Review Article

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Mechanosensing Piezo channels in tissue homeostasis including their role in lungs

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Abstract

Piezo channels are deemed to constitute one of the most important family of mechanosensing ion channels since their discovery in 2010. With recent advances in identifying their topological structure and the discovery of the agonist Yoda1 as well as the specific inhibitor GsMTx4, it is now possible to study the mechanisms by which Piezo channels are involved in physiological and pathophysiological processes. During embryonic cardiovascular development, Piezo1 senses shear stress and promotes vasculature growth. In adult mice, Piezo1 mediates the release of nitric oxide and ATP from endothelial cells to regulate blood pressure. Piezo channels also play a crucial role in cell differentiation and tissue homeostasis by exquisite mechanical force sensing.

Piezo channels are also abundantly expressed in lung tissues. As the lung is exposed to complex pulmonary hemodynamics and respiratory mechanics, cells in the lung, such as microvascular endothelial cells, bear mechanical forces from blood flow shear, pulsatile strain, static pressure, and cyclic stretch due to respiratory movement. These mechanical stimuli are involved in a serial of physiological function and pathophysiological processes of the lung, many of which Piezo channels may be the key player. Mutation of genes encoding Piezo channels are also associated with hereditary human diseases, thus highlighting the critical role of Piezo channels in both tissue homeostasis and disease.

Keywords

calcium signaling, endothelial cells, inflammation, stretch reflex, tension sensing

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Role of Piezo channels in mechanotransduction

Mechanotransduction is defined as a process by which cells convert a mechanical stimulus into electrical or chemical signals which allow the cells to adapt to mechanical changes in the *milieu*. Mechanosensitive channels are transmembrane proteins that respond to mechanical stress over a wide dynamic range of external mechanical stimuli. Coste et al., in 2010,[1] discovered a novel ion channel family—the Piezo family with its members Piezo1 (Fam38a) and Piezo2 (Fam38b), which are 2500 and 2700 amino acids, respectively. These proteins have a surprisingly large number of 18 transmembrane domains as recently demonstrated by CryoEM[2] and biochemical mapping of the intracellular and extracellular domains.[3] Piezo and its homologues are found in all multicellular organisms except fungi and brown algae. Recent evidence suggests that Piezo may have been necessary for evolution of multicellularity and cell–cell communication by allowing for the monitoring of mechanical stimuli and sensing of neighboring cells.[4] Another general means by which Piezo1 may control cell size during evolution may have been through orchestrating mechanical signal transduction with the Hippo pathway through activating YAP/TAZ transcription factors.[5]

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Structure of Piezo channels

Piezo1 forms a three-blade, propeller-shaped architecture (Fig. 1). The transmembrane region contains three extended and twisted arrays of transmembrane helices. In addition to the transmembrane helical array, three thick distal blades are placed superficially with a helicoidal surface, respectively. A single central cap is located above the transmembrane core surface. Piezo1 is activated by changes of membrane curvature, both convex and concave.[2] The blade region of Piezo1 is highly flexible and could serve as a force sensor which then responds to mechanical stimuli by regulating the gating of cations.

Activation and inhibition of Piezo channels using drugs

After screening nearly 3,200,000 substrates, the small molecule Yoda1 was identified as a specific activator of Piezo1.[6] In vitro assays showed that Yoda1 functioned by slowing the inactivation of Piezo1 and thus shifting the activation curve leftward. Yoda1 can also gate Piezo1 channels at resting levels of tension. Piezo channels can be inhibited non-specifically by Ruthenium Red and Gd^{3+}.[1] A peptide GsMTx4 isolated from tarantula venom also inhibits endogenous stretch channels, including Piezo.[7] Unlike Ruthemium Red, GsMTx4 is a specific inhibitor of Piezo1 and inhibited mechanically activated Piezo1 currents in patch clamp studies.[8]

Piezo channels in vasculature development

Before mid-embryogenesis in mammalian development, the onset of vascular shear stress due to the emergence of cardiac contraction is sensed by Piezo1 in the vasculature.[9] Global and endothelial-specific deletion of Piezo1 leads to disrupted embryonic vasculature development and noticeable whole embryo growth lag as well as lethality. Even though endothelial cells (ECs) differentiate, physiological vessel formation and remodeling of vessels is compromised in the absence of Piezo1. These data suggest that endothelial Piezo1 plays a crucial role in sensing shear stress. Piezo1 is required for Ca^{2+} influx and generation of non-selective cation current in response to shear stress. After Piezo1 activation, extracellular Ca^{2+} enters into the cells, activates the protease calpain-2, and remodels the extracellular matrix, thus facilitating polarity of ECs and other adaptive responses of cells in response to shear stress[9] (Fig. 2).

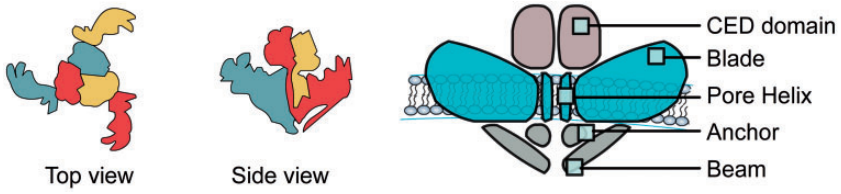

Fig. 1. The schematic structure (PDB 3JAC) of Piezo1 (left) shows possible structural domains (right) that may play a role in mechanosensing and channel activation. Reproduced with permission from Elsevier.[12]

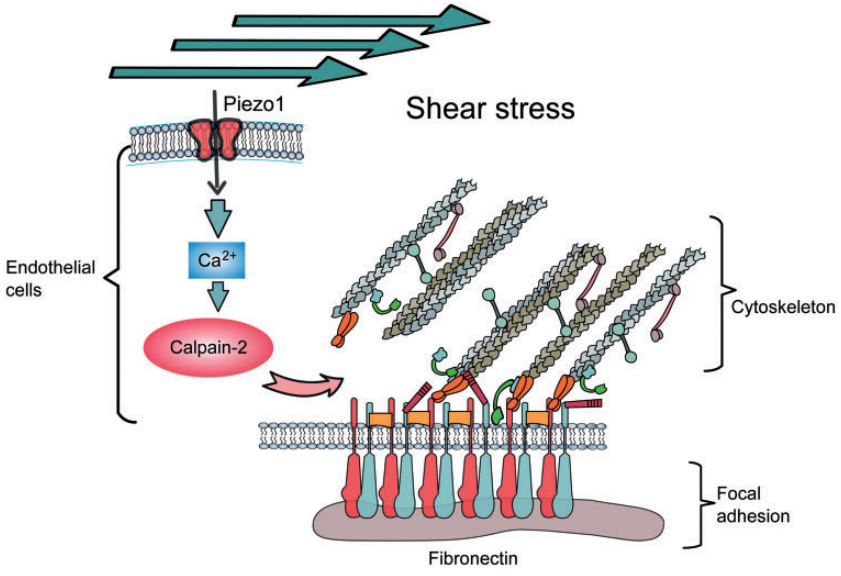

Fig. 2. In response to shear stress, Piezo1 gates Ca^{2+} influx and activates Calpain-2, which proteolyzes cytoskeletal and focal adhesion proteins, resulting in the alignment of endothelial cells along shear direction.

Role of Piezo1 in blood pressure regulation

Piezo1 is also required for blood pressure control. EC-specific deletion of Piezo1 in mice decreases nitric oxide and ATP generation in response to either shear stress or the Piezo1 agonist Yoda1.[10] These mice showed persistent hypertension throughout the observation period of 2–3 weeks. Vascular smooth muscle-specific deletion of Piezo1, on the other hand, disrupted arterial remodeling in response to hypertensive stimuli,[11] indicating a potential pathological role for Piezo1 in hypertensive vascular remodeling.

Piezo1 in cellular homeostasis

Cells in many tissues are continuously exposed to traction forces which are sensed by Piezo channels. Pathak et al. analyzed the differentiation of neural stem cells (hNSPC) on different substrates.[5] Both Piezo1 inhibition by GsMTx4 and Piezo1 siRNA knockdown increased astrocyte differentiation as compared to neuronal differentiation, thus suggesting a role for Piezo1 in the lineage choice of differentiating neural stem cells. Furthermore, Piezo1 is critical for sensing substrate stiffness in neural cells and retinal ganglion cells.[13,14] Eisenhoffer et al. found that overcrowding of epithelial cells could generate extrusion of cells to maintain homeostasis. Knockdown of Piezo1 expression or channel blockade using Gd^{3+} was shown to repress cell extrusion.[15] However, low density of epithelial cells induces cell division through activation of Piezo1 by mechanical stretch.[16] Thus, Piezo1-dependent Ca^{2+} influx appears to activate two opposing processes dependent on where and how Piezo1 is activated. In regions with sparse epithelial cells, Piezo1 accumulates in the plasma membrane to activate epithelial cell division, whereas Piezo1 localizes in cytoplasm in cell dense regions allowing cell extrusion to maintain cell numbers at a stable homeostatic level.

Diseases associated with mutations of Piezo1

Loss-of-function mutations of the Piezo1 gene in humans are linked to non-immune hydrops fetalis and lymphedema[17] and congenital lymphatic dysplasia.[18] A gain-of-function mutation of Piezo1 occurs in autosomal dominant hereditary xerocytosis (HX) (also termed as dehydrated stomatocytosis), which is a hereditary hemolytic anemia of normocytic or macrocytic type.[19–21] Hyperactivation of Piezo1 is assumed to elevate intracellular $[Ca^{2+}]_I$ in HX erythrocytes, resulting in upregulation of the intermediate conductance of Ca^{2+}-activated K^+ channel KCNN4, which in collaboration with the erythrocyte Cl^- channel gives rise to cell shrinkage and dehydration.

Role of Piezo2 in mechanical force sensing

Humans sense their mechanical environment partly through touch sensors in the skin. Merkel cells, which are abundant in fingertips and other specialized regions of the skin, make up touch domes responsible for touch sensation. Studies showed that Merkel cells depend on Piezo2 to sense mechanical forces[22–24] (Fig. 3). In the gut epithelium, enterochromaffin cells function in a manner similar to Merkel cells by sensing acute intraluminal mechanical force that distorts the epithelium and thereby regulates mucosal secretion in response to pressure changes in the gut to maintain normal gastrointestinal function.[25] Piezo2 is expressed in enterochromaffin cells and is assumed to be the primary mechanical sensor that adjusts gut mechanical sensitivity, 5-HT release, and downstream physiological processes.[25]

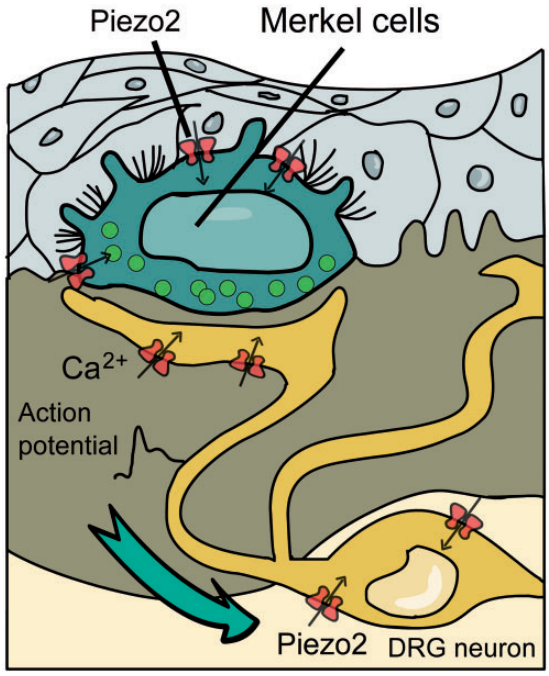

Fig. 3. Piezo2 contributes to the constitution of the Merkel cell-neurite complex in DRG neurons to mediate gentle touch sensation and mechanotransduction. Reproduced with permission from Elsevier.[12]

Diseases associated with Piezo2 mutations

Global knockout of mouse Piezo2 leads to lethality in the perinatal period,[26] whereas a bi-allelic loss-of-function mutation of Piezo2 is associated with congenital and progressive arthrogryposis syndrome manifested with variable clinical phenotype, which is not lethal. Piezo2 mutation-associated arthrogryposis syndrome is manifested as distal sensory neuropathy characterized by defective proprioception,[27] muscular atrophy, and scoliosis,[28] without evidence of central neural system abnormality. Bi-allelic gain-of-function results in a group of syndromes related to distal arthrogryposis; these syndromes include Gordon syndrome (distal arthrogryposis type 3), distal arthrogryposis type 5, and Marden–Walker syndrome.[29,30]

Role of Piezos in cartilage biosynthesis

Synergy between Piezo1 and Piezo2 contributes to normal function of mouse articular cartilage.[31] Both Piezo1 and Piezo2 mRNA are abundantly expressed in primary articular chondrocytes of mammals, which are responsible for cartilage biosynthesis and remodeling. Chemical inhibition or knockdown of either Piezo1 or Piezo2 suppressed mechanically induced calcium influx in chondrocytes, whereas cell death due to high strain mechanical injury was reduced by GsMTx4.

Role of Piezo channels in lung physiology and pathophysiology

Piezo2 plays a critical role in the regulation of the Hering-Breuer reflex which prevents over-inflation of the lung.[26] The reflex is activated by pulmonary stretch receptors in the smooth muscle of airways that respond to excessive stretching of the lung during large inspirations. Piezo2 deletion in vagal, spinal sensory, and dorsal root ganglion neurons led to reduced vagal nerve activity in response to lung inflation, increased tidal volume, prolongation of expiratory airflow, and blunted Hering–Breuer reflex (Fig. 4), thus establishing the critical role of Piezo2 in the Hering–Breuer reflex. While global knockout of Piezo2 did not impair embryonic lung development, respiratory distress was seen in newborn mice shortly after birth leading to death. Selective deletion of Piezo2 in neurons of the jugular and thoracic dorsal root ganglia replicated the symptoms seen in global knockout newborn mice. Selective knockout Piezo2 in the nodose ganglion, on the other hand, allowed mice survive to adulthood, but these mice also showed an impaired Hering–Breuer reflex with increased tidal volume.

Both the lung epithelium and lung endothelium are exposed to repeated mechanical stretch due to the cyclic inflation of alveoli.[32] Spontaneous breathing increases alveolar surface area by 25% or ~5% increase in circumferential stretch (CS) of epithelium and endothelium. Increasing lung volume increases from 40% to 100% can result in an increase of the alveolar surface area of 35%, corresponding to as high as an ~18% increase in CS. Although the precise role of Piezos in mediating lung inflation responses has not yet been examined, they may be crucial for the adaptation to stretch of both the lung epithelium and endothelium (Fig. 5). As Piezo1 regulates kidney epithelial cell homeostasis,[16] it is also likely an important mechanosensor for lung epithelial cell integrity through its promotion of proliferation and repair after alveolar injury. Piezo1 deficiency resulted in impaired epithelial cell adhesion and increased cell migration,[33] which may be a factor contributing to tissue repair in ARDS. Although this question has not been examined, alveolar epithelial-expressed Piezo1 and Piezo2 may regulate the response to stretch-induced or ventilation-induced lung injury.

Role of Piezo channels in pulmonary circulation and heart

Thus far, there is little known about the role of Piezo channels in mechanotransduction in the pulmonary circulation. However, Piezo1 mRNA is expressed in right heart[1,34] and pulmonary ECs (Zhong et al, unpublished data). Piezo1 shares some similar electrophysiological characters with non-selective stretch-activated ion channels of the heart, including sensitivity to GsMTx4. In a murine heart ischemia-reperfusion model, GsMTx4 pretreatment was protective as manifested by decreased infarction area and improved cardiac dynamics.[35] In EC-specific Piezo1 knockout adult mice, the heart and aorta appeared normal as assessed by histological staining and echocardiography.[36] Besides the mediating of vascular relaxation,[10] endothelial Piezo1 is also crucial for mesenteric vasoconstriction and blood pressure elevation during physical activity, which may re-direct blood from gastrointestinal system to muscle tissue to facilitate physical performance. The authors concluded that endothelial Piezo1 regulates anti-EDH (endothelium-derived hyperpolarization) through flow-sensing.[36] It is unknown whether Piezo expressed in lung ECs also functions in redirecting blow to better ventilated regions. These publications suggest a role for Piezos in physiological and

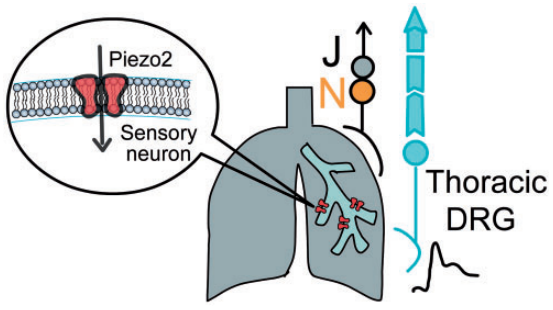

Fig. 4. Piezo2 in airway vagal sensory neuron senses lung inflation and activates afferent impulses through the jugular-nodose (J-N) ganglia complex and thoracic dorsal root ganglia (DRG) to form the Hering–Breuer reflex. Adapted by permisison from Elsevier.[26]

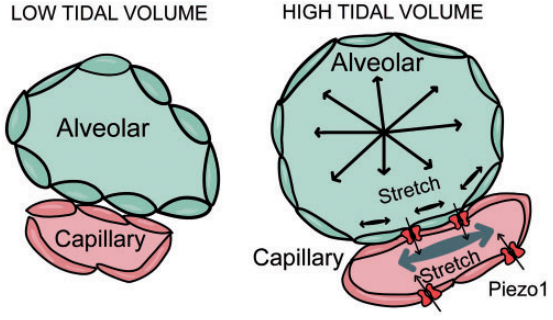

Fig. 5. Both alveolar and pulmonary capillary are exposed to mechanical stretch due to cyclic inflation of alveolar. The higher the tidal volume, the higher the level of stretch borne by epithelium and endothelium, which could be sensed and mediated by Piezo1.[37]

pathological phenotypes, such as pulmonary arterial hypertension and pulmonary vascular and right heart remodeling but the evidence is inclusive.

Pulmonary circulation is a low-pressure vascular bed in which pulmonary ECs are exposed to mechanical force during lung inflation. A fundamental question, therefore, is how Piezo1 expressed in ECs responds to stretch of ECs and the role of Piezo1 in regulating endothelial barrier function. This question remains unaddressed. Another related question relates to the role of Piezos in transmigration of leukocytes and cancer cells; specifically, whether activation of EC expressed Piezo1 contributes to the migration of cells across the endothelial barrier. Studies using breast cancer cell lines showed a relationship between malignancy and Piezo1 expression.[38]

Conclusion

Since the discovery of the Piezo channel family,[1] our understanding of the mechanisms of mechanotransduction has advanced, yet many fundamental questions remain regarding the regulatory role of Piezo channels in the lung. Since alveolar epithelial and endothelial cells in the lung are subjected to mechanical forces during each breath, Piezos likely play a fundamental role in regulating multiple aspects of lung biology from controlling the barrier properties of alveolar epithelium and endothelium to controlling lung inflation itself via the Hering–Breuer reflex. It is safe to say that many questions about Piezos are outstanding and that there are far more unknowns than knowns as of now, but that studying Piezo channels in the lung epithelium and endothelium will yield significant insights into lung physiology and pathophysiology.

Conflict of interest

The author(s) declare that there is no conflict of interest.

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