Mechanosensing Piezo channels in tissue homeostasis including their role in lungs

Ming Zhong, Yulia Komarova, Jalees Rehman and Asrar B. Malik
Department of Pharmacology and Center of Lung and Vascular Biology, University of Illinois, College of Medicine, Chicago, IL, USA

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

Date received: 13 November 2017; accepted: 7 March 2018

Pulmonary Circulation 2018; 8(2) 1–6
DOI: 10.1177/2045894018767393

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 CryoEM2 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

Corresponding author:
Asrar B. Malik, University of Illinois, College of Medicine, 835 S Wolcott Avenue, E403, MC 868, Chicago, IL 60612, USA.
Email: abmalik@uic.edu
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. 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. 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 nonspecifically by Ruthenium Red and Gd$^{3+}$. A peptide GsMTx4 isolated from tarantula venom also inhibits endogenous stretch channels, including Piezo. Unlike Ruthenium Red, GsMTx4 is a specific inhibitor of Piezo1 and inhibited mechanically activated Piezo1 currents in patch clamp studies.

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. 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 (Fig. 2).
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 Gd³⁺ 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 Ca²⁺ 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 lymphedema17 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 [Ca²⁺]i in HX erythrocytes, resulting in upregulation of the intermediate conductance of Ca²⁺-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 forces22–24 (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

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. 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. 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. 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, 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, 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 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 ischaemia-reperfusion model, GsMTx4 pretreatment was protective as manifested by decreased infarction area and improved cardiac dynamics. In EC-specific Piezo1 knockout adult mice, the heart and aorta appeared normal as assessed by histological staining and echocardiography. Besides the mediating of vascular relaxation, 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. 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...
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.

Conclusion
Since the discovery of the Piezo channel family, 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.

Funding
This work has been funded in part by R01HL045638 to ABM and YK.

References
1. Coste B, Mathur J, Schmidt M, et al. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science 2010; 330(6000): 55–60.
2. Ge J, Li W, Zhao Q, et al. Architecture of the mammalian mechanosensitive Piezo1 channel. Nature 2015; 527(7576): 64–69.
3. Coste B, Murthy SE, Mathur J, et al. Piezo1 ion channel pore properties are dictated by C-terminal region. Nat Commun 2015; 6: 7223.
4. Hung WC, Yang JR, Yankaskas CL, et al. Confinement sensing and signal optimization via Piezo1/PKA and Myosin II pathways. Cell Rep 2016; 15(7): 1430–1441.
5. Pathak MM, Nourse JL, Tran T, et al. Stretch-activated ion channel Piezo1 directs lineage choice in human neural stem cells. Proc Natl Acad Sci U S A 2014; 111(45): 16148–16153.
6. Syeda R, Xu J, Dubin AE, et al. Chemical activation of the mechanotransduction channel Piezo1. Elife 2015; 4: e07369.
7. Suchyna TM, Johnson JH, Hamer K, et al. Identification of a peptide toxin from Grammostola spatulata spider venom that blocks cation-selective stretch-activated channels. J Gen Physiol 2000; 115(5): 583–598.
8. Bae C, Sachs F and Gottlieb PA. The mechanosensitive ion channel Piezo1 is inhibited by the peptide GsMTx4. Biochemistry 2011; 50(29): 6295–6300.
9. Li J, Hou B, Tumova S, et al. Piezo1 integration of vascular architecture with physiological force. Nature 2014; 515(7526): 279–282.
10. Wang S, Chennupati R, Kaur H, et al. Endothelial cation channel PIEZO1 controls blood pressure by mediating flow-induced ATP release. J Clin Invest 2016; 126(12): 4527–4536.
11. Retailleau K, Duprat F, Arhatte M, et al. Piezo1 in smooth muscle cells is involved in hypertension-dependent arterial remodeling. Cell Rep 2015; 13(6): 1161–1171.
12. Wu J, Lewis AH and Grandl J. Touch, Tension, and Transduction: The Function and Regulation of Piezo Ion Channels. Trends Biochem Sci 2017; 42(1): 57–71.
13. Blumenthal NR, Hermanson O, Heimrich B, et al. Stochastic nanoroughness modulates neuron-astrocyte interactions and function via mechanosensing cation channels. Proc Natl Acad Sci U S A 2014; 111(45): 16124–16129.
14. Koser DE, Thompson AJ, Foster SK, et al. Mechanosensing is critical for axon growth in the developing brain. Nat Neurosci 2016; 19(12): 1592–1598.
15. Eisenhoffer GT, Loftus PD, Yoshigi M, et al. Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. Nature 2012; 484(7395): 546–549.
16. Gadipaty SA, Lindblom J, Loftus PD, et al. Mechanical stretch triggers rapid epithelial cell division through Piezo1. Nature 2017; 543(7643): 118–121.
17. Fotiou E, Martín-Almedina S, Simpson MA, et al. Novel mutations in PIEZO1 cause an autosomal recessive generalized lymphatic dysplasia with non-immune hydrops fetalis. Nat Commun 2015; 6: 8085.
18. Lukacs V, Mathur J, Mao R, et al. Impaired PIEZO1 function in patients with a novel autosomal recessive congenital lymphatic dysplasia. Nat Commun 2015; 6: 8329.
19. Albuissson J, Murthy SE, Bandell M, et al. Dehydrated hereditary stomatocytosis linked to gain-of-function mutations in mechanically activated PIEZO1 ion channels. Nat Commun 2013; 4: 1884.
20. Andolfi I, Alper SL, De Franceschi L, et al. Multiple clinical forms of dehydrated hereditary stomatocytosis arise from mutations in PIEZO1. Blood 2013; 121(19): 3925–3935, S1–12.
21. Bae C, Gnanasambandam R, Nicolai C, et al. Xerocytosis is caused by mutations that alter the kinetics of the mechanosensitive PIEZO1 channel. Proc Natl Acad Sci U S A 2013; 110(12): E1162–1168.
22. Ikeda R and Gu JG. Piezo2 channel conductance and localiziation domains in Merkel cells of rat whisker hair follicles. Neurosci Lett 2014; 583: 210–215.
23. Woo SH, Ranade S, Weyer AD, et al. Piezo2 is required for Merkel-cell mechanotransduction. Nature 2014; 509(7520): 622–626.
24. Maksimovic S, Nakatani M, Baba Y, et al. Epidermal Merkel cells are mechanosensory cells that tune mammalian touch receptors. Nature 2014; 509(7520): 617–621.
25. Wang F, Knutson K, Alcaino C, et al. Mechanosensitive ion channel Piezo2 is important for enterochromaffin cell response to mechanical forces. *J Physiol* 2017; 595(1): 79–91.

26. Nonomura K, Woo S-H, Chang RB, et al. Piezo2 senses airway stretch and mediates lung inflation-induced apnoea. *Nature* 2016; 541(7636): 176–181.

27. Mahmud AA, Nahid NA, Nassif C, et al. Loss of the proprioception and touch sensation channel PIEZO2 in siblings with a progressive form of contractures. *Clin Genet* 2017; 91(3): 470–475.

28. Chesler AT, Szcot M, Bharucha-Goebel D, et al. The role of PIEZO2 in human mechanosensation. *N Engl J Med* 2016; 375(14): 1355–1364.

29. Coste B, Houge G, Murray MF, et al. Gain-of-function mutations in the mechanically activated ion channel PIEZO2 cause a subtype of distal arthrogryposis. *Proc Natl Acad Sci U S A* 2013; 110(12): 4667–4672.

30. McMillin MJ, Beck AE, Chong JX, et al. Mutations in PIEZO2 cause Gordon syndrome, Marden-Walker syndrome, and distal arthrogryposis type 5. *Am J Hum Genet* 2014; 94(5): 734–744.

31. Lee W, Leddy HA, Chen Y, et al. Synergy between Piezo1 and Piezo2 channels confers high-strain mechanosensitivity to articular cartilage. *Proc Natl Acad Sci U S A* 2014; 111(47): E5114–5122.

32. Birukov KG. Cyclic stretch, reactive oxygen species, and vascular remodeling. *Antioxid Redox Signal* 2009; 11(7): 1651–1667.

33. McHugh BJ, Murdoch A, Haslett C, et al. Loss of the integrin-activating transmembrane protein Fam38A (Piezo1) promotes a switch to a reduced integrin-dependent mode of cell migration. *PLoS One* 2012; 7(7): e40346.

34. Gottlieb PA, Bae C and Sachs F. Gating the mechanical channel Piezo1: a comparison between whole-cell and patch recording. *Channels (Austin)* 2012; 6(4): 282–289.

35. Wang J, Ma Y, Sachs F, et al. GsMTx4-D is a cardioprotectant against myocardial infarction during ischemia and reperfusion. *J Mol Cell Cardiol* 2016; 98: 83–94.

36. Rode B, Shi J, Endesh N, et al. Piezo1 channels sense whole body physical activity to reset cardiovascular homeostasis and enhance performance. *Nat Commun* 2017; 8(1): 350.

37. Wang T, Gross C, Desai AA, et al. Endothelial cell signaling and ventilator-induced lung injury: molecular mechanisms, genomic analyses, and therapeutic targets. *The American Physiological Society* 2017; 312(4): L452–L476.

38. Li C, Rezania S, Kammerer S, et al. Piezo1 forms mechanosensitive ion channels in the human MCF-7 breast cancer cell line. *Sci Rep* 2015; 5: 8364.