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Review

# Cadherins in vascular smooth muscle cell (patho)biology: Quid nos scimus?

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ABSTRACT

Vascular smooth muscle cells (SMCs) phenotypes span a reversible continuum from quiescent/contractile (differentiated) to proliferative/synthetic (dedifferentiated) enabling them to perform a diversity of functions that are context-dependent and important for vascular tone-diameter homeostasis, vasculogenesis, angiogenesis or vessel reparation after injury. Dysregulated phenotype modulation and failure to maintain/regain the mature differentiated and contractile phenotypic state is pivotal in the development of vascular diseases such as atherosclerosis and restenosis after angioplasty and coronary bypass grafting. Many functions of SMCs such as adhesion, migration, proliferation, contraction, differentiation and apoptosis are regulated by a broad spectrum of cell-cell and cell-matrix adhesion molecules. Cadherins represent a superfamily of cell surface homophilic adhesion molecules with fundamental roles in morphogenetic and differentiation processes during development and in the maintenance of tissue integrity and homeostasis in adults. The cadherins have major inputs on signalling pathways and cytoskeletal assemblies that participate in regulating processes such as cell polarity, migration, proliferation, survival, phenotype and differentiation. Abnormalities in these processes have long been recognized to underlie pathological SMC-driven reparation, but knowledge on the involvement of cadherins is remarkably limited. This article presents a comprehensive review of cadherin family members currently identified on vascular SMCs in relation to their functions, molecular mechanisms of action and relevance for vascular pathology.

## 1. Introduction: why cadherins expressed on smooth muscle cells should be important for vascular health

The smooth muscle cell (SMC)[1] constitutes the most abundant resident cell type within the blood vessel wall and plays a major role in both vascular health and the pathogenesis of vascular disease. Unlike the majority of differentiated cells within adult animals SMCs can undergo profound and reversible changes in phenotype, a process referred to as phenotype switching. SMCs can transition between a mature 'differentiated' contractile phenotype that proliferates at an extremely low rate and exhibits low synthetic activity and an immature 'dedifferentiated' phenotype that loses contractile functions and acquires synthetic, migratory and proliferative properties [1–6]. The two phenotypes are not mutually exclusive and a continuum of intermediate SMC phenotypes between the 'differentiated' and 'dedifferentiated' extremes exists. While important for development and normal homeostasis, phenotype reversibility renders SMCs particularly susceptible to both physiological and non-physiological stimuli. Deregulated SMC

*Abbreviations:* ADAM, a disintegrin and metalloproteinase; AJ, adherens junction; Akt, AKT8 virus oncogene cellular homolog/protein kinase B; AMPKα2, AMP-activated protein kinase α2; Ang II, angiotensin II; APC, adenomatosis polyposis coli; APN, adiponectin; ApoE, apolipoprotein E; Bad, Bcl-2-associated death promoter; Bcl2, B-cell lymphoma 2; bFGF, basic fibroblast growth factor; $Ca_i^{2+}$, intracellular calcium; CArG box, [CC(A/T)$_6$GG]; Cas, Crk-associated substrate; Cdc42, cell division control protein 42 homolog; CDH, cadherin gene symbol; EC, extracellular; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; EMT, epithelial-to-mesenchymal transition; Erk, extracellular signal-regulated kinase; FA, focal adhesion; FAK, focal adhesion kinase; FGFR, fibroblast growth factor receptor; GPI, glycosylphosphatidylinositol; GSK3β, glycogen synthase kinase 3β; HB-EGF, heparin-binding epidermal growth factor-like growth factor; HEK293, human embryonic kidney; IGF, insulin like growth factor; IL2R-FAT1$_{1C}$, interleukin 2 receptor-FAT1 intracellular domain fusion protein; ILK, integrin-linked kinase; IRS, insulin receptor substrate; kDa, kilodalton; LC3, microtubule-associated protein 1A/1B–light chain 3; LDL, low density lipoprotein; MAPK, mitogen-activated protein kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/Erk kinase; MLC$_{20}$, myosin light chain 20; MMP, matrix metalloproteinase; MRTF, myocardin related transcriptional factor; MSC, mesenchymal stem cell; MTOC, microtubule-organizing center; mTORC1, mammalian target of rapamycin complex 1; MYH11, myosin heavy chain 11; MYOCD, myocardin; MYPT1, myosin phosphatase targeting subunit 1; Nox1, NADPH oxidase 1; p38 MAPK, p38 mitogen activated protein kinase; PDGF, platelet-derived growth factor; PI3K, phosphoinositide 3-kinase; Rac, Ras-related C3 botulinum toxin substrate; Raf, rapidly accelerated fibrosarcoma; Ras, retrovirus-associated DNA sequences; RhoA, Ras homolog gene family, member A; ROCK, Rho-associated protein kinase; ROCK, Rho-associated, coiled-coil containing protein kinase; ROS, reactive oxygen species; S6K1, (p70) S6 kinase 1; SMC, smooth muscle cell; SNC-Fc, soluble N-cadherin fusion protein; Src, Rous sarcoma oncogene cellular homolog; SRF, serum response factor; Tcf, T-cell factor; TGFβ, transforming growth factor β; TNFα, tumour necrosis factor α; VLDL, very low density lipoprotein; αSMA, alpha-smooth muscle actin; 3D, 3-dimensional

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phenotype switching and failure to maintain/regain its mature differentiated phenotypic state is a pivotal detrimental contributor to the evolution of vascular remodelling-associated diseases such as pulmonary hypertension, chronic obstructive pulmonary disease, atherosclerosis, post-angioplasty restenosis, transplant arteriopathy and vein graft disease. Even after decades of research, there are still fundamental gaps in our understanding of the cellular and molecular mechanisms that cause/control critical transitions in SMC phenotype or that participate in mediating altered SMC behaviour as the consequence of loss of the differentiated phenotype. Areas of active research with respect to SMC phenotype transition include epigenetic control mechanisms, transcriptional regulation of differentiation marker genes, embryonic stem cell pluripotency, signal transduction, cell-cell and cell-matrix interactions. A relatively unexplored area of research in SMC (patho) biology concerns the cadherins.

Cadherins are a superfamily of cell surface adhesion molecules long recognized for their crucial roles in important morphogenetic and differentiation processes during development, and in maintaining tissue integrity and homeostasis in adults. Cadherins mediate $Ca^{2+}$-dependent trans-junctional homophilic cell-cell interactions that function not only to establish tight cell-cell adhesion but also to define adhesive specificities of cells. The role of cadherins is not limited to homophilic adhesion; they are capable of heterophilic interactions with numerous extracellular and intracellular proteins and have major inputs on signalling pathways and cytoskeletal assemblies involved in regulating processes such as cell polarity, migration, proliferation, survival, phenotype and differentiation. Since abnormalities in these processes contribute toward pathological SMC-driven reparation, it can be expected that disruption of cadherin function and/or inappropriate cadherin expression on SMCs has significant implications in many vascular diseases. This expectation notwithstanding, investigations aiming at delineation of the specific functions and mechanisms of action of cadherins in SMCs are remarkably scarce. Further, and because SMC phenotype switching is so pivotal to development and progression of vascular disease, research on the role of cadherins in SMC (patho)biology has largely been conducted in the context of behavioural and cellular processes that characterize the differentiated/contractile or dedifferentiated/synthetic phenotypes.

This article appraises the current state of knowledge on the relevance of cadherins to SMC (patho)biology. Chief molecular characteristics of the different cadherin subfamily members identified to date on SMCs are outlined. We comprehensively review vascular disease-associated alterations in cadherin expression on SMCs, the contribution of cadherins to molecular control of SMC phenotype, behavioural and cellular functions of SMCs regulated by the cadherins and mechanisms of action, the significance of ecto-domain shedding of cadherins from SMCs and the participation of cadherins in mechanotransduction.

## 2. Key molecular characteristics of cadherin family members expressed on smooth muscle cell

The cadherin superfamily is large and heterogeneous. It comprises more than 350 members found in various species; more than 100 members have been described in vertebrates [7]. The foremost common structural feature of cadherins is the presence of $Ca^{2+}$-binding cadherin extracellular (EC) repeats in their ectodomain, the number of these repeats varying from two in calsyntenins to 34 in FAT-like cadherins. The majority of cadherins are transmembrane proteins, although there are exceptions (see below). Based on their structure and phylogenetic analysis of the protein sequences, the superfamily was traditionally divided into six large subfamilies: classical or type-I cadherins, atypical or type-II cadherins, desmogleins, desmocollins, Flamingo cadherins and protocadherins. Some cadherins with unique structures did not belong to any of the subfamilies, such as cadherin-13 (T-cadherin), -15, -16, -17, Dachsous, RET and several others. Based on an in-depth review and analysis of amassed data on molecular evolution and genomic sequencing, Hulpiau and van Roy proposed a novel classification that better incorporates the growing number of atypical and 'orphan' members into structurally related groups [8]. According to this classification, the superfamily is divided into two main branches, namely a cadherin major branch and a cadherin-related major branch. The cadherin major branch is comprised of two families C-1 (with type-I, type-II, desmocollins, desmogleins, 7D and solitary cadherin subfamilies) and C-2 (with Flamingo, type III and type IV cadherin subfamilies). The cadherin-related branch is subdivided into in four families: Cr-1a (protocadherins), Cr-1b (RET, FAT, Dachsous), Cr-2 (CDHR and μ-CDHR) and Cr-3 (FAT-like, CDHR28, CDHR15 and calsyntenins) [8]. Readers are referred to a range of excellent recent articles that variously review the evolution of cadherin family members, their diverse functions in tissue development and maintaining tissue integrity, and their many molecular mechanisms of action in cell types other than SMCs [9–34].

Cadherin proteins formally identified in SMCs include the type I E-cadherin (CDH1), N-cadherin (CDH2) and R-cadherin (CDH4), type II cadherin-6B and cadherin-11 (CDH11/OB-cadherin), solitary T-cadherin (CDH13/H-cadherin), and cadherin-related FAT1 (CDHR8). Key molecular characteristics of these cadherin family members (depicted in Fig. 1) are considered in the following.

### 2.1. E-cadherin

The prototype member of the superfamily, type I E-cadherin (CDH1), plays a central role in epithelial behaviour and tissue formation. Its presence is essential for maintenance of epithelial structures. Loss of E-cadherin expression is a key feature of epithelial-to-mesenchymal transition (EMT), which is also associated with induction of 'mesenchymal' cadherins (*e.g.* N-cadherin) [20]. E-cadherin is a transmembrane glycoprotein with an EC structure composed of five EC repeats (EC1-EC5), each approximately 110 amino acid residues long, folded into β-sheets composed of seven β strands. As for all members of the classical/type-I subfamily, E-cadherin is characterized by the presence of a conserved HAV peptide in EC1 domain which plays a major role in adhesive interactions and regulation of the ligand specificity. Conserved sequences DXNDN, DXD and LDRE localized at regions between adjacent EC repeats bind $Ca^{2+}$ ions, which stabilize the ectodomain structure. Adhesive interactions between two neighboring cells (*trans*-contacts) are established by bridging the membrane-distal EC1 domains of two E-cadherin molecules through the so-called 'strand swapping' mechanism: the hydrophobic region of the most N-terminal part of the β-A-strand containing tryptophan at position 2 is inserted into a hydrophobic pocket on EC1 domain of a partner E-cadherin molecule [29,35,36]. Adhesive *trans*-contacts are preferentially homophilic and are established between cadherin molecules of the same type, although heterophilic binding between members of the same superfamily (for example, between different type I cadherins) is sometimes observed. In addition to *trans*-contacts, cadherins present on the surface of the same cell form *cis*-dimers stabilized by lateral binding between EC1 and EC2 domains of two partner molecules [35,37]. Fully functional homophilic contacts are formed by cooperative alternate organization of *cis*- and *trans*-dimers into multimeric zipper-like structures between the surfaces of adjacent cells. A single membrane-spanning domain links cadherin ectodomains to the cytoplasmic domain which binds to cytoplasmic proteins β-catenin and plakoglobin and through them to α-catenin and the actin cytoskeleton, thus providing strong mechanical coupling of cells [38]. Besides their essential role in mechanical adhesion, cadherin–catenin proteins regulate diverse signalling pathways important for fundamental processes such as cell division, migration, differentiation and apoptosis [39,40].