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Endothelial Piezo1: Life depends on it

Jing Li, Bing Hou, and David J Beech*
School of Medicine; University of Leeds; Leeds, UK

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*Correspondence to: David J Beech; Email: d.j.beech@leeds.ac.uk

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Endothelial cells are fundamental to almost all physiology: foundation stones of an ancient vascular system, essential for the development and survival of animals. They are exposed to many physical forces: from external insult, the beating heart, fluid dynamics, and tissue remodelling. They have pronounced sensitivity to the frictional force of shear stress, which in physiology arises because of blood flow. In humans the force ranges from 1 to 70 dyn.cm^{-2} (0.1–7 Pa). It is actively sensed by endothelial cells to enable vascular development and maintain an efficient and healthy vasculature thereafter. The mechanisms by which physical forces regulate endothelial cells to determine the complexities of vascular structure and function have nevertheless been enigmatic. Important studies have revealed multiple participating proteins and sensing via Ca^{2+}-permeable non-selective cationic channels but the nature of the sensor itself and molecular basis of the channel have been controversial and elusive.[1]

We recently made an intriguing discovery about endothelial mechanical sensitivity.[2] *Piezo1* gene, previously without known vascular relevance, was found to be important for normal shear stress-evoked Ca^{2+} signaling and non-selective cationic channel activity in endothelial cells. What was especially striking was that Piezo1 knock-out in the mouse was embryonic lethal just after the time when the heart started to beat and when substantial arteries should first emerge to support expansion of the embryo. We demonstrated that the lethality reflected a specific requirement for endothelial Piezo1: with endothelial-specific *Piezo1* disruption, endothelial cells were present but did not remodel to form mature vascular architecture. Significance of Piezo1 in the adult mouse was also indicated through studies of haploinsufficient animals carrying 50% *Piezo1* expression: there were disturbances in both nitric oxide synthase and alignment of endothelial cells to the direction of blood flow. Proteomic and other studies led us to find that downstream of Piezo1-dependent Ca^{2+} signaling was protease (calpain) activation, focal adhesion turnover, and spatial re-alignment of endothelial cells to the polarity of the applied force (**Fig. 1**). This work therefore revealed a gene of critical importance for the vascular field, new fundamental understanding of how complex life develops and new ideas for addressing health problems such as cardiovascular disease and cancer where changes in blood flow are common and often unwanted.

In 2010, relationships of Piezo1 to ion channels, transmembrane Ca^{2+} flux, and mechanical sensitivity were first suggested.[3,4] The primary molecular hypothesis for Piezo1 has since been that it assembles as a tetramer to form a mechanically-activated machine with a central ion pore that allows transmembrane Na^+ and Ca^{2+} flux into cells.[5] A role in Ca^{2+} release has been suggested,[4] although it could not be detected in endothelial cells.[2] Amino acid sequence analysis suggests no relationship to other ion channel or putative ion channel proteins, except Piezo2, and there is currently no clear insight into the amino acid residues participating in the ion permeation pathway or mechanical sensitivity. Structural information for an extracellular loop has been obtained at 2.5Å resolution, supporting the suggestion that Piezo proteins lack close relatives.[6]

Piezo1 is at least 286 kDa, so the tetramer is over a million Daltons. It is tempting to think that such a large assembly

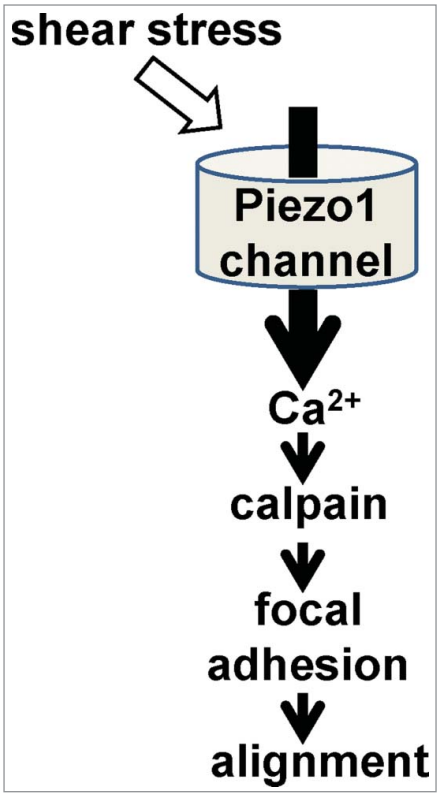

Figure 1. Simplified model for Piezo1 signaling in endothelial cells.

might be capable of ion channel and non-ion channel functions, although all of the effects linked to Piezo1 in endothelial cells were also sensitive to the spider toxin GsMTx4,[2] an inhibitor of Piezo1 channels.[7] There is however evidence of Piezo1 activity in the absence of applied exogenous mechanical force: regulation of β1-integrin in epithelial cells[4] and regulation of cell migration and nitric oxide synthase in endothelial cells.[2] Such effects may reflect independence of mechanical force or relationships of Piezo1 to intrinsic forces in cells, or between cells and their substrates.

Mutations in human *Piezo1* gene (*PIEZO1*) are linked to hereditary hemolytic anaemias.[8] They are found to alter the kinetics of Piezo1 channel activation and inactivation.[8] Although often considered to be gain-of-function mutations, the M2225R mutation slowed activation in response to mechanical strain[8] and inhibited activation by shear stress.[2] The finding of Piezo1 sensitivity to shear stress[2] could have important implications for understanding Piezo1 in erythrocytes, which routinely experience shear stress.

Endothelial Piezo1 stands out in being critical for vascular development but contributions of other proteins as additional shear stress sensors or sensors of other mechanical forces in endothelial cells is likely and there is good evidence for it.[1] The breadth of forces experienced by endothelial cells is substantial and so we can expect involvement of other proteins as sensors, back-up mechanisms, regulators and amplifiers, or components of integrated multi-protein complexes with Piezo1. A central player in shear stress sensing has been documented as the CD31 protein.[1] There are also other Ca^{2+}-permeable channels to consider: notably those formed from TRPP2 (polycystin 2), TRPV4 and P2X4 proteins.[1]

Determination of the reason for lethality in *Piezo1*-disrupted mice and exposure of a critical role specifically for endothelial Piezo1[2] provide important opportunities for achieving better understanding of the processes underlying maturation of arteries during vascular development and for revealing relationships between seemingly subtle physiological forces and the architecture and function of the vasculature. There is much work ahead to understand this profoundly important ion channel protein.

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