transmembrane anchor, and a cytoplasmic intracellular region (Fig. 1). The monitoring of cell aggregation by the binding of cells to immobilized cadherin ectodomains, or the binding of beads coated with purified cadherins, have led to the concept that cadherins function as homotypic cell adhesion molecules (Gumbiner, 2005). Cadherin molecules form homodimers on the cell surface; homotypic adhesion forms zipper-like adhesion, which may progress to extensive multimer formation (Boggon et al., 2002; Gumbiner, 2000). Functional features of homotypic adhesion might provide the barrier properties; for example, in mouse lung endothelial cells, a mutant of VE-cadherin lacking the extracellular domain, ΔEXD, increases vascular permeability (Broman et al., 2006).

Several groups have determined the three dimensional structures of the type I cadherin extracellular domain (Fig. 2) (Haussinger et al., 2004; Nagar et al., 1996; Pertz et al., 1999; Shapiro et al., 1995; Tamura et al., 1998). The extracellular domain consists of five ectodomains with immunoglobulin-like topology, ranging from the membrane-distal EC1 domain to the membrane-proximal EC5 domain (Boggon et al., 2002; Gumbiner, 2000; Harrison et al., 2010) Structural studies (Al-Amoudi and Frangakis, 2008; Nose et al., 1990; Shan et al., 1999), binding affinity measurements (Parisini et al., 2007), sequence analysis (Kitagawa et al., 2000), and molecular simulations (May et al., 2005; Tamura et al., 1998) have provided a detailed picture of the trans dimerization process that mediates cell-cell interaction. Trans dimerization is mediated by an interface formed between two cadherin molecules from opposing cells that swap the N-terminal β-strands of their EC1 domains, anchored by binding of the highly conserved Trp2 (Fig. 2). Cadherins show an exquisite specificity in their homophilic interactions by almost exclusively binding the same type of cadherin on the adjacent adherence cell. Binding between cadherin extracellular domains is relatively weak, but cell-cell adhesion may be strengthened by lateral clustering of cadherins mediated by protein linkages between the cadherin cytoplasmic domain and the actin cytoskeleton (Jamora and Fuchs, 2002). Thus, intracellular faces of these contacts are associated with the actin cytoskeleton in AJs (Fig. 1).

CADHERIN-CATENIN-ACTIN INTERACTION

The intracellular domain of classical cadherins, which is lacking in non-classical cadherins and protocadherins, interacts with various catenin proteins to form the cytoplasmic adhesion complex (Parthasarathi, 2009). In mice and humans, 5 type I cadherins and 13 type II cadherins have been described (Posy et al., 2008). Type I and type II cadherins share several common structural features. They both contain an ectodomain region, which is composed of five tandem extracellular cadherin domains, each of about 110 amino acids (Boggon et al., 2002; Nagar et al., 1996). Classical cadherins are anchored by a single-transmembrane region and have a short cytoplasmic domain with conserved binding sites for β/γ-catenins and p120 catenins (Ishiyama et al., 2010; Lampugnani et al., 1995), which help to mediate attachment to the cytoskeleton and to control cadherin trafficking (Liu et al., 2007; Reynolds and Carnahan, 2004). The cytoplasmic C-terminus of cadherin binds to intracellular proteins; β-catenin and p120 catenin (Type I cadherin) or γ-catenin (Typ II cadherin). β-Catenin binds to both the C-terminus of the cadherin intracellular domain and the N-terminus of α-catenin (Fig. 1). The E-cadherin cytoplasmic domain forms a high affinity, 1:1 complex with β-catenin, which binds with lower affinity to α-catenin (Aberle et al., 1994; Hinck et al., 1994; Huber and Weis, 2001; Pokutta and Weis, 2000). α-Catenin binds directly to F-actin of the cytoskeleton (Fig. 1) also through number of actin binding proteins, such as α-actinin and vinculin. Absence of α- or β-catenin results in defective cell adhesion

and failure of cadherin-catenin complexes to associate with the actin cytoskeleton. The Nelson group has challenged this view and suggested alternative roles for α-catenin in the junction (Drees et al., 2005; Yamada et al., 2005). They demonstrated with purified recombinant proteins that α-catenin cannot bind to β-catenin and actin simultaneously (Fig. 1), even in the presence of actin binding proteins. In fluorescence recovery after photobleaching (FRAP) experiments, E-cadherin, β-catenin, and α-catenin displayed very similar diffusional behaviors on the membrane, whereas actin associated with cell-cell contacts diffused more rapidly and was more mobile (Yamada et al., 2005). Moreover, deletion of the cadherin cytoplasmic domain or the actin-binding domain of α-catenin, which would break the link to actin, did not significantly alter the dynamics of the cadherin-catenin complex (Yamada et al., 2005). This evidence independently confirms the lack of a stable linkage between the cadherin-catenin complex and actin through α-catenin at cell-cell contacts. The Nelson group also has shown that the mammalian α-catenin isoform binds to actin preferentially as a dimer, and the α-catenin monomer binds to β-catenin (Drees et al., 2005). Thus, α-catenin may not be able to bind β-catenin and actin at the same time (Fig. 1), suggesting that α-catenin is not directly involved in the linkage between the E-cadherin–β-catenin complex and actin filaments (Drees et al., 2005). How cadherin cytoplasmic domains contribute to the stabilization of cell adhesion and endothelial barrier regulation is not clear.

REGULATION OF CADHERIN COMPLEX THROUGH SIGNALING MOLECULES

Cadherins are considered structural proteins, but there is evidence that cadherins are targets for signaling pathways that regulate adhesion, but also signaling molecules may themselves that regulate basic cellular processes, such as migration, proliferation, apoptosis and cell differentiation (Barth et al., 1997; Hulsken et al., 1994; Morin et al., 1997). Cadherin does not exhibit any enzymatic activity; therefore, their ability to function as signal transducing receptors depends on their physical interactions with other effectors. For example, phosphoinositide 3'-kinase (PI3K) is recruited to cell-cell contacts (Singleton et al., 2005; Sovova et al., 2004), activated by cadherin (Singleton et al., 2005; Sovova et al., 2004) and E-cadherin interacts with receptor tyrosine kinases, such as epidermal growth factor receptor (Andl and Rustgi, 2005).

Role of receptor tyrosine kinase (RTK) signaling

Tyrosine phosphorylation has been implicated in the regulation of cadherin function resulting in the disassembly of the cytoplasmic adhesion complex and, subsequently, the disruption of cadherin-mediated cell-cell adhesion. This includes phosphorylation of receptor tyrosine kinases, RTKs, which are frequently activated in cancer cells: epidermal growth factor receptor, hepatocyte growth factor receptor (c-MET), and fibroblast growth factor receptor. Converse to the regulation of E-cadherin function by RTKs, functional adhesion junctions can also affect the RTKs activity. For example, E-cadherin-mediated, cell-cell adhesion has been shown to repress EGF-induced epidermal growth factor receptor activation (Takahashi and Suzuki, 1996). Ligated E-cadherin also recruits epidermal growth factor receptor and induces its ligand-independent activation, leading to the activation of signal transduction cascades, including the PI3K and mitogen activated protein kinase (MAPK) pathways and to tumor cell survival (Kovacs et al., 2002; Pece and Gutkind, 2000). E-cadherin-mediated cell adhesion also induces the activation and phosphorylation of the RTK, resulting in the repression of cell-matrix adhesion (Zantek et al., 1999). However, the functional implication of this mechanism in barrier function is not known.

Role of nonreceptor tyrosine kinase (Src)

Disassembly of cadherin includes phosphorylation of the non-RTK, Src, which phosphorylates E-cadherin, neuronal (N)-cadherin, β-catenin, γ-catenin and p120-catenin (Fig. 3), resulting in the disruption of cadherin-mediated cell–cell adhesion (Behrens et al., 1993; Fujita et al., 2002; Hamaguchi et al., 1993). Cadherin molecules are not stably exposed at the cell surface; rather, they cycle on and off the plasma membrane in a highly dynamic fashion by exo- and endocytic events (Akhtar and Hotchin, 2001; Xiao et al., 2005). Internalization of E-cadherin from AJs is initiated by the Src-mediated tyrosine phosphorylation of E-cadherin (McLachlan et al., 2007; Papkoff, 1997). This posttranslational modification induces the dissociation of p120 from E-cadherin (Fig. 3), and the binding of the cbl-like ubiquitin-ligase, Hakai, which results in the ubiquitination of E-cadherin and internalization within clathrin-coated endosomes (Fujita et al., 2002; Palacios et al., 2005; Pece and Gutkind, 2002). p120 is a Src substrate and member of the catenin family (Anastasiadis and Reynolds, 2000) that binds to the juxtamembrane domain of E-cadherin (Fig. 3). p120 is involved in the maintenance of E-cadherin at the plasma membrane (Anastasiadis and Reynolds, 2000), (Ireton et al., 2002; Xiao et al., 2005). In an E-cadherin-bound state, p120 prevents the internalization of E-cadherin (Fujita et al., 2002; Pece and Gutkind, 2002). How the endocytic machinery regulates adherens junction formation or opening is an issue that requires further investigation.

Role of small GTPases

E-cadherin, once engaged in cell-cell adhesion, suppresses Rho activity by activating p190 Rho-GAP, probably through Src-family kinases, indicating that active signals are induced by the formation of cell junctions (Fig. 3). In addition to interacting with RhoGTPases through p190 Rho-GAP and p120-catenin, cadherin also communicates with these molecules through PI3K signaling. Ligation of cadherin molecules between two neighboring cells recruits PI3K to the cytoplasmic adhesion complex (Fig. 3), thereby generating phosphatidylinositol-(3,4,5)-triphosphate (PIP3) at the plasma membrane. Guanine nucleotide exchange factors (GEFs) that contain phosphatidylinositol-(3,4,5)-triphosphate (PIP3) binding pleckstrin-homology domains, such as TIAM1, are then recruited to the membrane, activating Rac1, and possibly activateing CDC42 (Fig. 3). Disrupting Rac1 or Rho interrupts AJ assembly (Braga, 2002; Yap and Kovacs, 2003), whereas CDC42 seems to regulate AJ maintenance (Kouklis et al., 2004). The Malik group demonstrated in mouse lung endothelial cells that the mutant of VE-cadherin lacking the extracellular domain (ΔEXD) increases vascular permeability, and coexpression of dominant-negative CDC42 (N17CDC42) prevents the increase of permeability induced by ΔEXD (Broman et al., 2006). This was attributed to inhibition of the α-catenin association with the ΔEXD-β-catenin complex, suggesting that CDC42 regulates AJ permeability by controlling the binding of α-catenin with β-catenin and the consequent interaction of the VE-cadherin/catenin complex with the actin cytoskeleton. Rho GTPases RhoA, Rac1, and CDC42 are important in regulating AJ assembly (Fukata and Kaibuchi, 2001; Zigmond, 2004). Cadherin-catenin interactions specifically activate Rac1, as seen in response to cadherin-based cell-cell adhesion (Lampugnani et al., 2002; Noren et al., 2001) and in cells binding to cadherin-coated substrates (Kovacs et al., 2002; Noren et al., 2001). Rac1 and CDC42 may support E-cadherin function. There seems to be a fine balance between Rac1 and Rho activity during AJ assembly. As cells make contact, Rac1 activation occurs at cell-cell contacts, whereas Rho acts at later contractile cables (Yamada and Nelson, 2007). Crosstalk between Rac1 and Rho helps the actin reconfiguration during AJ assembly.

Unfortunately, the overall picture of Rho proteins and barrier function is still not clear. For example, changing the composition of the ECM changed the function of Rac1 from a proadhesive to an anti-adhesive molecule (Sander et al., 1998). Rho family GTPases are

certainly involved in many different aspects of the various stages of cell-cell adhesion formation; however, details of their actual functional roles remain to be determined.

Role of the WNT signaling pathway

Assembly of the cadherin, β-catenin, and γ-catenin complex, which mediates cell adhesion, also has important functions in the canonical WNT signaling pathway (Bienz and Clevers, 2000) (Fig. 4). Non-sequestered, free β-catenin and γ-catenin are rapidly phosphorylated by glycogen synthase kinase 3β (GSK-3β) in the adenomatous polyposis coli (APC)-axin-GSK-3β complex and are subsequently degraded by the ubiquitin-proteasome pathway. If the tumor suppressor APC is non-functional, as in many colon cancer cells, or if the activated WNT-signaling pathway blocks GSK-3β activity, β-catenin accumulates at high levels in the cytoplasm (Fig. 4). The WNT ligand ultimately results in the stabilization of cytoplasmic β-catenin, which is then free to enter the nucleus. Subsequently, β-catenin translocates to the nucleus, where it binds to members of the transcription factor TCF/LEF1 and modulates the expression of their target genes. This dual function of β-catenin raised the question of whether the loss of cadherin function would subsequently lead to the activation of the WNT signaling pathway. In various cellular systems, it has been demonstrated that sequestration of β-catenin by E-cadherin competes with the β-catenin/TCF-mediated transcriptional activity of the canonical WNT signaling pathway (Fig. 4). The fact that E-cadherin does not completely deplete cytoplasmic catenin indicates that β-catenin exists in different functional pools (Gottardi et al., 2001; Orsulic et al., 1999; Stockinger et al., 2001). Since the activation of transcription factor Kruppel-like factor (KLF4), regulates VE cadherin expression (Cowan et al. 2010), and also interacts with C-terminal domain of β-catenin (Evans et al. 2010), might inhibits Wnt signaling (Fig. 4). Hence maintains the integrity of AJs, preventing vascular leakage in response to inflammatory stimuli.

CROSS TALK BETWEEN FAK AND CADHERIN-MEDIATED ADHESIONS

FAK is a critical bidirectional linkage between the actin cytoskeleton and the cell-matrix interface, thus providing stability that maintains endothelial cell barrier integrity. FAK activation and enhancement of AJs associated with RhoGTPase (Birukov et al., 2002; Shikata et al., 2003). RhoGTPase activity is subject to regulation by GEFs, guanine nucleotide dissociation inhibitors (GDIs), or GTPase activating proteins (GAPs). RhoGDI-1 (RhoGDIα) represses RhoA activation and thus protects endothelial cell junctions from disassembly (Gorovoy et al., 2007). RhoA activity also inhibited through the activation of p190RhoGAP (Holinstat et al., 2006). FAK activates p190RhoGAP after thrombin stimulation to inhibit the increase in permeability facilitated by RhoA (Fig. 3) and reassembles disrupted endothelial cell junctions, (Holinstat et al., 2006).

In a barrier protective effect, cAMP directly activates Epac, a Rap1GEF, (de Rooij et al., 1998) enhancing VE-cadherin junctional integrity and actin reorganization to decrease endothelial permeability (Kooistra et al., 2005). Rap1 decreases basal endothelial permeability by enhancing distribution of both AJs and tight junctions (Cullere et al., 2005; Kooistra et al., 2005), and Rap1 antagonizes thrombin-induced increased permeability by inhibiting activation of RhoA (Cullere et al., 2005; Kooistra et al., 2005). Since activation of FAK and CDC42 (Fig. 3) also parallels the time course of re-formation of AJs and endothelial barrier protection following thrombin challenge (Kouklis et al., 2004; Schilling et al., 1992), it is likely that Rap-1, FAK, and CDC42 act in concert to down regulate RhoA activity and to promote the reformation of endothelial cell junctions.

The effect of FAK, barrier strengthening or weakening, varies depending on the nature of stimuli and the physical or chemical states of surrounding matrices. For example, inhibiting signaling through FAK or decreasing FAK expression can promote assembly or disassembly

of cadherin-mediated cell-cell adhesions, respectively, depending on cell context and cadherin type (Avizienyte et al., 2002; Yano et al., 2004). In one case, loss of FAK or paxillin from HeLa cells leads to increased peripheral Rac1 activity and deregulation of N-cadherin-mediated cell–cell adhesion. In contrast, it is reported that integrin-induced activation of FAK can also result in activation of Rac1 via a p130Cas/CrkII/DOCK180 complex with DOCK180 acting as a Rac1 GEF (Cheresh et al., 1999; Hsia et al., 2003) (Fig. 3). This suggests that FAK can signal to Rac1 via different effectors and that these signaling pathways may have distinct, and probably localized, biological consequences.

From our studies using rat lung microvascular endothelial cells, it is evident that E-cadherin acts as a switch to either increase or decrease barrier strength through FAK signaling, which in turn regulates cadherin accumulation or clustering (Quadri and Bhattacharya, 2007). Moreover, H_2O_2 exposure induces an immediate loss of surface E-cadherin that then progressively increases with time. This response may be due to focal adhesions driving E-cadherin toward the surface. Thus, inhibition of FAK activation may block the signal for E-cadherin translocation to the surface, thereby compromising the integrity of the microvascular barrier. This suggests that in ECs, FAK activation is required for proper localization of E-cadherin to the cell periphery and for consequent strengthening of the endothelial cell barrier (Quadri and Bhattacharya, 2007). By contrast in other cell types, as in KM12C colon cancer cells, Src induced deregulation of E-cadherin requires αv/β1 integrin and Src-dependent tyrosine phosphorylation of FAK, suggesting Src–FAK has a negative influence on cadherin-mediated intercellular adhesion in motile phenotypes (Avizienyte et al., 2002). Since p120 is a Src substrate (Anastasiadis and Reynolds, 2000), cytoplasmic p120 binds the Vav2 exchange factor (Fig. 5) and regulates the activity of the small G-proteins Rac1, CDC42 and RhoA (Noren et al., 2001); could explain the cadherin-mediated intercellular deregulation, but E-cadherin-bound p120 prevents the internalization of E-cadherin (Fujita et al., 2002; Pece and Gutkind, 2002). This diversity of responses of FAK to Src could be due to some cell- or context-dependent signaling from FAK to RAC1 and on other upstream signaling inputs, such as Src activity. These findings suggest that FAK's activation induced signaling positively regulates intercellular adhesion; however, the Src-induced signaling pathway negatively regulates cell–cell adhesion.

Downstream of FAK, paxillin is also important for endothelial barrier regulation. Paxillin is a multidomain adapter, FA protein that functions as a molecular scaffold for protein recruitment to FAs and thereby facilitates protein networking and efficient signal transmission (Turner, 2000; Turner and Brown, 2001). Evidence shows that in mouse lungs and in HUVEC cells, loss of VE-cadherin junctional assembly in microvessels causes permeability and the reversal of the loss of barrier function after VE-cadherin junctions were reannealed in Ca^{2+} switch assay in the intact mouse lung (Gao et al., 2000). Reported findings has shown that human pulmonary endothelial cells undergo S1P-induced enhancement of VE-cadherin and association of β-catenin with paxillin (Fig. 3), which is critically dependent on Rac and CDC42 activities (Birukova et al., 2007) and is abolished by pharmacological or small interfering RNA (siRNA)-mediated inhibition of Rac and CDC42. It is also showed that enhancement of the VE-cadherin interaction with α-catenin and β-catenin was associated with the increased formation of FAK-β-catenin complexes. Depletion of β-catenin by siRNA resulted in loss of S1P-mediated, VE-cadherin association with FAK as well as paxillin rearrangement (Birukova et al., 2007) (Sun et al., 2009). Since β-catenin does not contain FAK or paxillin binding sites, possibly FAK and paxillin indirectly interacts with β-catenin and VE cadherin. Paxillin interacts with signaling proteins Crk, p60Src-kinase, FAK, (Fig. 3), and structural FA-associated proteins such as vinculin, actopaxin, and tubulin (Turner, 2000; Turner and Brown, 2001). Paxillin also binds to paxillin kinase linker (PKL/GIT2). GIT2 is a member of ADP-ribosylation factor GTPase activation factors (ARF GAP) family, and participate in Rac- and Rho-mediated signaling

events at FAs (Mazaki et al., 2001; Turner, 2000; Turner and Brown, 2001). Enhancement of cadherin and association of β-catenin with paxillin is critically dependent on Rac and CDC42 activities (Fig. 3). These findings suggested that Rac and CDC42 GTPases have been implicated in the assembly of these complexes.

In addition to the effects of cadherin-mediated adhesion on Rho GTPase activity, cytoskeleton-associated signaling proteins also have an effect on the stability of the cytoplasmic adhesion complex. GTPase-activating protein, IQGAP1, a downstream effector of Rac1 and CDC42 (Fig. 5) is known to negatively regulate E-cadherin mediated cell-cell adhesion by interacting with β-catenin and displacing α-catenin from the cytoplasmic adhesion complex (Kuroda et al., 1998). Activated GTP-bound forms of Rac1 and CDC42 sequester IQGAP1 and prevent its binding to β-catenin, thereby stabilizing cadherin-mediated cell adhesion (Fukata et al., 1999). Indeed, IQGAP1 expression or function has been observed during tumor progression in gastric cancer cells, for example (Takemoto et al., 2001). However, it remains to be determined whether IQGAP1-mediated disruption of cadherin function is a general process in barrier disruption. The understanding that the linkage between the cadherin-catenin complex and the actin cytoskeleton (Conacci-Sorrell et al., 2002; Gumbiner, 1996) is important for barrier regulation comes from findings that barrier-deteriorating stimuli deplete both the cadherin-catenin complex (Rabiet et al., 1996) and actin (Ehringer et al., 1999) from the cell periphery, thereby raising the possibility that FAK and cadherin-mediated, cell-cell contacts communicate with each other.

SUMMARY

As described above, FAK and VE/E-cadherin are able to associate with actin and signal transduction pathways by interacting with molecules such as receptor tyrosine kinases, Rho-family GTPases and components of the WNT signaling pathway. The expression of FAK and cadherin affect not only the adhesive properties of a cell, but also the signal transduction status. Conversely, signaling pathways can modulate the function of FAK and cadherin, altering the interactions between cells and their environment. Although many different examples of signaling mediated by FAK and cadherin have been reported, the functional implications of signaling molecules between FAK and cadherin crosstalk will certainly be a key focus of future research.

FUTURE PERSPECTIVE

In this review, I have discussed topics that appear to be crucial for understanding the structure and function of adhesions, including the molecular complexity of these sites, their heterogeneity, and their dynamics. The molecular complexity of FAs is probably considerably greater since many of these components are still unknown and others can be post-translationally modified or proteolytically processed, undergoing conformational changes. To provide an insight into the local molecular architecture of adhesion sites, advanced ‘multi-dimensional microscopy’ is needed; this will allow the simultaneous localization of multiple components at a high spatial and temporal resolution. Imaging of molecular interactions using fluorescence resonance energy transfer will be needed for studies of these complex molecular interactions *in situ*. Such approaches may help uncover not only the molecular architecture of adhesion sites but also the ways in which they function in matrix rearrangement, adhesion-mediated signaling, and endothelial barrier regulation.

Highlights

FAK and cadherin signaling coordinates appropriate changes at the cell-cell contact.

Cadherin organization and endothelial barrier function.

Cadherin-catenins-actin interactions are in question.

The role of small GTpases in the FAK and cadherin mediated cross talk.

The signaling molecules between FAK and cadherin cross talk will be a key focus of future.

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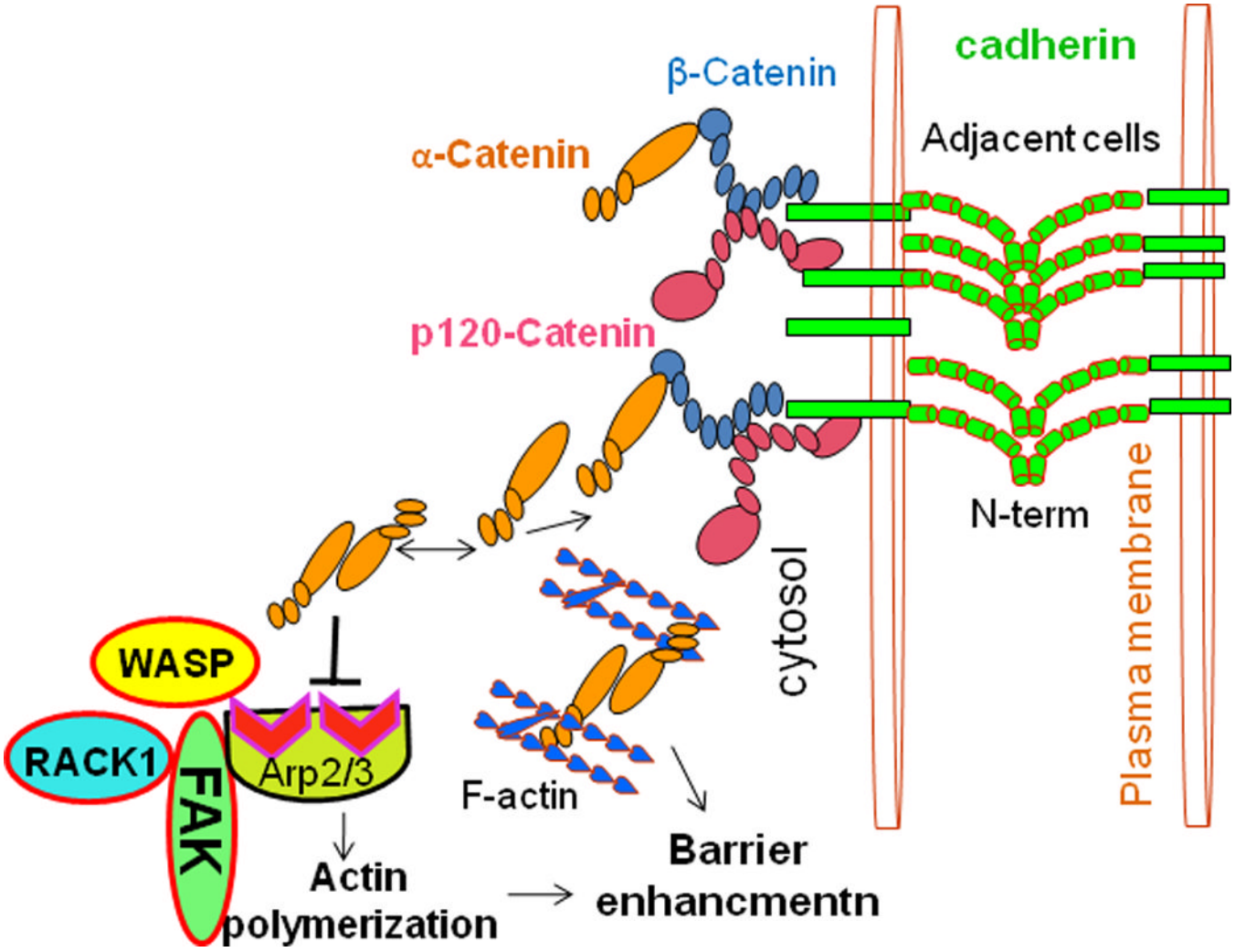

Fig. 1. Model of FAK and the cadherin complex function in actin polymerization

FAK signals to Arp2/3 in modulating cell adhesion and actin polymerization. The FAK N-terminal domain binds directly to Arp3, which promotes nucleation by bringing an actin monomer to Arp2/3 via the WASP-homology 2 domain, thereby enhancing actin polymerization. The α-catenin isoform binds to actin preferentially as a dimer, and the α-catenin monomer binds to β-catenin; therefore α-catenin does not bind to β-catenin and actin simultaneously.

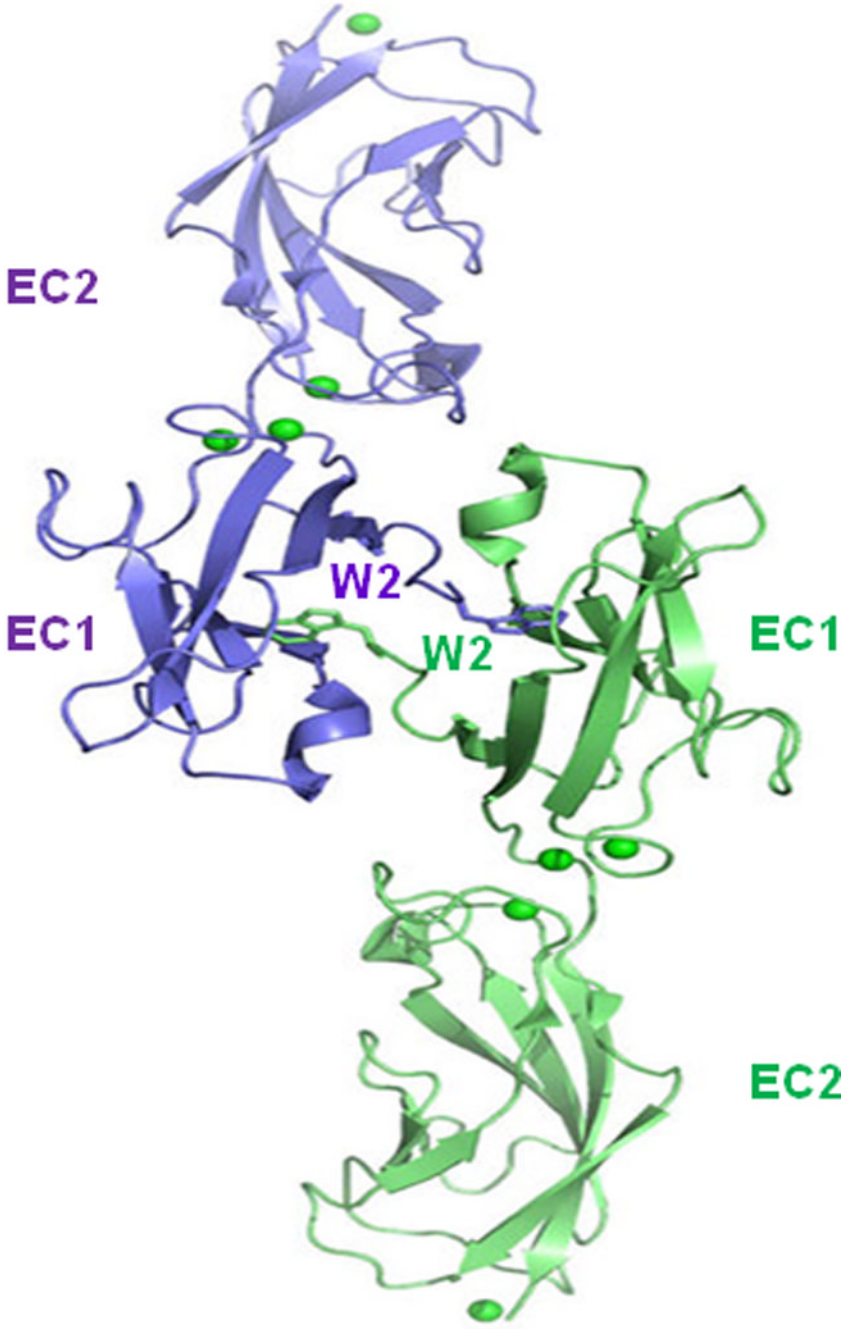

Fig. 2. Structure of strand-swapping in wild-type E-cadherin fragments (trans-dimer)

Ribbon diagram shows the strand-swapped dimer formed between protomers of wild-type E-cadherin EC1 in the crystal. Side chain atoms are shown for Trp2 residues (W2) and calcium ions are displayed as green spheres (reproduced with permission of the Nature Structure Molecular Biology Harrison, 2010).

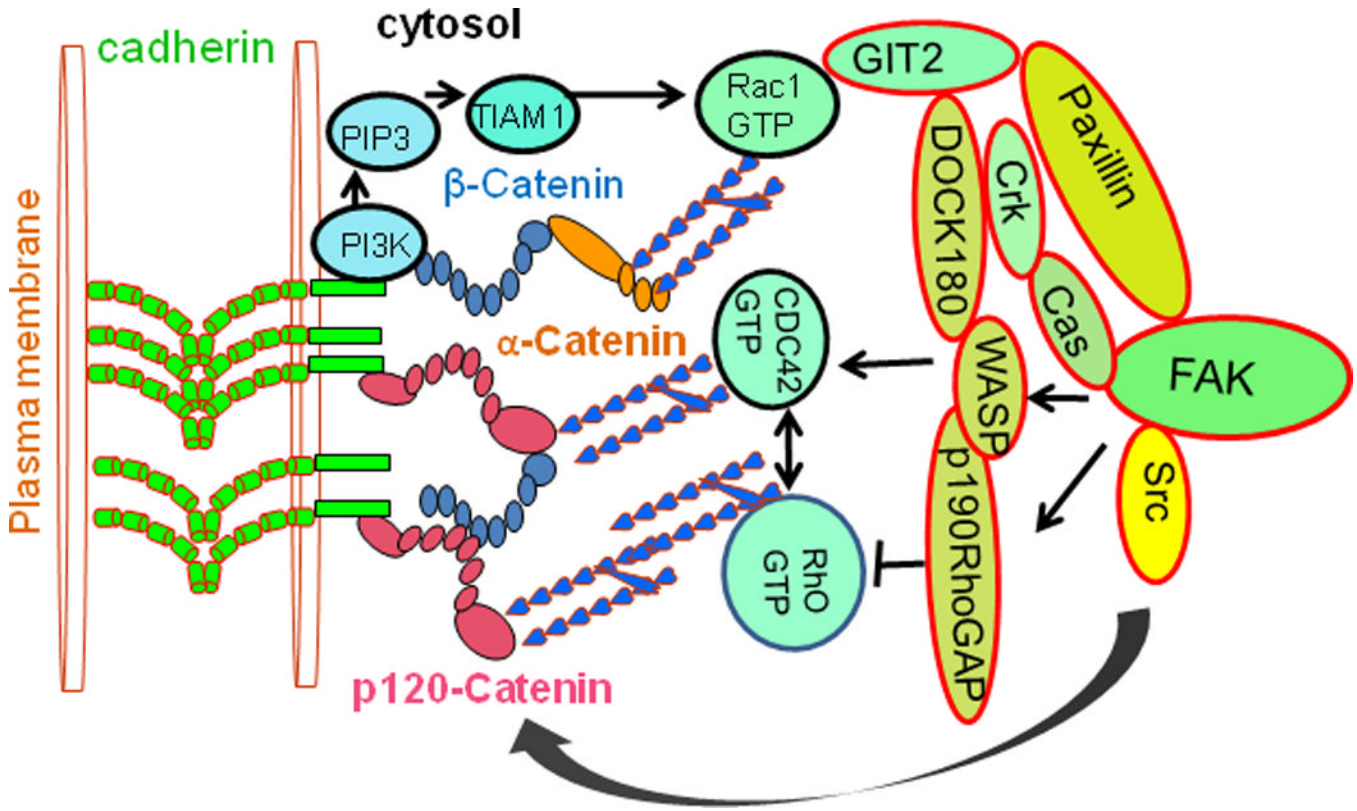

Fig. 3. Interactions between FAK and the cadherin complex

Signal from FAK to Rac1 plays a role in modulating cell adhesion and actin polymerization. PI3K is recruited to the membrane by intact E-cadherin AJs, where it generates PIP3, resulting in the activation of the Rho-GEF, TIAM1 and subsequently of Rac1 and CDC42. Activated FAK enhances Rac1 activity via a Cas/CrkII/DOCK180 complex. Paxillin binds to GIT2, participate in Rac- and Rho-mediated signaling events at FAs. Association of β-catenin with paxillin is depends on Rac and Cdc42 activities. Src family Activates p190 Rho-GAP, activated p190 Rho-GAP suppresses Rho activity. p120 is a Src substrate and is involved in the maintenance of cadherin at the plasma membrane.

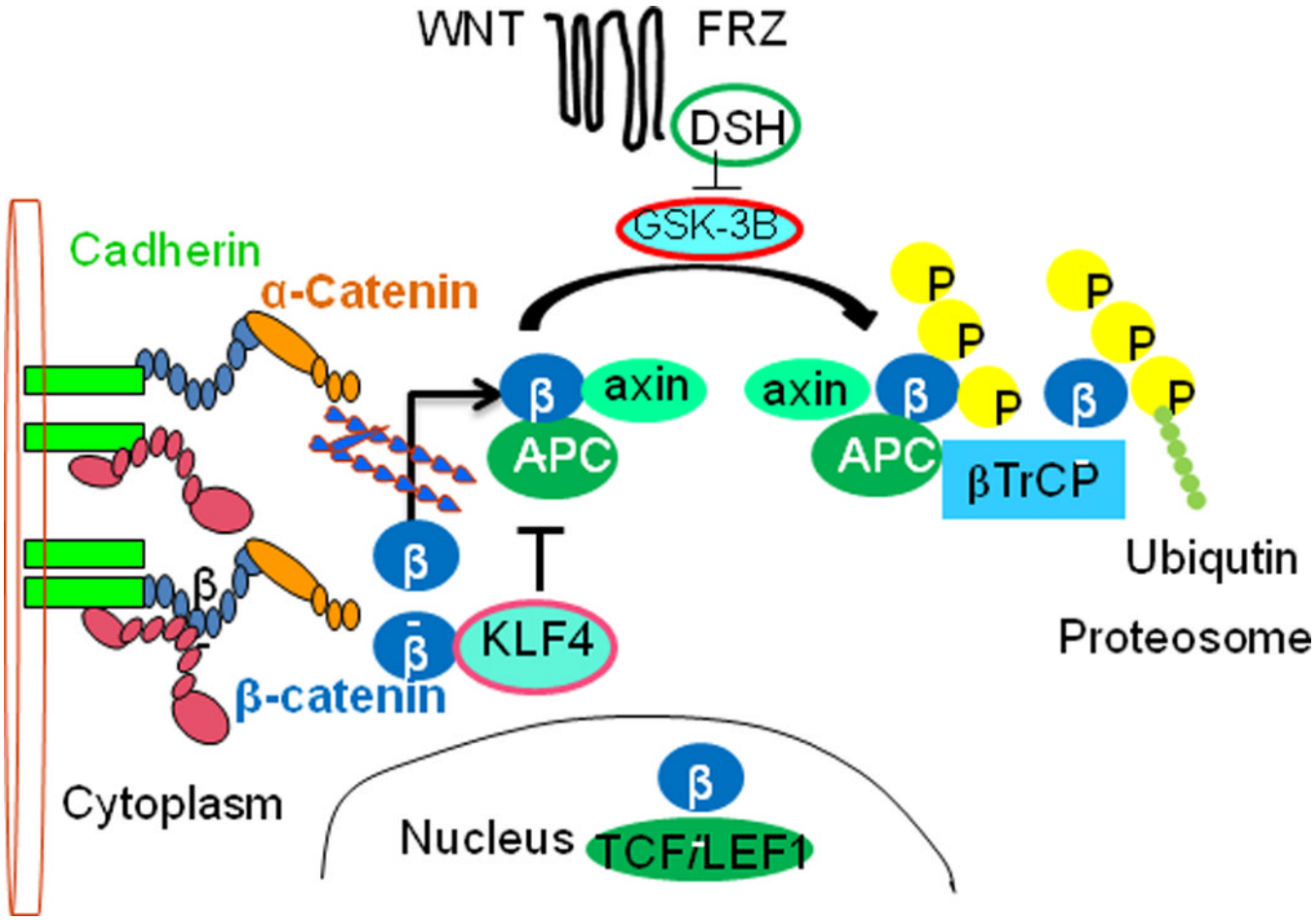

Fig. 4. Signaling pathways affected by loss of cadherin function

Upon disassembly of the cytoplasmic cell-adhesion complex, catenins are released and accumulate in the cytoplasm. β-Catenin (β) is then sequestered by the adenomatous polyposis coli (APC)–axin–glycogen synthase kinase 3β (GSK-3β) complex and phosphorylated by GSK-3β. Phosphorylated β-catenin is specifically bound by βTrCP, a subunit of the E3 ubiquitin-ligase complex, which ubiquitylates β-catenin and thereby marks it for rapid proteosomal degradation. However, on activation of the WNT signalling pathway, GSK-3β is repressed, and β-catenin is no longer phosphorylated. β-catenin translocates to the nucleus where, together with the TCF/LEF1 transcription factors, it modulates the expression of several target genes. Transcriton factor KLF4 interacts with β-catenin and inhibits Wnt signaling pathway.

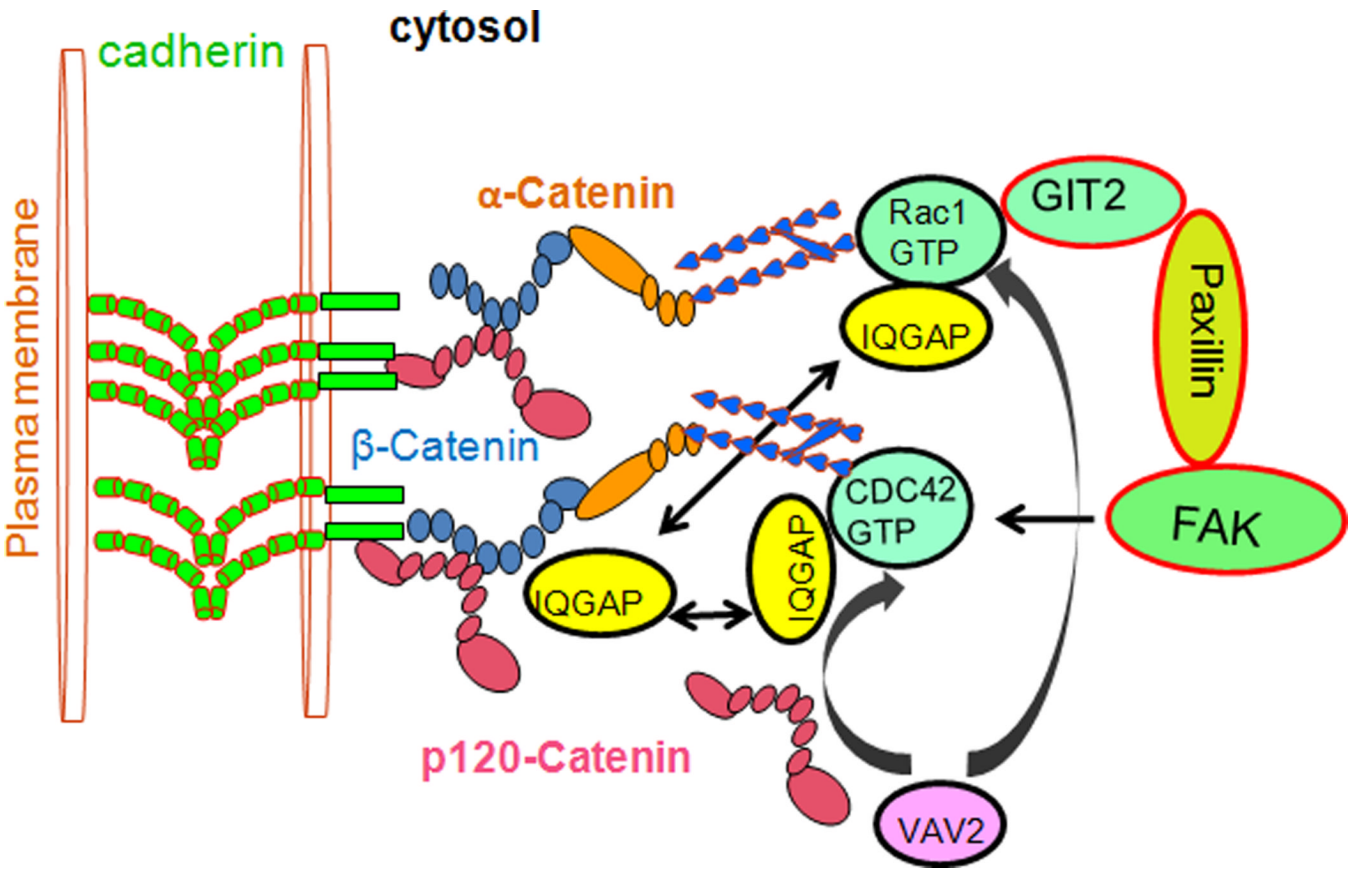

Fig 5. Cross talk between FAK and cadherin

Since β-catenin does not contain FAK or paxillin binding sites, FAK signals to Rac, activated Rac1 and CDC42 sequester the GTPase-activating protein IQGAP1, and prevent its binding to β-catenin, thereby stabilizing cadherin-mediated cell adhesion. Otherwise in free form IQGAP1 binds to β-catenin, thereby displacing α-catenin from the cytoplasmic adhesion complex and disrupting the anchoring of the cytoplasmic complex to the cytoskeleton. Cytoplasmic p120-catenin (p120) activates the Rho-family GTPases Rac1 and CDC42 through the VAV2 (Rho-GEF), and represses Rho by an unknown mechanism.