BIOLOGICAL REVIEWS
Cambridge Philosophical Society
Biol. Rev. (2022), **97**, pp. 604–614.
doi: 10.1111/brv.12814

Mechanosensing by Piezo1 and its implications for physiology and various pathologies

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ABSTRACT

Piezo1 is a mechanosensitive ion channel with essential roles in cardiovascular, lung, urinary, and immune functions. Piezo1 is widely distributed in different tissues in the human body and its specific roles have been identified following a decade of research; however, not all are well understood. Many structural and functional characteristics of Piezo1 have been discovered and are known to differ greatly from the characteristics of other mechanosensitive ion channels. Understanding the mechanisms by which this ion channel functions may be useful in determining its physiological roles in various organ systems. This review provides insight into the signalling pathways activated by mechanical stimulation of Piezo1 in various organ systems and cell types. We discuss downstream targets of Piezo1 and the overall effects resulting from Piezo1 activation, which may provide insights into potential treatment targets for diseases involving this ion channel.

Key words: mechanotransduction, Piezo1, shear stress, mechanoreceptor, mechanosensitive ion channel

CONTENTS

I. INTRODUCTION

Mechanosensitive ion channels play important roles in the transduction of physical stimuli from a cell's surrounding environment and the translation of these stimuli into biochemical responses. These ion channels translate mechanical cues into biological signals and activate intracellular signalling pathways which lead to a variety of cellular responses (Martinac, 2004). The Piezo family of mechanosensitive ion channels was discovered in 2010 (Coste *et al.*, 2010) and has garnered abundant interest in the field of mechanobiology for its unique properties and functions. Piezo1, previously known as FAM38A, is expressed in cells present in various tissues, especially those that are highly exposed to mechanical stimuli, such as the colon, kidneys, skin, bladder and lungs (Coste *et al.*, 2010) and, in particular, endothelial cells in

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blood vessels (Douguet *et al.*, 2019). In vascular endothelial cells, these ion channels are in direct contact with mechanical forces, including shear and strain. Piezo1 has been demonstrated to exhibit inherent mechanosensitivity, as it gates in direct response to physical forces when reconstituted into lipid bilayers or membrane blebs (Cox *et al.*, 2016; Syeda *et al.*, 2016).

Piezo1 is cation selective and permeable to monovalent and divalent ions (Coste *et al.*, 2010, 2012), forming a homotrimer and adopting a triple-blade propeller-like structure attached to a central ion-conducting pore with a cap (Guo & Mackinnon, 2017; Saotome *et al.*, 2018; Zhao *et al.*, 2018). The transmembrane helices that form the curved blades or 'arms' on the ion channel are embedded in the membrane and produce localised curvature of the membrane, which may contribute to force sensing (Guo & MacKinnon, 2017; Saotome *et al.*, 2018; Zhao *et al.*, 2018). Furthermore, it is hypothesised that mechanotransduction is likely triggered by a lever-like mechanism that controls the movement of the blades (Zhao *et al.*, 2018). The ion channel functions as a whole unit, as the expression of both N-terminal and C-terminal regions is necessary for channel activity (Bae *et al.*, 2016; Zhao *et al.*, 2016). The structure and function of Piezo1 are described in more detail elsewhere (Saotome *et al.*, 2018; Wang *et al.*, 2019).

Phosphoinositides (PIs) are membrane phospholipids that are often required for the function of membrane proteins. Of particular interest, several functional studies have identified a role for phosphoinositides in the mechanical gating of Piezo1 (Buyan *et al.*, 2020). For example, depletion of PI(4,5)P2 and PI(3,4,5)P3, the most abundant phosphoinositides in the membrane, reduces the electrical current *via* Piezo1 (Borbiro, Badheka & Rohacs, 2015). PI(4,5)P2 and PI(3,4,5)P3 are substrates for phospholipase C enzymes and contribute to the regulation of actin-dependent cellular processes and integrin signalling (Wu *et al.*, 2014). In addition to phophoinositides, a host of other lipid types have been shown to modify Piezo1 function (Tsuchiya *et al.*, 2018; Romero *et al.*, 2019; Ridone *et al.*, 2020).

Piezo1 is located at arginine-glycine-aspartic acid-containing matrix protein–integrin (e.g. $\alpha_V\beta_3$) adhesion sites in human foreskin fibroblast cells (Yao *et al.*, 2020), where it is suggested to regulate focal adhesion maturation, disassembly and directional migration of these cells. This recruitment is blocked by a Rho-associated kinase (ROCK) inhibitor. Given the contribution of Rho family members to mechanical signal transduction, this finding suggests that the localisation of Piezo1 at focal adhesion sites might be force-dependent (Lessey, Guilluy & Burridge, 2012; Ohashi, Fujiwara & Mizuno, 2017). However, whether the local activity of the channel changes in response to adhesion-mediated mechanical signalling (Ellefsen *et al.*, 2019) at focal adhesion sites and whether this mechanism also applies to other Piezo1-expressing cell types has not yet been determined.

In most cell types, two main signalling pathways have been identified downstream of Piezo1 activation: induction of adenosine triphosphate (ATP) release (Wei *et al.*, 2019) and activation of calpains (Li, Hou & Beech, 2015). In mammals, ATP signalling consists of the release of ATP in the extracellular environment, subsequent activation of purinergic P2X and P2Y receptors, and removal of ATP by ATP-scavenging ectonucleotidases. ATP signalling pathways play essential roles in cellular function and cell–cell communication, while alterations in these pathways contribute to a range of pathologies, such as inflammatory disease, neurodegenerative disorders, pain, hypertension and cancer metastasis (Burnstock & Boeynaems, 2014; Alves, Beamer & Engel, 2018; Di Virgilio *et al.*, 2018; Wei *et al.*, 2018, 2019). Calpains are Ca^{2+}-dependent intracellular cysteine proteases that are activated by localised Ca^{2+} influx and catalyse controlled proteolysis of target proteins (Suzuki *et al.*, 2004). Calpain activation is linked to several Ca^{2+}-dependent processes, such as cell cycle progression, cell proliferation, signal transduction, apoptosis, platelet activation and angiogenesis (Suzuki *et al.*, 2004).

Alterations in calpain activity have been linked to several pathologies, such as stroke, traumatic brain injury, Alzheimer's disease, cancer and type 2 diabetes (Vanderklish & Bahr, 2000; Huang & Wang, 2001). Both of these signalling pathways subsequently mediate the transduction of mechanical stress in physiological responses.

Piezo1-mediated mechanotransduction has been determined to be a key regulator linking the extracellular physical environment to different signal transduction cascades. Therefore, this review summarises the currently known and potential signalling pathways activated by Piezo1 stimulation that play roles in regulating physiological functions in different organ systems.

II. PIEZO1 IN ENDOTHELIAL PHYSIOLOGY AND VASCULAR FUNCTION

Blood flow as a result of cardiac function generates two main forces, shear stress (induced by movement of blood through the vessel) and cyclic stretch (determined by luminal pressure). Shear stress is a frictional force that is experienced mainly by endothelial cells and circulating blood cells. By contrast, mechanical stretch, which is directly proportional to blood pressure, is experienced by all cell types of the blood vessel wall. These haemodynamic forces are major determinants of vascular development, morphogenesis and function (Baratchi *et al.*, 2017).

Among the different classes of mechanoreceptors expressed in endothelial cells, there has been a recent appreciation of the role of Piezo1 in vascular physiology. This began in 2014 with a report showing that global knockout of Piezo1 is embryonically lethal (Li *et al.*, 2014). In that study, it was demonstrated that deficiency in Piezo1 in mouse embryos led to the impaired development of major blood vessels at mid-gestation despite normal vasculogenesis until embryonic day 9.5 (E9.5). Global Piezo1-knockout mice

exhibited major pericardial effusion as a result of poor circulation and died before E14.5 (Li *et al.*, 2014; Ranade *et al.*, 2014), showing that Piezo1 is vital to early vascular development. Piezo1 expression in the mouse endothelium and endocardium has been detected on E9 and E10.5 (Li *et al.*, 2014; Ranade *et al.*, 2014) and in adult mice Piezo1 is expressed in endothelial cells and smooth muscle cells (Retailleau *et al.*, 2015). However, it still remains to be unequivocally determined whether the loss of Piezo1 in endothelial cells drives the pericardial effusion phenotype. In humans, Piezo1 expression has been detected in fetoplacental (Morley *et al.*, 2018), aortic (Lai *et al.*, 2020), and umbilical vein endothelial cells (Wang *et al.*, 2016; Lai *et al.*, 2020).

In addition to contributing to vascular development, Piezo1 is a regulator of blood pressure. Piezo1 and Piezo2 together act as the molecular pressure sensors of baroreceptors, illustrated by the fact that deletion of these ion channels in mice leads to the complete absence of barosensation and baroreceptor reflex function (Zeng *et al.*, 2018). Baroreceptor nerve endings in the walls of the aortic arch and carotid sinus play a pivotal role in the regulation of blood pressure by sensing blood pressure and transferring the signal to the central nervous system to maintain appropriate tissue perfusion (Stocker, Sved & Andresen, 2019). Impaired cardiac baroreceptor sensitivity is associated with increased mortality after acute ischaemic stroke (Robinson *et al.*, 2003). Genetic ablation of Piezo1 and Piezo2 in the nodose and petrosal ganglia, where baroreceptor cell bodies are located, leads to aortic depressor nerve activity and increased blood pressure variability (Zeng *et al.*, 2018). Furthermore, optogenetic activation of Piezo2-positive sensory afferents in animals is sufficient to increase the baroreflex in mice (Zeng *et al.*, 2018), suggesting that both Piezo1 and Piezo2 contribute to the regulation of blood pressure. The contribution of Piezo1 to the regulation of blood pressure is not limited to its role in barosensation and baroreceptor reflex function.

One of the key roles of Piezo1 in the endothelium is the regulation of nitric oxide (NO) release, as mice with endothelial-specific Piezo1 deficiency lack flow-induced NO formation and vasodilation (Wang *et al.*, 2016). Flow-induced NO production is important in the adaptation of vessel diameter in response to haemodynamic forces and the consequent control of vascular tone and blood pressure. Endothelial Piezo1 contributes to the regulation of blood pressure by controlling flow-induced ATP release and subsequent P2Y2/G protein alpha-subunits Galphaq and Galpha11 (G_q/G_{11}) activation, which leads to activation of platelet endothelial cell adhesion molecule-1 (PECAM-1)/vascular endothelial (VE)-cadherin/vascular endothelial growth factor receptor 2(VEGFR2) and phosphorylation and activation of protein kinase B (AKT) and endothelial nitric oxide synthase (eNOS). This process is partially mediated by the activation of pannexin channels (Wang *et al.*, 2016). Similarly, Piezo1 channel gating after shear stress stimulation activates adrenomedullin, which in turn activates its receptor consisting of calcitonin receptor like receptor (CALCRL) and receptor activity modifying protein 2 (RAMP2); this activation activates the G protein Gs, leading to increases in cyclic adenosine monophosphate (cAMP), phosphorylation of AKT and eNOS and subsequent production of NO (Iring *et al.*, 2019).

As explained above, Piezo1 contributes to endothelial responses to laminar flow and controls the activation of atheroprotective signalling pathways by mediating the activation of eNOS. A recent study revealed the interconnectivity between PECAM-1/VE-cadherin/VEGFR2 and Piezo1 (Chuntharpursat-Bon *et al.*, 2019). This study elegantly demonstrated that Piezo-1 interacts with VE-cadherin and PECAM-1, but not VEGFR2 at cell–cell adhesion sites in a flow-dependent manner, and that the interaction of Piezo1 with PECAM-1 reduces the force sensitivity of Piezo1, thereby limiting overactivation of the channel (Chuntharpursat-Bon *et al.*, 2019).

In addition to laminar flow, Piezo-1 is activated in response to disturbed flow, which is generally observed under atherogenic conditions such as at branch points and curvatures of coronary and carotid arteries, and is linked to activation of the inflammatory phenotype in endothelial cells (Albarran-Juarez *et al.*, 2018). Both pathways, those induced by laminar and disturbed flow, initially involve stimulation of the cell adhesion complex (PECAM-1, VE-cadherin, VEGFR2), Src family kinases and phosphoinositide 3-kinase (PI3K) but diverge downstream. In the presence of laminar flow, PI3K lipid products allosterically activate AKT (Yudushkin, 2019) to phosphorylate eNOS (Fulton *et al.*, 1999) and produce NO, thereby promoting vasodilation. However, mice with endothelium-specific deficiency in Piezo1 or Gαq/Gα11 show decreased activation of integrin and expression of inflammatory markers, and consequent progression of atherosclerosis in atherosclerosis-prone areas exposed to disturbed flow. Under disturbed flow, the activation of PI3K activates α5 integrins present in lipid rafts (Sun *et al.*, 2016) and associated focal adhesion kinase (FAK) (Petzold *et al.*, 2009). This activation results in phosphorylation of the inhibitory kappa B (IκB) subunit of the nuclear factor kappa B (NFκB) transcription factor complex by IκB kinases (Nakajima & Mochizuki, 2017). The subsequent activation of NFκB (Baeriswyl *et al.*, 2019) induces inflammatory pathways while also downregulating eNOS, thus contributing to the development of an atherogenic phenotype (Albarran-Juarez *et al.*, 2018).

This process suggests that the expression, function and cellular localisation of mechanosensitive ion channels such as Piezo1 (Li *et al.*, 2014) can be modulated by flow dynamics and the signalling pathways activated by these conditions (see Fig. 1).

In addition to laminar and disturbed flow, Piezo1 function has been linked to endothelial responses to elevated blood pressure during physical activity (Rode *et al.*, 2017). Ca^{2+} influx *via* Piezo1 post-stimulation with elevated shear stress depolarises the membrane potential of mesenteric artery endothelial cells, leading to activation of voltage-sensitive Ca^{2+} channels of neighbouring smooth muscle cells and consequent vasoconstriction. This process is compromised in

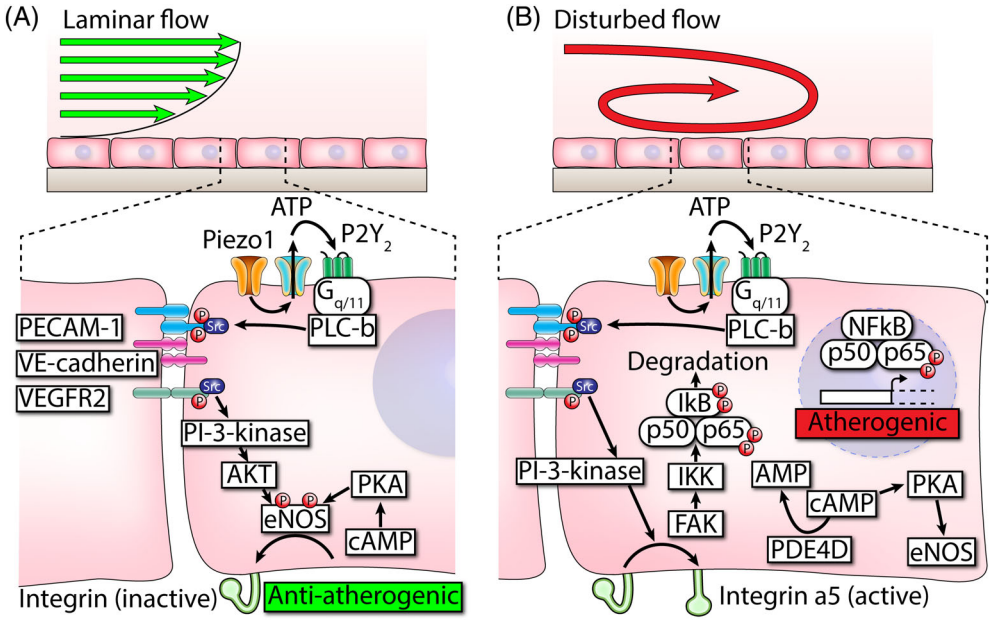

Fig. 1. Mechanotransduction of (A) laminar flows; and (B) disturbed flows *via* Piezo1. Both flow patterns initially activate the same signalling process involving activation of $P2Y_2$, G_q/G_{11} and PECAM-1/VE-cadherin/VEGFR2 signalling. However, integrins remain inactive in response to laminar flow, and atheroprotective signalling pathways are activated, while activation of integrin in response to disturbed flow triggers atherogenic signalling pathways. ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; eNOS, endothelial nitric oxide synthase; FAK, focal adhesion kinase; Gq/11, Gq protein alpha subunit; Ikb, inhibitor of nuclear factor-kB; IKK, inhibitor of nuclear factor-kB kinase; NFkB, nuclear factor kappa B; PDE4D, phosphodiesterase 4; PECAM-1, platelet endothelial cell adhesion molecule-1; PI3-kinase, phosphoinositide 3-kinase; PKA, protein kinase A; PLC: phospholipase C; VE-cadherin, vascular endothelial cadherin, VEGFR2, vascular endothelial growth factor receptor 2. Adapted from Albarran-Juarez *et al.* (2018).

mice lacking Piezo1 expression in their endothelial cells (Rode *et al.*, 2017).

In addition to its above-described roles in regulating endothelial cell function in blood vessels, a role of Piezo1 in regulating cardiomyocyte responses to cyclic stretch has also been reported (Wong *et al.*, 2018); however, how Piezo1 contributes to the regulation of cardiac contractility has yet to be determined. Overall, these studies indicate that Piezo1 mechanotransduction contributes to cardiovascular physiology.

III. PIEZO1-MEDIATED FUNCTIONS IN THE PULMONARY SYSTEM

The pulmonary system functions closely with the cardiovascular system to regulate the circulation of blood and its vital substances. Pulmonary circulation is specific and different from systemic circulation, as it conducts 100% of the cardiac output for the purpose of respiratory gas exchange; to facilitate this function, the pulmonary arterial pressure is much lower than the systemic arterial pressure (Fishman, 2004). Both lung endothelial cells and lung epithelial cells are exposed to cyclic mechanical stretch due to repeated alveolar inflation (Zhong *et al.*, 2018).

The role of Piezo1 in pulmonary circulation has recently been confirmed *via* demonstration of its role in intrapulmonary artery vascular tone in a study using mice with specific endothelial deletion of Piezo1 (Friedrich *et al.*, 2019). This study demonstrated that Piezo1 controlled the degradation and internalisation of VE-cadherin, an endothelial adherens junction protein, by triggering calcium-dependent activation of calpain and consequent proteolytic breakdown of VE-cadherin, β-catenin and catenin delta-1 (p120-catenin) complexes in response to hydrostatic pressure (Friedrich *et al.*, 2019). In endothelial cells, increased internalization of VE-cadherin *via* this mechanism leads to endothelial tight junction disruption and lung vascular hyperpermeability (Friedrich *et al.*, 2019). Similarly, activation of calpain *via* Piezo1 in alveolar capillary endothelial cells, in response to cyclic stretch exerted by the alveoli, can lead to cleavage and inactivation of Src family kinases to stabilise the lung endothelial barrier (Zhong *et al.*, 2020). This highlights the role of Piezo1 signalling in lung vascular permeability.

In addition to contributing to pulmonary circulation, Piezo1 is highly expressed in human lung tissue and knockdown of Piezo1 in lung epithelial cells *in vitro* reduces adhesion and consequently accelerates cellular migration. Furthermore, loss of Piezo1 expression has been shown to accelerate the rates of migration and cancer progression in non-small cell lung cancer (Huang *et al.*, 2019) as well as

breast cancer cells (Luo *et al.*, 2019). Local Ca^{2+} influx mediated by Piezo1 is proposed to modulate calpain activity, resulting in reduced cell migration and invasion of lung epithelial cells, while Piezo1 knockdown produces the opposite effect (Huang *et al.*, 2019). As mentioned earlier, calpain is a major downstream target of Piezo1 in different tissues including the lungs (Li *et al.*, 2014) and given the roles of calpain in several aspects of cell migration, including adhesion and spreading (Franco & Huttenlocher, 2005), the effects of loss of Piezo1 and its sensory mechanics may indicate the importance of mechanotransduction in lung cancer metastasis.

IV. PIEZO1-MEDIATED FUNCTIONS IN THE URINARY TRACT

The urinary tract functions to filter blood and store/evacuate urine, and consists of the kidneys, renal pelvis, bladder, ureters and urethra. The mechanosensitivity of various organs of the urinary tract system allows for their proper functioning in response to wall tension and shear stress (Dalghi *et al.*, 2019). The expression and response of Piezo1 to uniaxial stretch were first demonstrated in mouse urothelial cells, in which it was shown that stretch led to Piezo1-dependent increases in intracellular calcium concentrations and ATP release (Miyamoto *et al.*, 2014). Extracellular ATP then binds to P2X receptors expressed on afferent sensory neurons to transmit signals associated with bladder volume (Cockayne *et al.*, 2000).

Later, an elegant study demonstrated that in rats Piezo1 regulates bladder activity during cyclophosphamide (CYP)-induced cystitis (Liu *et al.*, 2018*b*). Interstitial cystitis is a clinical syndrome characterised by bladder pressure and pain. Interstitial cells of Cajal (ICC)-like cells (ICC-LCs) have an important role in regulating bladder activity *via* non-neural sensory signal transduction from the urothelium to the detrusor muscle (Gevaert *et al.*, 2014). This study showed that treatment of rats with CYP for 48 h increased the expression of Piezo1 in ICC-LCs leading to bladder hyperactivity. This effect was averted by pharmacological inhibition of Piezo1 using a spider venom peptide that selectively inhibits cation-permeable mechanosensitive channels (GsMTx-4) (Liu *et al.*, 2018*b*). Hypotonically induced Piezo1 expression and activation were associated with increased Na^+ and Ca^{2+} influx into ICC-LCs. Furthermore, Piezo1 functionally interacted with the reverse mode of Na^+/Ca^{2+} exchanger 1, and this interaction led to Ca^{2+} overload in bladder ICC-LCs that resulted in bladder hyperactivity and subsequently caused cystitis (Liu *et al.*, 2018*b*) (see Fig. 2).

The principal cells of collecting ducts have a major role in Na^+ and water regulation (Rao, Bhalla & Pastor-Soler, 2019) and Piezo1 expression in the principal cells of the kidney collecting ducts has also been demonstrated to be important in controlling urine osmolarity upon dehydration (Martins *et al.*, 2016). The collecting duct system is the final part of the nephron that contributes to salt and water absorption and fine tunes the osmotic balance. In particular, the collecting duct filters blood and predominantly expresses Piezo1 compared to Piezo2 (Martins *et al.*, 2016).

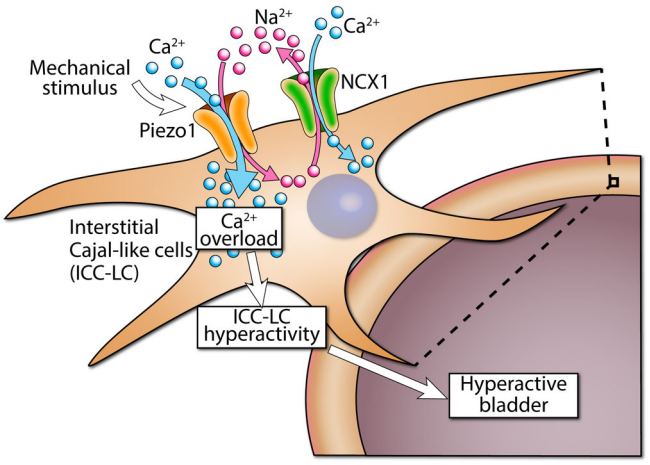

Fig. 2. Activation of Piezo1 resulting in bladder hyperactivity. Increased stretch and hypotonic swelling of bladder cells during cyclophosphamide (CYP)-induced chronic cystitis lead to the activation of Piezo1 in bladder ICC-LCs and the consequent influx of Ca^{2+} and Na^+ into ICC-LCs. The activation of Na^+/Ca^{2+} exchanger 1 (NCX1) in the reverse mode leads to the efflux of three Na^+ ions and the influx of one Ca^{2+} ion. This cooperative effect leads to increased intracellular calcium concentration ($[Ca^{2+}]_i$) and Ca^{2+} overload in ICC-LCs which results in bladder hyperactivity. Adapted from Liu *et al.* (2018*b*).

It has been determined that Piezo1 may play a role in osmoregulation by affecting urine dilution and urea levels *via* aquaporin 2 trafficking which can be explained by several mechanisms (Martins *et al.*, 2016). Firstly, Piezo1-mediated calcium influx decreases cAMP production, resulting in reduced water reabsorption and subsequent urine dilution during dehydration. Secondly, changes in cAMP levels mediated by Piezo1 may affect the trafficking of urea transporters, which require protein kinase A activation, and lastly, Piezo1 influences the synthesis and release of osmoregulatory hormones such as prostaglandins (Martins *et al.*, 2016).

Recently, Piezo1 expression and distribution in the mouse urinary tract were systematically assessed using a transgenic mouse model in which the COOH-terminal of Piezo1 was fused to a fluorophore (tandem-dimer Tomato) (Dalghi *et al.*, 2019). Using this approach, Piezo1 was shown to be expressed in different tissues of the urinary tract such as the kidneys, ureters, bladder and urethra, as well as in other organs in close proximity including the prostate, seminal vesicles and ducts, ejaculatory ducts, and vagina (Dalghi *et al.*, 2019). In terms of cell-type expression, Piezo1 expression has been detected in the basolateral membranes of epithelial cells covering the entire urinary tract as well as in the parietal cells of the renal corpuscle, the interstitial cells of the bladder and ureters and in smooth and striated muscle

cells (Dalghi *et al.*, 2019). These findings collectively provide evidence that mechanotransduction *via* Piezo1 is important in the physiology of the urinary tract.

V. PIEZO1 AND MECHANICAL FORCES IN BLOOD CELLS

Blood cells, including erythrocytes, leukocytes and platelets, are responsive to haemodynamic forces. For example, erythrocyte deformability in response to mechanical forces is physiologically and pathologically important, as shear stress deforms erythrocytes and allows them to pass through the microcirculation (Wan, Forsyth & Stone, 2011). Furthermore, mechanical stress improves the release of oxygen from erythrocytes, facilitating oxygen/CO_2 exchange (Rao *et al.*, 2009). Mechanosenstive ion channels play an unprecedented role in erythrocyte iron homeostasis and physiology. In 2013, it was shown for the first time that Piezo1 gain-of-function mutations are associated with the hereditary erythrocyte-dehydrating disease, xerocytosis, in which cation exchange is affected by delayed inactivation of Piezo1, suggesting that mechanotransduction *via* Piezo1 is required for volume regulation of erythrocytes (Zarychanski *et al.*, 2012; Bae *et al.*, 2013).

Following that, the role of Piezo1 in erythrocytes was studied in more detail using a mouse model with specific deletion of Piezo1 in haematopoietic cells. This study showed that deletion of Piezo1 in red blood cells leads to a volume increase due to overhydration which leads to erythrocyte fragility (Cahalan *et al.*, 2015). Piezo1-mediated calcium influxes after exposure to mechanical stretch result in downstream activation of the calcium-activated potassium KCa3.1 Gardos channel, which is implicated in erythrocyte dehydration and sickle cell disease (Cahalan *et al.*, 2015) (see Fig. 3). A common human gain-of-function mutation in Piezo1, E756del, in the African population is linked to hereditary xerocytosis and a reduction in the risk of malaria infection (Ma *et al.*, 2018).

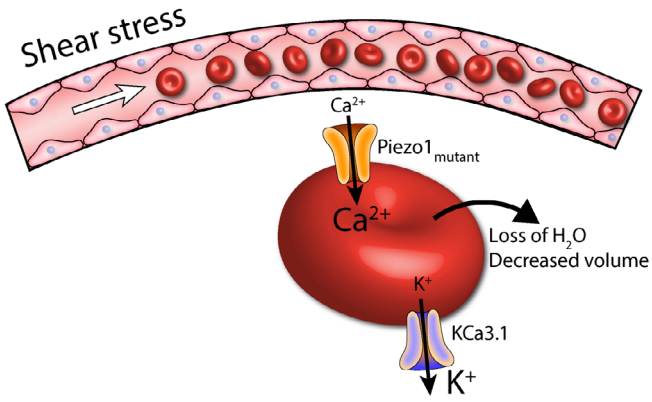

Fig. 3. Piezo1 contributes to the volume regulation of red blood cells. Calcium influx *via* Piezo1 post-exposure to shear stress leads to the activation of calcium-activated potassium KCa3.1 Gardos channels. The consequent efflux of K^+ from KCa3.1 leads to the loss of water and reduced volume of red blood cells. Adapted from Cahalan *et al.* (2015).

In addition to contributing to hereditary xerocytosis, Piezo1 contributes to the normal physiology of erythrocytes by performing mechanotransduction to induce the release of ATP after exposure to shear stress, which may be followed by activation of P2Y or P2X receptors on endothelial cells to influence vascular function (Cinar *et al.*, 2015).

In addition to their contribution to red blood cell physiology, there is an emerging interest in the effects of mechanical forces on the innate and adaptive immune systems. Although the field of immunomechanobiology is still in its infancy, progress in this field and elucidation of the fundamental mechanobiology of the immune system could inspire discoveries for immune therapy. For example, haemodynamic forces affect the ability of T-cells to migrate to sites of infection by affecting characteristics including crawling behaviour and extravasation across endothelial cells (Schreiber *et al.*, 2007; Steiner *et al.*, 2010). T-cells are master regulators of adaptive immunity, and the interactions between T-cell receptors and antigens presented by antigen-presenting cells in the form of the antigen-major histocompatibility complex (Ag-MHC) are crucial for the activation and clonal expansion of T-cells. In this regard, it is well accepted that the strength of the interactions between T-cell receptors and the Ag-MHC complex affects efficient T-cell receptor triggering. The role of Piezo1 in the optimal activation of T-cells has been demonstrated recently in a study showing that Piezo1 knockdown using small interfering RNA (siRNA) impairs the efficient priming of T-cells by antigen-presenting cells (Liu *et al.*, 2018*a*). Furthermore, Piezo1 activation with its agonist Yoda1 potentiates T-cell activation *via* regulation of calpain activity and actin cytoskeleton reorganisation (Liu *et al.*, 2018*a*). This finding provides evidence that Piezo1 contributes to the mechanosensitivity of T-cells in response to their microenvironment and as such allows for optimal T-cell activation and binding to antigen-presenting cells (Liu *et al.*, 2018*a*). However, no report has yet shown a direct role of Piezo1 in the T-cell response to haemodynamic forces.

In addition to contributing to adaptive immunity, mechanotransduction is important for the function of monocytes/macrophages, the master regulators of the innate immune response (Zhang *et al.*, 2020). In this regard, we have recently demonstrated in a clinical study that mechanical stress as a result of aortic valve stenosis directly affects several monocyte functions, including adhesion, phagocytosis, oxidized low-density lipoproteins (Ox-LDL) uptake and expression of proinflammatory cytokines. Furthermore, we have demonstrated that reducing pathological shear stress levels *via* transcatheter aortic valve implantation (TAVI) reduces these effects. Using a microfluidic model emulating high and low levels of shear stress in clinical samples, we showed that Piezo1 controls shear-induced calcium influx, along with adhesion and activation of cluster of differentiation molecule 11b (CD11b), after exposure to high shear stress in monocytes (see Fig. 4) (Baratchi *et al.*, 2020), which confirms that

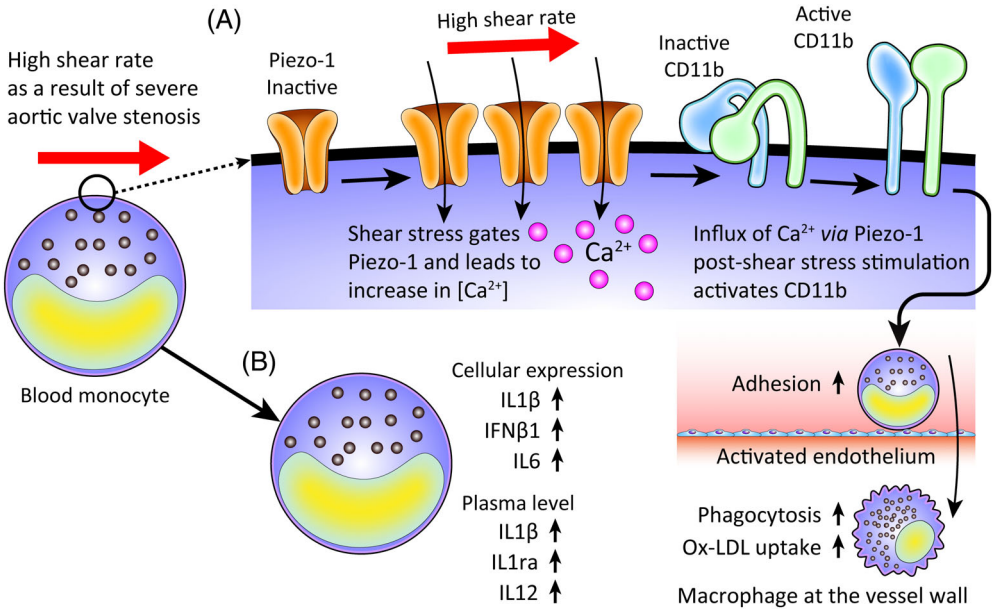

Fig. 4. Piezo1 sensitivity to shear stress contributing to the activation of monocytes in patients with severe aortic valve stenosis. High shear stress resulting from aortic valve stenosis: (A) activates Piezo1 expressed in monocytes and leads to the activation of cluster of differentiation molecule 11b (CD11b), increased monocyte adhesion to endothelial cells, increased phagocytosis and oxidized low-density lipoprotein (Ox-LDL) uptake by differentiated macrophages; and (B) increases the expression of inflammatory cytokines. IFN, interferon; IL, interleukin. Reproduced with permission from Baratchi *et al.* (2020) by AHA Journals.

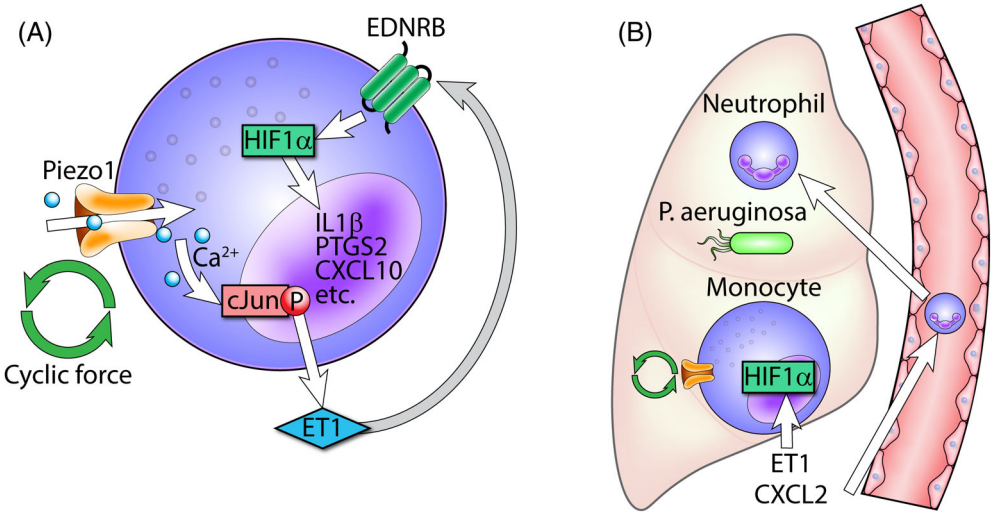

Fig. 5. Piezo1 sensitivity to cyclic stretch in monocytes. (A) Activation of Piezo1 in response to cyclic stretch leads to calcium influx, phosphorylation of transcription factor cJun and expression of endothelin 1 (ET1) which in turn activates hypoxia-inducible factor 1α (HIF1α) *via* the endothelin receptor type B (EDNRB). Activation of HIF1α leads to increased expression of proinflammatory genes, for example genes for interleukin 1β (IL1β), prostaglandin-endoperoxide synthase2 (PTGS2) and C-X-C motif chemokine ligand 10 (CXCL10) in myeloid cells. (B) Mechanotransduction *via* Piezo1 in monocytes that have infiltrated lung tissue leads to the activation of ET1, stabilisation of HIF1α and expression of the neutrophil chemoattractant molecule CXCL2, which induces neutrophilia to clear bacteria. Adapted from Solis *et al.* (2019).

Piezo1 is the key mechanosensor controlling several important functions in monocytes. In addition to circulating monocytes, expression of Piezo1 with the gain-of-function mutation E756del in macrophages has recently been reported to induce iron overload and regulation of phagocytosis, which elegantly links mechanotransduction with iron metabolism (Ma *et al.*, 2021).

In addition to being sensitive to shear stress, monocyte-derived-macrophages are sensitive to cyclical hydrostatic pressure in lung tissue. In a recent study using a mouse model

Table 1. Potential Piezo1 functions in different physiological systems in response to mechanical stimulation

Physiological system	Mechanical stimuli	Experimental models	Physiological functions
Cardiovascular	Shear stress (15 dyne/cm^2)	HUAECs, BAECs, HUVECs	Blood pressure regulation (Wang *et al.*, 2016)
	Shear stress (12 dyne/cm^2) for 24 h	HUVECs	Angiogenesis (Ranade *et al.*, 2014)
	5/25% cyclic stretch, 1 Hz, 24 h	AC16 human cardiomyocytes	Cardiac contractility (Wong *et al.*, 2018)
Pulmonary/ respiratory	Hydrostatic pressure (16 cm H_2O)	*Piezo1*$^{iEC-/-}$ mice	Vascular permeability (Friedrich *et al.*, 2019)
	Alveolar stretch (18% cyclic stretch) for 30 min	*Piezo1*$^{iEC-/-}$ mice, HLMVECs	Lung endothelial barrier stabilisation (Zhong *et al.*, 2020)
Urinary	Patch clamp (−80 mV)	Mouse IMCD-3 cells	Osmoregulation (Martins *et al.*, 2016)
	Uniaxial stretch (stretch distance: 200 μm, stretch speed: 100 μm/s)	Mouse urothelial cells	Bladder volume (Miyamoto *et al.*, 2014)
Blood	Patch clamp (−25 mm Hg) + chemical (Yoda1)	Mouse erythrocytes	Erythrocyte morphology/dehydration (Cahalan *et al.*, 2015)
Immune	45–60 mm Hg cyclic hydrostatic pressure, 2 cycles/s, 6 h	Bone marrow- derived macrophages	Inflammation *via* HIF1α (Solis *et al.*, 2019)
	45–60 mm Hg cyclic hydrostatic pressure, 2 cycles/s, 6 h	Alveolar monocytes	Neutrophil recruitment (Solis *et al.*, 2019)

BAEC, bovine aortic endothelial cells; HIF1α, hypoxia-inducible factor 1-alpha; HLMVEC, human lung microvascular endothelial cells; HUAEC, human umbilical artery endothelial cells; HUVECs, human umbilical vein endothelial cells; IMCD-3 cells, mouse inner medullary collecting duct-3 cells.
1 mm Hg = 133.322 Pascal.

lacking Piezo1 in myeloid cells, mechanotransduction *via* Piezo1 was shown to be important for immunity to bacterial infection (Solis *et al.*, 2019). Bone-marrow-derived macrophages highly express Piezo1 and their exposure to cyclical hydrostatic pressure results in calcium influx-mediated accumulation and stabilisation of hypoxia-inducible factor 1α (HIF1α), a known biomarker and regulator of hypoxia (Solis *et al.*, 2019). The pathway that activates this response involves activation of the c-Jun N-terminal kinases (JNK) and phosphorylation of its target c-Jun, a component of transcription factor activator protein 1 (AP-1), which in turn promotes transcription of endothelin-1 (see Fig. 5) (Solis *et al.*, 2019). Furthermore, mice lacking Piezo1 in their myeloid cells exhibit increased susceptibility to intranasal infection by *Pseudomonas aeruginosa*, as exposure to cyclical hydrostatic pressure activates Piezo1 expressed on monocytes, resulting in endothelin-1-mediated stabilisation of HIF1α to induce increased secretion of C-X-C motif chemokine ligand 2 (CXCL2) from monocytes. This is a key immune response, as CXCL2 recruits neutrophils to sites of infection, contributing to the major innate immune response to the pathogen (see Fig. 5) (Solis *et al.*, 2019).

VI. CONCLUSIONS

(1) Mechanoreceptors such as Piezo1 are responsive to intrinsic and extrinsic mechanical forces that influence the activation of different downstream signalling pathways in a cell/tissue- and stress-dependent manner. In this review, we have summarised the downstream mechanisms activated by Piezo1 in different tissues. As outlined in Table 1, mechanotransduction by Piezo1 is implicated in vascular development and maintenance of blood pressure, lung function, osmoregulation, and immune responses.

(2) The responses of Piezo1 to forces present under normal and under pathological conditions differ and may contribute to pathogenesis. For example, flow types such as laminar flow and disturbed flow activate different pathways downstream of Piezo-1 to induce atheroprotective and atherogenic responses, respectively.

(3) Common signalling mechanisms observed downstream of Piezo1 activation in most cell types include ATP release (Wei *et al.*, 2019) and calpain activation (Li *et al.*, 2015), which then trigger downstream signalling pathways to induce different cellular processes such as gene transcription, proliferation, adhesion and morphological alterations for migration.

(4) The signalling mechanisms activated by Piezo1 are diverse, with similarities and differences across organ systems including those not yet known or investigated. Examining Piezo1 signalling pathways will provide insights into the many functions of Piezo1 and identify potential targets for novel therapeutic approaches.

(5) Despite the progress made in this field so far, many questions still remain. For example, what is the functional interplay between Piezo1 and other mechanosensitive channels in different tissues? Are the

molecular mechanisms that drive stretch activation and shear stress-induced activation of Piezo1 the same or do they have structural differences? Does post-translational modification regulate Piezo1 mechanosensitivity? What is the sub-cellular localisation of Piezo1 in adherent cell types and how do Piezos interact with other force-sensing systems like integrin-mediated focal adhesion and cadherin-based cell–cell junctions? How is the localisation and expression of Piezo1 regulated in response to acute or chronic changes in mechanical force?

(6) Targeting Piezo1 holds great promise as a clinical intervention for a wide range of pathologies. Based on the importance of Piezo1 in health and disease and our progress in better understanding of the biology of Piezo1, it is now possible to develop strategies for Piezo1 drug discovery. One of the important challenges that need to be addressed is the development of potent and selective agonists and antagonists for Piezo1. In addition, the development of animal and cellular models and the ever-increasing number of *PIEZO1* variants being identified through whole-exome and genome sequencing will help to address the long-term goal of understanding the role of Piezo1 in pathologies and developing pharmacotherapies for these Piezo1-related disorders.

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(*Received 8 April 2021; revised 21 October 2021; accepted 4 November 2021; published online 15 November 2021*)