

Contributed Mini Review

Mechanosensitive ion channels in apoptosis and ferroptosis:
focusing on the role of Piezo1Yong-Jae Kim^{1,2} & Jeongeun Hyun^{1,2,3,*}¹Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan 31116, ²Department of Nanobiomedical Science & BK21 NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan 31116, ³Mechanobiology Dental Medicine Research Center, College of Dentistry, Dankook University, Cheonan 31116, Korea

Mechanosensitive ion channels sense mechanical stimuli applied directly to the cellular membranes or indirectly through their tethered components, provoking cellular mechanoresponses. Among others, Piezo1 mechanosensitive ion channel is a relatively novel Ca^{2+} -permeable channel that is primarily present in non-sensory tissues. Recent studies have demonstrated that Piezo1 plays an important role in Ca^{2+} -dependent cell death, including apoptosis and ferroptosis, in the presence of mechanical stimuli. It has also been proven that cancer cells are sensitive to mechanical stresses due to higher expression levels of Piezo1 compared to normal cells. In this review, we discuss Piezo1-mediated cell death mechanisms and therapeutic strategies to inhibit or induce cell death by modulating the activity of Piezo1 with pharmacological drugs or mechanical perturbations induced by stretch and ultrasound. [BMB Reports 2023; 56(3): 145-152]

INTRODUCTION

Mechanotransduction is a process whereby cells respond to mechanical forces (e.g., static pressure, membrane stretch, shear flow, substrate stiffness, tissue compression, and osmotic stress) and transduce them into biochemical signals, thereby influencing cell behavior and fate (1, 2). Among a variety of proteins involved in mechanotransduction, mechanosensitive (MS) ion channels, also known as mechanically gated ion channels, execute the initial mechanosensing step. MS ion channels change their conformations from a closed to an open form via directly sensing the mechanical stresses applied to the cellular membranes or through the associated nonmembrane components

such as cytoskeleton (3-6). Diverse MS ion channel superfamilies, including the epithelial sodium channel/degenerin (ENaC/DEG), Piezo-type nonselective cation channels (e.g., Piezo1 and Piezo2), two pore-domain potassium ion channels (K2P/KCNK) (e.g., TREK/TRAAK), transient receptor potential (TRP) nonselective cation channels, and transmembrane protein 16/Anoctamin (TMEM16/Ano) Ca^{2+} -activated chloride channels, are known (7). These channels possess different structures and mechanotransduction mechanisms with distinct biological roles. Notably, MS ion channels are not only expressed in sensory cells but also in non-sensory cells (8, 9), playing important roles in a broad range of cellular functions.

One of the most important roles of MS ion channels is to properly regulate cell death as dysregulated intracellular ion homeostasis results in cell death by triggering cell shrinkage (i.e., apoptosis) or swelling (i.e., necrosis) (10). Recent studies have demonstrated that the activation of MS ion channels mediates cell deaths led by mechanical stimulation (11-13). Uniaxial cyclic tensile strain induced apoptosis in mesenchymal stem cells by opening L-type voltage-gated Ca^{2+} channels that subsequently activated calpain protease and c-Jun N-terminal kinase (JNK), causing caspase-dependent DNA fragmentation (14). In addition, intracellular accumulation of Ca^{2+} was strain-dependent and correlated with apoptosis in aortic valve interstitial cells (15). The mechanical onset of apoptosis was prevented upon a treatment with gadolinium (Gd^{3+}), which is an inhibitor of mechanically-gated cation channels (16, 17). As uncontrolled cell death can lead to multiple human diseases, including osteoarthritis, metabolic syndrome, and cancer (18, 19), targeting MS ion channels can have potential therapeutic effects. In this review, we focus on the mechanisms by which Piezo-type MS Ca^{2+} -permeable channels regulate apoptosis and ferroptosis, the most typical and newest type of cell death, respectively. Furthermore, therapeutic strategies to inhibit or induce cell death by modulating the activity of Piezo channels using pharmacological drugs or mechanical stimuli are discussed.

MECHANOSENSITIVE ION CHANNELS

MS ion channels sense and transduce external and internal mechanical stimuli into different biochemical responses (20,

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21). In response to the presence of mechanical forces in cell membranes, the MS ion channels open in a pore-like manner via a conformational change induced by membrane tension (i.e., stretch) or physical connections, the so-called spring-like tethers, with the extracellular matrix and cortical cytoskeleton. The bacterial large-conductance MS ion channel (MscL) is an example of one such channel, which opens by membrane tension to prevent cell lysis under osmotic stress (22). Eukaryotic MS ion channels were first documented in 1984 in the skeletal muscle of embryonic chicks using patch-clamp technique (23). About 37 years later, the 2021 Nobel Prize in Physiology or Medicine was awarded to David Julius and Ardem Patapoutian for their discovery of vertebrate thermo-sensory TRP channels and mechanosensory Piezo-type ion channels, respectively (24).

Piezo1 (a.k.a. *Fam38A*) and its paralog Piezo2 (a.k.a. *Fam38B*) were discovered in 2010 using a small interfering RNA (siRNA)-based screening and were named after the Greek “πιεση” (piesi) meaning pressure (25). There exists 47% identity in the amino acid sequence of the extracellular cap domain, which plays a decisive role in conferring the distinct kinetics of inactivation, between mouse Piezo1 and Piezo2 (25-27). The structure of Piezo channels resembles that of a three-bladed propeller and changes from a closed to an open state (i.e., pore-forming) upon mechanical stimulation, thereby allowing the influx of cations, particularly that of Ca^{2+} , into the cytosol (27). Piezo channels are primarily located in the plasma membrane as well as in the endoplasmic reticulum (ER) and nuclear membranes, wherein these channels sense the membrane displacement that occur owing to regional and overall mechanical pressure (28-31). Since the discovery of Piezo channels that convert mechanical stimuli to intracellular signaling (i.e., mechanotransduction) via ion flux, which is critical for various biological processes, it has been cumulatively reported that Piezo channels play important regulatory roles in a number of cellular functions, including proliferation, migration, and differentiation (32-35).

THE ROLE OF PIEZO1 IN APOPTOSIS

Apoptosis is a mode of programmed cell death with distinct morphological features, including cell shrinkage, chromatin condensation leading to pyknosis (during the early phase), and plasma membrane blebbing followed by karyorrhexis and separation of cell fragments into apoptotic bodies (during the late phase) (36). The apoptotic bodies are subsequently phagocytosed and degraded by macrophages and parenchymal or neoplastic cells. Apoptosis normally acts as a homeostatic mechanism that maintains the cell population during development and aging or as a host defense mechanism against viral infections. Hence, inappropriate apoptosis is closely related with many human diseases, including cancers.

Loss of MS ion channel-mediated Ca^{2+} homeostasis can lead to apoptosis as cellular Ca^{2+} overload and perturbation of intracellular Ca^{2+} compartmentalization are well-known to be

cytotoxic (37). Ca^{2+} released from stressed ER via ryanodine receptors (RyRs) and inositol-1,4,5-trisphosphate receptors (Ins(1,4,5)P₃Rs) into the cytosol is taken up by mitochondria through a mitochondrial calcium uniporter (MCU). Once Ca^{2+} binds to cardiolipin at the inner mitochondrial membrane, cytochrome c (Cyt c) is dissociated from cardiolipin and is released through mitochondrial permeability transition pore (MPTP) opening into the cytosol, wherein Cyt c forms the apoptosome complex with apoptotic protease activating factor-1 (APAF-1) and procaspase-9; this promotes the activation of caspase-9 and subsequently caspase-3, leading to apoptosis (38-40). Recently, the role of Piezo1 channel in apoptosis has been investigated and its implications for pathophysiology have been also reported (41).

At the steady state, Piezo1 functions to ensure cellular Ca^{2+} homeostasis, preventing p53-dependent senescence and apoptosis (42). Silencing of Piezo1 provoked compensatory upregulation of T-type voltage-gated Ca^{2+} channels, namely CaV3.1 and CaV3.2, in skeletal muscle stem cells. This led to Ca^{2+} -dependent activation of conventional protein kinase C (cPKC) to promote the expression of NOX4, a reactive oxygen species (ROS)-generating enzyme, causing p53-dependent senescence and apoptosis in Piezo1-deficient skeletal muscle stem cells. On the other hand, cells under abnormal mechanical stresses show increased expression and activation of Piezo1, thereby allowing substantial Ca^{2+} influx, which consequently leads to cytotoxicity. In human articular chondrocytes constantly exposed to hydrostatic pressure, the increase in intracellular Ca^{2+} levels was accompanied by the upregulated expression of p53 and cleaved caspase-3 and -9, which was inhibited upon the treatment with gadolinium (Gd^{3+}) or an siRNA for Piezo1 (17). Similarly, a recent study suggested that pancreatitis can be caused by chronic death of pancreatic acinar cells due to Piezo1-mediated Ca^{2+} overload following pressure on the gland (11). In mice, mechanical pressure within the gland led to the development of pancreatitis; however, the mice with pancreatic acinar cell-specific deletion of Piezo1 were protected from this. In addition, it was demonstrated that administration of Yoda1, a Piezo1 agonist, directly induced pancreatic acinar cell death. Hence, this study may explain why pancreatitis develops in humans after abdominal trauma, pancreatic duct obstruction, pancreatography, or pancreatic surgery, which can exert pressure on the gland.

Mechano-physical properties of the microenvironment in which cells reside can also control the activity of Piezo1 channel. Among others, stiffness is one of the most studied and critical physical features of various tissues. On a stiff substrate with high elastic modulus (25 kPa), human nucleus pulposus (NP) cells showed upregulated expression of MS Piezo1 channel as well as elevated levels of intracellular Ca^{2+} when compared to the cells on a soft substrate (1 kPa) (31). Excessive intracellular Ca^{2+} levels brought about an increase in the ROS levels and expression of ER stress markers, GRP78 and CHOP, subsequently leading to senescence and apoptosis

in Piezo1-activated NP cells on the stiff substrate. In a rat model of intervertebral disc degeneration, which is known to be developed by excessive mechanical loading on NP tissues, siRNA-mediated inhibition of Piezo1 effectively ameliorated the progression of intervertebral disc degeneration by reducing the elastic modulus of NP tissues and apoptotic cell population (31). Notably, a mechanical compression of human NP cells yielded similar results; more NP cells expressed Piezo1 and underwent cellular senescence or apoptosis when the extent of compression was higher or longer (43).

Taken together, increase in intracellular Ca^{2+} levels to the extent of cytotoxicity through the extensive activation of Piezo1 is a key step in initiating mitochondrial apoptotic pathway in the presence of mechanical stimuli, such as compression and substrate stiffness, which induce membrane displacement (Fig. 1). Thus, inhibition of Piezo1 or removal of abnormal mechanical stimulation are potentially therapeutic options for apoptosis-associated diseases.

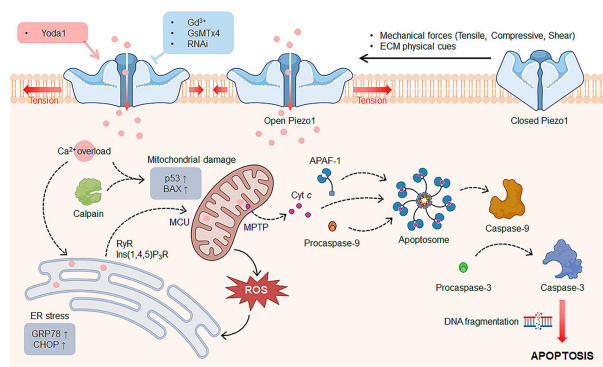


Fig. 1. Piezo1-mediated Ca^{2+} -dependent apoptosis mechanism. Piezo1, a mechanosensitive ion channel, opens in response to mechanical (tensile, compressive, shear) forces and physical cues in the extracellular matrix (ECM) that induce membrane tension, allowing Ca^{2+} to enter the cytosol. Mitochondrial damage caused by Ca^{2+} overload increases the levels of reactive oxygen species (ROS) that cause endoplasmic reticulum (ER) stress, indicated by upregulation of GRP78 and CHOP. Ca^{2+} is then released from the stressed ER via ryanodine receptors (RyRs) and inositol-1,4,5-trisphosphate receptors (Ins(1,4,5)P₃Rs) and taken up by mitochondria via mitochondrial calcium uniporters (MCU). In addition, cytosolic Ca^{2+} activates the calpain protease to cleave and translocate p53 and Bcl-2-associated X (BAX) proteins to the mitochondria. Once Ca^{2+} binds to cardiolipin at the inner mitochondrial membrane, cytochrome c (Cyt c) is dissociated from cardiolipin and the BAX protein weakens mitochondrial outer membrane integrity, then releases Cyt c into the cytosol through the mitochondrial permeability transition pore (MPTP). Subsequently, Cyt c forms an apoptosome complex with apoptotic protease activating factor-1 (Apaf-1) and procaspase-9 to promote the activation of caspase-9 and caspase-3 to initiate apoptosis. Piezo1-mediated apoptosis under mechanical stress can be inhibited by treatment with Gd^{3+} (an inhibitor of mechanically gated cation channels), GsMTx4 (a peptide blocker of Piezo1), or RNA interference (RNAi) to Piezo1, whereas the Piezo1 agonist Yoda1 promotes Piezo1-induced apoptosis by lowering the mechanical activation threshold of Piezo1.

THE ROLE OF PIEZO1 IN FERROPTOSIS

Ferroptosis is an iron-dependent form of programmed cell death characterized by the accumulation of lipid peroxides and unique morphological features (44). Under a microscope, ferroptotic cells show obvious mitochondrial shrinkage with increased mitochondrial membrane densities and reduced mitochondrial crista, mitochondrial outer membrane rupture, intact plasma membrane, and normal nucleus (45). The mechanisms underlying ferroptosis involve reduced intracellular glutathione (GSH) levels and decreased activity of glutathione peroxidase-4 (GPX-4), an enzyme that metabolizes lipid peroxides into non-toxic lipid alcohols (44). Otherwise, Fe^{2+} oxidizes lipids in a Fenton-like manner, resulting in a large amount of ROS, thereby promoting ferroptosis, which can be prevented by iron chelators (46). Several mechanisms have been proposed for how ferroptosis is induced, including the calcium-dependent mechanisms. Adding extracellular glutamate induces GSH depletion in cells by blocking the glutamate-cystine antiporter system Xc⁻ (transporter subunit is encoded by the *SLC7A11* gene), eventually resulting in ferroptosis. Henke et al. (47) observed an increase in extracellular Ca^{2+} influx in cells undergoing extracellular glutamate-induced ferroptosis. Inappropriate Ca^{2+} influx resulted due to a significant induction of store-operated calcium entry caused by the upregulation of a selective Ca^{2+} channel, namely the calcium release-activated calcium channel protein 1 (ORAI1).

Recent studies have indicated that Piezo1 as a MS Ca^{2+} channel also plays an important role in ferroptosis. As GPX-4 acts as a gatekeeper against ferroptosis, it is suppressed in ferroptosis and often used as a marker for its evaluation. When subjected to mechanical loading concentrated in knee joints by destabilizing the medial meniscus, chondrocyte-specific *Gpx-4* knockout mice developed more severe osteoarthritis compared with their wild-type littermates (48). Mechanical loading on human cartilage and mouse chondrocytes increased Piezo1 expression and triggered GPX-4-mediated ferroptosis, which was attenuated upon the suppression of Piezo1 activity using GsMTx4, a selective inhibitor of cationic MS channels, or preventing extracellular Ca^{2+} influx, thereby eventually reducing the severity of osteoarthritis. However, treatment with GsMTx4 was ineffective in terms of reversing the mechanical overload-induced chondrocyte damage in *Gpx-4* knockout mice, hinting at Piezo1 induced ferroptosis via GPX-4 inhibition.

Ionizing radiations induce lung injury by destroying pulmonary endothelial cell (PEC) functions (49). Recently, it was reported that PECs exposed to ionizing radiations underwent ferroptosis (49). In accordance with the finding that Piezo1 expression was increased in PECs after the exposure to ionizing radiations, Yoda1 treatment expanded ferroptotic cell population among non-radiated PECs while treatment with GsMTx4 reduced the number of ferroptotic cells among the radiated PECs. Mechanistically, it was demonstrated that elevated intracellular Ca^{2+} levels increased calpain protease activity, which degraded VE-cadherin to promote ferroptosis. Similarly, it was

reported that E-cadherin inhibited ferroptosis by activating the NF2-Hippo pathway, which suppresses pro-ferroptotic YAP activation in epithelial cells (50).

Taken together, the role of Piezo1 in ferroptosis depends on the regulation of GPX-4 (Fig. 2); future studies are required to analyze how Piezo1 controls the GPX-4 activity and whether Ca^{2+} ions are involved in the regulation of GPX-4.

POTENTIAL OF TARGETING MECHANOSENSITIVE ION CHANNELS FOR CANCER TREATMENT

Cancer cells acquire resistance to apoptosis due to their heterogeneity and mutations, leading to malignant progression and recurrence after anti-cancer therapies. Therefore, selective induction of apoptosis in cancer cells is of great importance for cancer treatment. As over- or extended activation of MS cation channels disrupts Ca^{2+} homeostasis, often causing cell deaths, targeting MS cation channels is a promising strategy for killing cancer cells. In early 2010, Kim *et al.* (51) developed disc-shaped magnetic microparticles (approximately 1 μm in diameter), which oscillate under an alternating magnetic field and transmit a cytotoxic level of mechanical force to cancer cells. Thus,

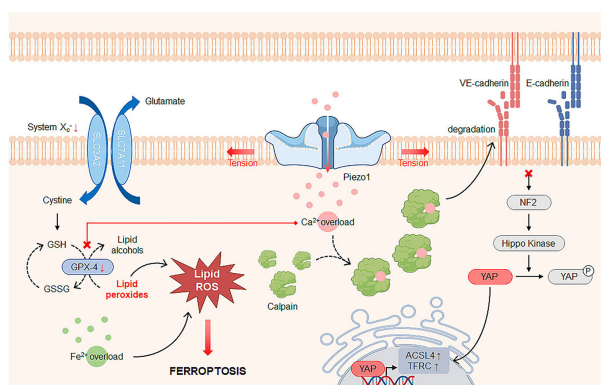


Fig. 2. Piezo1-mediated Ca^{2+} -dependent ferroptosis mechanism. Piezo1 is activated upon mechanical stimulation and increases the intracellular Ca^{2+} level, increasing the activity of the calpain protease that degrades (V)E-cadherin. Cells with loss of (V)E-cadherin and subsequent inactivation of NF2 and hippo kinase are susceptible to ferroptosis due to upregulation of multiple regulators of ferroptosis including ACSL4 and TFRC through YAP activation. The cystine/glutamate antiporter system Xc- (consisting of light chain SLC7A11 and heavy chain SLC3A2) mediates uptake of cystine for the production of cysteine and glutathione (GSH), which is converted to glutathione disulfide (GSSG) by glutathione peroxidase-4 (GPX-4), an antioxidant enzyme that metabolizes lipid peroxides into non-toxic lipid alcohols. However, Ca^{2+} suppresses the expression of GPX-4, which promotes accumulation of lipid peroxides that increase lipid ROS, consequently causing ferroptosis. Otherwise, Fe^{2+} binds to reactive oxygen species (ROS) to participate in iron-dependent lipid peroxidation and finally induce ferroptosis. Abbreviations: ACSL4, Acyl-CoA Synthetase Long Chain Family Member 4; TFRC, Transferrin Receptor 1.

interfacing with these microdiscs disturbed membrane integrity and increased intracellular Ca^{2+} levels, possibly through the activation of MS ion channels, which eventually induced programmed death in cancer cells (Fig. 3A). These microdiscs can be biofunctionalized with antibodies capturing cancer cell surface markers to enhance target specificity; they can further be scaled down to nanodiscs (approximately 100 nm in diameter).

To overcome the reduced sensitivity of cancer cells toward apoptosis, Wen *et al.* (52) induced non-apoptotic cell death in cancer cells by introducing a gain-of-function mutation of MscL. The gain-of-function mutant V23A-MscL constitutively opened, thus enabling intracellular Ca^{2+} overload, which resulted in non-apoptotic cell death characterized by cytoplasmic vacuolization in human hepatocellular carcinoma cells transduced with V23A-MscL; it also suppressed the tumor growth in a xenograft model of human hepatocellular carcinoma in mice. Notably, mutant G26C-MscL, which opened in response to [2-(trimethylammonium)ethyl] methane thiosulfonate bromide (MTSET), induced necrotic cell death with distinct morphological features such as disruption and aggregation of ER, membrane leakage following organelle-free membrane blebbing, impaired nuclei, and dispersed chromatin followed by clearing of the cytosol and plasma membrane rupture (52).

Recent studies have shown that cancer cells with higher sensitivity to mechanical stresses than normal cells undergo Ca^{2+} -dependent apoptosis upon mechanical perturbations. This distinct feature of cancer cells can be leveraged for their selective killing with minimal effect on normal cells by applying mechanical stresses via stretching or ultrasound.

Vulnerability of cancer cells to mechanical stress

Fundamental differences exist between normal and cancer cells, including biochemical signaling, metabolism, and anchorage (in)dependence. Besides, it has been proven that cancer cells also differ from normal cells in terms of mechanosensing, which influences cell fate under different mechanical stresses. To evaluate whether stretching reduces the tumor growth, primary mouse mammary tumor cells were implanted within third mammary fat pad tissues either subjected to stretching or non-stretched (53). Regular stretching markedly diminished tumor volume in mice compared to the non-stretched mice. Remarkably, with the significant upregulation of specialized pro-resolving lipid mediators (SPMs) RvD1 and RvD2, the proportion of IL-2⁺ CD8⁺ cytotoxic T cells increased while that of PD-1⁺ CD8⁺ exhausted T cell population decreased in the stretched mice, indicating an improvement in cytotoxic immune responses upon stretching. More directly, Tijore *et al.* (54) revealed that cyclic stretching lowered the growth rate and finally caused apoptosis in cancer cells, whereas it stimulated the growth of normal cells. The stretch-induced apoptosis occurred in cancer cells through Piezo1-mediated Ca^{2+} influx that activates calpain-2, leading to the cleavage and translocation of Bcl-2-associated X (BAX) proteins into mitochondria where they weaken mitochondrial outer membrane integrity followed

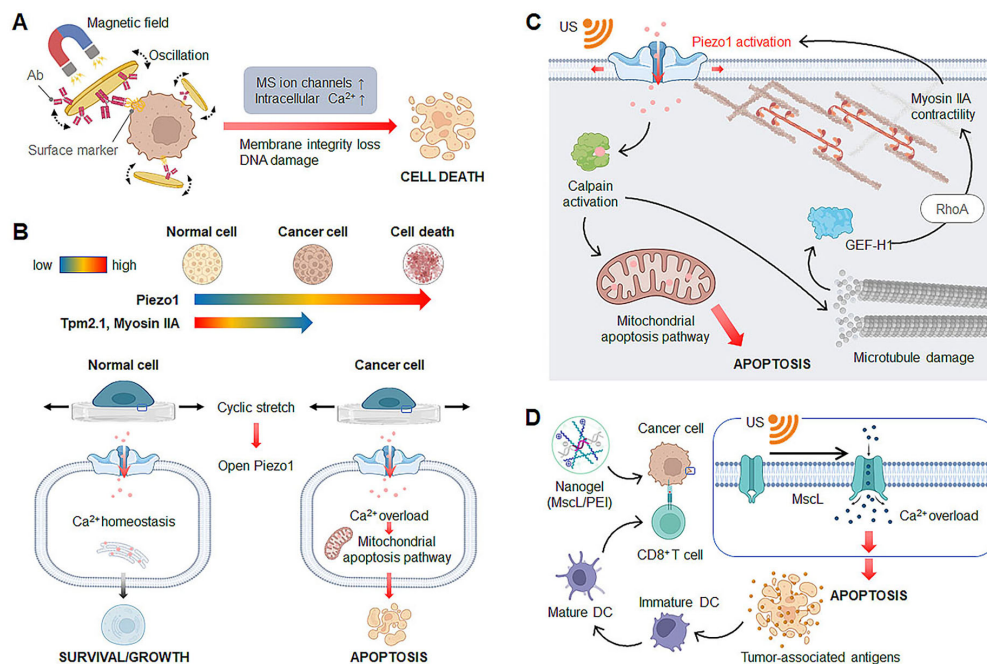


Fig. 3. Cancer treatment strategies through the induction of mechanosensitive ion channel-mediated Ca^{2+} -dependent cell death by mechanical perturbations. (A) Microdiscs biofunctionalized with an antibody (Ab) that captures cancer cell surface marker vibrate (i.e., oscillation) under a magnetic field to deliver cytotoxic levels of mechanical force to cancer cells. Interfacing with these microdiscs disrupts membrane integrity and increases intracellular Ca^{2+} levels, presumably through activation of mechanosensitive (MS) ion channels, eventually leading to programmed cell death in cancer cells. (B) Schematic diagram highlighting the different expression of mechanosensing proteins including Piezo1, tropomyosin 2.1 (Tpm2.1) and myosin IIA between normal and cancer cells. Cancer cells have higher levels of Piezo1 than normal cells, making them more susceptible to apoptosis due to the increase in intracellular Ca^{2+} levels to the extent of cytotoxicity upon mechanical stimulation. Cyclic stretching stimulates normal cell growth while slowing growth and eventually causing apoptosis in cancer cells. Stretch-induced apoptosis in cancer cells occurs through the Piezo1-mediated Ca^{2+} -dependent mitochondrial apoptosis pathway. Tpm2.1 is eliminated from cancer cells, allowing them to grow anchorage-independently. Cancer cells recovered with Tpm2.1 survive and proliferate upon cyclic stretching, whereas normal cells depleted of Tpm2.1 become susceptible to stretch-induced apoptosis. In addition, restoration of myosin IIA inhibits cancer cell growth, which can induce apoptosis through induction of Piezo1 activation (see Fig. 3C). (C) Piezo1 is activated by ultrasound (US)-mediated mechanical force, triggering Ca^{2+} influx to activate calpain proteases, which activates the mitochondrial apoptosis pathway and induces microtubule disassembly. Destabilized microtubules enhance myosin IIA contractility through activation of GEF-H1, a Rho guanine nucleotide exchange factor that can increase RhoA activity. Increased contractility, in turn, promotes Piezo1 expression and localization to peripheral adhesions where Piezo1 channel opening allows Ca^{2+} influx as a positive feedback loop. (D) Cancer cells in contact with nanogels carrying the MscL plasmid and polyethylenimine (PEI) as a transfection reagent are induced to express and activate MscL MS ion channels upon mechanical US waves, resulting in sustained Ca^{2+} entry to cause apoptosis when ultrasound was applied. Apoptotic cell fragments act as tumor-associated antigens to promote dendritic cell (DC) maturation and subsequent anti-cancer CD8⁺ T cell activation. Schematic illustrations were recomposed based on key finding in refs. (51) Kim *et al.* Nat Mater (2010) for (A), (54) Tijore *et al.* Biomaterials (2021) for (B), (61) Singh *et al.* Bioeng Transl Med (2021) for (C) and (65) He *et al.* Chem Eng J (2021) for (D).

by the leakage of Cyt c into the cytosol to activate caspase-3 (Fig. 3B). Interestingly, tropomyosin 2.1 (Tpm2.1)-restored cancer cells survived and proliferated upon cyclic stretching, while Tpm2.1-depleted normal cells were vulnerable to stretch-induced apoptosis. Tpm2.1 was previously shown as a rigidity sensing protein that disappears in malignantly transformed cells, enabling them to grow on soft matrices (i.e., anchorage-independent growth or anoikis resistance) (55). These findings indicate that proteins involved in mechanosensing are critical for cell survival.

Non-invasive induction of cancer cell death using ultrasound

More recently, ultrasound has been used for non-invasive cancer treatment. Tijore *et al.* (56) have reported that cancer cells, but not normal cells, undergo Ca^{2+} -dependent apoptosis through Piezo1 channel by activating a calpain-dependent mitochondrial apoptosis pathway in response to mechanical perturbation by low-frequency ultrasound. The Piezo1 channels on pancreatic ductal adenocarcinoma (PDAC) cells were gated by ultrasound with microbubbles that generated mechanical forces via cavitation, which triggered mitochondrial BAX-Cyt c apoptosis pathway, possibly through the activation of Ca^{2+} -dependent calpain proteases (57). The anti-cancer effect of ultra-

sound with microbubbles was reduced in *Piezo1*-knockdown PDAC xenograft models. The involvement of Piezo1 in ultrasound-mediated cancer cell apoptosis may also explain how the application of ultrasound with microbubbles was therapeutically effective in other types of cancers, including breast, colon, and liver cancers (57-60).

Combination of ultrasound and chemotherapeutic drugs is synergistic in treating cancer. Singh *et al.* (61) demonstrated that ultrasound-mediated mechanical forces disrupt microtubules via the activation of calpain proteases. Destabilized microtubules enhanced myosin IIA contractility through activation of GEF-H1, a Rho guanine nucleotide exchange factor that can increase RhoA activity. Consistently, myosin IIA was reported as a rigidity sensing protein, and its restoration inhibited cancer cell growth (55). Increased contractility, in turn, promoted Piezo1 expression and localization to the peripheral adhesions where opening of Piezo1 channel allowed Ca^{2+} influx as a feedback loop (Fig. 3C), suggesting that ultrasound can enhance cancer cell death when combined with microtubule destabilizing agents (61).

Indeed, mechanical induction of Ca^{2+} -dependent apoptosis in cancer cells relies on higher expression of Piezo1 in cancer cells than in normal cells (Fig. 3B). Piezo1 is highly expressed in human PDAC and gastric cancer cells and tissues (57, 62). Inhibiting Piezo1 decreased the viability of cancer cells, while activation of Piezo1 with Yoda1 at optimal concentration promoted colony formation (62), thereby suggesting the presence of distinct control mechanisms of Ca^{2+} signaling between normal and malignant cells (63). However, higher concentration of Yoda1 led to the apoptosis of cancer cells, which might be due to extreme Ca^{2+} levels (62). Supportively, metastatic malignant cells with a lower expression of Piezo1 channel than primary cancer cells were resistant to Piezo1-mediated apoptosis when exposed to fluid shear stress; this is possibly because metastatic cells must survive aberrant fluid shear stress in the circulation (64).

Hence, Ca^{2+} -dependent apoptosis can be achieved by introducing ectopic MS ion channels in cancer cell. He *et al.* (65) adopted a sonogenetic nanosystem, which was consisted of iron alginate nanogel as a carrier, MscL plasmids and polyethylenamine (PEI) as a transfection reagent; upon exposure to ultrasound, the MscL MS ion channel was specifically expressed and activated in nanogel-contacted cells. In cancer cells treated with this sonogenetic nanosystem, MscL was expressed on cell surfaces and was activated to allow continuous Ca^{2+} entry, causing apoptosis when ultrasound was applied. Furthermore, in mice inoculated with mouse melanoma cells, ultrasound triggered not only melanoma cell apoptosis but also anti-cancer immune responses; this was because apoptotic cell fragments acted as tumor-associated antigens to promote dendritic cell maturation and subsequent CD8^+ T cell activation (Fig. 3D).

CONCLUSION

Programmed cell death due to intracellular Ca^{2+} overload after exposure to mechanical stimuli is called “mechanoptosis”, which is regulated by MS ion channels such as Piezo1 (61). Overexpression or hyperactivation of Piezo1 induces uncontrolled Ca^{2+} -dependent cell deaths, including apoptosis and ferroptosis, causing a plethora of human diseases. Hence, Piezo1 is promising as a therapeutic target. For example, Piezo1 blockade using RNA interference or a peptide inhibitor (e.g., GsMTx4) may relieve pancreatitis and osteoarthritis by preventing Ca^{2+} -dependent apoptosis/ferroptosis of pancreatic acinar cells and chondrocytes, respectively. However, the therapeutic potential of Piezo1 inhibition in other human disorders, such as developmental disorders, immunodeficiency, autoimmune diseases, and neurodegeneration, remains to be investigated.

On the other hand, cancer cells are intrinsically resistant to programmed cell death because they differ from normal cells in terms of expression of mechanosensing proteins, including Tpm2.1, myosin IIA, and Piezo1. Because the basal levels of Piezo1 expression are higher in most cancer cells than those in normal cells, the induction of Piezo1 may have potential anti-cancer therapeutic effects. Considering this, Piezo1 agonist (e.g., Yoda1) or mechanical stimulation (e.g., stretch or ultrasound) has been applied to cause Ca^{2+} -dependent cancer cell death, while promoting the ability of the normal cells to regenerate. In conclusion, information pertaining to Piezo1 expressional levels and subcellular localization at different developmental and/or environmental stages will help in developing therapeutic strategy against cancer. Advances in biomedical engineering would assist in developing anti-cancer devices that locally generate a mechanical stimulus as well as in designing cancer-specific induction of Piezo1-mediated genetic circuit upon mechanical perturbations.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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