Piezo channels in the urinary system

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INTRODUCTION
Mechanosensitive ion channels (MSCs) convert mechanical signals into electrochemical signals, which are essential for almost all mammalian cells¹. The urinary system is important in the regulation of water and salt metabolism, the maintenance of acid-base balance, and hormone production²–⁴. As key mechanotransducers, MSCs are widely present in the urinary system in response to mechanical stimuli, including shear stress, bladder wall stretching, and urine flow⁵–⁷, resulting in a wide range of biological effects. Given the crucial physiological and pathological roles of MSCs in the urinary system, revealing their potential mechanotransduction mechanisms and characteristics as new targets for disease management is imperative.

Since the discovery of MSCs by Corey and Hudspeth in their analysis of the ionic basis of the receptor potential in a vertebrate hair cell in 1979, which led to the first recording of electrical currents activated by mechanical force, studies exploring these types of ion channels have emerged¹¹,¹². However, the identification criteria of MSCs have long been controversial. In 2007, Christensen AP et al. summarized the existing research and proposed a series of criteria for identifying candidate channels as MSCs, which facilitated the development of this field¹³. In 2010, researchers in the Ardem Patapoutian laboratory made major progress in the investigation of MSCs¹⁴. In particular, a glass microprobe was used to apply force to the surface of the candidate cells, and the current model of the cells was recorded via patch-clamp¹⁴. It was found that the Neuro2A (N2A) mouse neuroblastoma cell line expressed stable and sustained mechanically activated (MA) currents¹⁴. Then, we tested candidate genes in N2A cells with siRNA knockdown, discovered the existence of Piezo, and confirmed that there are two members of the Piezo family, Piezo1 and Piezo2¹⁴. These molecules are a type of evolutionarily conserved transmembrane (TM) protein with little sequence homology to other known ion channel families¹⁴. For over a decade, since the discovery of Piezo channels, a substantial number of related studies have been published that have extensively elucidated the physiopathology related to these molecules. Moreover, Piezo channels are expected to become a new target for drug discovery. Therefore, the Nobel Prize in Physiology or Medicine 2021 was awarded to Ardem Patapoutian in recognition of his outstanding contribution to the field of MSCs.

In this review, we summarize the data on Piezo channels, including their structure, mechanogating mechanisms, pharmacological properties, and physiological functions in human body systems. By emphasizing the known physiological and pathophysiological roles of Piezo channels in the urinary system, we highlight the potential implications of targeting this cation channel family for therapy to expand the horizons for the treatment of urinary system diseases.

OVERVIEW OF PIEZO CHANNELS
Structure of Piezo channels
Piezo1. Analyzing structure is the key to understanding functionality. Due to advancements in cryoelectron microscopy (cryo-EM) and X-ray crystallography, the high-resolution three-dimensional structure of the Piezo1 channel has been clearly presented¹⁵–¹⁷. With the trimeric three-blade, propeller-like medium-resolution cryo-EM structure of the mouse Piezo1 channel¹⁸, many pieces of information about TM regions were illuminated¹⁹–²⁰ (Fig. 1), promoting understanding of the structure-function relationship. The mouse Piezo1 channel consists of three modules containing 2547 residues¹⁸–²⁰. The central ion-conducting pore module is responsible for ion permeability and selectivity and includes the outer helices (OHs), inner helices (IHs), intracellular C-terminal domains (CTDs), and extracellular C-terminal domains (CEDs)¹⁸–²⁰.

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The mechanosensing module includes peripheral propeller blades. The mechanotransduction module consists of 90 Å long intracellular beams, anchors, and CTDs. A single blade contains nine repeating transmembrane helical units (THUs) comprising four TM helices each. Together with OH and IH, these structures form a highly curved, nonplanar arrangement 38-TM topology. The joint linking the three groups of THUs and the adjacent three groups of THUs is a physical bend, which is approximately 100 degrees from the extracellular view. Importantly, the conformation of this unusually curved blade structure might change with the tension and curvature of the membrane. Between the intracellular surface of THU7-THU9 and CTD, the beam structure connecting TM28 and the central ion-conducting pore was identified. It is possible that this structure utilizes two leucine residues, L1342/L1345, as a pivot to transfer the fine mechanical force felt by the blade to the central ion-conducting pore module through a lever-like mechanism and thereby selectively exposing the cations. Three sets of such topologies are assembled into Piezo1, which possesses a total of 114 TM helices, making it one of the most complex mechanosensors. Each blade twists in a clockwise direction to form a propeller-like structure from the extracellular view. Under the stimulation of mechanical force, the clockwise movement of the cap might open the TM gate. Through the leverage of the beam, the lateral plug gate might be partially unplugged, and the lateral portal might be opened, thus allowing cations to flow in.

Piezo2. Structurally, Piezo2 has approximately 42% sequence homology to Piezo1 in the cryo-EM three-dimensional structure. Piezo1/2 is a homotrimer assembled from three blades with a total of 114 TM helices. This finding not only indicates that Piezo channels are the membrane proteins with the largest number of transmembrane passes discovered thus far but also suggests that they might mediate mechanotransduction functions.

Fig. 1 The 38-TM topology model, and mechanogating mechanisms from the extracellular view of the mouse Piezo1 channel (adapted from 15, 23). a A 38-TM topology structure of Piezo1. The dark red THU1—THU3 indicates unresolved areas. The linker region is the key area in which SERCA2 suppresses the MA current. The yellow dashed box is the extracellular loops EL15-16 and EL19-20, which are the key mechanotransduction sites for the hydrophilic small molecule agonist Jedi. The beam might utilize two leucine residues, L1342/L1345, as the pivot to transfer the fine mechanical force felt by the blade to the central ion-conducting pore module through a lever-like mechanism and thereby selectively exposing the cations. Three sets of such topologies are assembled into Piezo1, which possesses a total of 114 TM helices, making it one of the most complex mechanosensors. b Each blade twists in a clockwise direction to form a propeller-like structure from the extracellular view. Under the stimulation of mechanical force, the clockwise movement of the cap might open the TM gate. Through the leverage of the beam, the lateral plug gate might be partially unplugged, and the lateral portal might be opened, thus allowing cations to flow in.
by similar mechanogating mechanisms. The completely resolved Piezo2 structure shows that under the interaction of the hydrophobic belts formed by TM helices, three blades curved into a nanobowl configuration with a midplane opening diameter of 24 nm and a depth of 9 nm, which fully wrapped the extracellular cap (Fig. 2a). Nanobowls are potentially the structural basis for Piezo channels to be highly mechanosensitive. The distal and proximal ends of the beam are connected to the clasp and latch, respectively, by hydrogen bonds, which are the key structures for the lever-like transduction of the beam. The highly conserved IHs in the Piezo family are an integral part of the central pore module. Of interest, in contrast to Piezo1, the two transmembrane constriction sites (L2743, F2754 and E2755) in Piezo2 IHs indicate that the central pore is relatively narrow. Therefore, it is postulated that the two constriction sites might be the upper and lower transmembrane gates, controlled by the upward and clockwise movement of the cap (Fig. 2). In addition, three lateral portals were found in the central pores of Piezo1 and Piezo2. Mutating residues of lateral portals will alter the ion permeability, indicating that they are most likely part of the ion-conducting pathway. In the cytoplasmic region of the central pore module, there is a 10 Å long constriction neck consisting of three amino acid residues (Fig. 2a). This structure has also been identified in Piezo1, yet whether it is part of the ion-conducting pathway is unclear.

Mechanogating mechanisms of Piezo channels

Considering the important physiological and pathological functions of Piezo channels and their potential as new pharmacological targets for the future treatment of diseases, exploring the mechanogating mechanisms of Piezo channels is particularly crucial. A 14-residue intracellular linker sequence between the anchor and OHS is a key region where Piezo1 interacts with sarcoplasmic/endoplasmic-reticulum Ca2+-ATPase 2 (SERCA2). The aforementioned findings suggest that Piezo1 utilizes peripheral blades to gate the central pore module. These discoveries regarding the linker region provide a direction for uncovering the ingenious mechanogating and chemical regulatory mechanisms of Piezo channels.

Lever-like mechanogating mechanism. The proposal of the lever-like mechanogating mechanism of Piezo channels is important for understanding the basic life process of the conversion of mechanical stimulation into electrical signals. The blades composed of THUs, sense stimulation from mechanical forces or biomolecules, and the beam-constituted lever-like device transmits mechanical or chemical signals to the central pore module, further opening the intracellular lateral pathways. THU1-6 cells at the end of the blades are highly curved; consequently, the presence of the lever-like apparatus is also conducive to responding to large-scale changes in membrane curvature.

Cap-motion mechanogating mechanism. The cap structure, a homotrimer formed by CEDs, is located on top of the central pore module, which controls the opening of the extracellular fenestration sites and allows the penetration of cations. Compared with Piezo2, the cap of Piezo1 has obvious clockwise movement. Deleting the α1 and α2 helical structures of the cap in both Piezo1 and Piezo2 completely eliminated the whole-cell currents caused by mechanical stimulation, indicating that cap motion controls the opening of the upper and lower transmembrane gates.

Plug-and-Latch mechanogating mechanism. Geng J et al. confirmed the conjecture that the three intracellular lateral portals of Piezo channels are cation-permeating entrances and identified the “lateral plug” domain that physically blocks the lateral ion-conducting routes and the “latch” structure that controls the lateral plug. Initially, the nine residues of the lateral pathways were subjected to combined site-directed mutation, and the permeability of Cl– over Na+ (pCl/pNa) of the Piezo1 mutants was significantly increased. When all nine residues were mutated to lysine residues, the Piezo1 mutant became a completely anion-selective channel, suggesting that the Piezo1 channel employs three lateral portals to allow permeation of cations. Subsequently, the RNA sequencing data of Piezo1 obtained with the Trinity method showed that it undergoes reassembly, and the spliced variant Piezo1.1 was identified. Intriguingly, Piezo1.1 lacks an exon that encodes the lateral plug structure, suggesting that Piezo1 utilizes the alternative splicing mechanism for the expression of this key exon. Additionally, further studies were carried out on Piezo1.1. Compared with Piezo1, Piezo1.1 has greater single-channel conductance, lower Ca2+ permeability, and greater mechanosensitivity. In brief, the lateral plug structure affects the ion permeability and mechanosensitivity of Piezo1. Therefore, an ingenious plug-and-latch mechanogating mechanism was developed. When the mechanical force acts on the blade, the three lateral plugs are anchored to the intracellular central axis under the leverage of the beam so that the physical obstruction is lifted and the lateral pathways are opened, allowing cations to flow in (Fig. 2).

A dual mechanogating mechanism. A dual mechanogating mechanism that can be used to explain the complex gating system of Piezo channels has been proposed, in which the transmembrane gates are gated by cap motion, and the intracellular routes are controlled by a series of key elements of the blade-beam-plug-latch through a lever-like mechanism and a plug and latch mechanism. Exploring the delicate mechanogating mechanisms of Piezo channels not only allows us to better understand how the Piezo family exerts biological effects but also, more importantly, will help us to use it as a new effective target for related drug development.

Biophysical properties of Piezo channels

Inactivation. Piezo1 responds to various forms of mechanical stimuli, including poking, stretching, shear stress, and changes in local membrane tension. In contrast, Piezo2 is less responsive to changes in stretching and local membrane tension. Despite their differences, both Piezo1 and Piezo2 nonselectively allow the penetration of cations in the following order: Ca2+ > K+ > Na+ > Mg2+. They also generate an MA current. In addition to showing different sensitivities to specific mechanical stimuli, Piezo1 and Piezo2 also have different inactivation characteristics, with significant voltage dependence. Specifically, Piezo2 exhibits faster inactivation kinetics than Piezo1, which slows down with depolarization.

Site-directed mutagenesis of residues L2475 and V2476 in the IH domain that physically blocks the lateral ion-permeating routes and the “latch” structure that controls the lateral plug. Initially, the nine residues of the lateral pathways were subjected to combined site-directed mutation, and the permeability of Cl– over Na+ (pCl/pNa) of the Piezo1 mutants was significantly increased. When all nine residues were mutated to lysine residues, the Piezo1 mutant became a completely anion-selective channel, suggesting that the Piezo1 channel employs three lateral portals to allow permeation of cations. Subsequently, the RNA sequencing data of Piezo1 obtained with the Trinity method showed that it undergoes reassembly, and the spliced variant Piezo1.1 was identified. Intriguingly, Piezo1.1 lacks an exon that encodes the lateral plug structure, suggesting that Piezo1 utilizes the alternative splicing mechanism for the expression of this key exon. Additionally, further studies were carried out on Piezo1.1. Compared with Piezo1, Piezo1.1 has greater single-channel conductance, lower Ca2+ permeability, and greater mechanosensitivity. In brief, the lateral plug structure affects the ion permeability and mechanosensitivity of Piezo1. Therefore, an ingenious plug-and-latch mechanogating mechanism was developed. When the mechanical force acts on the blade, the three lateral plugs are anchored to the intracellular central axis under the leverage of the beam so that the physical obstruction is lifted and the lateral pathways are opened, allowing cations to flow in (Fig. 2).

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inactivation and subsequent calcium ion flux of the Piezo1 channel. Mutations in Piezo2 can cause a subtype of distal arthrogryposis. These changes might cause pathology by accelerating the recovery of Piezo2 channels from inactivation.

Fig. 2 Structure and mechanogating mechanisms of the mouse Piezo2 channel. a Piezo2 channel in the closed state. The clockwise movement of the cap might control the opening of the extracellular fenestration sites and allows the penetration of cations. The cyan nanobowl structure is located in a three-dimensional space formed by three highly curved blades. The yellow structure is the constriction neck, and it is not clear whether it is part of the ion-conducting path. The green dashed line represents the ion-conducting routes where cations enter the central pore from the extracellular fenestration sites and then flow in the intracellular space through three lateral portals. The black arrow indicates that under mechanical stimulation, the blades move toward the plasma membrane and tend to be flat. b The nanobowl also changes as the blades become completely flat. The in-plane membrane area might expand from 450 nm² in the closed state to 700 nm² in the open state, which might be the structural basis for the mechanosensitivity of Piezo channels. At the same time, the mechanical force transmitted by the beam might unplug the lateral plug gates, contributing to opening the intracellular ion conduction routes so that the cations can enter.

Inactivation kinetics, as the pore characteristics of ion channels, show variable performance on Piezos. Therefore, many studies are still needed to explain the specific inactivation mechanism of Piezo channels.
Pharmacological regulation. Various Piezo channel blockers have been investigated recently. The small molecules ruthenium red and gadolinium can nonspecifically block Piezo channels. The peptide toxin grammostola spatulate mechanotoxin 4 (GmTX4) reduces the membrane tension near the Piezo1 channel by inserting into the lipid bilayer, consequently exerting a non-specific inhibitory effect. In addition, polycystin-2 (PC2) and SERCA are a class of proteins that can interact with Piezo1 and weaken the MA current by binding directly. Recently, the potential of the dietary fatty acid margaric acid (MA) to act on the beam structure to increase the mechanical force threshold level of Piezo2 channels has been shown to counteract the Piezo2 hypersensitivity caused by bradykinin. This finding might help alleviate tactile allodynia caused by inflammation. In summary, the lack of Piezo channel-selective blockers is a roadblock in our understanding of their specific roles in physiological processes. Thus, further research on the pharmacology of Piezo channels is needed.

Currently, there are two known Piezo1 agonists, Yoda1 and Jedi1/2. Both agonists have been suggested to lower the mechanical force threshold of Piezo1, prolong the inactivation time and increase its mechanosensitivity. The key site for the interaction between Yoda1 and Piezo1 is at the proximal end of the blade (residues 1961-2063). After binding, Yoda1 promotes membrane tension-induced blade motion through a wedge-like effect. Additionally, Yoda1’s analog Dooku1 and the traditional Chinese medicine extract Tansmoeis A can competitively neutralize the agonistic effect of Yoda1. Notably, both Jedi1/2 and Yoda1 interact with the blade, but the activation of the Piezo1 channel by Jedi1/2 is faster, more reversible and less persistent, suggesting a different activation mechanism from Yoda1. Consistent with the previous conclusions, Piezo1 becomes insensitive to Yoda1 and Jedi by mutating the beam pivot residues L1342/L1345. Accordingly, the beams are indispensable for downstream transduction pathways. Recent studies have shown that fecal ssRNA might function as a natural and intestines. Together, these studies provide new insights for the blade structure to increase the mechanical force threshold level of Piezo2 channels has been shown to counteract the Piezo2 hypersensitivity caused by bradykinin. This finding might help alleviate tactile allodynia caused by inflammation. In summary, the lack of Piezo channel-selective blockers is a roadblock in our understanding of their specific roles in physiological processes. Thus, further research on the pharmacology of Piezo channels is needed.

Expression and distribution of Piezo channels

Piezo1. Piezo1 channels allow mammalian cells to sense mechanical signals and consequently govern diverse physiological responses. Piezo1 senses shear stress and promotes vascular formation during the embryonic period; mouse embryos lacking Piezo1 die in the second trimester. Furthermore, repressing the expression of the Piezo1 gene in neural stem cells suppressed neurogenesis but enhanced astrogenesis. Therefore, it was proposed that the Piezo1 channel determines the mechanosensitive lineage choice in neural stem cells. The Piezo1 channel promotes T-cell activation by optimizing the process of TCRs recognizing MHC, which provides supportive evidence for the involvement of Piezo1 in human immunoregulation. In addition, specifically knocking down Piezo1 in CD4+ T cells enhanced the proliferation of Tregs and consequently alleviated autoimmune encephalomyelitis in mice, which may be beneficial to the treatment of autoimmune diseases. Moreover, other mechanobiological roles of Piezo1 require further investigation in other physiological processes, such as lymphatic development, red blood cell volume regulation, maintenance of intestinal and bone homeostasis, and jumping performance in humans.

Piezo2. The Piezo2 channel is a known indispensable factor in touch, proprioception, and tactile pain, but recent studies have indicated its important role in regulating urination and maintaining skeletal integrity. Humans with Piezo2 mutations showed abnormal voiding behaviors. Loss of Piezo2 expression in proprioceptive neurons led to deformity and dysplasia of the spine and hip joints, suggesting the crucial role of Piezo2 in maintaining skeletal integrity. Knockout of the Piezo2 gene in tumor endothelial cells inhibited tumor growth by suppressing angiogenesis. Additionally, Piezo2 in cochlear outer hair cells is an essential molecule for mediating ultrasonic hearing in mice, but it is not important for low-frequency hearing. It was concluded that ultrasonic hearing and low-frequency hearing might use distinct auditory pathways.

Roles of Piezo channels in the urinary system

The urinary system is an essential human metabolic route and plays an essential role in maintaining homeostasis of the body’s internal environment. Piezo channels, as multipurpose mechanotransducers in mammals, govern physiological processes of the urinary system by sensing mechanical stimuli such as shear stress and bladder wall stretching. Hence, it is indispensable to determine the expression and distribution of Piezo channels in the urinary system. Previous immunoblotting results on the lysates obtained from transgenic reporter mice revealed that Piezo1 was expressed in the following rank order: kidney > ureter > bladder > urethra. Immuno-fluorescence studies showed that Piezo1 was predominantly expressed in glomerular endothelial cells, parietal cells of Bowman’s capsule, distal convoluted tubule endothelial cells, principal cells of collecting ducts and urothelial cells of the renal pelvis in the kidney. Intriguingly, the conclusion of this study indicated that Piezo1 is almost undistributed in the proximal tubules, which is contrary to the study by Peyronnet R et al. It is still unclear whether antibody specificity gives rise to this discrepancy. Moreover, consistent with two other studies, Piezo1 was also expressed in the urothelial cells of the bladder and ureter, the interstitial cells of Cajal and smooth muscle cells but mainly in urothelial cells. Furthermore, Piezo1 was found in the urethra and surrounding tissues, including stromal cells and striated muscle cells of the rhabdosphincter, vaginal epithelium, prostate gland, seminal vesicles and ejaculatory ducts. Although various studies have comprehensively identified the distribution of Piezo1 in the mouse urinary system, its function has yet to be investigated. By comparison, Piezo2 in the mouse bladder is poorly understood. Marshall et al. reported that Piezo2 was expressed in 81.5% of CTB-labeled bladder afferent neurons and 74% of Krt20-positive umbrella cells, but their study used neither controls nor specific probes. Another study suggested that Piezo2 was mostly limited to a scattered population of umbrella cells, even when using Piezo2-Cre-dependent lineage tracing.
### Table 1. Piezo1 channel in cellular mechanotransduction.

| Cells                        | Pathway and Function                                                                 | References |
|------------------------------|--------------------------------------------------------------------------------------|------------|
| **Digestive System**         |                                                                                      |            |
| Intestinal ECs               | ROCK1/2→Claudin-1→Epithelial barrier function                                         | 128        |
| BC membranes                 | Ca\(^{2+}\) transfer→BC contraction→Bile secretion                                   | 129        |
| Enterochromaffin cells       | SSRNA→Piezo1→SHT release→Gut homeostasis                                             | 49         |
| Acinar cells                 | PLA2→TRPV4→Ca\(^{2+}\) influx→Pancreatitis                                          | 130,131    |
| Mouse G cells                | Antrum distension→Gastrin release→Control gastric activities                         | 132        |
| **Musculoskeletal System**   |                                                                                      |            |
| MLO-Y4 osteocytic cells      | YAP1/TAZ→Wnt1→Bone anabolism                                                         | 133        |
| IDG-SW3 osteocytic cells     | Akt→Sost→Bone formation                                                              | 134        |
| Mouse osteoblastic cells     | i. Ca\(^{2+}\) influx→AKT/GSK-3β/β-catenin→Runx2→Bone formation                     | 135–138    |
|                             | ii. Ca\(^{2+}\) influx→ERK1/2/Perinuclear F-actin→Bone regeneration                  |            |
|                             | iii. YAP1→COL2α1/COL9α2→Bone anabolism                                               |            |
| Mouse liver ECs              | Ca\(^{2+}\) influx→PI3K-AKT/Notch→Angiogenesis in bone fracture healing              | 139        |
| UE7T-13 MSCs                 | BMP2→Differentiation of MSCs→Bone homeostasis                                         | 140        |
| Mouse tenocytes              | Ca\(^{2+}\) influx→Collagen cross-linking→Tendon stiffness→Jumping performance       | 61         |
| Mouse primary adipocytes     | FGF1/FGFR1→Adipogenesis                                                              | 141        |
| **Cardiovascular System**    |                                                                                      |            |
| HUVECs                       | i. Vascular architecture and endothelial cell alignment                               | 142–145    |
|                             | ii. Ca\(^{2+}\) influx/ATP release→P2Y2/G\(_{15}\)/G\(_{11}\)→AKT/eNOS→NO release→Control blood pressure |            |
|                             | iii. Ca\(^{2+}\) influx→Adrenomedullin release→cAMP→PKA/eNOS→NO release→Vascular tone and blood pressure |            |
|                             | iv. Ca\(^{2+}\) /ATP→P2Y2/G\(_{15}\)/G\(_{11}\)→FAK/NF-κB→Endothelial inflammation and atherosclerosis |            |
| Mouse cardiomyocytes         | Ca\(^{2+}\) influx→Rac1/NOX2→ROS→Heart homeostasis                                   | 146        |
| Mouse ECs                    | i. S1P→Piezo1/Ca\(^{2+}\) influx→MT1-MMP→Angiogenesis                              | 55,147,148 |
|                             | ii. EDH(F)→Redistribution of blood flow                                              |            |
|                             | iii. Embryonic development and vascular remodeling                                   |            |
| Human primary LECs           | Lymphatic valve development and maintenance                                          | 149        |
| Zebrafish endothelial tip cells | Ca\(^{2+}\) transients→Calpain/NOS→Brain vascular pathfinding                      | 150        |
| **Blood System**             |                                                                                      |            |
| Zebrafish RBCs               | Erythrocyte volume homeostasis                                                        | 151        |
| Mouse RBCs                   | Ca\(^{2+}\) influx→KCa3.1 channel→Dehydration→Decreased cell volume                  | 60         |
| Human RBCs                   | i. Ca\(^{2+}\) influx/ATP release→Oxygen delivery, blood rheology, transfusion, etc. | 152,153    |
|                             | ii. Ca\(^{2+}\) influx→Ca-ATPase→Increased glycolysis→RBC mechanical distortion       |            |
| Human platelets and megalakaryocytes | Thrombogenesis                                                                     | 154        |
| Human erythroblast cells     | Ca\(^{2+}\) influx→NFATc2/EpoR→Erythropoiesis                                        | 155        |
| **Nervous and Endocrine System** |                                                                                      |            |
| Human neural stem/progenitor cells | Yap/Taz→Specification of neurons and gliocytes                                    | 56         |
| Xenopus RGCs                 | Axon growth in the brain                                                             | 156        |
| Drosophila sensory neuron    | CaMKII/NOS/PKG→Inhibition of axon regeneration                                        | 157        |
| Rat OPCs                     | CNS regeneration                                                                    | 158        |
| Rat β-cell lines             | Ca\(^{2+}\) influx→Insulin release                                                  | 159        |
| **Immune System**            |                                                                                      |            |
| Human T cells                | Ca\(^{2+}\) influx→F-actin scaffold→T cells activation                                | 57         |
| Mouse BMDMs                  | i. AP-1→EDN1→HIF1α→Innate immune activation                                          | 160–162    |
|                             | ii. NF-κB/STAT6→IFNγ/LPS→IL4/IL13→Proinflammatory and healing response              |            |
|                             | iii. LPS→TLR4/Piezo1→CaMKII-Mst1/2-Rac1→Innate immune activation                    |            |
| Mouse T\(_{reg}\) cells     | TGFβ/SMAD→Restrained T\(_{reg}\) cells                                              | 58         |
| Mouse myeloid cells          | HDAC2/Rb1→Myelopoiesis→Cancer and infectious disease                                  | 163        |
The Piezo1 channel plays an important role in mechanical transduction in different types of cells, although some have not been verified in humans. For example, in the digestive system, the Piezo1 channel is essential for intestinal epithelial barrier function, bile secretion, intestinal homeostasis, and gastric activity. In the musculoskeletal system, the Piezo1 channel is mainly involved in bone metabolism, bone formation, and bone regeneration. In the respiratory system, the Piezo1 channel regulates pulmonary vascular permeability, lung epithelial homeostasis, release of alveolar surfactant, and other physiological processes. Interestingly, an increase in pulmonary microvascular pressure caused by head trauma or high altitude will open the Piezo1 channel, leading to the degradation of VE-cadherin/p120-catenin, which in turn destroys AJs and decreases the lung endothelial barrier function. However, the opening of the Piezo1 channel caused by alveolar stretching will make AJs more stable and prevent the endothelial barrier from being destroyed. The author ascribed these opposing results to the difference in the type, direction, and magnitude of mechanical force.

### Table 2. Piezo2 channel in cellular mechanotransduction.

| Cells                              | Pathway and Function                                                                 | References |
|------------------------------------|--------------------------------------------------------------------------------------|------------|
| Respiratory System                 | i. GTP/Piezo2 → Increased RA current amplitude                                      | 170,171    |
|                                   | ii. Visceral sensation                                                              |            |
| Respiration                        | i. Epa1/Piezo2 → Allodynia                                                          | 62–64,66,172–174 |
|                                   | ii. Touch sensation                                                                 |            |
|                                   | iii. STOML3/Cholesterol → Control the membrane mechanics                              |            |
|                                   | iv. Sensitivity of Piezo2 → Tactile allodynia                                        |            |
|                                   | v. Proprionception → Skeletal integrity                                             |            |
|                                   | vi. Mtmr2/PIP2 → Decreased RA current → Somatic sensation                           |            |
| Nervous System                     | i. Mouse Merkel cells                                                               | 175        |
|                                   | ii. Rat Merkel cells                                                                |            |
|                                   | i. Calpain–VE-cadherin/i-catennin/p120-catenin−Disassembly of AJs−Lung vascular hyperpermeability | 164,165    |
|                                   | ii. Calpain−Src/VE-cadherin−AJs stabilization−Endothelial barrier homeostasis       |            |
|                                   | i. Rat ATI and ATII cells                                                           | 166        |
|                                   | ii. Ca^2+ influx−ATP release from ATI cells−P2Y2 on ATII cells−Surfactant secretion |            |
| Reproductive System               | i. Ca^2+ influx−NO, EDHF, prostacyclin release−Vasodilation during pregnancy        | 167        |
| Sense Organs                      | i. Ca^2+ influx−Focal adhesions−Intraocular pressure regulation                     | 168        |
|                                   | ii. Mouse trabecular meshwork cells and SC endothelial cells                         | 169        |
|                                   | Aqueous humor outflow                                                               |            |
|                                   | i. Lung volume regulation and the Hering–Breuer reflex in adult mice                 | 182        |
|                                   | ii. Lung expansion and efficient respiration in newborn mice                         |            |
| Digestive System                  | S-HT release and epithelial fluid secretion                                          | 183,184    |
|                                   | i. Lung expansion and efficient respiration in adult mice                            |            |
|                                   | ii. Lung volume regulation and the Hering–Breuer reflex in adult mice                |            |

The Piezo2 channel exerts physiological effects on the nervous system, respiratory system, and digestive system. In the nervous system, the Piezo2 channel might be a key sensor for light touch, pain, and visceral sensation. The Piezo2 and Piezo1 channels of the baroreceptive nerve endings in the carotid sinus coordinate to control blood pressure. In addition, the Piezo2 channel is not only an indispensable sensor for proprioception but also important in maintaining the integrity of bones. Mice lacking Piezo2 will develop scoliosis and hip dysplasia. In the respiratory system, the Piezo2 channel is essential to establish effective breathing in newborn mice and maintain normal breathing in adult mice. The Piezo2 channel might set the mechanosensitivity of enterochromaffin cells and convert mechanical stimulation of the intestinal lumen into the release of serotonin.

Additionally, Piezo2 was detected in suburothelial fibroblasts, blood vessels and dorsal root ganglia cells. Although the sites of Piezo2 expression in the bladder are controversial, a multitude of studies have investigated Piezo2-mediated mechanotransduction in bladder (patho)physiology.

### Physiological Roles of Piezo Channels in the Urinary System

**Piezo1 Affects the Regulation of Urinary Osmolarity**

The kidney regulates body fluid equilibrium by concentrating and diluting urine, which is the basis for maintaining a relatively stable internal physiological environment. For a long time, researchers have focused on polycystins in the kidney. Polycystins, present on the primary cilia of various cell types, such as renal epithelial cells and endothelial cells, are considered flow sensors and play a crucial role in the mechanosensory transduction of the kidney and blood vessels. Nevertheless, achievements in scientific research on polycystins cannot wholly clarify the mechanobiology in the kidney. Fortunately, the discovery of Piezo channels provides researchers with a promising direction. Piezo1 is also a key mechanotransduction molecule that senses mechanical stimuli in the kidney and is important for regulating urinary osmolarity. In adult mice, Piezo1 was preferentially expressed in the collecting ducts.
duct principal cells of the inner medulla, while Piezo2 was minimally expressed. Impaired urinary dilution and urea concentration after dehydration or fasting have been observed in mice with conditional renal epithelium Piezo1 KO. A possible model is that during dehydration, Piezo1 on the medullary principal cells of the kidney might increase cAMP production by improving the activity of adenylyl cyclase-6, facilitating aquaporin- principal cells of the kidney might increase cAMP production by improving the activity of adenylyl cyclase-6, facilitating aquaporin-2 lipid membrane targeting to accelerate water reabsorption. However, this hypothesis still needs to be verified by rigorous experiments. Animal models, such as zebrafish, which is the optimal model for studying kidney function, can be used to find their upstream and downstream targets. Notably, polycystin-2 and its mutant PC2-740X can directly or indirectly interact with Piezo1 in renal tubular epithelial cells through their N-terminal domain to inhibit its mechanosensitivity, affecting the regulation of intrarenal pressure. Together, these findings will provide a strong foundation for further studying kidney diseases related to increased intrarenal pressure.

Piezo is vital for micturition. The bladder urothelium not only serves as a physical barrier but also functions as a "collector" of bladder urination signals, sensing mechanical bladder wall stretching, thereby causing Ca\(^{2+}\) influx and releasing adenosine triphosphate (ATP) which is the stimulant for the afferent nerves of the bladder. This molecule acts on P2X3 and other purinergic receptors on sensory neurons to communicate information regarding the degree of filling, initiate the micturition reflex and promote voiding.

In vitro experiments showed that Piezo1 is indispensable for primary cultured urothelial cells evoking Ca\(^{2+}\) influx and the release of ATP after sensing direct stretch stimulation (Fig. 3). Surprisingly, ATP release from the native urothelium was not dramatically reduced in conditional urothelial Piezo1 KO mice that also displayed normal voiding function and behavior, suggesting potential compensatory mechanisms to overcome the loss of Piezo1. However, to date, there is still insufficient evidence to clarify whether Piezo2 overexpression can compensate for the loss of Piezo1. Recently, a study reported that Piezo2 is a key mechanoreceptor for urinary function, renders the lower urinary tract sensitive to stretching, and initiates a duly timed micturition reflex in mouse models. Marshall et al. monitored bladder pressure and sphincter activity in UPKI-cre expressing Piezo2 mice, whose Piezo2 in urothelial cells was widely deleted. Knockout mice displayed a higher bladder stretch threshold, required higher bladder pressure to accomplish voiding and exhibited attenuated urethral reflexes. However, these researchers used the same mouse strain to generate a urothelial Piezo2 KO model, and most of the studies used a small number of animals. Moreover, the researchers treated replicates as individual events instead of showing the mean/aggregate data for each individual animal. This method makes it unclear whether any of the results they show for cystometry or electromyography analysis are significantly different between the control and KO animals. Furthermore, they did not evaluate whether urothelial mechanotransduction and voiding behavior were affected in Piezo2 KO mice. Significantly, although Piezo2 KO mice and patients with Piezo2 deficiency reported voiding anomalies, their voiding function and behavior were not lost. Dalghi et al. argued that conditional urothelial Piezo1 and Piezo2 KO mice exhibited near-normal mechanotransduction and voiding behavior. In other words, knocking out urothelial Piezo1 or Piezo2 alone was insufficient, and instead, both channels must be knocked out simultaneously to reveal the true phenotype of urothelial Piezo channel-deficient mice. Specifically, the dual Piezo1/2 KO mice exhibited urinary incontinence during the active dark phase. Thus, the role of Piezo1/2 in the lower urinary tract needs to be further studied. Moreover, the specific functional roles of Piezo1 in the urethra and detrusor remain largely unknown. Whether Piezo2 in blood vessels and fibroblasts also contributes to normal or abnormal LUT function deserves attention. Overall, Piezo1- and Piezo2-mediated ATP release in the urothelium is essential for normal urination function and behavior. These studies deepen our understanding of the connection between Piezo-dependent mechanotransduction and behavior, and more importantly, they lay the foundation for interrogating how urothelial Piezo1 and Piezo2 collaborate to control urination and determine how they affect abnormal LUT function.
The urinary function of the bladder is controlled by clock genes with circadian rhythms\(^\text{80-82}\). The chief function of the bladder during the day is to urinate and ensure regular and efficient urination frequency, whereas the urine storage function at night can facilitate better rest and sleep\(^\text{83-85}\). In the case of disruption of rhythmic homeostasis, diseases such as nocturia develop\(^\text{86}\). Recently, studies have claimed that the time-dependent gene expression pattern is applicable to Piezo1, as well as transient receptor potential vanilloid 4 (TRPV4), which is another well-known mechanosensitive ion channel\(^\text{82,87,88}\). Accordingly, the urethra has a circadian rhythm of sensing bladder filling\(^\text{89}\). More specifically, the expression of Piezo1 and TRPV4 in the mouse urethral increased during the active phase and decreased during the sleep phase\(^\text{87}\). Moreover, a mutation in the clock gene can keep the Piezo1 expression level constant in the bladder\(^\text{87}\). Consistently, in primary mouse urothelial cells, the oscillation of Ca\(^{2+}\) influx in response to Piezo1 and TRPV4 has a circadian rhythm, which might be a significant cause of circadian rhythm for the sensation of bladder filling\(^\text{89}\). Experimental evidence by chromatin immunoprecipitation suggested that the clock protein binds to the promoters of Piezo1 and TRPV4 to regulate transcription\(^\text{87}\). More critically, the role of GsMTx4 in lowering urine sensation in wild-type mice is related to the expression levels of the circadian clock and Piezo1\(^\text{90}\). The maximum effect is exerted during the sleep phase\(^\text{87}\). In this sense, reshaping the circadian rhythm of urinary function by inhibiting the Piezo1 channel might become a new treatment strategy for nocturia. However, it is worth noting that GsMTx4-mediated inhibition of the Piezo1 channel is nonspecific and affects other MSCs. Thus, we must account for this factor before drawing any conclusions that GsMTx4 benefits Piezo1-associated disorders.

Pathophysiological roles of Piezo channels in the urinary system

**Urological cancer.** Piezo1 channels are directly involved in Ca\(^{2+}\) signal transduction or indirectly maintain the requisite electrochemical gradients for Ca\(^{2+}\) entry to affect the developmental process of tumors, including tumor cell proliferation, angiogenesis, migration, extracellular matrix remodeling, and the tumor microenvironment\(^\text{91-94}\). For instance, downregulating the expression of Piezo1 lowered intracellular Ca\(^{2+}\) levels in esophageal squamous cell carcinoma and then repressed tumor cell migration and invasion through the epithelial-mesenchymal transition pathway\(^\text{95}\). Knockout of Piezo1, which is highly expressed in gastric cancer tissues, significantly counteracted carcinogenic effects\(^\text{96}\). In addition, Piezo1 expression was upregulated in breast cancer, osteosarcoma, and glioma\(^\text{97-99}\). In contrast, there are few studies on the biological effects of Piezo2 in tumors. In gliomas, knocking down the expression of Piezo2 reduced the intracellular Ca\(^{2+}\) concentration, inhibited tumor angiogenesis and lowered vascular permeability through the Wnt/β-catenin pathway\(^\text{87}\). Thus, these discoveries have encouraged the study of the pathophysiological roles of Piezo channels in urological tumors.

Over 90% of bladder cancer (BCa) occurs in the urothelium; this is the fourth most common malignant tumor in men\(^\text{100}\). In an experiment comparing the expression of Piezo1 between human and mouse BCa, real-time PCR (RT–PCR) analysis found that the mRNA expression of Piezo1 and Piezo2 increased significantly in both human BCa tissues and mouse BCa tissues\(^\text{101}\). Furthermore, the expression of Piezo1 is related to tumor stage, grade and size, while Piezo2 expression is correlated only with tumor stage\(^\text{101}\). Therefore, the activation of Piezo channels appears to contribute to the pathophysiology of BCa. These studies initially examined the correlation between Piezo channels and BCa and provided a new direction for the investigation of BCa. Future studies will be needed to elucidate whether Piezo channels are associated with tumor cell growth and apoptosis, pyrolysis, autophagy, the immune microenvironment, and metabolism. Anatomically, the bladder urothelium is a layer of tightly connected cells covering the inner surface of the bladder and has a strong regenerative capability\(^\text{102}\). Understanding the cellular and molecular mechanisms underlying the homeostatic maintenance and repair of the urothelium is key to designing strategies against BCa. In zebrafish, Piezo1 senses cell crowding, promoting live cell extrusion to maintain homeostatic cell numbers in epithelia\(^\text{103}\). Gupte\(^\text{a}\) et al. also found that Piezo1 in epithelial cells recognizes two different mechanical signals, crowding and stretching\(^\text{104}\). Thus, Piezo1 controls cell division and apoptosis by affecting the intracellular Ca\(^{2+}\) concentration to modulate the number of epithelial cells in tissues and organs\(^\text{105}\). It can be inferred from these findings that Piezo1-mediated live cell extrusion might be a BCa suppressive mechanism that prevents the accumulation of excess epithelial cells. Moreover, Piezo1 might be required for urothelial repair and regeneration in BCa, and intensive mechanistic studies are urgently required to promote the scientific understanding of this issue. Piezo1 might be a promising target for the early prevention and management of BCa.

Prostate cancer (PCa) is an epithelial malignant tumor that occurs in the prostate gland. Urinary system tumors have a relatively high morbidity and mortality rate, which seriously endangers the life and well-being of men\(^\text{106}\). PCa is related to a variety of signaling pathways, including the PI3K-AKT-mTOR/FOXO/NF-kB pathway, Wnt/β-catenin pathway, TGF-β/Smad pathway, and Notch signaling pathway\(^\text{106-110}\). Among these signaling pathways, the Akt/mTOR pathway has been shown to promote the development of PCa by its coupling with Piezo1\(^\text{111}\) (Fig. 4). Immunohistochemistry performed on 70 human PCa tissue specimens showed that the expression of Piezo1 was significantly upregulated, which was consistent with the results obtained by culturing PC3 and DU145 PCa cells in vitro\(^\text{111}\). A lentiviral vector expressing Piezo1 shRNA was applied to knock down Piezo1 in DU145 PCa cells. Then, the cells were injected subcutaneously to induce xenograft prostate tumors in immunodeficient mice\(^\text{111}\). It was found that the proliferation and migration of cancer cells in vivo were suppressed, suggesting a tumor-suppressor role of Piezo1 in prostate tumors\(^\text{111}\). Further studies confirmed that repressing the expression of Piezo1 would impair intracellular Ca\(^{2+}\) signaling, hinder the phosphorylation of Akt and mTOR, and restrain the activation of cyclin D1 and CDK4, thereby inhibiting tumor growth\(^\text{111}\) (Fig. 4). However, most experiments have been based on cultured cells in vitro, which provides valuable information, and in vivo studies are lacking. Compared with the complex tumor microenvironment, artificially controlled conditions in vitro are suboptimal, and therefore, additional studies are required to investigate the consistency of the effects of Piezo1 in vivo and in vitro. In addition to immunocompromised animals, other immune-sound murine models should be considered for comprehensive evaluation of the role of Piezo1. Currently, the sole pathway identified for the mechanism of action of Piezo1 in prostate cancer progression is the Akt/mTOR pathway. Whether other pathways cooperate with Piezo channels to promote tumor progression remains unclear. Additionally, it cannot be discounted that cytosolic Ca\(^{2+}\), as the most abundant second messenger, plays a pivotal role in tumorigenesis, angiogenesis and metastasis\(^\text{112}\). The natural characteristic of preferential permeability to Ca\(^{2+}\) gives Piezo channels the potential to become biomarkers and new drug targets for the diagnosis and treatment of urological cancer. More investigations will focus on the tissue specificity of Piezo gene expression in bladder and prostate cancer and the downstream pathways stimulated by Piezo-mediated Ca\(^{2+}\) signaling.

**Lower urinary tract dysfunction.** Neurogenic and myogenic mechanisms can be used to explain overactive bladder (OAB). The neurogenic mechanism involves increased excitability of the
central nervous system, and the sensitization of bladder afferent nerves causes dysfunctional bladder control. The myogenic mechanism distinguishes OAB from unstable detrusor contraction. In recent years, the urothelial-derived mechanism has gradually been accepted, opening up new horizons for the study of OAB. This theory states that the urothelium, acting as a nonneuronal interoceptor, improperly senses intravesical changes in pressure and chemistry, leading to OAB. Accumulating evidence suggests that changes in the expression/sensitivity of urothelial mechanoreceptors might affect mechanosensitivity to stretching, resulting in abnormal bladder activity. For instance, the overexpression of TRPV4 on urothelial cells will increase the sensitivity of the urothelium to the sensation of bladder filling, inducing increased Ca\(^{2+}\) influx and subsequent ATP release in the suburothelium and detrusor increased observably on Day 7 after urethral ligation and reported that Piezo1 mRNA in the suburothelium was associated with detrusor overactivity in rats with BOO. Michishita M et al. produced a rat model of pBOO through partial urethral ligation and reported that Piezo1 mRNA in the suburothelium and detrusor increased observably on Day 7 after pBOO. Moreover, a decrease in neurofilament expression with increased Piezo1 mRNA was observed. This result suggests that Piezo1 participates in the compensatory mechanism of bladder denervation; however, the underlying molecular mechanisms remain unknown. Thus, evidence has implicated (although not verified) Piezo1 in detrusor–sphincter dysynergia. BOO awaits further investigation, and additional evidence of Piezo1 involvement in the urethra and bladder outlet is needed before any conclusion can be drawn.

Bladder outlet obstruction. Bladder outlet obstruction (BOO), defined from the perspective of clinical urodynamics, is difficulty in voiding. BOO is characterized by increased resistance of the urine outflow tract caused by a variety of pathogeneses, including benign prostatic hyperplasia in men, bladder neck obstruction in women and urethral stricture. The clinical manifestations are mainly lower urinary tract symptoms, including storage symptoms (urination frequency, urgency and nocturia) and voiding symptoms (feelings of incomplete voiding, weak stream and hesitancy). Nevertheless, the pathological mechanism of bladder dysfunction caused by this obstructive disease has yet to be fully elucidated. Studying abnormal mechanotransduction in the lower urinary tract is a promising strategy. Bladder hyperactivity in mice with partial bladder outlet obstruction (pBOO) was linked to downregulated expression of the mechano-sensitive TREK-1 potassium channel in the detrusor. TRPV4 was associated with detrusor overactivity in rats with BOO. Michishita M et al. produced a rat model of pBOO through partial urethral ligation and reported that Piezo1 mRNA in the suburothelium and detrusor increased observably on Day 7 after pBOO. Moreover, a decrease in neurofilament expression with increased Piezo1 mRNA was observed. This result suggests that Piezo1 participates in the compensatory mechanism of bladder denervation; however, the underlying molecular mechanisms remain unknown. Thus, evidence has implicated (although not verified) Piezo1 in detrusor–sphincter dysynergia. BOO awaits further investigation, and additional evidence of Piezo1 involvement in the urethra and bladder outlet is needed before any conclusion can be drawn.
CONCLUSIONS
In recent years, publications related to Piezo channels have shown a strong increasing trend. This field has gradually become an intriguing research direction, deepening our understanding of mechanobiology in the urinary system. Considering the limited pharmacology of Piezo channels, it is still too early to discuss the underlying problems faced by their antagonists in clinical trials. However, the fact that inhibiting Piezo channels does not always induce beneficial results within the same tissue is unavoidable. One possible scenario is that Piezo1 antagonism in the management of bladder hyperactivity might be restricted. Although drugs that specifically target Piezo channels have a long way to go before reaching the clinic, we believe that studies on Piezo channels will undoubtedly change clinical practice to treat urinary system diseases.

REFERENCES
1. Bagriantsiev, S. N., Gracheva, E. O. & Gallagher, P. G. Piezo proteins: regulators of mechanosensation and other cellular processes. J. Biol. Chem. 289, 31673–31681 (2014).
2. Verschuren, E. H. J. et al. Sensing of tubular flow and renal electrolyte transport. Nat. Rev. Nephrol. 16, 337–351 (2020).
3. Bignon, Y. et al. Defective bicarbonate reabsorption in Kir4.2 potassium channel deficient mice implicates acid-base balance and ammonia excretion. Kidney Int. 97, 304–315 (2020).
4. Kurtz, A. Renin release: sites, mechanisms, and control. Annu. Rev. Physiol. 73, 377–399 (2011).
5. Xu, Y. et al. Effect of fluid shear stress on the internalization of kidney-targeted delivery systems in renal tubular epithelial cells. Acta Pharm. Sin. B 10, 680–692 (2020).
6. Roberts, M. W. G. et al. TRPV4 receptor as a functional sensory molecule in bladder urothelium: Stretch-independent, tissue-specific actions and pathologi cal implications. Am. J. Physiol. Ren. Physiol. 314, F22–F34 (2018).
7. Ferguson, D. R., Kennedy, I. & Burton, T. J. ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes-a possible sensory action and pathologic implication. J. Physiol. 352, 263–278 (2000).
8. Endlich, K., Kliewe, F. & Endlich, N. Stressed podocytes-mechanical forces, sensors, signaling and response. Pflug. Arch. 469, 937–949 (2017).
9. Srivastava, T. et al. Mechanotransduction signaling in podocytes from fluid flow shear stress. Am. J. Physiol. Ren. Physiol. 314, F22–F34 (2018).
10. Aw Yong, K. M., Sun, Y., Merajver, S. D. & Fu, J. Mechanotransduction-induced reversible phenotypic switching in prostate cancer cells. Biophys. J. 112, 1236–1245 (2017).
11. Corey, D. P. & Hudspeth, A. J. Ionic basis of the receptor potential in a vertebrate hair cell. Nature 281, 675–677 (1979).
12. Guharay, F. & Sachs, F. Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. J. Physiol. 352, 685–701 (1984).
13. Christensen, A. P. & Corey, D. P. TRP channels in mechanosensation: direct or indirect activation? Nat. Rev. Neurosci. 8, 510–521 (2007).
14. Coste, B. et al. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science 330, 55–60 (2010).
15. Zhao, Q. et al. Structure and mechanogating mechanism of the Piezo1 channel. Nature 554, 487–492 (2018).
16. Guo, Y. R. & MacKinnon, R. Structure-based membrane dome mechanism for Piezo mechanosensitivity. elife 6, e33660 (2017).
17. Saotome, K. et al. Structure of the mechanically activated ion channel Piezo1. Nature 545, 481–486 (2018).
18. Ge, J. et al. Architecture of the mammalian mechanosensitive Piezo1 channel. Nature 527, 64–69 (2015).
19. Xiao, B. Levering mechanically activated piezo channels for potential pharmacological intervention. Annu. Rev. Pharmacol. Toxicol. 60, 195–218 (2020).
20. Zhao, Q. et al. Ion permeation and mechanotransduction mechanisms of mechanosensitive piezo channels. Neuron 89, 1248–1263 (2018).
21. Wang, Y. et al. A lever-like transduction pathway for long-distance chemical and mechano-gating of the mechanosensitive Piezo1 channel. Nat. Commun. 9, 1300 (2018).
22. Wang, L. et al. Structure and mechanogating of the mammalian tactile channel PIEZO2. Nature 573, 225–229 (2019).
Minors, D. S. & Waterhouse, J. M. Circadian rhythms of urinary excretion: the role of the urinary bladder. *Neurourol. Urodyn.* 26, 307–316 (2017).

Jairaman, A. et al. Piezo1 channels restrain regulatory T cells but are dispensable for effector CD4+ T cell responses. *Sci. Adv.*, 6, eaab5895 (2021).

Lukacs, V. et al. Impaired PIEZO1 function in patients with a novel autosomal recessive congenital lymphatic dysplasia. *Nat. Commun.* 6, 8329 (2015).

Cahalan, S. M. et al. Piezo1 links mechanical forces to red blood cell volume. *elife* 4, e07370 (2015).

Passini, F. S. et al. Shear-stress sensing by PIEZO1 regulates tendon stiffness in rodents and influences jumping performance in humans. *Nat. Biomed. Eng.* 5, 1457–1471 (2021).

Runade, S. S. et al. Piezo2 is the major transducer of mechanical forces for touch sensation in mice. *Nature* 516, 121–125 (2014).

Woo, S. H. et al. Piezo2 is the principal mechanotransduction channel for proprioception. *Nat. Neurosci.* 18, 1756–1762 (2015).

Eijkelkamp, N. et al. A role for Piezo2 in EPAC1-dependent mechanical allodynia. *Nat. Commun.* 4, 1682 (2013).

Marshall, K. L. et al. PIEZO2 in sensory neurons and urothelial cells coordinates urination. *Nature* 588, 290–295 (2020).

Assaraf, E. et al. PIEZO2 expressed in proinflammatory neurons is essential for skeletal integrity. *Nat. Commun.* 11, 5168 (2020).

Yang, H. et al. PIEZO2 protein: A novel regulator of tumor angiogenesis and hyperpermeability. *OncoTarget* 7, 44630–44643 (2016).

Li, J. et al. PIEZO2 mediates ultrasonic hearing via cochlear outer hair cells in mice. *Proc. Natl. Acad. Sci. USA* 118, e2101207118 (2021).

Dalglish, M. A. et al. Expression and distribution of PIEZO1 in the mouse urinary tract. *Am. J. Physiol. Ren. Physiol.* 317, F303–F321 (2019).

Miyamoto, T. et al. Functional role for Piezo1 in stretch-evoked Ca2+ influx and ATP release in urothelial cell cultures. *J. Biol. Chem.* 289, 16565–16575 (2014).

Michishita, M., Yano, K., Tomita, K. I., Matsuzaki, O. & Kasahara, K. I. PIEZO1 expression increases in rat bladder after partial bladder outlet obstruction. *Life Sci.* 166, 1–7 (2016).

Dalglish, M. G. et al. Functional roles for Piezo1 and PIEZO2 in urothelial mechanotransduction and lower urinary tract interoception. *JCI Insight* 6, e152964 (2021).

Patel, A. & Honore, E. Polycystins and renovascular mechanosensory transduction. *Nat. Rev. Nephrol.* 6, 530–538 (2010).

Martins, J. R. et al. Piezo1-dependent regulation of urinary osmolarity. *PLoS Genet.* 12, 1197–1206 (2016).

Jerman, S. & Sun, Z. Using Zebrafish to study kidney development and disease. *Curr. Top. Dev. Biol.* 124, 41–79 (2017).

Dunning-Davies, B. M., Fry, C. H., Mansour, D. & Ferguson, D. R. The regulation of ATP release from the urethra by adenosine and transepithelial potential. *BJU Int.* 111, 505–513 (2013).

Mochizuki, T. et al. The TRPV4 cation channel mediates stretch-evoked Ca2+ influx and ATP release in primary urothelial cell cultures. *J. Biol. Chem.* 284, 21527–21526 (2009).

Wei, L. et al. Adenosine Triphosphate release and P2 receptor signaling in Piezo1 channel-dependent mechanoregulation. *Front. Pharmacol.* 10, 1304 (2019).

Vlasovskaya, M. et al. P2X3 knockout mice reveal a major sensory role for urothelially released ATP. *J. Neurosci.* 21, 5670–5677 (2001).

Noh, J. Y. et al. Circadian rhythms in urinary functions: possible roles of circadian clocks? *Int. Neurourol. J.* 15, 64–73 (2011).

Negoro, H. et al. Impaired PIEZO1 function in patients with a novel autosomal recessive congenital lymphatic dysplasia. *Nat. Commun.* 6, 8329 (2015).

Ihara, T. et al. The Circadian expression of PIEZO1, TRPV4, Connexin26, and VNUT, associated with the expression levels of the clock genes in mouse primary cultured urothelial cells. *Neurourol. Urodyn.* 37, 942–951 (2018).

Ihara, T. et al. The oscillation of intracellular Ca2+ influx associated with the circadian expression of PIEZO1 and TRPV4 in the bladder urothelium. *Sci. Rep.* 8, 5699 (2018).

Ihara, T. et al. Different effects of GtMtx4 on nocturia associated with the circadian clock and Piezo1 expression in mice. *Life Sci.* 278, 115555 (2021).

Petho, Z., Najder, K., Bulk, E. & Schwab, A. Mechanosensitive ion channels push cancer progression. *Cell Calcium* 80, 79–90 (2019).

Monteith, G. R., Prevorskaya, N. & Roberts-Thomson, S. J. The calcium-cancer signalling nexus. *Nat. Rev. Cancer* 17, 367–380 (2017).

Marchi, S. & Pinton, P. Alterations of calcium homeostasis in cancer cells. *Curr. Opin. Pharmacol.* 29, 1–6 (2016).

De Felice, D. & Alaimo, A. Mechanosensitive piezo channels in cancer: focus on altered calcium signaling in cancer cells and in tumor progression. *Cancers* 12, 1780 (2020).

Gao, L. et al. Suppression of esophageal squamous cell carcinoma development by mechanosensitive protein Piezo1 downregulation. *ACS Omega* 6, 12054–12061 (2021).

Zhang, J. et al. PIEZO1 functions as a potential oncogene by promoting cell proliferation and migration in gastric carcinogenesis. *Mol. Carcinog.* 57, 1144–1155 (2018).

Li, C. et al. Piezo1 forms mechanosensitive ion channels in the human MCF-7 breast cancer cell line. *Sci. Rep.* 5, 8364 (2015).

Jiang, L., Zhao, Y. D. & Chen, W. X. The function of the novel mechanically activated ion channel PIEZO1 in the human osteosarcoma cells. *Med. Sci. Monit.* 23, F303–F321 (2017).

Chern, X. et al. A feedforward mechanism mediated by mechanosensitive ion channel PIEZO1 and tissue mechanics promotes glioma aggression. *Neuron* 100, 799–815 e797 (2018).

Ren, A. T., Lee, P. C., Charnie, K. & Mshis, M. D. Bladder cancer: a review. *JAMA* 324, 1980–1991 (2020).

Etem, E. O. et al. The increased expression of Piezo1 and Piezo2 ion channels in human and mouse bladder carcinoma. *Adv. Clin. Exp. Med.* 27, 1025–1031 (2018).

Wang, C., Ross, W. T. & Mysovrek, I. U. Urothelial generation and regeneration in development, injury, and cancer. *Dev. Dyn.* 246, 336–343 (2017).

Eisenhoffer, G. T. et al. Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. *Nature* 484, 546–549 (2012).

Gudipaty, S. A. et al. Mechanical stretch triggers rapid epithelial cell division through Piezo1. *Nature* 543, 118–121 (2017).

Murtuza-Garzon, V. B. & Gupta, R. WNT signalling in prostate cancer. *Nat. Rev. Urol.* 14, 683–696 (2017).

Song, B. et al. Targeting FOXA1-mediated repression of TGF-beta signaling suppresses castration-resistant prostate cancer progression. *J. Clin. Invest.* 129, 569–582 (2019).

O’Brien, R. & Marigol, L. The Notch-1 receptor in prostate tumorigenesis. *Cancer Treat. Rev.* 36, 36–46 (2017).

Han, Y. et al. Mechanosensitive ion channel Piezo1 promotes prostate cancer development through the activation of the Akt/mTOR pathway and acceleration of cell cycle. *Int. J. Oncol.* 55, 629–644 (2019).

Cui, C., Merritt, R., Fu, L. & Pan, Z. Targeting calcium signaling in cancer therapy. *Neuron* 100, 1762 (2015).

Griffin, B. et al. The Notch-1 receptor in prostate tumorigenesis. *BJU Int.* 108, F303–F321 (2011).

Kimura, Y. et al. The circadian rhythm of bladder clock genes in the spontaneously hypertensive rat. *Plos One* 14, e0220381 (2019).

Minors, D. S. & Waterhouse, J. M. Circadian rhythms of urinary excretion: the relationship between the amount excreted and the circadian changes. *J. Physiol.* 327, 39–51 (1982).

Herrera, G. M. & Meredith, A. L. Dermal variation in urodynamics of rat. *Plos One* 5, e12298 (2010).

Parsons, M. et al. Normative bladder diary measurements: night versus day. *Neurourol. Urodyn.* 26, 465–473 (2007).
116. Birder, L. A. & de Groat, W. C. Mechanisms of disease: involvement of the urethral sphincter in bladder dysfunction. Nat. Rev. Urol. 4, 46–54 (2007).

117. Hawthorn, M. H., Chapple, C. R., Cock, M. & Chess-Williams, R. Urethral-derived inhibitory factor(s) influences on detrusor muscle contractility in vitro. Br. J. Pharm. 129, 416–419 (2000).

118. Lee, S. R., Kim, H. J., Kim, A. & Kim, J. H. Overactive bladder is not only overactive but also hypersensitive. Urology 75, 1053–1059 (2010).

119. Peyronnet, B. et al. A comprehensive review of overactive bladder pathophysiology: on the way to tailored treatment. Eur. Urol. 75, 988–1000 (2019).

120. Gevaert, T. et al. Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding. J. Clin. Invest. 117, 3435–3462 (2007).

121. Liu, Q. et al. Increased Piezo1 channel activity in interstitial Cajal-like cells induces bladder hyperactivity by functionally interacting with NCK1 in rats with cyclophosphamide-induced cystitis. Exp. Mol. Med. 50, 1–16 (2018).

122. D’Ancona, C. et al. The International Continence Society (ICS) report on the terminology for adult male lower urinary tract and pelvic floor symptoms and dysfunction. Neurourol. Urodyn. 38, 433–477 (2019).

123. Zhang, L. et al. Bladder outlet obstruction: progression from inflammation to fibrosis. BJU Int. 106, 1686–1694 (2010).

124. Kaplan, S. A. Re: EAU Guidelines on the Assessment of Non-Neurogenic Male Lower Urinary Tract Symptoms Including Benign Prostatic Obstruction. J. Urol. 196, 1712–1714 (2016).

125. Baker, S. A. et al. Role of TREK-1 potassium channel in bladder overactivity after cyclophosphamide-induced cystitis. Neurourol. Urodyn. 38, e4745 (2019).

126. Geng, J. et al. TLR4 signalling via Piezo1 engages and enhances the macrophage inflammatory response during bacterial infection. Sci. Rep. 9, 16876 (2019).

127. Song, J. et al. Fluid shear stress induces Runx-2 expression via upregulation of osteoblast-osteoclast crosstalk. Bone Res. 7, 709 (2019).

128. Xu, L. et al. The mechanosensitive ion channel Piezo1 mediates macrophage polarization and stiffness sensing. Nat. Commun. 12, 3256 (2021).

129. Kuchel, P. W. et al. Piezo2 is required for Merkel-cell mechanotransduction. Nature 579, 80–85 (2021).

130. Li, X. et al. Piezo1 channels sense whole body physical activity to reset cardiovascular homeostasis and enhance performance. Nat. Commun. 8, 350 (2017).

131. Rode, B. et al. Piezo1 channels sense whole body physical activity to reset cardiovascular homeostasis and enhance performance. Nat. Commun. 8, 350 (2017).

132. Ranade, S. S. et al. Piezo1, a mechanically activated ion channel, is required for vascular development in mice. Proc. Natl Acad. Sci. U.S.A. 111, 10347–10352 (2014).

133. Cho, D. et al. Piezo1 incorporates mechanical force signals into the genetic program that governs lymphatic valve development and maintenance. JCI Insight 4, e125068 (2019).

134. Liu, T. T. et al. Piezo1-mediated Ca2+ activities regulate brain vascular pathfinding during development. Neuron 108, 180–192 e185 (2020).

135. Faucherre, A., Kiss, K., Nargeot, J., Mangoni, M. E. & Jolimp, C. Piezo1 plays a role in lung cytoplasmic volume homeostasis. Haematologica 99, 70–75 (2014).

136. Kuchel, P. W. et al. Accelerating metabolism and transmembrane cation flux by distorting red blood cells. Sci. Adv. 3, eaa0106 (2017).

137. Chen, P. et al. Mechanosensitive Piezo1 in endothelial cells promotes angiogenesis to support bone fracture repair. Mol. Brain 11, 25923 (2016).

138. Florez-Paz, D., Bali, K. K., Kuner, R. & Gomis, A. A critical role for Piezo2 channels in the mechanotransduction of mouse proprioceptive neurons. Nature 573, 130–134 (2019).

139. Deivasikamani, V. et al. Piezo1 channel activation mimics high glucose as a stimulus of insulin release. Sci. Rep. 9, 16876 (2019).

140. Solis, A. G. et al. Mechanosensation of cyclical force by PIEZO1 is essential for innate immunity. Nature 573, 69–74 (2019).

141. Atcha, H. et al. Mechanically activated ion channel Piezo1 modulates macrophage polarization and stiffness sensing. Nat. Commun. 12, 3256 (2021).

142. Geng, J. et al. TR4 signalling via Piezo1 engages and enhances the macrophage mediated host response during bacterial infection. Nat. Commun. 12, 3519 (2021).

143. Ahn, S. et al. Targeting Piezo1 unleashes innate immunity against cancer and infectious disease. Sci. Immunol. 5, eabb5168 (2020).

144. Friedrich, E. E. et al. Endothelial cell Piezo1 mediates pressure-induced lung vascular hyperpermeability via disruption of adherens junctions. Proc. Natl Acad. Sci. U.S.A. 116, 12980–12985 (2019).

145. Zhong, M. et al. Alveolar stretch activation of endothelial piezo1 protects adherens junctions and lung vascular barrier. Am. J. Respir. Cell Mol. Biol. 62, 168–177 (2020).

146. Diem, K. et al. Mechanical stretch activates piezo1 in caveolae of alveolar type I cells to trigger ATP release and paracrine stimulation of surfactant secretion from alveolar type II cells. Faseb J. 34, 12785–12804 (2020).

147. John, L. et al. The Piezo1 cation channel mediates uterine artery shear stress mechanotransduction and vasodilation during rat pregnancy. Am. J. Physiol. Heart Circ. Physiol. 315, H1019–H1026 (2018).

148. Yarishkin, O. et al. Piezo1 channels mediate mesenchymal mesenchymal cell-to-cell communication processes in bladder dysfunction. J. Physiol. 599, 571–592 (2021).

149. Zhu, W. et al. Piezo1 channels promote alveolar cell differentiation via activation of FGF1/FGFR1 signaling pathway in mice. J. Clin. Invest. 125, 5912 (2015).

150. Narayanpan, P. et al. Myotubulin related protein-2 and its phospholipid substrate PIP2 control Piezo2-mediated mechanotransduction in peripheral sensory neurons. eLife 7, e32346 (2018).

151. Murphy, S. E. et al. The mechanosensitive ion channel Piezo2 mediates sensitivity to mechanical pain in mice. Proc. Natl Acad. Sci. U.S.A. 116, 12980–12985 (2019).

152. Young, N. et al. Membrane stiffness facilitates mechanosensation in sensory neurons. Nat. Commun. 6, 8512 (2015).

153. Kuchel, P. W. & Shishmarev, D. Accelerating metabolism and transmembrane cation flux by distorting red blood cells. Sci. Adv. 3, eaa0106 (2017).

154. Chen, P. et al. Mechanosensitive Piezo1 in endothelial cells promotes angiogenesis to support bone fracture repair. Mol. Brain 11, 25923 (2016).

155. Jia, Z., Ikeda, R., Ling, J. & Gu, J. G. GTP-dependent run-up of Piezo2-type mechanosensitive currents in rat dorsal root ganglion neurons. Mol. Brain 6, 57 (2013).

156. Yang, J. et al. The potential role of Piezo2 in the mediation of visceral sensation. Neurosci. Lett. 630, 158–163 (2016).

157. Yu, C. et al. Membrane stiffness facilitates mechanosensation in sensory neurons. Nat. Commun. 6, 8512 (2015).

158. Narayanpan, P. et al. Myotubulin related protein-2 and its phospholipid substrate PIP2 control Piezo2-mediated mechanotransduction in peripheral sensory neurons. eLife 7, e32346 (2018).

159. Murthy, S. E. et al. The mechanosensitive ion channel Piezo2 mediates sensitivity to mechanical pain in mice. Sci. Transl. Med. 10, eaat9897 (2018).

160. Florez-Paz, D., Bali, K. K., Kuner, R. & Gomis, A. A critical role for Piezo2 channels in the mechanotransduction of mouse proprioceptive neurons. Sci. Rep. 6, 25923 (2016).

161. Woo, S. H. et al. Piezo2 is required for Merkel-cell mechanotransduction. Nature 509, 622–624 (2014).

162. Ikeda, R. et al. Merkel cells transduce and encode tactile stimuli to drive Abeta-afferent impulses. Cell 157, 664–675 (2014).
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COMPETING INTERESTS
The authors declare no competing interests.