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Cadherins in cardiovascular disease

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Summary

Cardiovascular diseases encompass an enormous range of conditions arising through an equally diverse aetiology. The cadherin superfamily of cell surface adhesion molecules have long been recognised for their crucial roles in morphogenesis and controlled growth and turnover in adult tissues. Thus, their involvement in the development of cardiovascular diseases characterised by tissue remodelling can be predicted. However, given the diversity of cadherins expressed on resident cells in cardiac and vascular tissue and their assorted and frequently overlapping functions that extend beyond mere mediation of adhesive interactions, definition of specific roles in the progression of cardiovascular diseases can be confounding. Compared with the fields of embryogenesis and oncology, investigations targeted specifically toward delineation of the participation of cadherins in cardiovascular disease are remarkably scant. In this article we offer the reader a brief introduction to members of the cadherin superfamily, and review the involvement of cadherins in cardiac diseases (dilated and dysplastic cardiomyopathies) and vascular diseases (atherosclerosis and restenosis) in which prominent alterations in tissue architecture occur and ultimately cause the clinical manifestations and complications of the diseases. Putative functions of the different cadherins expressed in cardiomyocytes, smooth muscle cells and endothelial cells are discussed.

Key words: cardiovascular disease; atherosclerosis; restenosis; cadherin; cardiomyocyte; vascular smooth muscle cell; endothelial cell

The cadherin superfamily – an overview

Precise spatial and temporal regulation of intercellular and cell-extracellular matrix adhesive contacts is crucial to accurate execution of complex biological processes that occur during the development of new tissues and the controlled growth and turnover of adult tissues. Four major groups of cell adhesion proteins have been described, including integrins, immunoglobulin-domain containing cell adhesion molecules, selectins and cadherins. Cadherins are particularly important for dynamic regulation of intercellular adhesive contacts in diverse morphogenetic processes. Cadherins are integral membrane glycoproteins and represent a superfamily, which in vertebrates is comprised of more than 100 members divided into subfamilies based on amino acid sequence comparisons and structural features. The subfamilies are designated as classic Type I and Type II cadherins, desmosomal cadherins, protocadherins, Flamingo/CELSR and FAT cadherins. There are also a number of family members such as T-cadherin, LI-cadherin and the RET proto-oncoprotein, *inter alia*, which do not fit exactly into a defined subfamily. Several reviews have variously described the phylogenic and genomic organization and structure-function relationships of the cadherin superfamily [1–12].

Figure 1 illustrates some basic differences in the molecular characteristics of some subfamilies of the cadherin superfamily. Proteins are considered to be members of the cadherin family if their extracellular domain contains one or more repetitive subdomains termed cadherin repeats, which contain conserved sequences that are involved in calcium binding. Cadherin repeats are involved in

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Abbreviations:

ARVC/D	arrhythmogenic right ventricular cardiomyopathy/ dysplasia
DSG2	desmoglein 2
DSC2	desmocollin
EC	endothelial cell
GPI	glycosylphosphatidylinositol
VSMC	vascular smooth muscle cell

cis- or *trans*-interactions between the extracellular domains, which lead to homophilic binding (or heterophilic in some cases) between cadherin molecules. A variable number of cadherin repeats contributes to a divergent structural arrangement of the extracellular domain. With a few exceptions, cadherins are transmembrane proteins. The classical Type I (e.g., E-, N-, P-, R) and Type II (e.g., VE-) cadherins, the desmosomal cadherins (e.g., desmogleins, desmocollins), protocadherins (e.g., R-, CNR-, ARCADLIN-) and the FAT cadherins are single-pass transmembrane proteins. The Flamingo/CELSR cadherins have a seven-pass transmembrane domain that is homologous with G-protein-coupled receptors. T-cadherin has neither transmembrane nor cytosolic domains and is linked to the plasma membrane through a glycosylphosphatidylinositol (GPI) lipid anchor. Type-I cadherin members possess the HAV (histidine-alanine-valine) cell adhesion recognition sequence in their N-terminal extracellular module. The HAV sequence is absent on the extracellular domain of either type-II members or the non-type-I or -II cadherins. The intracellular domain is not conserved amongst the cadherin subfamilies.

The biological function of the cadherin superfamily is not limited to establishment of intercellular adhesion at cell-cell interfaces through their cadherin repeats [13]. The classical and desmosomal cadherins are well defined as adhesion molecules but most other cadherin members do not necessarily show strong adhesive activities. Cadherin functions extend to multiple aspects of tissue organisation and morphogenesis, including cell recognition and sorting, boundary formation in tissues, induction and maintenance of structural and functional cell and tissue polarity, cytoskeletal organisation, cellular phenotype modulation, cell migration, cell proliferation and cell survival. These diverse functions are facilitated by the ability of cadherins to trigger signal transduction in the cytoplasm and nucleus through interactions of the intracellular domain with an assort-

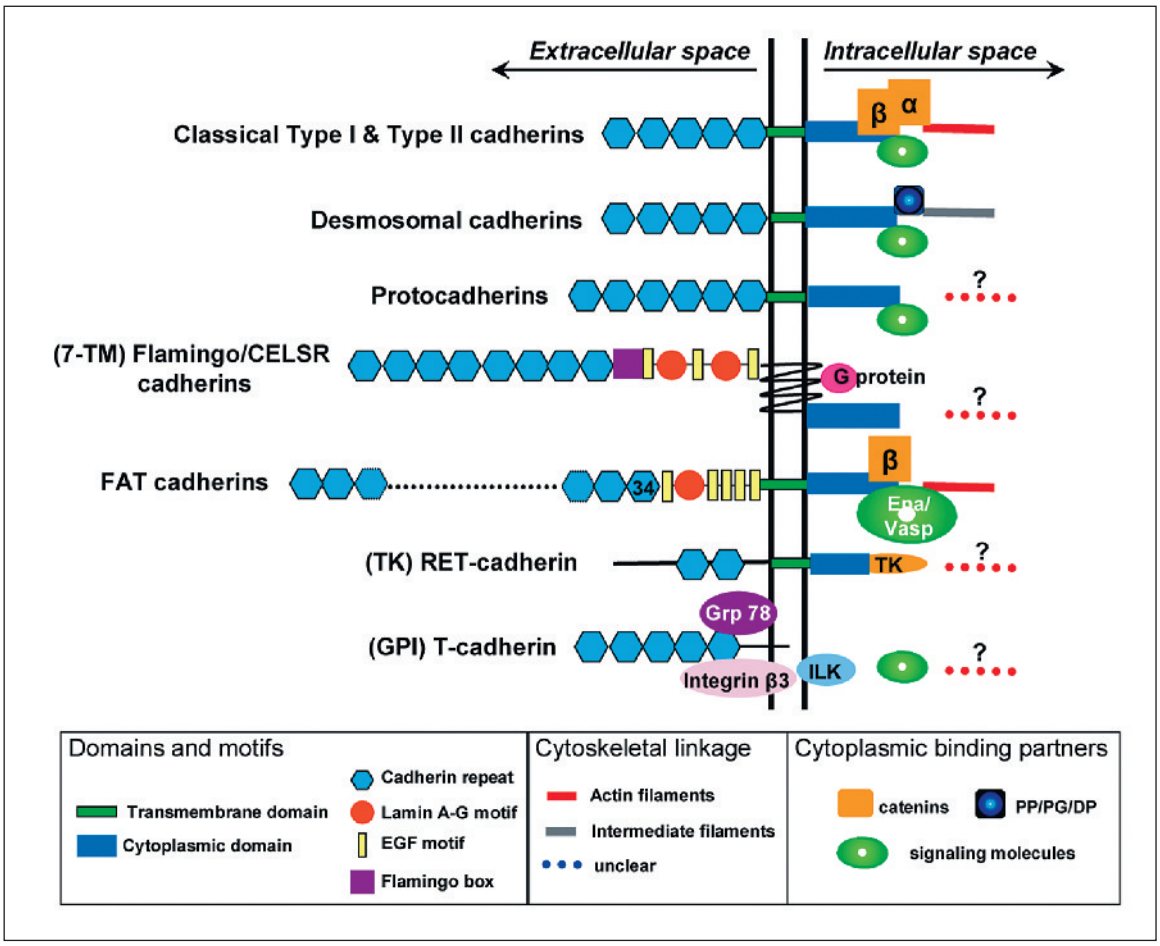

Figure 1

Schematic of basic structural features of cadherin superfamily members.
All cadherin superfamily members possess calcium binding "cadherin repeats" of varying number. FAT cadherin is the largest cadherin, with 34 cadherin repeats. Non-classical cadherins have some additional extracellular motifs such as laminin A-G and EGF domains and flamingo boxes. The cytoplasmic domain varies greatly amongst the subfamilies of the cadherins. Type I and II classical cadherins associate with the actin cytoskeleton through the β-catenin and α-catenin complex. Desmosomal cadherins associate with intermediate filaments through plakophilin (PP), plakoglobin (PG) and desmoplakin (DP). FAT interacts with β-catenin and the Ena/Vasp family of actin regulators. It is not clear how the other subfamilies interact with the cytoskeleton. The cytoplasmic domain of the cadherins can interact with signalling molecules; these include cytoplasmic and transmembrane proteins that participate in cellular signalling (e.g. growth factor receptors, receptor tyrosine kinases and phosphatases, PI3-kinase, Shc, small GTPases) or control of cytoskeletal dynamics (e.g. formin-1, Arp2/3, dynein). These interactions may be both cadherin and cell specific. 7-TM, seven transmembrane domain; TK, tyrosine kinase; GPI, glycosylphosphatidylinositol anchor; ILK, integrin-linked kinase.

ment of intracellular binding partners including cytoskeletal regulators, protein kinases and phosphatases, and transcriptional cofactors. Furthermore, cadherins can affect cell signalling and function through lateral interactions of the transmembrane domain with growth factor receptors and other plasma membrane-located signalling molecules. Some interactions of the cadherin superfamily with a variety of intracellular and membrane binding partners are depicted in figure 1.

Given the broad influence of cadherins on fundamental cellular processes, it is not surprising that defective cadherin expression or function is a feature of many pathological states. The role of cadherins in disease has mainly been studied in the context of disruption of normal tissue architecture and uncontrolled cell growth in neoplastic diseases. Aberrations in cell migration, proliferation and apoptosis/survival have long been recognised as characteristics of a range of cardiovascular diseases, but delineating the participation of cadherins in cardiovascular health and disease remains an astonishingly unchartered area of research. In the following we review currently known functions of cadherins in relation to the pathophysiology of cardiovascular diseases (see table 1 for summary).

Cadherins in the heart

Cardiomyocytes express the classical Type I N-cadherin, the desmosomal cadherins, desmoglein 2 (DSG2) and desmocollin 2 (DSC2), and GPI-anchored T-cadherin. In cardiac muscle, mechanical and electrochemical coupling integrity between cardiomyocytes is maintained by a unique junctional complex termed the intercalated disc. From a traditional viewpoint, the intercalated disc comprises a mosaic of two morphologically distinct intercellular adhering junctions – the adherens junction (*fasciae adhaerentes*) and the desmosome (*maculae adhaerentes*) – which are interspersed with some gap junctions. However, more recent immunoelectron microscopy studies have demonstrated that in fact the predominant intercellular adhering junction of the intercalated disc is a mixed-type junctional structure, termed "*area composita*" that contains both adherens junction and desmosomal molecular members [14–16]. N-cadherin (typically an adherens junction molecule) provides strong cell-cell adhesion through cadherin-catenin complex linkage to the actin cytoskeleton (illustrated in figure 2) and is also the site of attachment of the myofibrils, thus enabling transmission of contractile force across the plasma membrane. DSG2 and DSC2 (typi-

Table 1

Functions of cadherins relevant to the pathophysiology of cardiovascular diseases.

Cadherin	Expression in the cardiovasculature	Function	Implication in disease
VE-cadherin (vascular endothelial, CDH5)	endothelial cells	forms EC intercellular adherens junctions; controls vascular permeability, leukocyte transmigration, vasculogenesis and angiogenesis	regulates embryonic and cancer *angiogenesis*; expression in the arterial intima reflects the level of neovascularisation and is associated with atherosclerotic plaque severity, instability and clinical events; plasma levels of VE-cad are increased in patients with cardiovascular disease.
N-cadherin (neural, CDH2)	cardiomyocytes	forms myocardial intercellular adherens junctions; regulates myocardial development, cardiomyocyte differentiation, formation and function of the intercalated disc	involved in pathogenesis of cardiomyopathy and arrythmias
	smooth muscle cells	forms VSMC adherens junctions, modulates cell spreading, motility, growth and survival	expression altered during restenosis of the carotid artery
	endothelial cells	forms heterotypic adherens junctions between EC/VSMC or EC/pericytes; promotes angiogenesis, vessel maturation	plays a role in embryonic and cancer angiogenesis
T-cadherin	cardiomyocytes	not yet investigated	unknown
(H-cadherin, CDH13)	smooth muscle cells	regulates migration, proliferation, survival, acts as a receptor for heterophilic ligands (lipoproteins, adiponectin)	increased in atherosclerotic lesions and restenosis
	endothelial cells	regulates migration, proliferation, survival, angiogenesis, acts as a receptor for heterophilic ligands (lipoproteins, adiponectin)	promotes angiogenesis
R-cadherin (retinal, CDH4)	smooth muscle cells	inhibits proliferation	downregulated early during restenosis after arterial injury
desmosomal cadherins	cardiomyocytes	form desmosomal junctions that provide structural support in the cardiac tissue	DSC2 and DSG2 mutations cause arrhythmogenic right ventricular cardiomyopathy/dysplasia
FAT 1	smooth muscle cells	regulates proliferation and motility	expression is increased during neointimal formation after arterial injury

Abbreviations: CDH, cadherin; EC, endothelial cell; VSMC, smooth muscle cell; DSC2, desmocollin 2; DSG2 desmoglein 2.

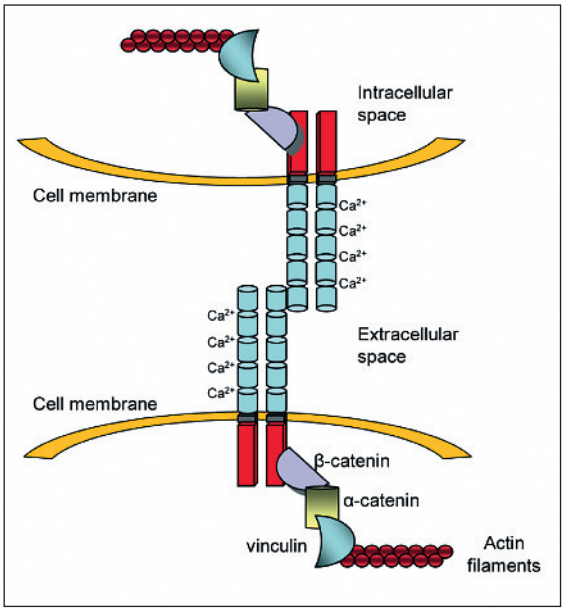

Figure 2

Cadherins in the adherens junction. The adherens junction contains *cis* homodimers of classical Type I (e.g. N-cadherin, P-cadherin, R-cadherin) or Type II (VE-cadherin) cadherins that interact in the extracellular space with *cis* homodimers of the neighbouring cell to form *trans* homodimers. Adhesion is conferred by interaction of the cadherin cytoplasmic domain with β-catenin, which binds to α-catenin to link the cadherin to vinculin/actin filaments. Type II VE-cadherin is unique amongst the classical cadherin in that its cytoplasmic domain can link not only to the actin cytoskeleton through the catenin interactions described above, but also to the intermediate filament cytoskeleton through interactions with plakoglobin, much like the desmosomal junction (fig. 3).

cally desmosomal molecules) provide structural support through cadherin-plakophilin/plakoglobin/desmoplakin complex linkage to the intermediate filaments (illustrated in fig. 3). The gap junctions, which are responsible for electrical coupling and signalling between cells, do not contain any cadherin molecules but are located in very close proximity to the adhering junctions and affected by changes therein. T-cadherin exhibits a global distribution over the myocyte surface and is not located within any specific junctional location [17].

N-cadherin

N-cadherin plays a critical role in myocardial development and function and is of paramount importance in maintaining the structural integrity of the heart. The involvement of N-cadherin in morphoregulation, stabilisation of cardiomyocyte differentiation and formation, and function of the intercalated disc is well established [18–24].

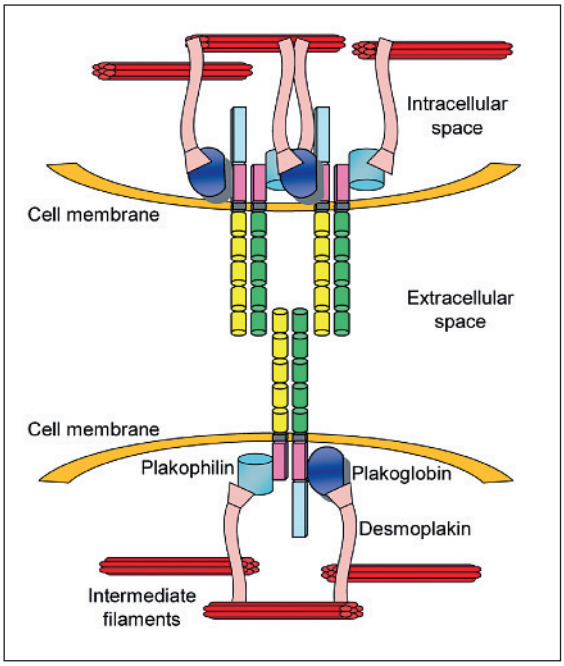

Figure 3

Cadherins in the desmosomal junction. The desmosome contains *cis* heterodimers of desmoglein (green) and desmocollin (yellow) which interact heterophilically with *cis* heterodimers on neighbouring cell to form *trans* heterodimers. Adhesion is conferred by interaction of the cytoplasmic domains of the desmosomal cadherins with plakoglobin and plakophilin, which in turn interact with desmoplakin for anchorage of the cadherins to intermediate filaments.

Structural perturbation of the intercalated disc accompanied by downregulation of N-cadherin occurs in a hereditary hamster model of dilated cardiomyopathy [25] and a mouse model of human desmin-related cardiomyopathy [26]. Genetically engineered mice with a germline deletion of N-cadherin experience embryonic lethality shortly after implantation accompanied by multiple embryonic abnormalities that include severe cardiovascular defects [27]. On the other hand, mice engineered to overexpress N-cadherin in the adult mouse myocardium suffer from a dilated cardiomyopathy due to cadherin-mediated modulation of intercalated disc function [28]. The importance of N-cadherin in myocardial cell-cell interactions was shown using chimeric mouse embryos derived from N-cadherin-deficient embryonic stem cells whereby N-cadherin-null cardiomyocytes are excluded from the myocardium during development [29]. Conditional knockout mice (N-cadherin CKO) possessing a cardiac-specific, inducible *Cre* transgene that allows specific deletion of N-cadherin in the adult myocardium after complete development have been generated [30, 31]. The myocardium of N-cadherin CKO animals display loss of the intercalated disc structure; this includes disruption of the cell-cell adherens and desmosomal contacts, and a parallel disassembly/destabilisation of gap junctions. The latter is due to a loss and heterogenous distribution of the gap junction protein connexin43 [30], which requires N-cadherin for trafficking and assembly into functional gap junctions [28, 32, 33]. In the hereditary hamster model of dilated cardiomyopathy downregulation of connexin43 also accompanies the downregulation of N-cadherin [26]. The findings with respect to connexin43 illustrate the importance of the integrity of adherens junctions on the integrity of gap junctions. N-cadherin CKO mice are phenotypically characterised by spontaneous ventricular tachycardia, slow conduction velocity, moderate biventricular cardiomyopathy and sudden cardiac death within two months after deletion of the N-cadherin gene.

To our knowledge there are no studies in humans that have directly investigated associations between altered expression of myocardial N-cadherin and heart disease. Nevertheless, the data obtained in animal models of cardiomyopathy have clearly identified N-cadherin as a critical determinant in the pathogenesis of cardiac arrhythmias, which has implications for the development of sudden death in many forms of heart disease where mechanical coupling has been compromised, including hypertrophic, ischaemic and dilated cardiomyopathies.

Desmosomal cadherins

The heart relies upon the integrity of desmosomal adhesion in order to withstand high mechanical stress. When this adhesion fails cardiac tissue architecture and function is compromised.

Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia (ARVC/D) is considered a desomsome cardiomyopathy. ARVC/D is an autosomal dominant disease characterised by ventricular arrhythmias, progressive myocardial atrophy with fibroadipocytic replacement of cardiomyocytes and sudden death, commonly in the young. Recent studies in both humans with ARVC/D [34] and a murine model of ARVC/D [35] indicate that ARVC/D is not exclusively a disease of the right ventricle and that the left ventricle is significantly affected. Approximately 50% of patients with symptomatic ARVC/D harbour a mutation in various components of the desmosome. Several studies in human ARVC/D have demonstrated heterozygous mutations of the desmomal cadherins DSC2 [34, 36–38] and DSG2 [34, 39, 40]. Mutations in plakophilin-2, plakoglobin and desmoplakin, proteins that link the desmosomal cadherins to the intermediate filaments (fig. 2), are also common in ARVC/D (reviewed in [41–43]). Mutations in the desmosomal components and resultant destabilisation of the desmosomal complex alter the integrity of cardiac cell-to-cell junctions in a manner sufficient to promote cardiomyocyte death under conditions of mechanical stress [44]. Subsequent tissue repair through fibro-fatty replacement/scar formation provides the anatomical basis for reentrant arrhythmias and progressive heart failure. Further, and as discussed below, the superimposition of gap junction remodelling may act synergistically with the characteristic structural defects in ARVC to promote a substrate for malignant arrhythmias.

Disruption of cell junctions associated with desmosomal protein abnormalities is believed to impair proper gap junction formation and function, leading to disturbances in cardiac electrical conduction and associated arrhythmias [44]. In Naxos disease and Carvajal syndrome, which are rare but severe autosomal recessive ARVC phenotypes caused by mutations in plakoglobin and desmoplakin, respectively, a significant reduction in connexin43 expression and gap junction formation in the heart has been reported [45, 46]. siRNA-mediated knock-down of plakophilin-2 expression in cardiomyocyte cultures was shown to result in decrease in total connexin43 content, a significant redistribution of connexin43 to the intracellular space and a decrease in dye coupling between cells [47, 48]. Decreased connexin43 expression and localisation to intercalated discs was also demonstrated in the myocardium of patients with autosomal dominant ARVC/D secondary to heterozygous plakophilin-2 mutations [48]. Abnormalities of connexin43 expression at intercalated discs occur in Boxer dogs with ARVC [49], although the specific gene mutation in this model has not been identified [50].

A novel signal-transduction-based mechanism underlying fibroadipocytic replacement of cardiomyocytes in ARVC/D has been postulated [51]. Using cultures of desmoplakin-silenced atrial myocytes and cardiac-restricted desmoplakin-deficient mice the loss of desmoplakin was demonstrated to lead to a nuclear localisation of plakoglobin (also known as γ-catenin). The nuclear plakoglobin competes with β-catenin, which is a signal transducer of the canonical Wnt signalling pathway. This competition with β-catenin causes a suppression of this pathway and ultimately enhances adipogenesis, fibrogenesis and myocyte apoptosis [51]. This transdifferentiation hypothesis, which has yet to be examined in ARVC patients, highlights the possibility that the mutation of other desmosomal proteins leads to ARVC through disruption of the same pathway.

Plakoglobin has been proposed to play an important role in ARVC development regardless of the underlying mutation [43]. Plakoglobin fails to localise at the intercalated discs in myocardial tissue from patients with plakoglobin [45] or desmoplakin [46] mutations, from plakophilin-2 gene ablated mice [52] and from Boxer dogs with ARVC [49], possibly indicating a final common pathway role for plakoglobin in the pathogenesis of ARVC. Functional studies of all the desmosomal molecules implicated in ARVC, including the desmosomal cadherins DSC2 and DSG2, will provide further insight into the molecular mechanisms that underlie the disease and will ascertain if ARVC is a disease of cell adhesion or cell signalling.

T-cadherin

Although T-cadherin is abundantly expressed in the heart (cardiomyocytes and blood vessels) [17, 53, 54] its function in this organ is completely unknown. T-cadherin was speculated to play a role in synaptic placement during cardiac development [17], as suggested for motor neurons and skeletal muscle [55, 56], or to modulate cardiac function through extracellular Ca^{2+} sequestration and accumulation on the surface of the plasma membrane [17]. These hypotheses have not yet been tested.

Cadherins in the blood vessel

Vascular remodelling is a critical part of the pathogenesis of clinically important hyperplastic vascular disorders in humans such as atherosclerosis, restenosis after angioplasty and saphenous vein graft disease [57, 58]. Investigations on the role of cadherins in vascular diseases are limited

and mostly restricted to immunohistochemical analyses of vascular tissue from experimental models of vascular diseases and to *in vitro* experimentation on cultures of vascular smooth muscle cells (VSMC) and endothelial cells (EC).

Smooth muscle cell cadherins

Cadherins currently identified in VSMC include classical Type I N- and R-cadherins, T-cadherin and FAT1 cadherin. The significance of these cadherin superfamily members in VSMC biology is not well defined, but accumulating evidence points to important roles in modulating cell migration, proliferation and apoptosis/survival. These are key cellular events in hyperplastic vascular disorders. Quite how VSMC spatially and temporally integrate the expression levels and functions of their complement of cadherins in the maintenance of vessel homeostasis or vessel architecture has not yet been studied and will remain elusive for some time to come.

N-cadherin

Studies in the rat vasculature have demonstrated that N-cadherin is present within adherens junctions in healthy, uninjured blood vessels and can mediate intercellular contacts between VSMC [59] and between VSMC and EC [60].

Upregulation of N-cadherin and β-catenin was reported to occur *in vivo* within the first week of neointimal formation following balloon catheter injury of the rat carotid artery (experimental restenosis), with a return to control levels following completion of arterial tissue repair/remodelling [59]. A contrasting observation was made in a porcine model of restenosis, whereby downregulation of N-cadherin, without an alteration in β-catenin, was found to occur during the early stage of restenosis [61]. Expression of N-cadherin has not yet been examined in human atherosclerotic tissue or in experimental models of atherosclerosis.

In vitro studies using cultures of porcine, rat and human VSMC have indicated that N-cadherin can modulate several aspects of VSMC behaviour important to vascular remodelling including migration, proliferation and survival, although some of the reported observations are contradictory. N-cadherin upregulation could be induced by wounding of porcine artery VSMC monolayers, with a return to control after reformation of the monolayer [59]. Antibody-mediated inhibition of N-cadherin homophilic binding suppressed spreading and migration of VSMC during repair of *in vitro* wounds and cell movement was disoriented [59], suggesting a stimulatory role for N-cadherin homophilic interactions in directed VSMC migration. In contrast, another study using human aortic VSMC demonstrated an inhibitory role for N-cadherin in VSMC migration [61]. Antibody-mediated inhibition of N-cadherin homophilic interactions increased migration potential [61]. Further, N-cadherin levels and RhoA activities were reduced in migratory VSMC phenotypes compared with the quiescent phenotype, while overexpression of N-cadherin in VSMC inhibited migration potential in association with elevated RhoA activity [61]. More recently it was demonstrated that N-cadherin redistribution to the posterior-lateral cell edge of VSMC is important for cell polarity during migration (see fig. 4) and that N-cadherin ligation upon encountering a layer of N-cadherin expressing cells, including EC, abrogated the polarised migration of VSMC [62]. This could be an important mechanism of maintaining normal vessel stability; in the face of endothelial denudation a lumenally-oriented migration of the VSMC might be unopposed.

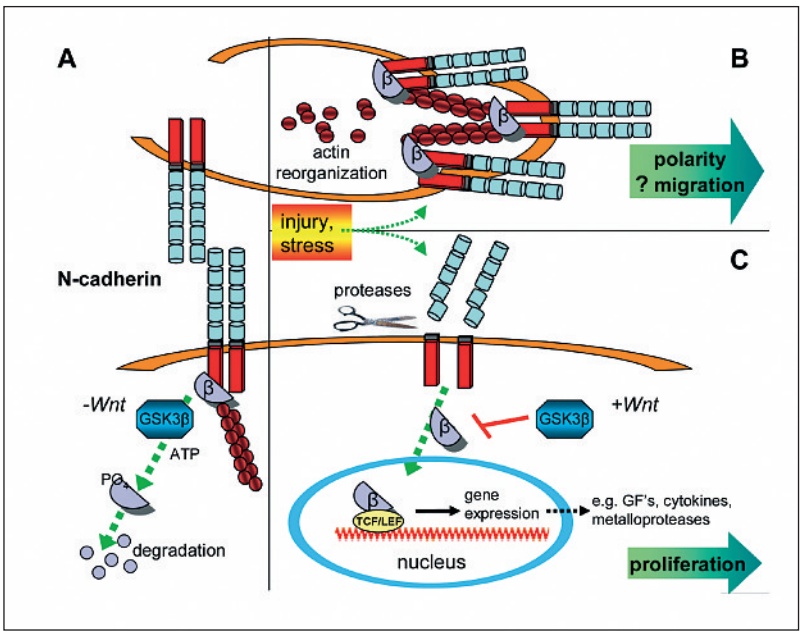

Figure 4

Hypothetical roles of N-cadherin in migration and proliferation of smooth muscle cells. Panel A: In the quiescent state N-cadherin homodimer interactions are intact and maintain cell-cell adhesion through stable linkage to the actin cytoskeleton via catenins/vinculin (only β-catenin shown in this schematic). Panel B: Under injurious or stressful conditions a redistribution of N-cadherin to the posterior-lateral cell edge may function to facilitate a polarised migration of VSMC during tissue reparation [61]. (The question mark indicates conflicting reports as to whether N-cadherin is upregulated [59] or downregulated [61] during VSMC migration, and whether its effects on migration are stimulatory [59] or inhibitory [61]). Panel C: During injury/stress (e.g., inflammation) locally produced proteases may cleave N-cadherin from the cell surface, with a resultant disassembly of the cadherin associated β-catenin. In the absence of Wnt signalling, any β-catenin entering the cytoplasm undergoes phosphorylation by GSK3β, followed by ubiquitination and proteasomal degradation (A). However, in the presence of Wnt signalling (or possibly other stimuli signalling through GSK3β) remains unphosphorylated and undergoes translocation into the nucleus where it associates with and transactivates transcription factors of the TCF/LEF family. This nucleoprotein complex can induce expression of a number of cell cycle regulatory genes (e.g. cyclin D1), leading to cell proliferation. Concomitantly released growth factors (GF's), cytokines, matrix proteins and proteases may sustain the initial reparative response to injury.

A loss of N-cadherin homophilic interactions during VSMC growth *in vitro* has been reported [63]. Downregulation of N-cadherin occurred in cultures of human saphenous vein VSMC stimulated to proliferate, while overexpression of dominant negative N-cadherin mutant that consisted of only transmembrane and cytosolic domains (i.e., absence of N-cadherin-mediated homophilic cell-cell adhesive interactions) increased VSMC proliferation [63]. The proliferation of VSMC resulting from loss of adherens junction N-cadherin was attributed to an accumulation of β-catenin in the nucleus [63], permitting convergence on the Wnt signalling pathway and activation of the transcription of TCF-/LEF-dependent genes (reviewed in [64, 65] and see fig. 4). Modulation of nuclear β-catenin accumulation by N-cadherin expression levels was confirmed in a study that demonstrated reduction of β-catenin transcriptional activity in VSMC overexpressing native N-cadherin [66]. N-cadherin also affects VSMC survival; overexpression of the dominant negative N-cadherin mutant in VSMC or neutralisation of N-cadherin function induced apoptosis, while overexpression of native N-cadherin increased VSMC survival [67].

R-cadherin

To date there is only a single study which has examined the influence of R-cadherin on VSMC behaviour [68]. R-cadherin was found to be downregulated 2,5, 24 and 48 hours after balloon catheterisation injury of the rat carotid artery and without a change in N-cadherin during these time periods. R-cadherin downregulation was accompanied by nuclear accumulation of β-catenin and upregulation of cyclin D1, and there was a correlation between R-cadherin negative and BrdU positive cells. The interpretation that R-cadherin inhibits proliferation was confirmed by neutralisation experiments whereby blockade of R-cadherin homophilic interactions increased proliferation, nuclear accumulation of β-catenin and cyclin D1 expression. Thus, like N-cadherin [59, 67], R-cadherin appears to exert anti-proliferative effects on VSMC by effectively sequestering β-catenin away from the nucleus and limiting transcriptional activity through the Wnt signalling pathway.

FAT1 cadherin

Expression of FAT1 protocadherin on VSMC was only recently identified in a study screening for molecules differentially expressed after balloon injury of rat carotid arteries [66]. This study also reported for the first time that the cytoplasmic domain of FAT1 can interact with β-catenin. FAT1 expression was increased in balloon injured rat carotid arteries during the proliferative phase and in VSMC cultures stimulated with serum, or with growth factors or chemokines known to promote VSMC activation and neointimal formation. Use of a chimeric construct (IL2R-Fat1$_{1C}$) to express only the cytoplasmic domain of FAT1 resulted in inhibition of VSMC proliferation and lowered cyclin D1 expression. Moreover in FAT1-silenced VSMC β-catenin transcriptional activity and proliferation were increased. These knock-in and knock-out experiments suggested that in spite of upregulation during restenosis and in proliferating cells FAT1 actually functions to oppose VSMC proliferation through β-catenin sequestration, as suggested for R-cadherin [68] and N-cadherin [59, 67, 69]. FAT1 silencing inhibits VSMC migration. Interestingly, in VSMC FAT1 localises to both cell-cell junctions and cell free edges [66]. Cell-cell localisation suggests that FAT1 might limit VSMC proliferation, while presence at cell free edges may signal directional migration cues during vascular remodelling. The dual location of FAT1 could serve an integrative function that opposes the formation of hyperproliferative cellular clusters [66].

T-cadherin

T-cadherin, which lacks transmembrane and cytosolic domains and instead attaches to the membrane through a GPI anchor, does not mediate strong intercellular adhesion; in VSMC it is globally distributed on the cell surface with only very minor enrichment at cell-cell borders [70].

The first indication of a positive relationship between T-cadherin expression on VSMC and stimulation of proliferation and motility emerged from immunohistological studies of diseased vessels. Analysis of human arterial tissue revealed a differential expression of T-cadherin in VSMC from different vascular layers as well as an inverse relationship between levels of T-cadherin and α-actin [53], which is a marker of the contractile phenotype and absent in the proliferative/secretory phenotype. Medial contractile VSMC, with high levels of α-actin, express less T-cadherin than the intimal VSMC. VSMC located to the subendothelial intimal layer expressed low levels of α-actin and high levels of T-cadherin, while VSMC in the deeper, muscular-elastic intimal layer displayed high levels of α-actin and low levels of T-cadherin [53]. Upregulation of T-cadherin in intimal VSMC occurs during atherosclerosis development and most prominently within lesions (fibroatheroma and fibrous plaque) of high disease severity [53]. Upregulation of T-cadherin expression in VSMC was also found during the period of tissue reparation/restenosis following balloon injury of rat carotid arteries; spatial and temporal changes in T-cadherin level coincided with cell migration and proliferation activities during neointima formation [71]. Interestingly, T-cadherin expression is higher in the internal mammary artery than the highly restenosis- and atherosclerosis-prone coronary artery [72]. This could be interpreted to imply that upregulation of T-cadherin in diseased coronary arteries and the reparative activities attributed to T-cadherin as

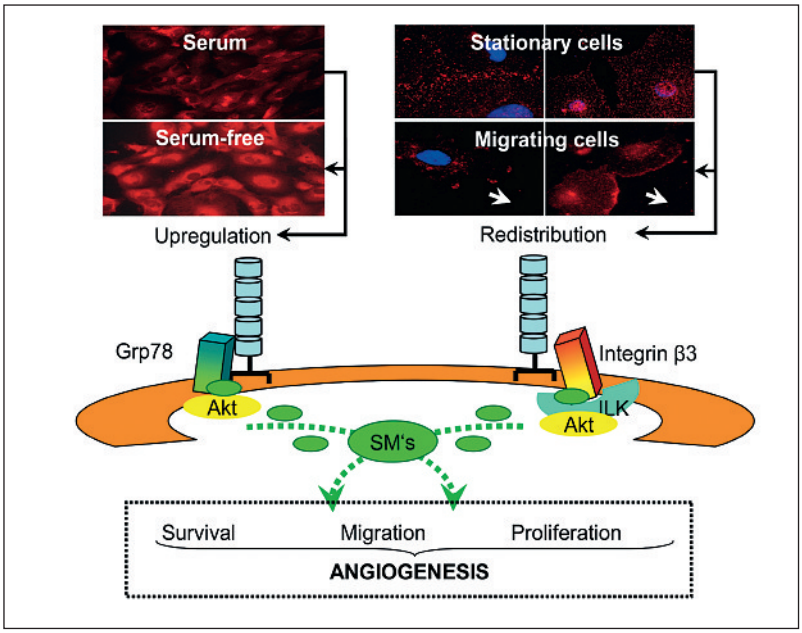

Figure 5

Modulation of T-cadherin-induced cell responses in EC through engagement of signalling "adaptors". The photomicrographs on the left illustrate upregulation of T-cadherin in near-confluent cultures of EC after subjection to oxidative stress (4 hours of serum-deprivation). Photomicrographs on the right illustrate the global distribution of T-cadherin in stationary EC cultures and the redistribution and polarisation of T-cadherin to the leading edge of migrating cells (white arrow indicates direction of migration). T-cadherin functions in promoting the survival, migration and proliferation of EC, all of which are key processes that occur during angiogenesis. To exert these functions GPI-anchored T-cadherin must necessarily engage molecular "adaptors" (e.g. Grp78, integrin β3, integrin linked kinase (ILK)) which facilitate inward signal transduction pathway activation (e.g. via Akt or other membrane complexed or cytoplasmic signalling molecules (SM's; indicated as green ovals).

discussed below may serve a protective rather than an exacerbating function.

T-cadherin null mice are viable and fertile and do not develop life-threatening vascular defects during embryogenesis [73]. However, it has not yet been investigated whether T-cadherin deficiency impacts the development of hyperplastic vascular disorders such as restenosis (i.e., following balloon catheterisation of the aorta) and atherosclerosis (i.e., use of apoE$^{-/-}$ x T-cadherin$^{-/-}$ mice). Specific effects of T-cadherin deletion on angiogenesis are discussed later in this article.

In vitro studies on human and rat aortic VSMC have provided evidence that T-cadherin modulates VSMC growth and motility. T-cadherin expression in subconfluent, growing VSMC cultures was greater than that in maintained confluent cultures [74], while overexpression of T-cadherin in VSMC stimulated growth and progression through the cell cycle [75]. Although T-cadherin homophilic ligation mechanisms appear not to participate in growth stimulation, they do affect motile properties of VSMC in a manner that suggests a function for T-cadherin in contact-dependent navigation. Induction of homophilic interactions by plating VSMC onto substratum containing immobilised functionally agonistic antibodies or recombinant T-cadherin protein limits spreading and adhesion of the cells [76]. In Boyden chamber assays T-cadherin-overexpressing VSMC migrate through, but fail to adhere to, substrata containing recombinant T-cadherin (own unpublished data). Because T-cadherin is absent from intercellular contacts of VSMC monolayers [70], its ability to induce cell deadhesion might not influence the behaviour of normally quiescent and contractile VSMC of healthy vessels. On the other hand, upregulation and polarisation of T-cadherin on phenotypically modulated VSMC of diseased vessels may enhance homophilic ligation and facilitate cell deadhesion and motility towards sites of injury. Molecular mechanisms involved in effects of T-cadherin on growth and motility in VSMC have not yet been studied.

A further aspect of T-cadherin in VSMC biology concerns interactions with serum-borne molecules. To date two heterophilic "ligands" have been identified. In VSMC T-cadherin was initially characterised as an atypical low density lipoprotein binding protein [77–79], and the GPI-anchor was shown to be required for lipoprotein binding [80]. Although low density lipoproteins have been found to disrupt T-cadherin dependent intercellular interactions in T-cadherin-transduced HEK293 cells [79], the consequences of T-cadherin-lipoprotein interactions in VSMC have not been studied. More recently, T-cadherin was identified as a receptor for high molecular weight forms of adiponectin [81], which are the predominant active forms in serum. Adiponectin has potent anti-inflammatory, anti-atherogenic, anti-angiogenic and anti-proliferative effects and a reduction in serum levels of adiponectin has been implicated in several cardiovascular diseases (reviewed in [82, 83]). Adiponectin is absent in healthy vessels but accumulates in the vessel wall after catheter injury with a histochemical localisation resembling that in regions where T-cadherin is upregulated [84]. Co-localisation of adiponectin and T-cadherin within the vessel wall has not yet been demonstrated. T-cadherin deficient mice have increased circulating levels of adiponectin [73], which provides some evidence for a relationship between these two molecules. However, the function of T-cadherin-adiponectin interactions is not yet understood and there have been no studies reporting on adiponectin-induced cellular responses through its association with T-cadherin. Adiponectin can form oligomeric complexes with several growth factors such as platelet-derived growth factor-BB, basic fibroblast growth factor, heparin-binding epidermal growth factor, and thrombospondin 1 [85–87]. The interaction of adiponectin with these growth factors was shown to preclude their binding to the membrane receptors and lead to the attenuation of their mitogenic actions in a number of cell type including VSMC [85]. Therefore it was proposed that adiponectin could co-ordinately control cell growth and tissue remodelling by regulating the local concentration and bio-availability of different growth factors.

Endothelial cell cadherins

EC express VE-cadherin, N-cadherin and T-cadherin. VE-cadherin is unique to EC and is the cadherin present in the adherens junctions of EC ([88, 89] for reviews). A specific feature of VE-cadherin as compared with all other classical cadherins is that it may also behave as a desmosomal-like cadherin, and through plakoglobin VE-cadherin can recruit desmosomal proteins desmoplakin and vimentin at the membrane. This desmosomal-like structure *(complexus adhaerentes)* is specific for EC [88]. In contrast to VSMC and cardiomyocytes, N-cadherin is excluded from adherens junctions in EC and is diffusely distributed on the cell membrane [90, 91]. It is also expressed at contact zones between pericytes and endothelial cells [92]. As for VSMC, T-cadherin in EC is globally distributed on the cell surface with only very minor enrichment at cell-cell borders and polarises to the leading edge of migrating EC [70]. Regulation of EC adhesive and signalling functions by VE-cadherin has been the subject of intensive study for decades. N-cadherin and T-cadherin are relative newcomers to investigations on EC biology and their roles in vascular diseases are poorly defined.

VE-cadherin

As reviewed in several excellent papers [11, 88, 89, 93–100] a wealth of *in vitro* and *in vivo* studies have established that VE-cadherin plays essential roles in controlling vascular permeability, vascular integrity, leukocyte transmigration, vasculogenesis and angiogenesis. Not surprisingly therefore, alterations in VE-cadherin expression and distribution contribute to atherogenesis. VE-cadherin is not expressed in the intima of healthy human arteries [101]. It is present within the intima of atherosclerotic lesions with a frequency and intensity that increases with lesion severity and reflects the level of neovascularisation [101, 102]. The expression of VE-cadherin was associated with plaque instability, degree of stenosis and clinical events [102]. Neovessels surrounded by inflammatory cells had irregular or reduced levels of VE-cadherin in association with a breakdown of endothelial integrity, favouring further infiltration of inflammatory cells into plaque tissue [101]. Atherosclerotic lesions are localised to arterial geometries (curvature, branches and stenosis segments) associated with blood flow disturbances [103]. *In vivo* VE-cadherin expression at cell-cell junctions is weaker in atherosclerosis-susceptible sites [104]. This was attributed to an effect of blood flow. *In vivo* and *in vitro* studies of the effect of flow on VE-cadherin distribution showed that whereas staining under laminar or pulsatile conditions net forward flow was normal, intermittent patterns of staining developed under conditions of reciprocating, low net flow [104]. Total cellular levels of VE-cadherin remained unaltered, implying redistribution within the membrane. "Soluble" VE-cadherin can be detected in human plasma [105, 106]. Plasma levels of VE-cadherin were higher in coronary sinus samples than peripheral samples and correlated with the degree of coronary atherosclerosis, independent of classical atherosclerotic risk factors [105]. The presence of "soluble" VE-cadherin in plasma possibly reflects the EC activation/dysfunction characteristic of diseased vessels and release of microparticles shed from the plasma membrane of EC [107–109]. Levels of VE-cadherin-positive EC microparticles were found to be elevated in supernatants from activated EC cultures and in plasma from patients with type 2 diabetes, and especially those with coronary artery disease [106]. On the other hand VE-cadherin can be proteolytically cleaved from EC by disintegrin and metalloprotease ADAM10, with resultant dissolution of adherens junctions and increased permeability [110]. Proteolytic cleavage of VE-cadherin may be a mechanism for stimulation of EC migration. Preliminary studies reported that ADAM10 is highly expressed in human atherosclerotic plaques and in association with plaque neovascularisation, and that ADAM10 activity is required for migration of EC [111].

N-cadherin

Expression of N-cadherin on EC has not yet been studied in human atherosclerosis or experimental restenosis. However, it is likely that an increase might be detected in association with neovascularisation, since the literature provides ample evidence for pro-angiogenic properties of N-cadherin. N-cadherin has been shown to play a critical role in angiogenesis, although its function differs from that of VE-cadherin. Whereas VE-cadherin mostly promotes the homotypic interaction between EC, N-cadherin is responsible for the formation of heterotypic ablumenal adherens junctions between EC and pericytes [92, 112, 113] or myoendothelial junctions between EC and underlying VSMC [114, 115]. N-cadherin-dependent pericyte coverage is critical for stabilisation and of maturation of newly-formed endothelial sprouts. N-cadherin blockage *in vivo* results in defective pericyte adhesion accompanied by vascular dysmorphogenesis and haemorrhage [113, 116]. Recent data suggest that N-cadherin may also influence angiogenesis by directly regulating EC function and influencing VE-cadherin expression levels. Knockdown of N-cadherin *in vivo* and *in vitro* caused a significant decrease in VE-cadherin [117]. A synthetic peptide capable of antagonising N-cadherin-mediated adhesion disrupted angiogenesis *in vitro* [118]. Furthermore, neutralisation of N-cadherin with a cyclic peptide containing the HAV motif was shown to induce apoptosis through inhibition of cadherin-medi-

ated activation of FGFR signalling [119]. Soluble N-cadherin (consisting of the extracellular domain) stimulated angiogenesis *in vivo* and migration *in vitro* [120]. Soluble N-cadherin did not affect intercellular adhesion, and its effects on EC migration are mediated through complex formation with fibroblast growth factor receptor (FGFR) [120], which has been implicated as an important partner of N-cadherin in a number of cell types, including EC. Possibly metalloproteinase-mediated shedding of soluble N-cadherin from VSMC during proliferation [63] may have a knock-on pro-angiogenic effect.

T-cadherin

Similarly to its expression on VSMC, and as illustrated in Figure 5, T-cadherin on EC is globally distributed on the cell surface with only very minor enrichment at cell-cell borders, and it polarises to the leading edge of migrating EC [70]. *In vivo* T-cadherin is up-regulated in atherosclerotic lesions [53], during restenosis [71] and on EC from tumour vasculature [121]. *In vitro* T-cadherin is upregulated on proliferating EC [75] or EC exposed to oxidative stress [122]. T-cadherin overexpression on EC *in vitro* induces proliferation and motility [76] and protects EC from oxidative stress-induced apoptosis [122, 123]. Together the data suggest that T-cadherin is both a marker of EC activation/stress and an inducer of an activated EC phenotype (fig. 5). Anti-adhesive/repulsive functions for T-cadherin in the vasculature emerged from studies showing that homophilic ligation in EC is rapidly followed by the acquisition of a less-adhesive, motile or pro-migratory, pro-angiogenic phenotype [76, 124]. These functions of T-cadherin require the activity of the small GTPases RhoA and Rac1 [124]. GPI-anchored T-cadherin lacks transmembrane and cytosolic domains, and thus its effects on EC behaviour require association with molecular adaptors to mediate inward signalling. A number of membrane adaptors including integrin β3, Grp78/Bip [123] and integrin linked kinase [125] have been identified (fig. 5). Proangiogenic properties for T-cadherin have been demonstrated using *in vitro* models of angiogenesis [126]. Using a model of myoblast-mediated gene transfer to mouse skeletal muscle delivery of soluble T-cadherin potentiated VEGF effects on neovascularisation in a manner that involved an increase in vessel calibre [126]. Another study using the Matrigel implant model reported that ectopic delivery of T-cadherin inhibited neovascularisation [127]. However, specific confirmation of proangiogenic functions for T-cadherin was recently provided through use of T-cadherin null mice whereby T-cadherin deficiency was found to limit angiogenic responses [73].

Concluding remarks

Data is accumulating to support the role of a number of cadherins in cardiovascular disease progression and in ways that extend beyond anomalies in cell-cell adhesion. The cadherins affect various aspects of cardiomyocyte, VSMC and EC biology such as proliferation, survival, migration and morphogenesis. Studies *in vitro* have demonstrated that these functions of the cadherins are facilitated by their ability to affect cytoplasmic and nuclear signal transduction through direct and indirect engagement of a wide variety of membrane and cytoplasmic proteins such as growth factor receptors, cytoskeletal regulators, kinases, phosphatases and transcriptional cofactors. Aberrations in cadherin expression on cardiomyocytes, VSMC and EC are implicated in some cardiovascular diseases including cardiomyopathies/dysplasia, atherosclerosis and restenosis. However, the functional contribution of cadherins to these diseases remains poorly understood. There is a need to further investigate how the multiple cadherin-dependent processes established *in vitro* translate to functions within cardiac and vascular tissues using suitable animal models and clinical samples.

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