RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2025.067

ScienceAsia 51 (4): 2025: ID 2025067: **1–10**

Emodin alleviates mechanical stress-induced chondrocyte apoptosis via the Piezo1 channel

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Received 13 Nov 2024, Accepted 17 Jun 2025
Available online 30 Aug 2025

ABSTRACT: This study investigates the protective effects of emodin on mechanical stress-induced chondrocyte apoptosis, focusing on the role of the Piezo1 channel. Rabbit articular chondrocytes were subjected to varying levels of mechanical stress, and the impact of emodin was evaluated using apoptosis assays, calcium measurements, Western blot analysis, and Reverse transcription–quantitative polymerase chain reaction. Under high mechanical strain, Piezo1 expression and intracellular calcium levels significantly increased, correlating with elevated apoptosis. Emodin treatment downregulated Piezo1 expression, decreased calcium influx, and reduced apoptosis rates. To clarify the involvement of Piezo1, we used gene silencing and pharmacological activation strategies. Piezo1 knockdown enhanced the protective effects of emodin, whereas its activation diminished them, resulting in increased apoptosis. These findings indicate that the therapeutic effects of emodin may be mediated by its ability to inhibit Piezo1 activity, thereby offering a potential multi-targeted approach for conditions like osteoarthritis, where excessive mechanical stress accelerates joint degeneration. The modulation of Piezo1 and calcium signaling by emodin presents a novel intervention for mechanical stress-related joint diseases.

KEYWORDS: emodin, chondrocytes, apoptosis, mechanotransduction, Piezo1 protein

INTRODUCTION

Mechanical stress is a key factor in the physiological and pathological processes of chondrocytes. While moderate mechanical stress helps maintain cartilage homeostasis by regulating programmed cell death, excessive stress has a detrimental effect. Zhang et al [1] demonstrated that moderate mechanical stress supports chondrocyte survival by modulating mitochondrial function and apoptosis. In contrast, excessive mechanical stress induces chondrocyte damage and apoptosis, contributing to cartilage degradation and inflammatory responses, thereby accelerating the progression of osteoarthritis [2].

Piezo1 is a recently identified mechanosensitive ion channel that plays a crucial role in the chondrocyte response to mechanical stress. This channel is activated under mechanical strain and plays a significant role in various intracellular signaling processes. Research shows that Piezo1 regulates calcium ion influx in chondrocytes during mechanical stress, influencing cellular metabolism and function [3]. Piezo1 works in concert with the mechanotransduction processes in chondrocytes when combined with other mechanosensitive channels such as Transient Receptor Potential Vanilloid 4 (TRPV4) [4]. During physiological activities like walking and exercise, chondrocytes experience strains of approximately 10–40%. This causes Ca^{2+} to enter the cells via activated TRPV4 channels, which then trigger anabolic responses. However, when cell strains exceed 50% due to injurious loads, the Piezo1 channel becomes active and responds to the high-strain mechanical stress [5]. Excessive Piezo1 activation beyond an optimal level can cause apoptosis, cartilage degeneration, and the progression of arthritis [6, 7].

Emodin (3-methyl-1, 6, 8-trihydroxyanthraquinone), an anthraquinone compound derived from the traditional Chinese herb *Rheum palmatum*, is a powerful agent with a range of biological activities, including anti-inflammatory [8], antioxidant [9], and anticancer activities [10]. Recent studies have proven that emodin offers significant protection to articular chondrocytes. Emodin effectively alleviates chondrocyte apoptosis induced by mechanical stress by inhibiting inflammatory factors and oxidative stress responses [11, 12]. Furthermore, emodin modulates signaling pathways, such as inhibition of the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Mitogen-activated protein kinase (MAPK) pathways, to protect chondrocytes [13, 14].

Mechanical stress-induced apoptosis is a hallmark of cartilage degeneration in osteoarthritis, and Piezo1 has recently been identified as a key mechanosensitive ion channel involved in this process. Piezo1 activation promotes calcium influx and downstream apoptotic signaling under high strain conditions. On the other hand, emodin, a plant-derived anthraquinone compound, has shown anti-inflammatory and anti-apoptotic effects in osteoarthritic models and has been reported to modulate ion channels and calcium sig-

naling [15]. However, whether emodin can modulate Piezo1 activity or expression in chondrocytes remains unknown. Therefore, we hypothesize that emodin may exert its protective effects on chondrocytes under mechanical stress by modulating Piezo1 expression or activity, thereby reducing intracellular calcium overload and apoptosis.

MATERIALS AND METHODS

Materials

All reagents were purchased from commercial suppliers. Rabbit chondrocytes and their culture medium (IMP-RA082-1, IMMOCELL, Xiamen, China) were used for cell culture. Emodin (HY-14393, MCE, NJ, USA), fetal bovine serum (FBS, 10270-106, Gibco, Grand Island, NY, USA), DMEM, and Opti-MEM media (Gibco) were included in the study. Phosphate-buffered saline (PBS, BL302A, Biosharp, Hefei, China) was applied for cell washing. Additionally, the rabbit monoclonal Piezo1 antibody (ab128245, Abcam, Cambridge, UK) was used for immunodetection. For RNA extraction and quantitative PCR, reagents including the RNA Isolator Total RNA Extraction Reagent (R401-01, Vazyme, Nanjing, China) and Taq Pro Universal SYBR qPCR Master Mix (Q712-02, Vazyme) were utilized. GelRed Nucleic Acid Gel Stain (10,000X, 41003, Biotium, San Francisco, USA) was employed for nucleic acid staining.

Cell culture and pressurization

Rabbit articular chondrocytes (IMP-RA082, IMMOCELL) were seeded at 1×10^5 cells/cm^2 in DMEM with 10% FBS (10270-106, Gibco) and 1% penicillin-streptomycin (Gibco) at 37 °C, 5% CO_2. The culture medium was renewed every 2–3 days, and cells were cultured to 80–90% confluence before treatment in Opti-MEM (Gibco). Second-passage (P2) cells were used for all experiments to preserve cartilage-specific characteristics and minimize dedifferentiation. Mechanical pressure was applied using the Programmable Mechanical Cell Compress System (Hangzhou surface power technology Co., Ltd., Hangzhou, China), which is equipped with an air pump that generates hydrostatic pressure, delivering compression to one or multiple groups of tissue samples. Chondrocytes were seeded into 45 mm Petri dishes and cultured to confluence before pressure application. Subsequently, the Petri dishes were positioned within the pressure chamber. The internal pressure was continuously calibrated and monitored, with a ± 2 kPa accuracy to ensure consistent and reproducible mechanical stress across groups.

Study design and drug treatment

To further elucidate the effects of specific pressure values on *in vitro* cultured articular chondrocytes, we referenced a previous study [16]. In light of these findings, the study was divided into 3 initial groups: NC Group (Normal Control): Chondrocytes cultured under standard conditions; Low-Strain Group: Chondrocytes exposed to mechanical pressure of 40 kPa for 6 h, applied at a frequency of 0.1 Hz; High-Strain Group: Chondrocytes exposed to mechanical pressure of 70 kPa for 6 h, applied at a frequency of 0.1 Hz. Following this intervention, we sought to observe the effects of emodin on chondrocyte apoptosis under conditions of mechanical stress. Chondrocytes were cultured on flexible-bottom plates and subjected to cyclic hydrostatic pressure using a programmable mechanical cell compression system. A pressure of 70 kPa was applied at a frequency of 0.1 Hz for 6 h, mimicking pathological mechanical loading associated with cartilage degeneration. The study was subsequently expanded to include 4 additional groups: Control Group (Ctrl Group): Rabbit chondrocytes cultured under mechanical stress without any treatment; Emodin Group: Rabbit chondrocytes cultured under mechanical stress and treated with 10 μM emodin [17]; Emodin + si-Piezo1 Group: Rabbit chondrocytes treated with siRNA to silence Piezo1, followed by treatment with 10 μM emodin under mechanical stress; Emodin + Yoda1 Group: Rabbit chondrocytes initially treated with the Piezo1 activator Yoda1 (5 μM) [18], followed by treatment with 10 μM emodin under mechanical stress. In the Emodin-related groups, chondrocytes were subjected to a mechanical pressure of 70 kPa for 6 h to simulate high-strain mechanical stress conditions. The emodin concentration of 10 μM was selected based on prior studies that demonstrated effective anti-apoptotic effects in chondrocytes without cytotoxicity. Concentrations above 20 μM have been shown to reduce chondrocyte proliferation [17, 19]. Emodin at a concentration of 10 μM was added to the culture medium 30 min before mechanical stress was applied. After 6 h of mechanical loading with emodin exposure, samples were immediately collected for analysis. The experimental design permitted an evaluation of the impact of mechanical stress on chondrocytes and an assessment of the potential protective effects of emodin against apoptosis under these conditions.

Piezo1 siRNA

The target sequence for si-Piezo1 (siB140821184040-1-5, Ribobio, Guangzhou, China) was 5′-CGGCCAACATAAAG AACAT-3′, and the si-NC (negative control) sequence was 5′-UUCUCCGAACGUGUCACGUTT-3′. Chondrocytes were transfected with either si-Piezo1 or si-NC using Lipofectamine 3000 (Invitrogen, California, USA) following the manufacturer's instructions. Following a 6-h incubation period, the Opti-MEM medium (Gibco) was replaced with DMEM medium (YOBIBIO, Shanghai, China).

Apoptosis assay

Chondrocytes were stained with Annexin V-FITC and propidium iodide (PI) according to the manufacturer's protocol (Beyotime, Shanghai, China). After staining, 2×10^5 cells per sample were acquired on a BD FACSCalibur flow cytometer (BD Biosciences, New Jersey, USA). Flow cytometric data were analyzed using FlowJo software (version X.0.7), and a consistent gating strategy was applied across all groups.

Measurement of intracellular calcium levels

Fluo-4 AM (Biyuntian, Shanghai, China) was used to measure intracellular calcium levels [20]. Following the manufacturer's protocol, Fluo-4 AM was diluted to a concentration of 1 µM in PBS. After washing the chondrocytes with HBSS, the chondrocytes were incubated with Fluo-4 AM for 30 min. Images were captured using a Nexcope NIB610-FL inverted microscope and quantified using ImageJ software (version 1.8.0). Mean Fluorescence Intensity (MFI) was calculated as the integrated fluorescence density within selected regions of interest (ROIs) divided by area and averaged across 3 independent replicates.

Western blot analysis

Total lysates of rabbit chondrocytes were prepared using RIPA buffer (Beyotime). After SDS-polyacrylamide gel electrophoresis, the proteins were transferred onto PVDF membranes using a semi-dry transfer method at 250 mA for 1 h with pre-chilled transfer buffer. After blocking with 5% skim milk powder for 1 h at room temperature, the membranes were incubated with primary antibodies against Piezo1 (1:2000; cat. no. ab128245; Abcam) and GAPDH (1:5000; cat. no. GB15002; Servicebio, Wuhan, China) overnight at 4 °C. The membranes were washed and incubated with HRP-conjugated goat anti-mouse IgG secondary antibody (1:15,000; cat. no. G1214; Servicebio) for 1 h at room temperature. Finally, the PVDF membrane was washed 3 times with TBST for 5 min each. Enhanced chemiluminescence (ECL) detection kit (Thermo Fisher Scientific, MA, USA) was utilized to visualize specific bands. Chemiluminescent signals were analyzed using a ChemiDoc XRS gel imaging system (Bio-Rad Laboratories, CA, USA), and the intensity of specific bands was measured using ImageJ software. This method allowed for precise quantification of Piezo1 protein expression in rabbit chondrocytes under various experimental conditions.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from rabbit chondrocytes using the RNA Isolator Total RNA Extraction Reagent (Vazyme) according to the manufacturer's protocol. To ensure the complete removal of genomic DNA, a DNase treatment step was included during the reverse transcription process. Specifically, 4 µg of total RNA was treated using the HiScript II Q RT SuperMix for qPCR with gDNA wiper (Vazyme), which included a gDNA wiper mix for eliminating any residual genomic DNA. The resulting cDNA was then used for RT-qPCR, which was performed with the Taq Pro Universal SYBR qPCR Master Mix (Vazyme) on a Bio-Rad Real-Time PCR system (Bio-Rad Laboratories). The relative expression levels of target genes, including *Piezo1* and *Bax*, were calculated using the $2^{-\Delta\Delta Ct}$ method, with GAPDH as the internal control. Fold changes in gene expression were determined by comparing each experimental group to a designated control group. For mechanical stress comparisons, fold expression was normalized to the Normal Control (NC) group. For drug treatment comparisons (e.g., Emodin, siRNA, Yoda1 groups), fold expression was normalized to the Mechanical Stress Control (Ctrl) group. All comparisons were specified in the respective figure legends. The primers used for these reactions are listed in Table S1.

Molecular docking

The 2D structure of emodin was obtained from the PubChem online database (https://pubchem.ncbi.nlm.nih.gov/) and converted to MOL2 format using OpenBabel software. Next, the PDB file of Piezo1 (PDB code 4RAX) was downloaded from the Protein Data Bank (https://www.rcsb.org/). Using PyMOL, water molecules and ligands were removed from the structure. The prepared files were uploaded into Autodock 4.2 software for molecular docking.

Statistical analyses

All experiments were independently repeated 3 times ($n = 3$). Data are expressed as the mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test for multiple group comparisons. The Bonferroni method was chosen for its ability to reduce false positives (Type I error) when comparing multiple groups. Statistical significance was set at $p < 0.05$. Graphs were generated using GraphPad Prism version 6.

RESULTS

Effect of different mechanical stress on chondrocyte apoptosis

Flow cytometry analysis revealed that different levels of mechanical stress resulted in varying degrees of chondrocyte apoptosis. The Normal Control (NC) group showed a baseline apoptosis level of 3.29% ± 0.14. In the Low Strain group, apoptosis increased moderately to a mean value of 7.73% ± 0.56, indicating that even low levels of mechanical stress significantly promote chondrocyte apoptosis. The High

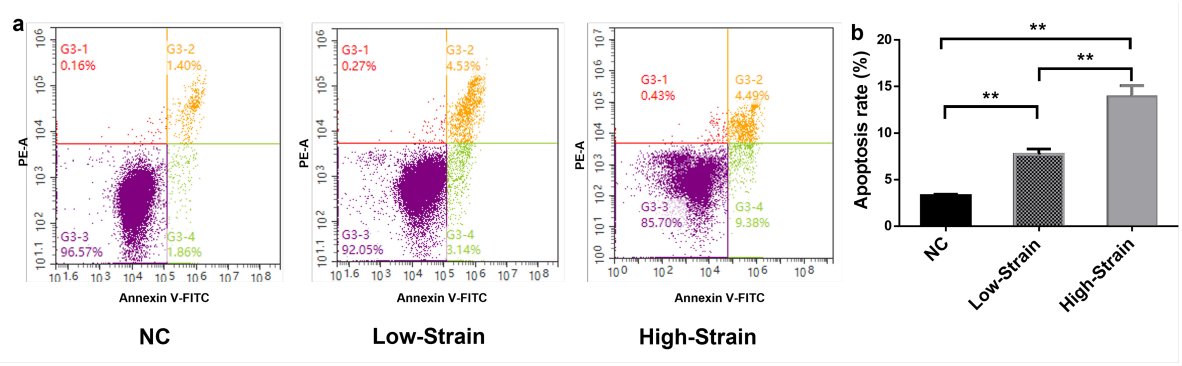

Fig. 1 Mechanical strain inducing the apoptosis in chondrocytes. (a, b) Flow cytometry analysis of chondrocyte stained with Annexin V-FITC and PI ($n = 3$). One-way ANOVA with Bonferroni's test for (b). * $p < 0.05$, ** $p < 0.01$.

Strain group exhibited a marked increase in apoptosis, with a mean value of 13.95% ± 1.14. Statistical analysis confirmed that the differences among these groups were highly significant ($p < 0.01$), demonstrating a dose-dependent relationship between mechanical stress and chondrocyte apoptosis (Fig. 1a,b). In particular, the apoptosis rate in the High Strain group was approximately 7 times higher than that in the Low Strain group, highlighting the severe impact of high mechanical stress on chondrocyte viability.

Piezo1 gene expression and intracellular calcium levels under different mechanical stress

In the NC group, Piezo1 RNA expression was low (0.32 ± 0.041), with similar levels observed in the Low Strain group (0.297 ± 0.0231). However, the High Strain group showed a marked increase in Piezo1 RNA expression (0.990 ± 0.036, $p < 0.01$) (Fig. 2a). Western blot analysis further confirmed this upregulation at the protein level, with Piezo1 protein levels increased from 0.49 ± 0.20 in the Low Strain group to 0.86 ± 0.22 in the High Strain group (Fig. 2b,c), indicating coordinated transcriptional and translational responses to high mechanical stress.

Intracellular calcium levels were also significantly elevated in the High Strain group (60.76 ± 6.19) compared to the NC (28.52 ± 4.688) and Low Strain (34.61 ± 3.77) groups ($p < 0.01$) (Fig. 2d,e). These findings support prior studies, including those by Lee et al [23], confirming that Piezo1 regulates calcium influx under high mechanical stress, with increased Piezo1 protein levels closely associated with elevated intracellular calcium, underscoring Piezo1's role in the cellular stress response.

Emodin inhibiting chondrocyte apoptosis under mechanical stress

Emodin significantly reduced chondrocyte apoptosis induced by mechanical stress. In the control group, which was not treated with emodin, the mean apoptosis rate was 13.32% ± 0.74. However, emodin significantly reduced apoptosis, lowering it to 11.53% ± 0.75 in the emodin group (Fig. 3a,b). Emodin treatment significantly reduced the apoptosis rate in chondrocytes under mechanical strain ($p < 0.01$). Moreover, the pro-apoptotic gene Bax exhibited a significant reduction in expression, from 5.26 ± 0.23 in the control group to 1.28 ± 0.13 in the emodin-treated group, further supporting emodin's role in reducing chondrocyte apoptosis ($p < 0.01$), (Fig. 3c).

Emodin suppressing chondrocyte apoptosis through Piezo1 channel inhibition

To further investigate whether the protective effects of emodin against mechanical stress-induced apoptosis are mediated by the Piezo1 channel, we compared the apoptosis rates among the Emodin, Emodin + si-Piezo1, and Emodin + Yoda1 groups. As shown in Fig. 3a,b, siRNA-mediated knockdown of Piezo1 further enhanced the anti-apoptotic effect of emodin, while activation of Piezo1 by Yoda1 significantly reversed this protective effect. Emodin-treated cells showed reduced Piezo1 RNA expression (0.89 ± 0.10) compared to controls (1.32 ± 0.12, $p < 0.01$). This reduction was further pronounced with Piezo1 silencing (0.327 ± 0.014), while activation led to a slight increase (0.95 ± 0.13, $p < 0.01$) (Fig. 4a). Western blot analysis confirmed similar trends in Piezo1 protein levels, decreasing from 0.75 ± 0.22 in controls to 0.49 ± 0.20 in emodin-treated cells, with further reduction in the silenced group (0.21 ± 0.10) and an increase upon activation (0.86 ± 0.22, $p < 0.05$) (Fig. 4b and c). Intracellular calcium levels were significantly lowered in the emodin group (35.05 ± 4.23) versus controls (54.37 ± 13.69), with even greater reduction upon Piezo1 silencing (15.59 ± 5.57). Conversely, Piezo1 activation raised calcium levels to 68.06 ± 14.74, surpassing control levels ($p < 0.01$) (Fig. 4d,e). Compared to the Ctrl group (mechanical stress only), Piezo1 expression was significantly reduced in the Emodin group, indicating that emodin downregulates Piezo1 expression under mechanical stress conditions.

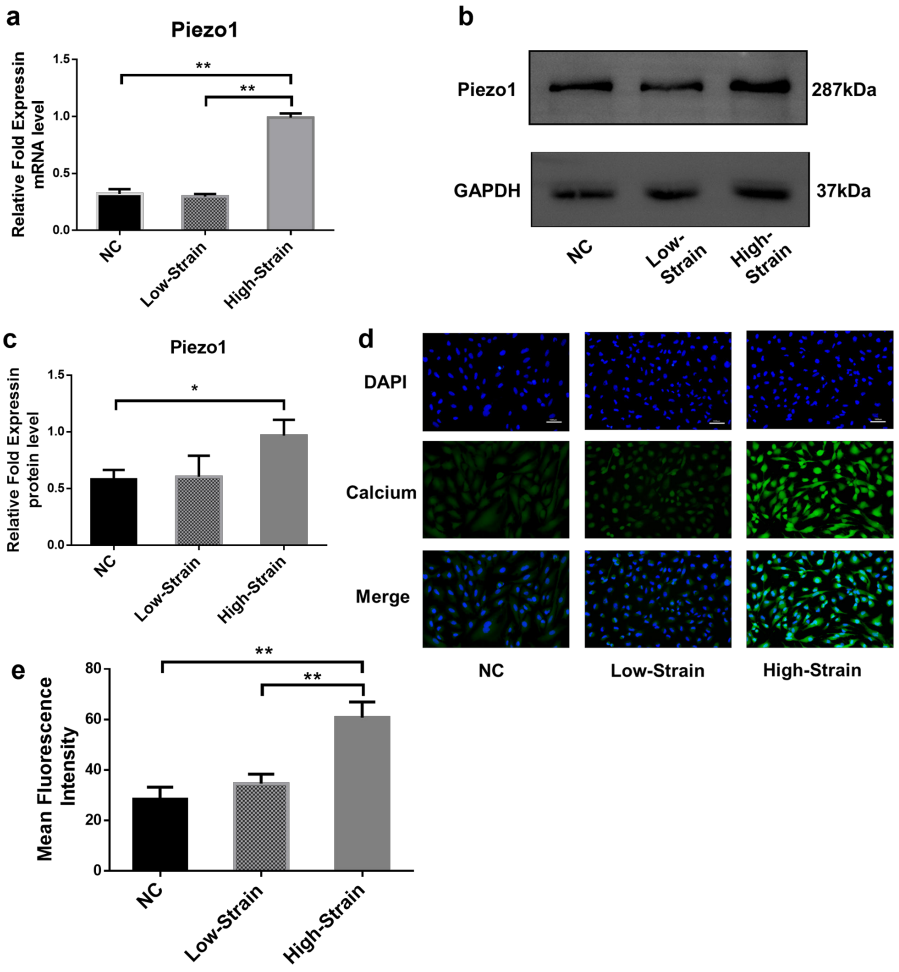

Fig. 2 Mechanical strain upregulating Piezo1 and increasing intracellular calcium in chondrocytes ($n = 3$). (a) RT-qPCR analysis of Piezo1 in chondrocytes. Gene expression in Low Strain and High Strain groups was normalized to the NC group. (b, c) Protein level of Piezo1 determined by Western blot analysis in chondrocytes. (d) Immunofluorescence staining of calcium in chondrocytes. (e) Quantified results of immunofluorescence. One-way ANOVA with Bonferroni's test for (a, c, e). * $p < 0.05$, ** $p < 0.01$.

Emodin-treated cells showed lower Piezo1 expression and intracellular calcium levels compared to the untreated group ($p < 0.01$). Conversely, Piezo1 activation may negate these effects, emphasizing Piezo1's central role in chondrocyte stress response.

Molecular docking simulation of emodin with Piezo1

Molecular docking involves binding a compound to a protein receptor and using computer simulations to calculate their matching patterns and affinity [21]. The binding affinity of emodin to Piezo1 was assessed through molecular docking simulations. The molecular docking schematics shown in Fig. 5a–c revealed that emodin binds stably to Piezo1, with combined free energy values of −3.0 and −3.3 kcal/mol (Fig. 5b,c). Generally, a lower binding energy indicates a more stable interaction between the ligand and the receptor. It is commonly accepted that a binding energy of $\leqslant -5.0$ kcal/mol suggests a strong affinity [22]. The binding energy of emodin to Piezo1 approaches this threshold, suggesting a moderate binding affinity rather than a strong one.

DISCUSSION

In 2010, Coste et al [24] made a groundbreaking discovery by identifying a family of mechanically activated ion channels, known as the Piezo family. Piezo1, a pivotal member of this family, has since been identified as a crucial component in responding to mechanical stress by altering the ion permeability of the cell membrane, which in turn rapidly regulates intracel-

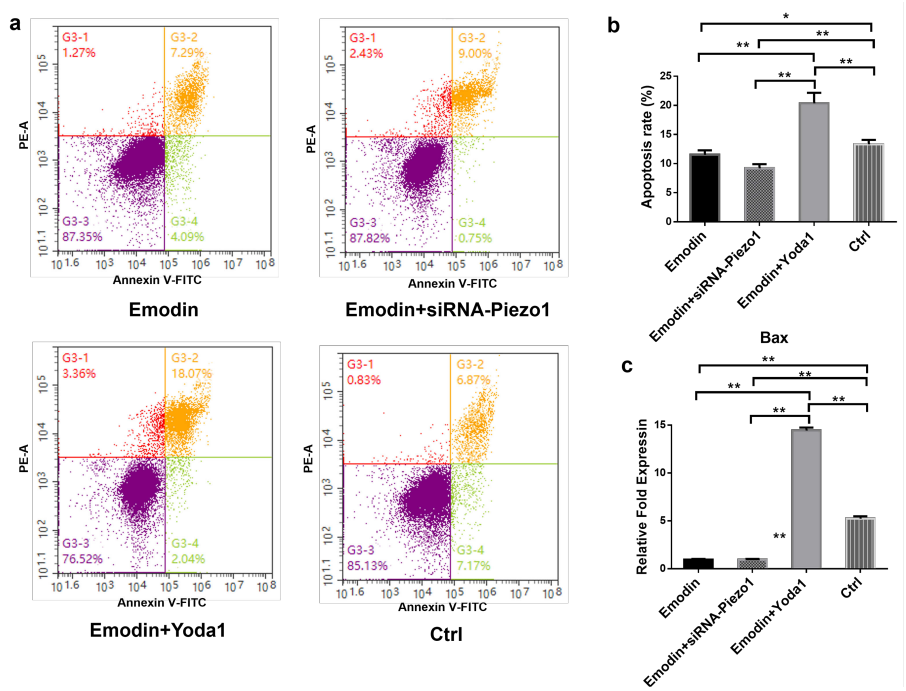

Fig. 3 Emodin inhibiting chondrocyte apoptosis under mechanical stress ($n = 3$). (a, b) Flow cytometry analysis of chondrocytes stained with Annexin V-FITC and PI. (c) RT-qPCR analysis of Bax in chondrocytes. One-way ANOVA with Bonferroni's test for (b, c). * $p < 0.05$, ** $p < 0.01$.

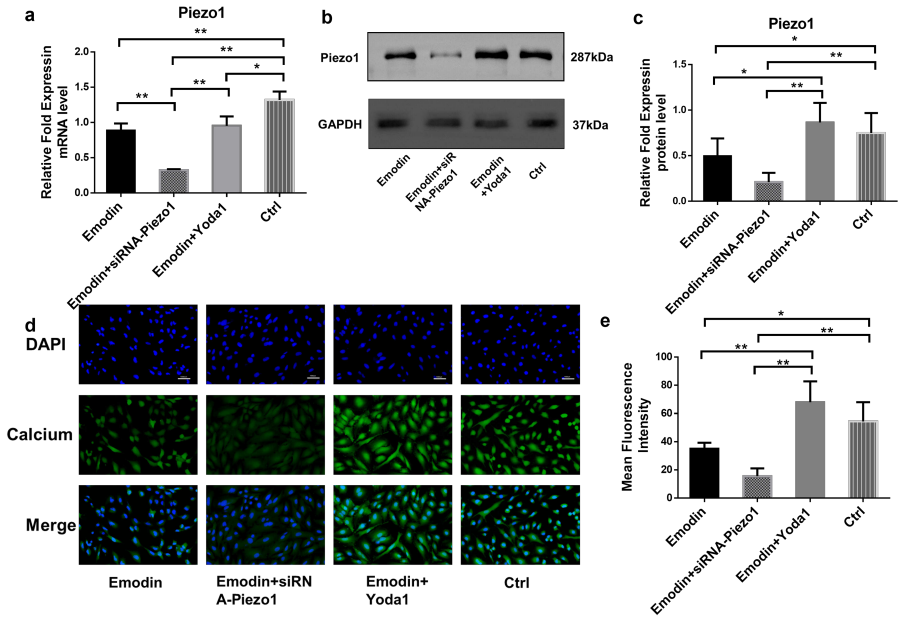

Fig. 4 Emodin inhibiting Piezo1 activity and reducing calcium influx in chondrocytes under mechanical strain ($n = 3$). The Ctrl group indicates chondrocytes under mechanical stress (70 kPa) without emodin; the Emodin group refers to cells under stress plus 10 μM emodin. (a) RT-qPCR analysis of Piezo1 in chondrocytes. Gene expression levels were normalized to the control group (mechanical stress only) using the $2^{-\Delta\Delta Ct}$ method. (b, c) Protein level of Piezo1 determined by Western blot analysis in chondrocytes. (d) Immunofluorescence staining of calcium in chondrocytes. (e) Quantified results of immunofluorescence. One-way ANOVA with Bonferroni's test for (a, c, e). * $p < 0.05$, ** $p < 0.01$.

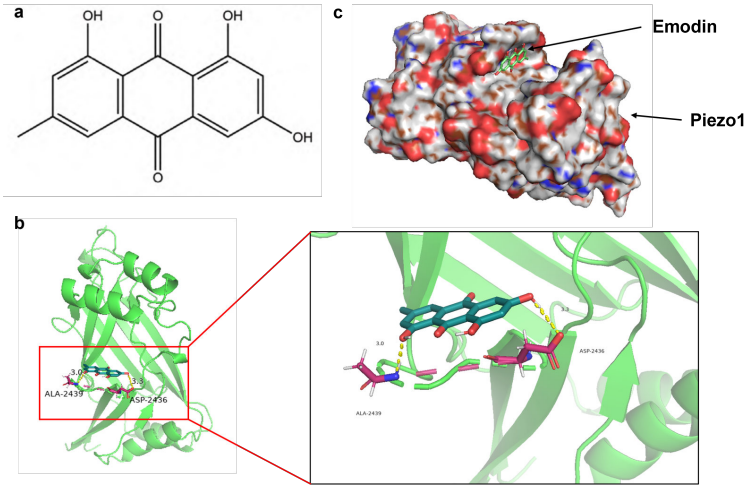

Fig. 5 Molecular docking simulation showing that emodin binds stably to the Piezo1 target. (a) Chemical structure of emodin. (b) Schematic diagram of molecular docking of emodin with Piezo1 proteins. Putative emodin binding site in mouse Piezo1 (PDB code 4RAX). Emodin is displayed in blue sticks, residues are depicted in red sticks, and H-bonds in the complex are depicted as yellow dashed lines. Emodin formed hydrogen bonds with ALA2439 and ASP2436. (c) Molecular docking surface diagram.

lular calcium concentrations [25]. This characteristic makes Piezo1 a central player in the mechanotransduction pathways of various cell types, including chondrocytes. Piezo1 channels have been shown to transmit harmful levels of biomechanical strain in articular chondrocytes and mediate apoptosis [26].

In the investigation of osteoarthritis, emodin has been reported to exhibit multiple pharmacological activities, including anti-inflammatory by reducing Interleukin-1β (IL-1β), Interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) levels [11, 27, 28], antioxidant effects that decrease nitric oxide (NO) and prostaglandin E2 (PGE2) production [27], inhibition of cartilage matrix metalloproteinases, including matrix metalloproteinase (MMP) 3, MMP-13, ADAM metallopeptidase with thrombospondin type 1 motif (ADAMTS) 4, and ADAMTS5 [29], and protective effects on chondrocytes. Liu et al [19] demonstrated that emodin promotes chondrocyte proliferation by inhibiting the ERK and Wnt/β-catenin pathways. Additionally, Li et al [17] reported that 10 µM emodin enhances chondrocyte viability, while concentrations above 20 µM significantly reduce viability, with 40 µM notably inhibiting chondrocyte proliferation.

In articular cartilage, chondrocytes are constantly exposed to complex mechanical stimuli such as tensile forces, compressive loads, and shear stress [1]. When subjected to mechanical load, Piezo1 regulates calcium signaling and activates pathways related to apoptosis, inflammation, and cartilage degeneration. This ultimately leads to chondrocyte apoptosis and extracellular matrix degeneration [31]. Changes in mechanical stress are critical factors in the pathogenesis of traumatic arthritis and osteoarthritis [32, 33], as they directly affect the biomechanical environment of chondrocytes, thereby accelerating the progression of articular pathology [33]. Post-traumatic and articular degeneration results in aberrant mechanical stress distribution, which impairs the repair process, making it difficult for chondrocytes to effectively self-repair and regenerate [34].

The specific conditions of mechanical stress applied to chondrocytes, such as pressure and duration, vary across studies. For instance, research conducted by Li et al [35] demonstrated that mechanical loading at 150 kPa is optimal for chondrocytes. Conversely, the incidence of necrosis and cartilage degeneration increased at pressures exceeding 200 kPa. Similarly, Chen et al [16] reported that chondrocyte proliferation remained unaltered under 90 kPa of pressure for 1 h but significantly decreased after 6 h of sustained pressure.

In this study, we investigated the effects of 3 distinct pressure levels on apoptosis in cultured chondrocytes *in vitro*, building on prior research findings [16, 36]. Our results demonstrated that low-strain exposure led to increased apoptosis compared to cells without mechanical strain, consistent with the finding by Chen et al [31], who observed elevated apoptosis following 6 h of pressure stimulation. Higher strain (70 kPa) resulted in a marked increase in apoptosis, aligning with studies linking compressive strain to Piezo1-mediated apoptosis in chondrocytes. Additionally, our study revealed that there were notable variations in Piezo1 expression in response to varying stress levels. While there was no significant change

in Piezo1 expression under low stress, there was a notable increase under high stress, when compared to both the control and low-strain groups. Furthermore, alterations in intracellular calcium concentration were also observed. While calcium levels exhibited a slight increase under low stress, they demonstrated a significant rise after 6 h of high-strain stimulation. As an essential second messenger within the cell, fluctuations in intracellular calcium levels trigger additional downstream signaling, regulating the process of chondrocyte apoptosis [37]. These findings indicate that alterations in Piezo1 expression and intracellular calcium levels in chondrocytes are pivotal factors in mechanical strain-induced apoptosis.

Flow cytometry results in our study showed that emodin significantly reduced apoptotic chondrocytes, aligning with prior research on its anti-apoptotic effects in various cell types [11, 12]. Emodin effectively downregulated Bax expression, a key apoptosis marker [38], contributing to its protective role. Previous studies also support emodin's protective effects on chondrocytes, especially in osteoarthritis. For instance, Ma et al [12] found that emodin attenuates oxidative stress-induced apoptosis in osteoarthritic chondrocytes via the Nuclear Factor Erythroid 2-Related Factor 2/Heme Oxygenase-1 (Nrf2/HO-1) pathway, while Liu et al [19] showed it promotes chondrocyte proliferation by inhibiting RAS-extra-cellular signal regulated kinase (ERK) and Wnt/β-catenin pathways. Although the present study confirmed apoptosis through flow cytometry and molecular markers, future work will incorporate morphological analyses (e.g., DAPI or TEM) to visually validate chondrocyte apoptosis under mechanical stress.

Western blot and RT-qPCR analyses demonstrated that emodin significantly downregulated Piezo1 expression, a critical mechanosensitive ion channel in chondrocytes responding to mechanical strain [5]. Upregulated Piezo1 is associated with apoptosis and cartilage degeneration, as shown in prior studies [7]. Piezo1 regulation may involve a negative feedback loop affecting both transcription and protein expression; Ren et al [20] reported that inhibition of Piezo1 modulates the Calcineurin/NFAT1 axis, while Chen and Zhang found that IL-1β-induced Piezo1 activation triggers apoptosis via the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mechanistic target of rapamycin (mTOR) pathway [39]. Inhibiting Piezo1 or using emodin reduces apoptotic signaling by downregulating Piezo1 and intracellular calcium influx, thus mitigating apoptosis through calcium modulation [40]. Collectively, our findings suggest protective effects of emodin on chondrocytes are mediated through Piezo1 downregulation and calcium signaling modulation.

To further elucidate the role of Piezo1 in emodin's protective mechanism, we employed both Piezo1 silencing and activation strategies. The silencing of Piezo1 enhanced the protective effects of emodin and reduced the influx of calcium in chondrocytes. Conversely, the activation of Piezo1, even in the presence of emodin, significantly increased chondrocyte apoptosis and elevated the influx of calcium. These findings highlight the pivotal role of Piezo1 in regulating the apoptotic response to mechanical strain, suggesting that emodin's protective effects are largely dependent on its capacity to inhibit Piezo1 activity.

Our findings identify Piezo1 as a key mediator of the deleterious effects of mechanical strain on chondrocytes. Ren et al [20] showed that Piezo1 inhibition via the Gsmtx4 inhibitor reduces chondrocyte apoptosis, promotes cartilage matrix synthesis, and delays osteoarthritis progression. Similarly, Feng et al [41] found that Piezo1 inhibition mitigates cartilage degradation by regulating the Yes-related protein (YAP) – matrix metalloproteinases MMP13/(ADAM Metallopeptidase with Thrombospondin Type 1 Motif 5 (ADAMTS5) pathway, alleviating mechanically induced joint diseases. Unlike Gsmtx4, which specifically targets Piezo1, emodin modulates multiple pathways—including anti-inflammatory, antioxidant, and anti-apoptotic signaling—providing broader therapeutic benefits. Although less selective, emodin significantly reduces intracellular calcium levels and apoptosis in chondrocytes under mechanical strain, potentially offering a more versatile treatment option for complex conditions like osteoarthritis, where diverse pathways drive disease progression. In this study, we found that emodin significantly reduced Piezo1 expression at both mRNA and protein levels under mechanical stress. To investigate whether emodin might act through direct interaction with Piezo1, we performed molecular docking analysis. The results revealed that although emodin could form hydrogen bonds with Piezo1 residues, the binding energy values (−3.0 to −3.3 kcal/mol) were above the threshold typically associated with strong ligand-receptor interactions. Therefore, it is more likely that emodin regulates Piezo1 expression indirectly, potentially through transcriptional or calcium-mediated feedback mechanisms. Further studies involving promoter activity assays and transcription factor pathway analysis would help clarify the exact regulatory mechanism.

Although this study was conducted *in vitro*, our results have important implications for *in vivo* applications. Previous animal studies have demonstrated that emodin reduces cartilage damage and inflammation in osteoarthritis models. The current findings suggest that emodin may also act by downregulating mechanosensitive channels such as Piezo1. This highlights a novel translational potential for emodin in treating mechanical stress-related cartilage degeneration, which warrants further validation in animal models and clinical studies.

However, several limitations remain in the present study. The experiments used cultured rabbit articular chondrocytes, which may not fully replicate the *in*

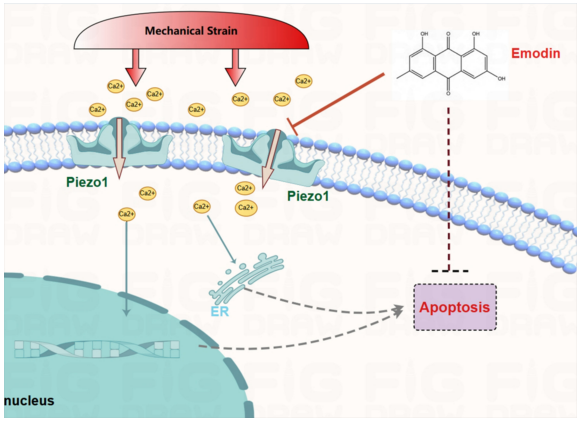

Fig. 6 The potential mechanism by which emodin inhibits chondrocyte apoptosis under mechanical stress through the Piezo1 channel. ER: endoplasmic reticulum.

vivo cartilage environment. While the study focuses on the immediate effects of emodin on apoptosis and Piezo1 activity under mechanical stress, its long-term therapeutic potential for cartilage degeneration and osteoarthritis progression remains unclear. Additionally, although we highlight Piezo1 as a key mediator of emodin's effects, the exact mechanism by which emodin interacts with Piezo1 and the downstream calcium signaling pathways is not fully explored. Future research should focus on *in vivo* validation using animal models of osteoarthritis, exploring the interactions between emodin and other mechanosensitive channels like TRPV4, and evaluating its effects in clinical trials.

CONCLUSION

In conclusion, our findings highlight the critical role of Piezo1 in mediating the effects of mechanical strain on chondrocyte apoptosis and underscore emodin's potential as a therapeutic agent for chondrocyte apoptosis. By targeting Piezo1, emodin not only reduces apoptosis but also modulates calcium signaling pathways (Fig. 6). These results advance our understanding of mechanotransduction mechanisms underlying cartilage degeneration and support the development of Piezo1-targeted therapies for cartilage diseases. These findings highlight the potential of emodin as a novel therapeutic agent for protecting chondrocytes against mechanical stress-induced apoptosis by targeting the Piezo1-mediated mechanotransduction pathway and contribute to a broader understanding of the molecular mechanisms underlying cartilage degeneration, thereby supporting the development of Piezo1-targeted strategies for cartilage-related diseases.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2025.067. The datasets generated during the current study are available from the corresponding author on reasonable request.

Acknowledgements: The authors wish to thank the Wuhan Knowledge Innovation Project 2023 (Project Number: 2023020201010179) and the Hubei Provincial Health Commission Translational Medicine Project (Project Number: WJ2021ZH0010) for their financial support, as well as the use of facilities during the course of this research work.

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Appendix A. Supplementary data

Table S1 RT-qPCR primer sequences used in the study.

Gene	Primer	Sequence (5′–3′)	PCR Product
Rabbit GAPDH	Forward Reverse	CAGGGCTGCTTTTAACTCTGG TGGAAGATGGTGATGGCCTT	177 bp
Rabbit Piezol	Forward Reverse	CAAAGGCTACTACGACCCCA CTTGAGGTTGGCTGCATTGT	223 bp
Rabbit bax	Forward Reverse	ATGAAGACAGGGGCCCTTTT GTCCAGTTCGTCGCCAATG	156 bp