Article

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Mechanical Stretch-Induced ATP Release from Osteocytes Promotes Osteogenesis of Bone Marrow Mesenchymal Stem Cells

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Background: Mandibular distraction osteogenesis (MDO) is a highly effective method for bone regeneration, commonly employed in treating craniofacial defects and deformities. Osteocytes sense mechanical forces in the pericellular space, relay external stimuli to biochemical changes, and send signals to other effector cells, including bone marrow mesenchymal stem cells (BM-MSCs), to regulate bone resorption and formation. Piezo1 potentially affects the secretion signal molecules of bone cells under mechanical stretch. The primary aim of this study was to enhance our comprehension of the molecular biology underlying this therapeutic approach and to identify specific signaling molecules that facilitate bone formation in response to stretch forces.
Methods: Mechanical stretching was applied to negative controls and Piezo1 knockdown osteocyte-like MLO-Y4 cells. Alkaline phosphatase and Alizarin Red S staining were used to survey the osteogenic potential of BM-MSCs. The production and secretion content of adenosine triphosphate (ATP) was measured using ATP content determination analysis. Pathway-related and osteo-specific genes and proteins were evaluated using real-time polymerase chain reaction (RT-PCR), Western blots, and immunofluorescence. Mitochondrial organization was examined with a transmission electron microscope.
Results: The conditioned medium of stretch-exposed MLO-Y4s significantly upregulated osteogenesis-related indicators of BM-MSCs ($p < 0.001$). The upregulation of BM-MSC osteogenesis was associated with ATP release from osteocytes. Mechanically induced calcium transfer and transcriptional coactivator with PDZ-binding motif (TAZ) nuclear translocation mediated by Piezo1 could promote mitochondrial fission and ATP release. Osteocytes detected stretch forces through Piezo1, triggering calcium influx, TAZ nuclear translocation, and ATP production.
Conclusions: The stretch stimulation of Piezo1 induces calcium influx, which in turn promotes calcium-related TAZ nuclear translocation, changes in mitochondrial dynamics, and the release of ATP in osteocytes. This signaling cascade leads to an up-regulation in the osteogenic capacity of BM-MSCs. Mitochondrial energy metabolism of mechanosensitive protein Piezo1-dependent and ATP release may provide a new effective intervention method for mechanically related bone remodeling.

Keywords: osteocytes; mechanical stretch; bone marrow mesenchymal stem cells; osteogenesis; ATP

Introduction

Distraction osteogenesis (DO) is an effective form of bone regeneration used to treat orthopedic disorders, bone defects, and craniofacial deformities [1,2]. The distraction device is engaged in separating the bone segments gradually and continuously until the desired length is reached. The gap between bone segments is filled with new bone tissue during this process, and the resulting callus is mineralized until bone regeneration is sufficient [3]. Mandibular distraction osteogenesis (MDO) has emerged as a commonplace surgical treatment of severe micrognathia, congenital mandibular retrognathia, and mandibular hypoplasia [4–6]. However, its application is limited because of the long clinical treatment period. In models of mandibular distraction osteogenesis, previous studies have highlighted the importance of Collagen Type II Alpha 1 (COL2A1), Collagen Type XI Alpha 1 (COL11A1), and Focal Adhesion Kinase (FAK) signals in new bone formation [7,8]. By gaining a deeper understanding of the mechanical principles underlying this therapy, it may be feasible to identify specific signaling molecules that promote bone formation under stretch tension. Such insights could contribute to developing strategies to reduce clinical treatment durations.

Osteocytes, which are terminally differentiated osteoblasts residing within the bone matrix [9], serve as ma-

jor sensors and transducers of mechanical stimuli [10,11]. Multiple studies have shown that osteocyte counts and expression of apoptotic markers decrease immediately after fracture and then increase gradually as bone healing [12]. During the combined treatment of MDO, the number of mature osteocytes changes significantly with the process of bone healing [13,14], indicating that osteocytes may play a vital role in the MDO progress. However, the specific role of osteocytes in MDO and the mechanism of influencing osteogenesis remain unclear. Recent research has reported a phenotype of cortical bone and bone trabecular mass reduction of Piezo1^{Dmp1Cre} mice in which Piezo1 protein is inactivated in osteocytes. Furthermore, Piezo1 was reported to be related to mandibular development [15]. Therefore, osteocytes may sense mechanical stimulations and participate in the regulation of bone formation during MDO progress.

Nonetheless, most earlier studies concentrated on the biological alterations that occur in osteocytes when subjected to fluid flow shear stress (FSS) [16,17]. The question of whether osteocytes react to other types of mechanical stimulation, such as mechanical stretching force, has only been partially investigated in research. Meanwhile, emerging evidence suggests that osteocytes and their secretory factors may play a regulatory role in bone repair [12]. Osteoclasts and osteoblasts are among the effector cells that osteocytes communicate with to control the creation and resorption of bone [18]. Osteocytes emit signaling molecules in response to mechanical stimuli, which coordinate mineral homeostasis, osteoblastic bone production, and osteoclastic bone resorption [19–22]. Bone marrow mesenchymal stem cells (BM-MSCs) are located in the bone marrow and play a critical role in maintaining bone homeostasis [23]. During osteoblastogenesis, mesenchymal stem cells (MSCs) differentiate into osteocytes [24]. MSCs receive biochemical stimulation from osteocytes in the surrounding natural environment, which affects their differentiation processes [25]. Here, we delved into whether there is an unknown mechanism under which osteocytes regulate MSC osteogenesis through secretion signals.

Mechanotransduction is the process of transducing the mechanical force from load to biological signals that induce cellular responses [26]. Mechanical stretching stimulates bone formation, but the mechanism underlying it remains unclear. Beginning with the discovery of Piezo proteins (Piezo1 and Piezo2) in 2010, accumulating evidence suggests that the mechanically activated Piezo1 channel serves as a critical mechanical sensor that converts mechanical stimulation into electrochemical signals in mammals [27–29]. As an ion channel with a high affinity for calcium, Piezo1 directly senses mechanical stretching signals and functions via Ca^{2+} signaling [29,30]. Mice with deleted Piezo1 from osteocytes and osteoblasts have developed decreased bone formation and bone mass phenotypes, with blunted skeletal responses to mechanical loads [31,32]. Recently, it has been reported that the Piezo1 channel mediates mechanically induced Ca^{2+} response and adenosine triphosphate (ATP) release due to its mechanical sensitivity in astrocytes [33]. However, whether Piezo1 regulates mitochondrial energy metabolism or ATP release in osteocytes remains unclear. Therefore, we reasoned that Piezo1 is also involved in mechano-chemo transduction in osteocytes, mediating calcium reaction and ATP release, thus affecting the function of bone cells and bone formation.

The Hippo pathway changes the nuclear location of transcription co-activators in response to extracellular cues. Yes-associated transcriptional regulator (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) are reportedly involved in the major gene regulation and biological functions of the vertebrate mechanical signal transduction pathways [34]. Previous studies have connected mechanical stretch stimulation with TAZ nuclear transport [35]. Furthermore, TAZ has been proven to play a role in the mechanical transduction of osteocytes mediated by Ca^{2+}-related ion channel Piezo1 [31,36]. TAZ has been reported to stimulate mitochondrial biogenesis [37], but the relevant mechanism of this process in osteocytes remains to be investigated.

Mitochondria maintain their homeostasis through a dynamic process of continuous fusion and division [38]. Fission and fusion play a crucial role in regulating the morphology and function of mitochondria. Dynamin-related protein 1 (DRP1) regulates mitochondrial fission, while Mitofusin-2 (MFN2) is the master regulator of mitochondrial fusion [39]. Since mitochondrial metabolism is closely related to ATP production and release, we hypothesized that osteocytes perceive stretching forces through the mechanosensitive Piezo1 channel and Hippo TAZ to promote further the generation of mitochondria and ATP release of osteocytes to modulate BM-MSC osteogenesis.

Here, we verified that more substantial ATP release from stretching-induced osteocytes accelerates BM-MSC osteogenesis. We also confirmed that TAZ is a critical downstream effector of Piezo1-mediated cytosolic calcium transport and mitochondrial energy metabolism.

Materials and Methods

Cell Culture

The osteocyte-like cell line, MLO-Y4 cells, were cultured in α-Minimum Essential Medium (α-MEM, #C12571500BT, Gibco, New York, NY, USA), which contained 5% calf serum (CS, #16010-159, Gibco, New York, NY, USA), 5% fetal bovine serum (FBS, #0025, Sciencell, Carlsbad, CA, USA), and 1% streptomycin-penicillin (#SV30010, Hyclone, Logan, UT, USA). The dishes were coated with rat tail collagen type I (#354236, BD Biosciences, Bedford, MA, USA). Prior to commencing the investigation, the MLO-Y4 cells underwent testing for mycoplasma contamination. BM-MSCs were isolated and collected using the previously described method [40]. Bone

marrow was obtained from femurs and tibias of 8-week-old C57BL/6J male mice purchased from Vitalriver (Beijing, China). Following the completion of behavior assessments, the mice were anesthetized with pentobarbital (100 mg/kg, intraperitoneal) followed by cervical dislocation, and bone-marrow cells were isolated from femur and tibia after the death of the mice was confirmed. BM-MSCs were cultured in Dulbecco's Modified Eagle's Medium (DMEM, #C11995500BT, Gibco, New York, NY, USA) supplemented with 10% fetal bovine serum (FBS, #0025, Sciencell, Carlsbad, CA, USA) and 1% streptomycin-penicillin (#SV30010, Hyclone, Logan, UT, USA). All cells were incubated at 37 ℃, 5% CO_2 in standard humidified CO_2 incubators.

Mechanical Stretching Load

MLO-Y4 cells were seeded in 6-well flexible silicone rubber BioFlex™ plates covered with collagen type I (Flexcell International Corp., Hillsborough, NC, USA) at a density of 5×10^4 cells/mL in 2 mL general growth medium. After the cells reached 70% confluence, the Flexcell® FX-5000™ Tension Plus™ unit (Flexcell International Corp., Hillsborough, NC, USA) was used to apply cyclic sinusoidal continuous mechanical tension (2% elongation, 0.5 Hz; sinusoidal waveforms). The control group was incubated under unanimous conditions without mechanical force load. Cells were collected at the same time point immediately after the mechanical stretching load application.

Cell Cycle Measurements

BM-MSCs were stained with the cell cycle detection reagent (#C543, Dojindo, Kumamoto, Japan) - PI/RNase and incubated for 30 minutes in the dark to mark the DNA content. The proportions of cells in the G0/G1, G2, and S phases were obtained using live cell overflow cytometry (BD Biosciences, San Jose, CA, USA). The cell cycle results were analyzed using FlowJo software (Version10.6.2, FlowJo, LLC, Ashland, Oregon).

RNA Isolation and RT-PCR

Total RNA of cells was extracted using the Minibest Universal RNA Extraction Kit (#9767, Takara, Shiga, Japan) and transcribed into cDNA using the Takara Primescript RT Master Mix Kit (#RR036A, Takara, Shiga, Japan). RNA isolation and quantitative real-time polymerase chain reaction (RT-PCR) were carried out using the protocol described previously with 7900 Real Time-PCR System (Applied Biosystems) using SYBR Green Master Mix (Takara, Shiga, Japan) [11]. The primer sequences are presented in **Supplementary Table 1**. The stability of Internal reference protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was analyzed, and all data were normalized to GAPDH expression.

Cell-Transfection

MLO-Y4 cells were transfected using lentivirus Piezo1 purchased from GeneChem (Shanghai, China) for silencing studies. Sequences of siRNA probes are as follows: Piezo1 Negative control (NC) siRNA, 5′- TTCTCCGAACGTGTCACGT -3′; Piezo1 siRNA [41], 5′- CACCGGCATCTACGTCAAATA -3′. After 24 hours of transfection at 50%–60% confluence, 6 μg/mL puromycin was added, and the cells were cultured for 24 hours to select positive cells. The infection efficiency exceeded 70%.

ATP Synthesis and Release

The ATP content in the culture medium and cells was assessed using fluorescein luciferase (#S0027, Beyotime, Beijing, China). The culture media or cell extracts and dilution buffer with luciferase were mixed in 96-well microplates. Synthetic luminescence detected with scintillation and bioluminescence detector (LB 960, Berthold, German) corresponds to ATP contents (nmol). The protein concentration of each sample was determined with the Coomassie Brilliant Blue R250 Protein Determination Kit (#P0017B, Beyotime, Shanghai, China) to normalize the ATP levels.

Alkaline Phosphatase Staining and ALP Activity Assay

BM-MSCs treated with a conditioned medium were fixed with 4% paraformaldehyde for 30 minutes. Alkaline phosphatase (ALP) staining was performed using BCIP/NBT Alkaline Phosphatase Color Development Kit (C3206, Beyotime, Shanghai, China) based on the manufacturer's protocol. Images of stained cells were taken by a scanner (EU-88, Epson, Suwa, Japan). The ALP activity of BM-MSCs was detected by the Alkaline Phosphatase Assay Kit (A509-2, Jian-Cheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. A microtiter plate spectrophotometer (Spectra Max M2e, Molecular Devices, San Jose, CA, USA) was used to record the absorbance value associated with ALP activity at 520 nm.

Alizarin Red S Staining

To examine the extent of matrix mineralization, BM-MSCs treated with conditioned medium for 21 days were first fixed with 4% paraformaldehyde for 30 minutes and then stained with 1% Alizarin Red S (DZ0207, Leagene, Beijing, China) at ph4.2. To quantify the relative amount of calcium, 10% (w/v) Cetylpyridinium Chloride (CPC, #C9002, Sigma, St. Louis, MO, USA) was diluted in H_2O and well-vortexed at 37 ℃. 200 μL CPC solution was added into each unit of the plate to ensure all calcium nodes were sufficiently dissolved, harvesting purple solution in different degrees. The absorbance of extracted dye was then determined at 562 nm using a Pan-wavelength microplate reader.

Measurements of Intracellular Ca^{2+} Concentration

MLO-Y4 cells were cultured in 6-well flexible silicone rubber plates and subsequently rinsed with a standard buffer solution (SBS) containing calcium ions. The cells were then exposed to SBS supplemented with the fluorescent probe Fluo-4/AM manufactured by Invitrogen Thermo Fisher Scientific at a concentration of 4 μM, along with 0.04% pluronic acid F-127. This incubation process lasted for 45 minutes at a temperature of 37 °C while being kept in darkness. After incubation, the cells were maintained in phosphate-buffered saline (PBS) with 5% fetal bovine serum and stimulated with a mechanical stretching load. The intracellular calcium concentration was monitored by a FACScan flow cytometer (FACSCaliburTM, BD Biosciences, San Jose, CA, USA) and a laser scanning confocal microscope (LSM 710, Zeiss, Oberkochen, Baden-Wurttemberg, German). The ratio of fluorescence intensity emitted at 510 nm (F340/F380) was used to reflect the calcium concentration The mean data was presented by F340/F380 expression as the percentage of F340/F380 from cells under control conditions.

Western Blot

The Western blot procedure followed the established Western blot procedure (https://www.westernblotprotocol.com). Primary antibodies: anti-GAPDH, 1:1000 (#5174, Cell Signaling Technology, Danvers, MA, USA), anti-Piezo1, 1:1000 (#NBP1-78537, NOVUS, Littleton, CO, USA), anti-TAZ, 1:1000 (#4883, Cell Signaling Technology, Danvers, MA, USA), anti-p-TAZ, 1:1000 (#59971, Cell Signaling Technology, Danvers, MA, USA), anti-Lamin B1, 1:1000 (#16048, Abcam, CA, UK), MFN2, 1:1000 (#124773, Abcam, CA, UK) and DRP1, 1:1000 (#184247, Abcam, CA, UK). Secondary antibodies: HRP-goat anti-rabbit, 1:10000 (#111-035-003, Jackson ImmunoResearch, PA, USA); HRP-goat anti-mouse, 1:10000 (#111-035-174, Jackson ImmunoResearch, PA, USA).

Immunofluorescence

The MLO-Y4 cells that had undergone pretreatment were washed three times with PBS. Subsequently, the cells were subjected to fixation by immersing them in a 4% paraformaldehyde solution for 30 minutes. Following that, the cells were rendered permeable by treating them with a 0.3% solution of Triton X-100 (Beyotime, Shanghai, China) for 15 minutes. After blocking with goat serum for 1 hour, the cells were incubated with the primary antibody against Piezo1 (1:100, NOVUS#NBP1-78537) at 4 °C overnight and the secondary antibody HRP-goat anti-rabbit (1:100, #SA00013-4, Proteintech, Beijing, China) for 1 hour. DAPI (1:1000, #R37605, Invitrogen, Carlsbad, CA, USA) was used to stain the nucleus, while phalloidin (Cytoskeleton, Denver, CO, USA) was used to stain the cytoskeleton. A fluorescence microscope (DMi8, Leica, German) was used to observe Piezo1 expression.

Transmission Electron Microscope (TEM) Images

MLO-Y4 cells were harvested using a cell scrapper and centrifuge, treated with 4% glutaraldehyde for 1 hour at room temperature, and stored overnight at 4 °C. Samples were dehydrated in graded ethanol concentrations, and subsequently incubated in 100% ethanol and propylene oxide with two separate changes of propylene oxide. The samples were embedded in epoxy resin and polymerized. Ultrathin sections (50–60 nm) were prepared, mounted on a 300-mesh copper grid, and stained with uranyl acetate and uranyl citrate. Mitochondrial organization was examined with a transmission electron microscope (JEM-1400, JEOL Ltd., Musashino, Akishima, Tokyo, Japan).

Data Presentation and Statistical Analysis

Statistical analysis of data uses SPSS 22.0 (IBM Corp., Chicago, IL, USA). All data are expressed as ± standard deviation (SD). Appropriate parameters were evaluated using one-way analysis of variance (ANOVA) with Turkey post-test, two-way ANOVA with Turkey post-test, or Student's *T*-test, where $p < 0.05$ was statistically significant.

Results

Osteocytes Loaded by Mechanical Stretching Promote BM-MSC Osteogenesis

It has been reported that mesenchymal stem cells receive biochemical stimulation from surrounding cells throughout the process of bone homeostasis maintenance and bone remodeling [25], so we set out to verify whether mechanical stretching could affect osteocytes and then regulate the proliferative and osteogenic capacity of BM-MSCs. BM-MSCs were cultured with no-stretch and 6h-stretched negative control medium (NCM) and conditioned medium (CM) derived from no-stretch and 6h-stretch treated MLO-Y4s. Cell Counting Kit-8 (CCK8) results revealed that there was no significant difference ($p > 0.05$) in the proliferation of BM-MSCs between the stretched-CM and non-stretched-CM groups (Fig. 1a). Likewise, the flow cytometry findings indicated that there were no statistically significant variations ($p > 0.05$) in the indices of cell proliferation at 3rd, 5th, and 7th day time points (Fig. 1b,c). RT-PCR results suggested that the mRNA and protein expression levels of osteogenic-specific markers Runt-related transcription factor 2 (RUNX2) and osteopontin (OPN) in BM-MSCs were up-regulated under CM from MLO-Y4s treated by stretching (Fig. 1d). Additionally, ALP and Alizarin Red S (ARS) staining confirmed that osteogenesis was inhibited after culturing with CM, whereas the stretched CM group showed enhanced osteogenesis compared to the unstretched group (Fig. 1e). The alkaline phosphatase staining area and cellular alkaline phosphatase activity were then assessed to evaluate whether CM from MLO-Y4s affected BM-MSC osteogen-

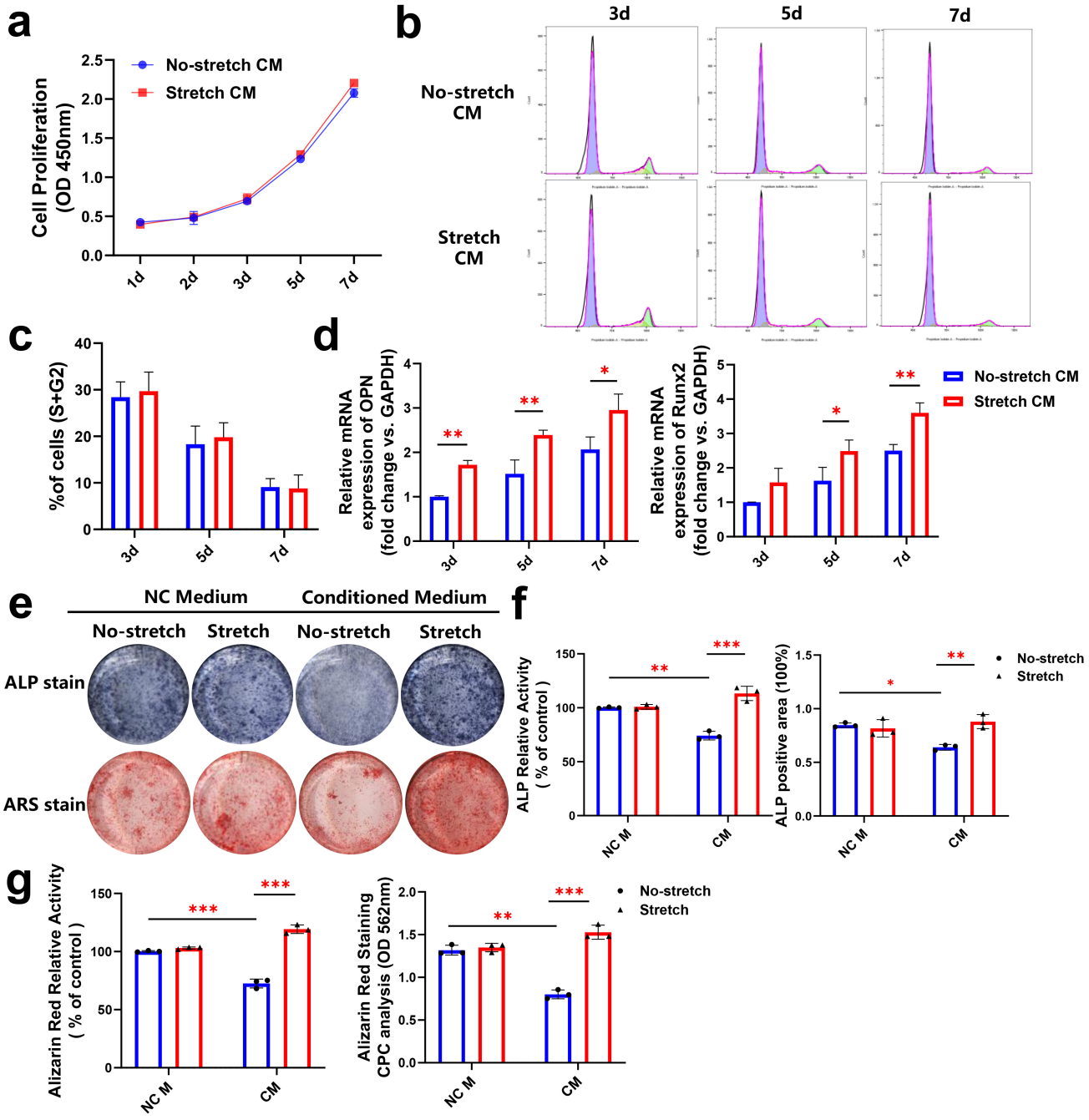

Fig. 1. Conditioned medium of stretch-exposed MLO-Y4s induces the osteogenic differentiation of bone marrow mesenchymal stem cells (BM-MSCs). (a) The proliferation ability of BM-MSCs cultured with conditioned medium (CM) from no-stretch and 6 h-stretch treated MLO-Y4s was tested using Cell Counting Kit-8 assays on days 1, 2, 3, 5, and 7. (b) Flow cytometry cell cycle analysis of BM-MSCs from the stretched-CM and non-stretched-CM groups was performed on days 3, 5 and 7. (c) The total percentage of cells in S and G2 phase was calculated and analyzed. (d) After culturing BM-MSCs with stretched-CM and non-stretched-CM for 3, 5, and 7 days, mRNA levels of the osteogenic marker osteopontin (OPN) and RUNX2 were detected using real-time polymerase chain reaction (RT-PCR). (e) Alkaline phosphatase (ALP) and Alizarin Red S (ARS) staining were performed to visualize the osteogenic abilities of BM-MSCs. (f) The ratio of ALP^+ areas was measured and analyzed. Cellular alkaline phosphatase activity of the no-stretch and 6 h-stretched negative control medium (NCM) and CM groups was detected using colorimetry. (g) Quantification of ARS staining using Cetylpyridinium Chloride (CPC) analysis. Values are the mean ± standard deviation (SD) of three wells. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

esis. As expected, the results demonstrated that the ALP activity of the stretched-CM group gradually increased, which was also significantly different ($p < 0.001$) from that of the non-stretched-CM group (Fig. 1f). Alizarin Red S staining showed a similar trend to ALP staining (Fig. 1g). To assess whether CM from MLO-Y4s affected BM-MSC proliferation, we used CCK8 and flow cytometry during the culture process. Thus, we determined that the osteogenic differentiation potential of BM-MSCs cultured with CM derived from mechanical stretch-exposed MLO-Y4s was elevated compared with that from the no-stretch group, while the proliferation ability was not significantly changed.

Mechanical Stretching-Related ATP Release of Osteocytes is Involved in Regulating BM-MSC Osteogenesis

To evaluate whether mechanical stretching loads affected osteocyte-mediated BM-MSC osteogenesis through released ATP, we measured the production and secretion content of ATP in MLO-Y4 cells and culture-medium supernatant. The results of ATP content determination showed that ATP synthesis and release increased after 0.5, 1, 2, 4, and 6 hours of stretching, and the production of ATP reached the peak at 1 hour (Fig. 2a,b). To determine if released ATP was involved in osteocyte-mediated BM-MSC osteogenesis, we applied gradient concentration exogenous ATP to a conditioned medium from negative control and mechanical stretching-treated MLO-Y4s. RT-PCR results showed that exogenous ATP and stretching load could increase the mRNA expression of RUNX2 and OPN in BM-MSCs (Fig. 2c). It has been reported that RUNX2 triggered the osteogenesis in the early stage, and OPN was closer to phenotype as a late osteogenesis biomarker [42]. We detected the expression of RUNX2 and OPN on day 7 after culturing with CM and observed that the overall mRNA level increased in the group with exogenous ATP. To verify the osteogenic phenotype, we added 0.5 µM exogenous ATP into the conditioned medium from the no-stretch and stretch groups and carried out staining on BM-MSCs. Alkaline phosphatase staining and ALP activity analysis revealed that conditioned medium with exogenous ATP improved the osteogenic potential of BM-MSCs, whose effect was similar to that derived from MLO-Y4s exposed to mechanical stretching. Meanwhile, Alizarin Red S staining showed the same tendency as ALP staining (Fig. 2d–f). Thus, we concluded that ATP released by osteocytes like MLO-Y4 cells treated with mechanical stretching force played a pivotal role in inducing BM-MSC osteogenesis. Next, to test whether released ATP regulates osteogenesis, we used apyrase to degrade ATP in stretched CM and further examined the mRNA expression of RUNX2 and OPN in BM-MSCs. We found that after the degradation of ATP by apyrase, although the osteogenic indexes in the stretch-CM group were up-regulated compared with the no-stretch-CM group, they were significantly weaker ($p < 0.001$) than those in the apyrase-free group (Fig. 2g). These results suggested that ATP released by osteocytes was involved in regulating osteogenesis of BM-MSCs. At the same time, other extracellular molecules may also potentiate this process.

Mechanical Stretch Sensing by Piezo1 in Osteocytes

A class of proteins known as mechanosensory ion channels (MSICs) harness mechanical stimuli to send electrochemical signals [43]. To investigate osteocyte mechanosensation, we first examined whether osteocyte-like MLO-Y4 cells express mechanosensory ion channels. RT-PCR analysis in MLO-Y4 cells showed high mRNA levels of Piezo1, while levels of other reported mechanosensory ion channels were negligible (Fig. 3a). Immunofluorescence results also showed Piezo1 expression in the nuclear envelope, plasma membrane, nucleus and cytoplasm in MLO-Y4 (Fig. 3b). To determine the role of Piezo1 in osteocyte mechanosensation, we transfected Piezo1-specific siRNA in MLO-Y4 cells. RT-PCR and Western blot results showed that the knockdown efficiency of Piezo1 was approximately 80% (Fig. 3c,d). To investigate the physiological reaction of osteocytes to mechanical force, a series of cyclic mechanical stretching experiments (**Supplementary Fig. 1a,b**) were conducted on MLO-Y4 cells. It has previously been shown that Piezo1 is an ion channel with a high affinity for calcium and functions through Ca^{2+} signaling [29,30]. The effect of siRNA-mediated genetic depletion of the Piezo1 on calcium transients was examined. The findings indicated that the amount of intracellular Ca^{2+} in the cells of the control group and negative control siRNA-infected group increased after pretreatment with mechanical stretching. However, the intracellular Ca^{2+} increases caused by stretching loads were suppressed in the Piezo1 knockdown group (Fig. 3e,f). Calcium transient in real time was evaluated by treating negative control and Piezo1-specific siRNA infected cells with Yoda1, a small molecular chemical agonist of Piezo1 (Fig. 3g). The intracellular Ca^{2+} increase caused by Yoda1 was similarly suppressed in the Piezo1 knockdown group. These results indicated that Piezo1 contributed to the MLO-Y4 responses to mechanical stretching and caused dependent calcium influx.

Mechanical Stretching Induces Piezo1-Related TAZ Nuclear Translocation in Osteocytes

Previous research has indicated a potential association between TAZ and the enhancement of bone density linked to the Piezo1 protein [31]. Furthermore, prior studies have demonstrated that TAZ alters cellular activity in response to mechanical stress, such as stretching, by inducing alterations in localization and phosphorylation [44,45]. To explore the molecular signaling pathways involved in intercellular communication, we conducted an analysis of possible transcription factors that may elucidate the mechanisms governing protein secretion regulation. The measurement

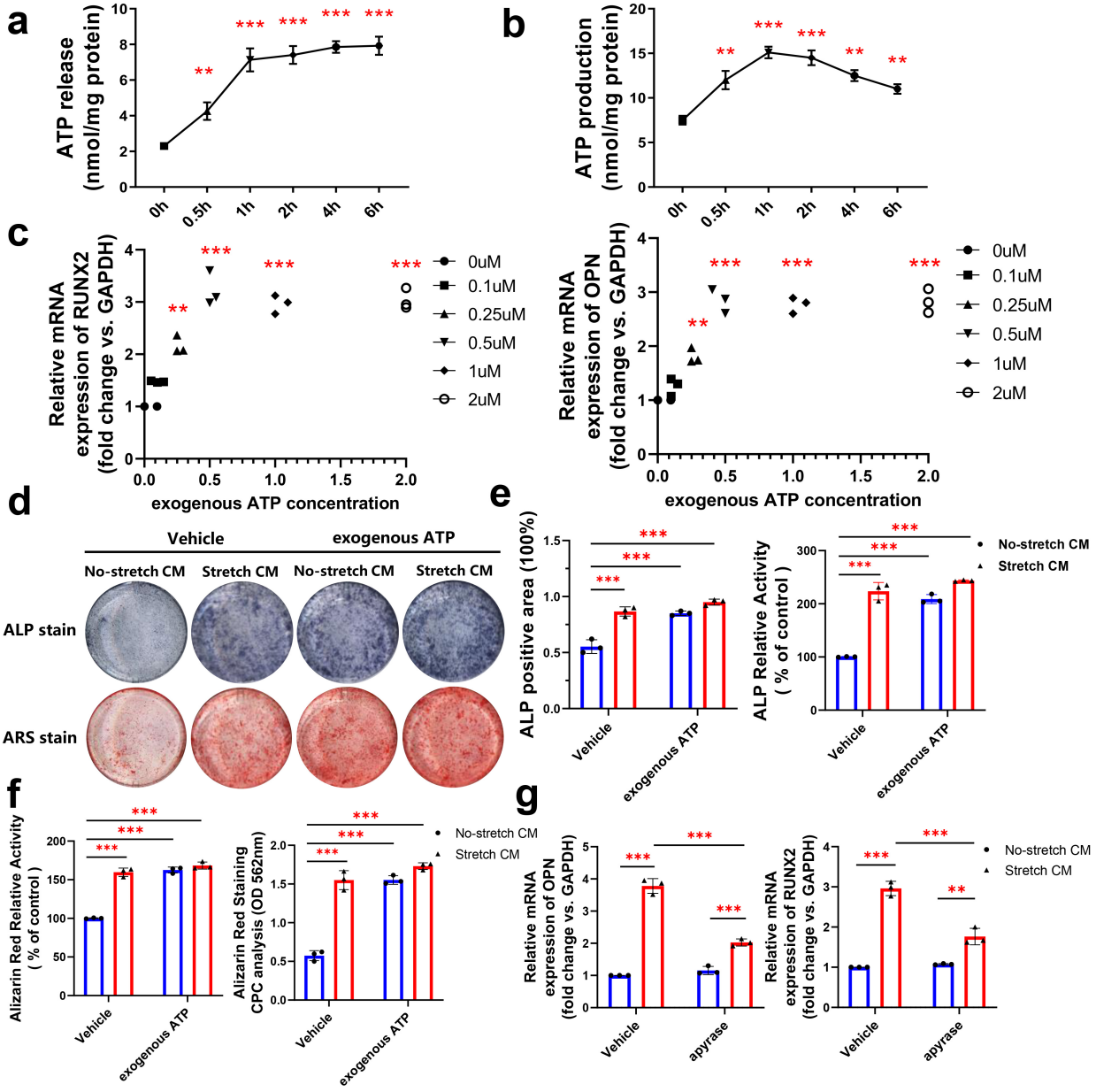

Fig. 2. Mechanical stretching-related adenosine triphosphate (ATP) release of osteocytes is involved in upregulating BM-MSC osteogenesis. (a) ATP content determination analysis of the ATP release contents in medium supernatant of MLO-Y4s after 0.5, 1, 2, 4, and 6 hours of mechanical stretching load. (b) ATP content determination analysis of the ATP synthesis in MLO-Y4s cells. (c) RT-PCR analysis of Runt-related transcription factor 2 (RUNX2) and OPN mRNA expression levels in BM-MSCs treated with exogenous ATP of gradient concentration. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalization. (d) Alkaline phosphatase and Alizarin Red S staining were used to examine the osteogenic ability of BM-MSCs cultured with conditioned medium with 0.5 uM exogenous ATP. Images of stained cells were taken through a scanner and an inverted microscope. (e) The ratio of ALP^{+} areas was measured and analyzed using ImageJ. Cellular alkaline phosphatase activity was detected using colorimetry after 7 days of culture. (f) Quantification of ARS staining by CPC analysis. (g) The osteogenic marker OPN and RUNX2 mRNA levels in BM-MSCs cultured with stretch-CM treated with apyrase were detected using RT-PCR. All cell experiments were done three times, and each time, they were done in at least three replicate wells. All data are presented as mean ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$.

of nuclear localization of TAZ is facilitated by phosphorylation [46]. Consequently, the levels of TAZ and phosphorylated TAZ expression in the nucleus, as well as the total protein levels in MLO-Y4 cells subjected to mechanical stretching were quantified. The results obtained from Western blot analysis demonstrated a statistically significant increase ($p < 0.001$) in the expression levels of nuclear and total TAZ proteins, while the expression of total phosphory-

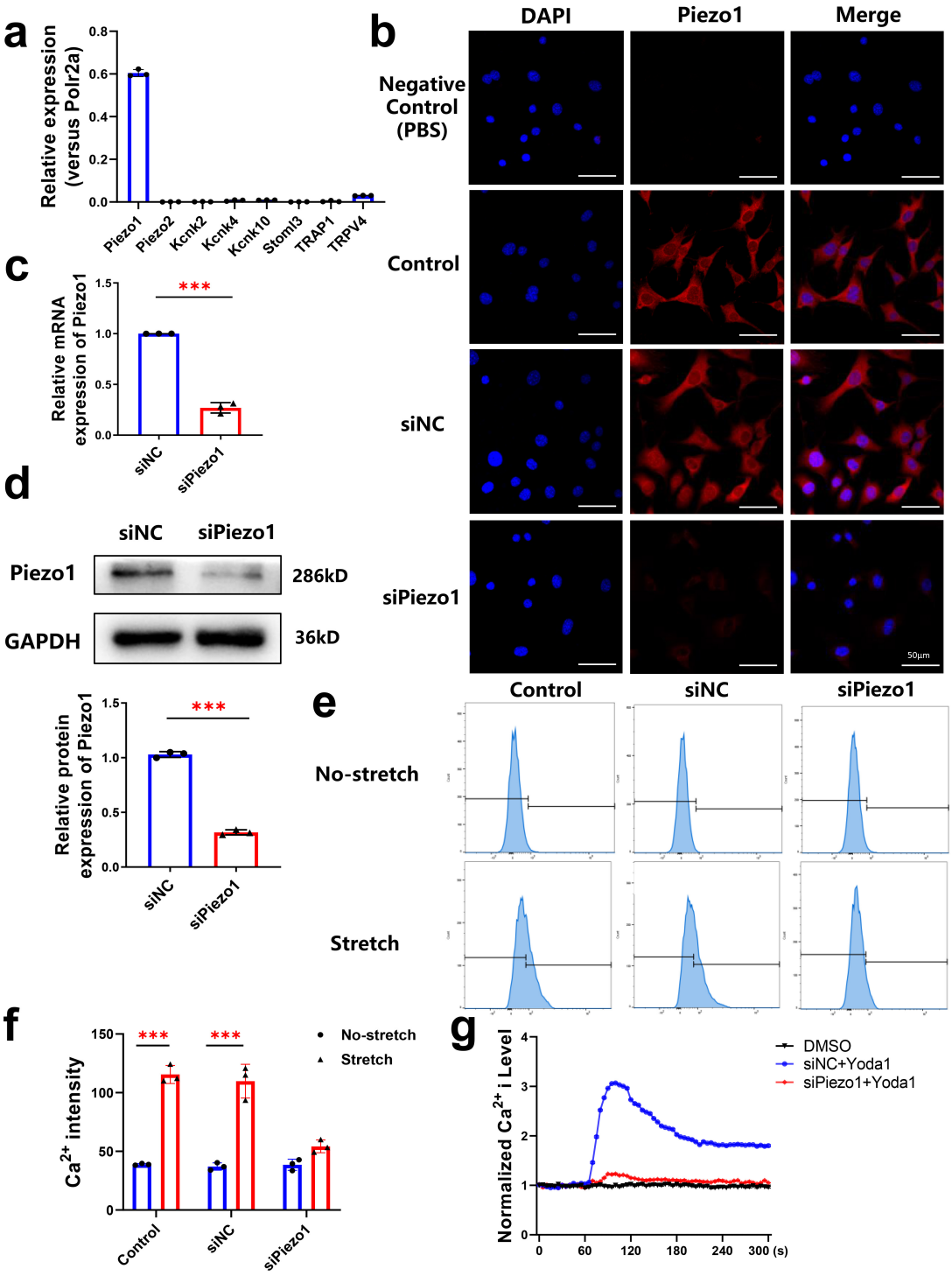

Fig. 3. Mechanical stretch sensing by Piezo1 in osteocytes. (a) RT-PCR analysis of reported mechanosensory ion channels from the osteocyte-like cell line MLO-Y4. (b) Immunofluorescence of Piezo1 (red) in MLO-Y4. DAPI shows the nucleus (blue). (c) RT–qPCR analysis of Piezo1 expression levels in negative control MLO-Y4 cells and cells transfected with siPiezo1. (d) Western blot and quantification analysis of Piezo1 expression levels in siPiezo1-transfected cells. (e) Intracellular [Ca^{2+}] of the control group without siRNA transfection, the negative control siRNA, and the Piezo1-specific siRNA transfected cells subjected to mechanical stretching. (f) Quantification of mean Ca^{2+} intensity at the same time points in parallel experiments. (g) Calcium transient in real time of negative control and Piezo1-specific siRNA infected cells treated with Yoda1. All cell experiments were repeated three times. Values are presented as mean $\pm$ SD. ***$p < 0.001$ vs. control.

lated TAZ proteins exhibited a notable decrease in response to mechanical stretching conditions. The nuclear translocation of TAZ was observed to be significantly increased ($p < 0.001$), with the maximum levels observed at the 1-hour time point (Fig. 4a). Immunofluorescence was employed to assess the subcellular localization of TAZ. The findings revealed that following a 1-hour application of mechanical stretching, a significant quantity of cytoplasmic TAZ was observed to undergo translocation to the nucleus (Fig. 4b).

To confirm whether Piezo1 and Piezo-dependent calcium influx were involved in stretch-induced TAZ upregulation, negative controls and siPiezo1-transfected MLO-Y4 cells were subjected to mechanical stretching for 1 hour. Western blots showed that the transfection of PIEZO1-specific siRNA inhibited mechanical-induced TAZ nuclear translocation (Fig. 4c). To determine whether Piezo1 and Ca^{2+} participated in the regulation of this progress, we treated MLO-Y4s with the Piezo1-specific chemical inhibitor GsMTx4 and the calcium ion chelator BAPTA before applying mechanical stretching. Western blot results revealed that the nuclear translocation of TAZ in MLO-Y4s treated with GsMTx4 and BAPTA exposed to mechanical stretching was inhibited compared to the control group (Fig. 4d). The expression of TAZ was monitored within the nucleus to detect the transfer of osteogenic signals mediated by Piezo1. Furthermore, we investigated the global phosphorylation status of TAZ to ascertain if the down-regulation of Piezo1 leads to a decrease in dephosphorylation or nuclear transport mechanisms. The study's findings indicated a down-regulation in the expression of p-TAZ, whereas the expression of TAZ exhibited a small up-regulation. However, the level of TAZ in the nucleus was decreased after knocking down Piezo1. Interfering Ca^{2+} with GsMTx4 delivered the same result that nuclear transport was blocked, as shown in our study. The nuclear transport of TAZ could be regulated by Piezo1 in Ca^{2+} dependent way.

Piezo1-Dependent TAZ Nuclear Translocation Promotes Mitochondrial Fission and ATP Release in Osteocytes in Response to Stretch Stimuli

Lately, research has reported that the Piezo1 channel mediates mechanically induced Ca^{2+} response and ATP release in astrocytes [33]. To determine whether mechanical stimulation regulates mitochondrial energy metabolism or ATP release in osteocytes, we applied stretching force to MLO-Y4s and observed the changes in mitochondria under a transmission electron microscope (TEM). It is interesting to note that we found a significantly increased number of mitochondria in stretch-treated osteocytes (Fig. 5a). Dynamin-related protein 1 (DRP1) regulates mitochondrial fission, and Mitofusin-2 (MFN2) is the master regulator of mitochondrial fusion [39]. The protein expression of mitochondrial dynamic indexes DRP1 and MFN2 was up-regulated in stretching-treated osteocytes (Fig. 5b). In addition, mitochondria fluorescence labeling and measurements were conducted. We found that the area of fluorescence of mitochondria was significantly elevated after stimulation of stretch ($p < 0.001$), with semiquantitative analysis verified. The fluorescence result confirmed that stretch stimulation promoted the generation of mitochondria in osteocytes (Fig. 5c). To determine whether the fission of mitochondria was responsible for the increased ATP release, we treated no-stretch and stretched MLO-Y4s with DRP1 inhibitor Mdivi-1. After blocking the effect of DRP1 with Mdivi-1, the stretched group produced considerably less ATP than the no-stretch group (Fig. 5d). Mitochondrial fission and generation in response to stretching force correlated with the release of ATP in osteocytes.

Next, to determine whether Piezo1 influenced mechanical stretching-mediated mitochondrial metabolism, negative control, and siPiezo1-transfected MLO-Y4 cells were subjected to mechanical stretching for 1 hour. The Western blot analysis yielded findings indicating that the transfection of PIEZO1-specific siRNA effectively suppressed the up-regulation of DRP1 in response to mechanical stimulation. However, the increase of MFN2 was not affected by the knockdown of Piezo1 (Fig. 6a). These results suggested that Piezo1 promoted mitochondrial fission through DRP1 to cause ATP production and release rather than mitochondrial fusion through MFN2. Corresponding to DRP1 expression, the production and release content of ATP in the si-Piezo1 transfected group was significantly reduced (Fig. 6b,c), $p < 0.001$. Furthermore, we examined whether TAZ participated in mechanical sensitive channel Piezo1-induced mitochondrial fission. Negative controls and MLO-Y4 cells transfected with siPiezo1 were treated with TM-25659, an agonist of TAZ. The expression of DRP1 did not exhibit a significant increase ($p > 0.05$) following the application of stretching stress in MLO-Y4 cells transfected with siPiezo1. However, TM-25659 could rescue the reduced expression of DRP1 (Fig. 6d). TM-25659 could also rescue the ATP production and release content in the Piezo1-knockdown group exposed to mechanical stretching (Fig. 6e,f).

Taken together, these findings illustrated that Piezo1-mediated mechanically induced TAZ nuclear translocation could improve osteocyte mitochondrial fission and ATP release, facilitating BM-MSCs osteogenesis (Fig. 7).

Discussion

The maintenance of bone homeostasis, bone remodeling, and tissue healing all require the initiation of cellular mechanical transduction mechanisms in response to various mechanical stimuli, including fluid shear force, compression, and tension [47]. The major interest in the role of osteocytes in regulating bone metabolism stems from their status as the most abundant resident cells in bone. It was suggested that osteocytes possess the ability to perceive me-

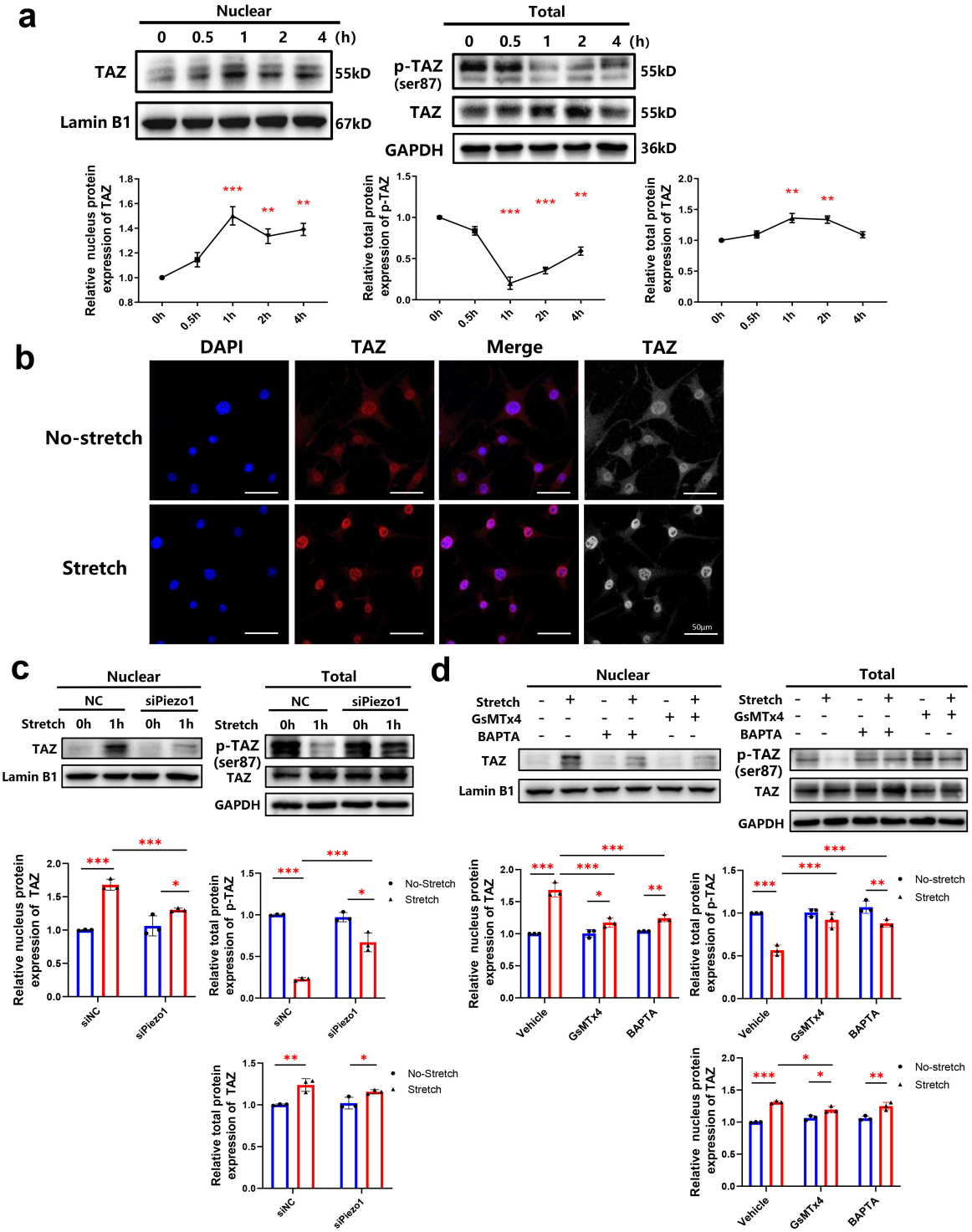

Fig. 4. Mechanical stretching induces Piezo1-related transcriptional coactivator with PDZ-binding motif (TAZ) nuclear translocation in osteocytes. (a) Nuclear and total protein TAZ and phosphorylated TAZ levels in MLO-Y4s were measured by Western blots after 0.5, 1, 2, and 4 hours of mechanical stretching. Quantification of TAZ and p-TAZ protein expression. Lamin B1 and GAPDH were used for normalization. (b) Immunofluorescence of subcellular localization of TAZ in MLO-Y4s exposed to stretching for 1 hour. (c) Western blot analysis of the TAZ and phosphorylated TAZ expression levels in the nucleus and total protein levels of negative control and siPiezo1-transfected MLO-Y4 cells subjected to 1-hour of mechanical stretching. Quantification of TAZ expression in the nucleus. (d) Western blot analysis of the TAZ and phosphorylated TAZ expression levels of negative control and siPiezo1-transfected groups treated with GsMTx4 and BAPTA. Quantification of TAZ and p-TAZ protein expression. All data are shown as mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

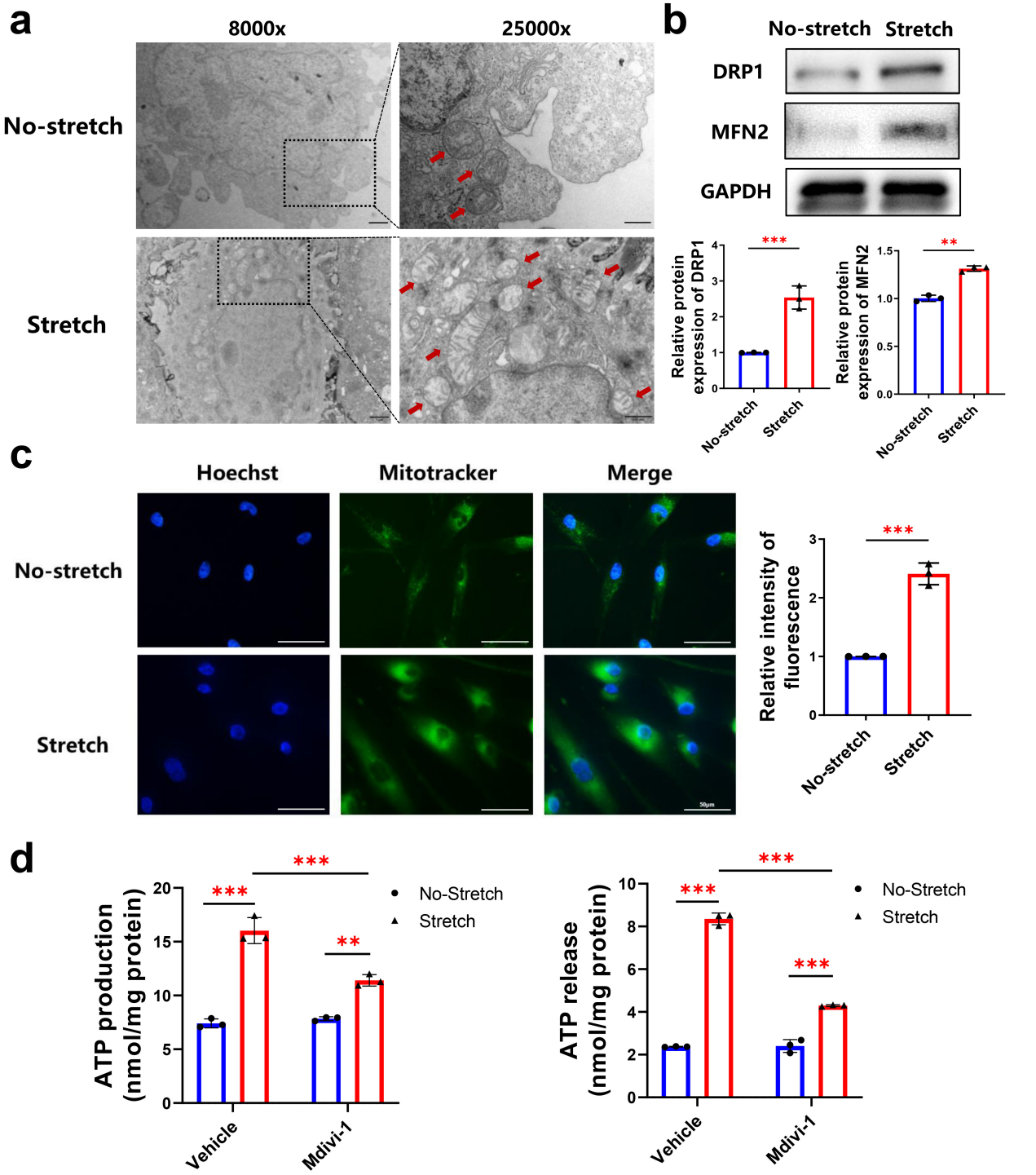

Fig. 5. Mitochondrial generation in response to stretch stimuli regulates the release of ATP in osteocytes. (a) Mitochondrial morphology of no-stretch and stretch-treated MLO-Y4s was observed by transmission electron microscope (TEM). Scale bar = 1 μm (8000×) and 500 nm (25,000×). (b) Western blot and quantification analysis of the Dynamin-related protein 1 (DRP1) and Mitofusin-2 (MFN2) protein expression levels in MLO-Y4s after 1-hour mechanical stretching. (c) Mitochondria (green) fluorescence labeling and determination. The nuclei were stained with Hoechst (blue). (d) ATP content determination analysis of the ATP release and ATP synthesis contents in medium supernatant of MLO-Y4s treated with Mdivi-1. All data are shown as mean ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$.

chanical stimuli and transmit them into biochemical signals [48,49]. However, how they might perceive mechanical loads is still a subject of ongoing research. We observed that CM from stretch-exposed MLO-Y4 cells enhanced the osteogenesis of BM-MSCs. Piezo1 senses mechanical stretch force and regulates osteocyte secretion. Mechanical tension

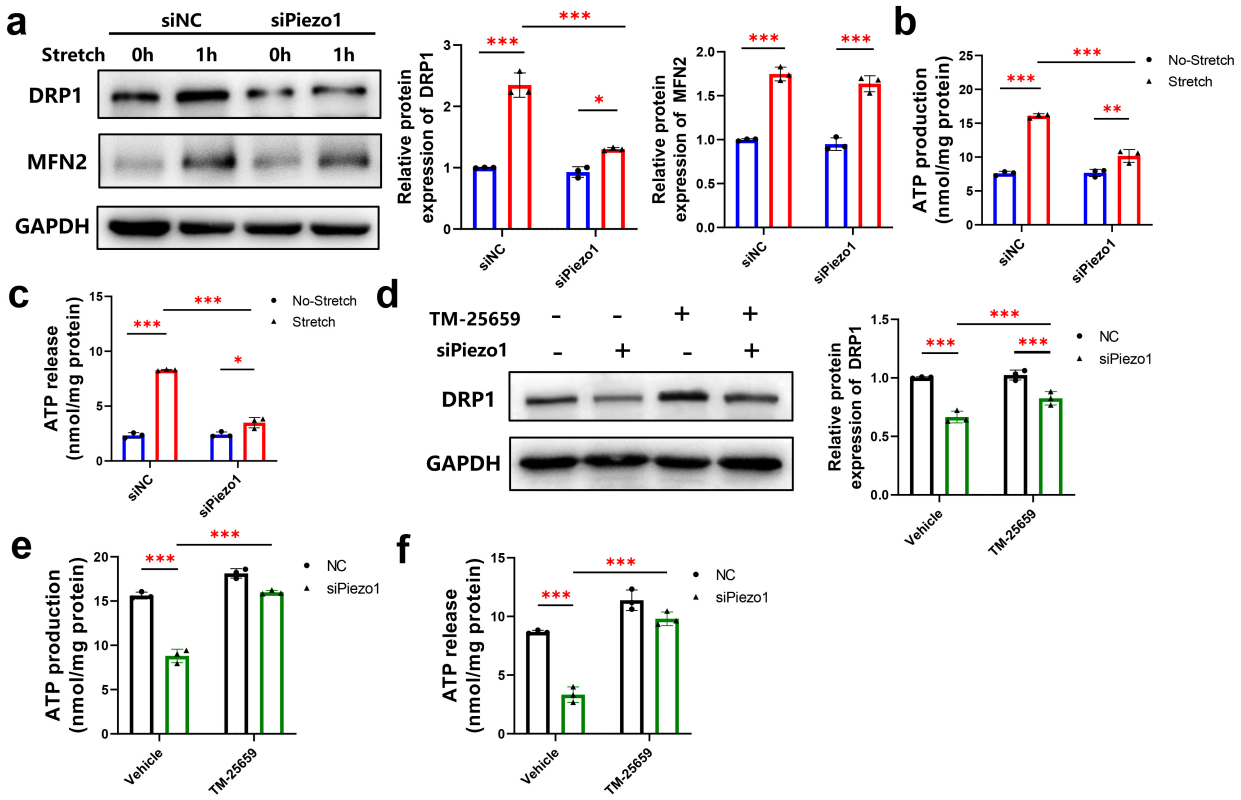

Fig. 6. Piezo1-dependent TAZ nuclear translocation promotes mitochondrial fission and ATP release in osteocytes. (a) Western blot and quantification analysis of the DRP1 and MFN2 protein expression levels of negative control and siPiezo1-transfected MLO-Y4 cells subjected to 1-hour of mechanical stretching. (b) ATP content determination analysis of the ATP release contents in medium supernatant of MLO-Y4s after 1-hr mechanical stretching load. (c) ATP content determination analysis of the ATP synthesis in MLO-Y4s cells. (d) Western blot and quantification of DRP1 expression in TM-25659-treated negative control and siPiezo1-transfected groups. (e) ATP content determination analysis of the ATP release contents in medium supernatant of negative control and siPiezo1-transfected MLO-Y4s treated with TM-25659. (f) ATP content determination analysis of the ATP synthesis in MLO-Y4s cells. All data are shown as mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

can activate Piezo1 and cause calcium influx and TAZ nuclear translocation, thereby inducing mitochondrial fission and ATP release in osteocytes and promoting BM-MSCs osteogenesis. Our results reveal a new mechanism that Piezo1 mediates ATP release in osteocytes under mechanical force to modulate bone defect repair.

The Piezo protein family, including Piezo1 and Piezo2, was discovered in 2010, thus opening a new field of mechanical transduction research [28]. Piezo1 is a mechanically activated ion channel protein that links mechanical force with biological signals. Extensive research has demonstrated its involvement in the control of diverse biological processes [28,50]. Several of these studies have demonstrated Piezo1 expression in osteocytes and its potential role in sensing mechanical force. Li *et al.* [31] discovered that the bones of Piezo1-osteocyte knockout mice were small and weak. The findings from *in vitro* experimentation demonstrated that the protein piezo1 stimulated increased bone formation and decreased bone resorption in osteocyte-like MLO-Y4 cells. This effect was achieved by boosting the production of Wnt1 by activating YAP1 and TAZ, specifically under the influence of fluid shear force [31]. However, relatively few studies show how osteocytes sense stretch force through Piezo1. Hence, it was deemed imperative to thoroughly investigate the intricate mechanism under which bone cells perceive and respond to mechanical stretching stimuli. A previous study by Sasaki revealed that Piezo1 participates in the transduction of mechanical stretch by activating downstream Akt signaling, which down-regulates the sclerostin expression and leads to decreased bone formation [31]. TAZ regulates the osteogenic differentiation through nuclear transport subsequent to dephosphorylation [51]. In this study, mechanical stretching loads were administered to osteocyte-like MLO-Y4 cells, leading to the observation that the MLO-Y4 cells responded to cyclic mechanical stretching forces via the activation of piezo1. Applying mechanical stretching stresses can potentially increase the overall expression of TAZ and significantly enhance its nuclear translocation through Piezo1-dependent calcium influx.

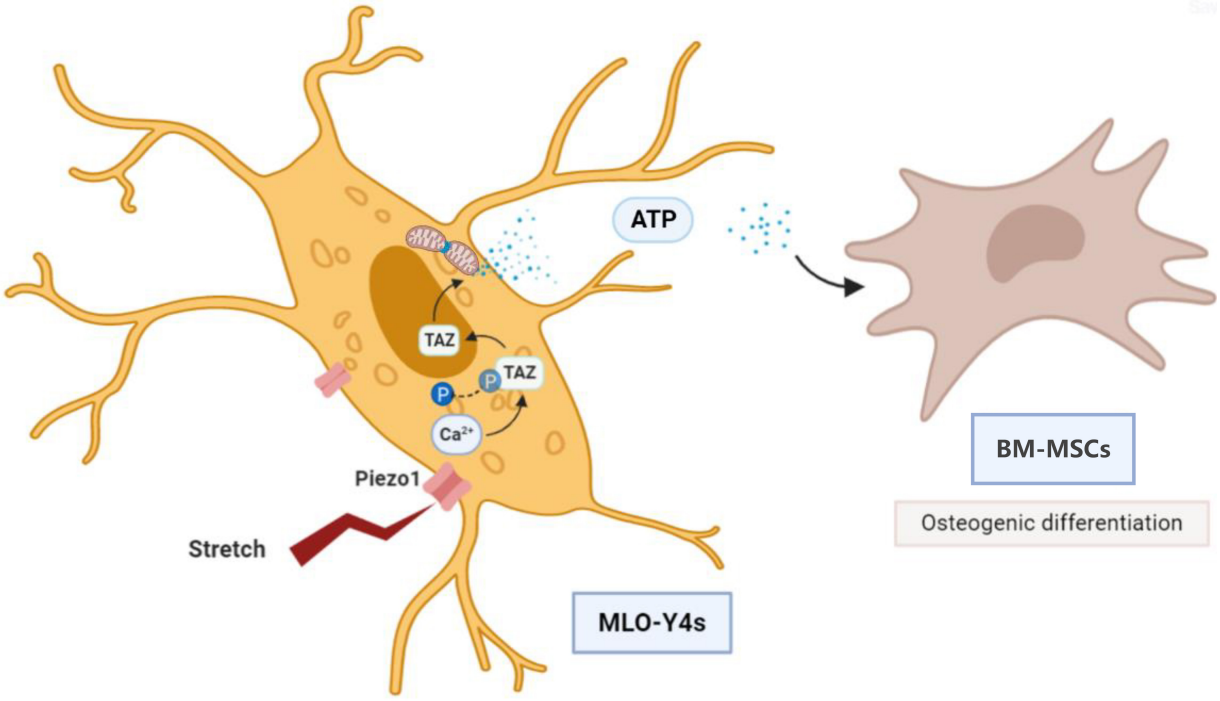

Fig. 7. Piezo1 recognition of stretch force in osteocytes drives MSCs' osteogenic differentiation. Mechanical stretch force signals, transferred via Piezo1 in osteocyte-like MLO-Y4 cells, resulted in Ca^{2+} influx and TAZ-mediated mitochondrial fission and ATP release. This signaling cascade then drove up-regulation in the osteogenic capacity of BM-MSCs. Figure created with BioRender.com.

Zhang's results [52] indicated fluid shear stress-induced ATP release and synthesis in osteocytes. Our study found that the mechanical stretching force can also promote the release and generation of ATP. The principle of the stretching force loading system is to use the vacuum space between the orifice elastic film and the loading element to stretch and contract the elastic film. However, the medium in the elastic orifice plate may flow under the loading stretching force. The distinction between cellular perception and response to fluid shear and stretching force of osteocytes may need further exploration. Huang *et al.* [53] reported that TAZ inhibited mitochondrial dysfunction under inflammatory conditions by improving ATP levels. Similarly, we observed that TAZ agonists promoted the release of ATP from osteocytes. Piezo1 was recently reported to mediate astrocytes' Ca^{2+} response and ATP release [33]. Here, we demonstrated the response that Piezo1-induced Ca^{2+} influx regulated mitochondrial fission and ATP production in osteocytes treated with stretching mechanical force. Of note, Piezo1 can sense both stretching and extrusion pressures, according to Gudipity's research. Additionally, the subcellular localization and levels of Piezo1 may influence its capacity to detect various forms of tension [30]. Therefore, whether piezo1 senses other types of mechanical stress in osteocytes, such as extrusion and compression force, and/or potentially mediates different biochemical processes may be worth further exploration.

As a powerful method to reconstruct segmental bone defects, distraction osteogenesis has the main disadvantage of being a time-consuming treatment course. Numerous studies focus on accelerating bone formation during distraction osteogenesis to shorten consolidation [54,55]. Mitochondrial energy metabolism of mechanosensitive protein Piezo1-dependent and ATP release were identified to play an important role in the transmission of mechanical loads and the regulation of bone formation here, which may provide a new effective intervention method for bone remodeling and regulation. In the future, we will try to promote bone anabolism by targeting pharmaceutical agents that activate these pathways. At the same time, it is also possible to combine related drug formulations with biological materials to accelerate the therapeutic process of distraction osteogenesis.

Conclusions

The osteogenic differentiation property of BM-MSCs cultivated with CM derived from mechanical stretch-exposed osteocytes is improved compared to that from the no-stretch group. Piezo1 regulates calcium transfer and mechanical transduction processes caused by mechanical stretching in osteocyte-like MLO-Y4 cells. Mechanically induced calcium transfer and TAZ nuclear translocation mediated by Piezo1 promote mitochondrial fission and ATP release in osteocytes under mechanical force to modulate bone defect repair and regeneration during craniofacial skeleton distraction osteogenesis.

Availability of Data and Materials

Data available on request from the corresponding authors.

Author Contributions

YWR, WBZ and LW designed the research study; YWR and HG performed the research; YWR, HG and LS acquired and analyzed the data. YWR and HG have been involved in drafting the manuscript and all authors have been involved in revising it critically for important intellectual content. All authors give final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

The animal research procedures obtained approval from the Animal Ethics Committee of Nanjing Medical University (IACUC-1803015).

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202436182.46.

References

[1] Ransom RC, Carter AC, Salhotra A, Leavitt T, Marecic O, Murphy MP, *et al*. Mechanoresponsive stem cells acquire neural crest fate in jaw regeneration. Nature. 2018; 563: 514–521.

[2] Yang S, Wang N, Ma Y, Guo S, Guo S, Sun H. Immunomodulatory effects and mechanisms of distraction osteogenesis. International Journal of Oral Science. 2022; 14: 4.

[3] Mi J, Xu J, Yao H, Li X, Tong W, Li Y, *et al*. Calcitonin Gene-Related Peptide Enhances Distraction Osteogenesis by Increasing Angiogenesis. Tissue Engineering. Part a. 2021; 27: 87–102.

[4] McCarthy JG, Stelnicki EJ, Grayson BH. Distraction osteogenesis of the mandible: a ten-year experience. Seminars in Orthodontics. 1999; 5: 3–8.

[5] McCarthy JG, Stelnicki EJ, Mehrara BJ, Longaker MT. Distraction osteogenesis of the craniofacial skeleton. Plastic and Reconstructive Surgery. 2001; 107: 1812–1827.

[6] Brody-Camp S, Winters R. Craniofacial Distraction Osteogenesis. StatPearls. 2023.

[7] Jones RE, Salhotra A, Robertson KS, Ransom RC, Foster DS, Shah HN, *et al*. Skeletal Stem Cell-Schwann Cell Circuitry in Mandibular Repair. Cell Reports. 2019; 28: 2757–2766.e5.

[8] Swanson D, Ba'th F, Zavala H, Chinnadurai S, Roby BB. Incidence of mandibular distraction osteogenesis in Stickler Syndrome: Variation due to COL2A1 and COL11A1. International Journal of Pediatric Otorhinolaryngology. 2021; 146: 110749.

[9] Bonewald LF. The amazing osteocyte. Journal of Bone and Mineral Research: the Official Journal of the American Society for Bone and Mineral Research. 2011; 26: 229–238.

[10] Li MCM, Chow SKH, Wong RMY, Qin L, Cheung WH. The role of osteocytes-specific molecular mechanism in regulation of mechanotransduction - A systematic review. Journal of Orthopaedic Translation. 2021; 29: 1–9.

[11] Li W, Zhao J, Sun W, Wang H, Pan Y, Wang L, *et al*. Osteocytes promote osteoclastogenesis via autophagy-mediated RANKL secretion under mechanical compressive force. Archives of Biochemistry and Biophysics. 2020; 694: 108594.

[12] Choy MHV, Wong RMY, Chow SKH, Li MC, Chim YN, Li TK, *et al*. How much do we know about the role of osteocytes in different phases of fracture healing? A systematic review. Journal of Orthopaedic Translation. 2019; 21: 111–121.

[13] Kang SY, Deshpande SS, Zheutlin AR, Donneys A, Rodriguez JJ, Nelson NS, *et al*. Role of parathyroid hormone in regeneration of irradiated bone in a murine model of mandibular distraction osteogenesis. Head & Neck. 2017; 39: 464–470.

[14] Urlaub KM, Lynn JV, Carey EG, Nelson NS, Polyatskaya Y, Donneys A, *et al*. Histologic Improvements in Irradiated Bone Through Pharmaceutical Intervention in Mandibular Distraction Osteogenesis. Journal of Oral and Maxillofacial Surgery: Official Journal of the American Association of Oral and Maxillofacial Surgeons. 2018; 76: 2660–2668.

[15] Tao H, Zhu M, Lau K, Whitley OKW, Samani M, Xiao X, *et al*. Oscillatory cortical forces promote three dimensional cell intercalations that shape the murine mandibular arch. Nature Communications. 2019; 10: 1703.

[16] Deepak V, Kayastha P, McNamara LM. Estrogen deficiency attenuates fluid flow-induced $[Ca^{2+}]_i$ oscillations and mechanoresponsiveness of MLO-Y4 osteocytes. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 2017; 31: 3027–3039.

[17] Liao C, Cheng T, Wang S, Zhang C, Jin L, Yang Y. Shear stress inhibits IL-17A-mediated induction of osteoclastogenesis via osteocyte pathways. Bone. 2017; 101: 10–20.

[18] Lau E, Al-Dujaili S, Guenther A, Liu D, Wang L, You L. Effect of low-magnitude, high-frequency vibration on osteocytes in the regulation of osteoclasts. Bone. 2010; 46: 1508–1515.

[19] Chen H, Senda T, Kubo KY. The osteocyte plays multiple roles in bone remodeling and mineral homeostasis. Medical Molecular Morphology. 2015; 48: 61–68.

[20] Yan Y, Wang L, Ge L, Pathak JL. Osteocyte-Mediated Translation of Mechanical Stimuli to Cellular Signaling and Its Role in Bone and Non-bone-Related Clinical Complications. Current Osteoporosis Reports. 2020; 18: 67–80.

[21] Sapir-Koren R, Livshits G. Osteocyte control of bone remodeling: is sclerostin a key molecular coordinator of the balanced bone resorption-formation cycles? Osteoporosis International: a Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2014; 25: 2685–2700.

[22] Sasaki F, Hayashi M, Mouri Y, Nakamura S, Adachi T, Nakashima T. Mechanotransduction via the Piezo1-Akt pathway underlies Sost suppression in osteocytes. Biochemical and Biophysical Research Communications. 2020; 521: 806–813.

[23] Heggli I, Blache U, Herger N, Mengis T, Jaeger PK, Schuepbach R, *et al*. FGF2 overrides key pro-fibrotic features of bone marrow stromal cells isolated from Modic type 1 change patients. European Cells & Materials. 2022; 44: 101–114.

[24] Chen Q, Shou P, Zheng C, Jiang M, Cao G, Yang Q, *et al*. Fate decision of mesenchymal stem cells: adipocytes or osteoblasts? Cell Death and Differentiation. 2016; 23: 1128–1139.

[25] Liao C, Zhang C, Jin L, Yang Y. IL-17 alters the mesenchymal stem cell niche towards osteogenesis in cooperation with osteocytes. Journal of Cellular Physiology. 2020; 235: 4466–4480.

[26] Spyropoulou A, Karamesinis K, Basdra EK. Mechanotransduction pathways in bone pathobiology. Biochimica et Biophysica Acta. 2015; 1852: 1700–1708.

[27] Xiao B. Levering Mechanically Activated Piezo Channels for Potential Pharmacological Intervention. Annual Review of Pharmacology and Toxicology. 2020; 60: 195–218.

[28] Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, *et al*. Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science (New York, N.Y.). 2010; 330: 55–60.

[29] Coste B, Xiao B, Santos JS, Syeda R, Grandl J, Spencer KS, *et al*. Piezo proteins are pore-forming subunits of mechanically activated channels. Nature. 2012; 483: 176–181.

[30] Gudipaty SA, Lindblom J, Loftus PD, Redd MJ, Edes K, Davey CF, *et al*. Mechanical stretch triggers rapid epithelial cell division through Piezo1. Nature. 2017; 543: 118–121.

[31] Li X, Han L, Nookaew I, Mannen E, Silva MJ, Almeida M, *et al*. Stimulation of Piezo1 by mechanical signals promotes bone anabolism. eLife. 2019; 8: e49631.

[32] Hendrickx G, Fischer V, Liedert A, von Kroge S, Haffner-Luntzer M, Brylka L, *et al*. Piezo1 Inactivation in Chondrocytes Impairs Trabecular Bone Formation. Journal of Bone and Mineral Research: the Official Journal of the American Society for Bone and Mineral Research. 2021; 36: 369–384.

[33] Chi S, Cui Y, Wang H, Jiang J, Zhang T, Sun S, *et al*. Astrocytic Piezo1-mediated mechanotransduction determines adult neurogenesis and cognitive functions. Neuron. 2022; 110: 2984–2999.e8.

[34] Hong W, Guan KL. The YAP and TAZ transcription co-activators: key downstream effectors of the mammalian Hippo pathway. Seminars in Cell & Developmental Biology. 2012; 23: 785–793.

[35] Li W, Zhao J, Wang J, Sun L, Xu H, Sun W, *et al*. ROCK-TAZ signaling axis regulates mechanical tension-induced osteogenic differentiation of rat cranial sagittal suture mesenchymal stem cells. Journal of Cellular Physiology. 2020; 235: 5972–5984.

[36] Chakraborty M, Chu K, Shrestha A, Revelo XS, Zhang X, Gold MJ, *et al*. Mechanical Stiffness Controls Dendritic Cell Metabolism and Function. Cell Reports. 2021; 34: 108609.

[37] Hwang JH, Kim KM, Oh HT, Yoo GD, Jeong MG, Lee H, *et al*. TAZ links exercise to mitochondrial biogenesis via mitochondrial transcription factor A. Nature Communications. 2022; 13: 653.

[38] Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu SS. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. American Journal of Physiology. Cell Physiology. 2004; 287: C817–C833.

[39] Westermann B. Mitochondrial fusion and fission in cell life and death. Nature Reviews. Molecular Cell Biology. 2010; 11: 872–884.

[40] Eslaminejad MB, Nadri S. Murine mesenchymal stem cell isolated and expanded in low and high density culture system: surface antigen expression and osteogenic culture mineralization. In Vitro Cellular & Developmental Biology. Animal. 2009; 45: 451–459.

[41] Sun W, Chi S, Li Y, Ling S, Tan Y, Xu Y, *et al*. The mechanosensitive Piezo1 channel is required for bone formation. eLife. 2019; 8: e47454.

[42] Komori T. Regulation of Proliferation, Differentiation and Functions of Osteoblasts by Runx2. International Journal of Molecular Sciences. 2019; 20: 1694.

[43] Delmas P, Hao J, Rodat-Despoix L. Molecular mechanisms of mechanotransduction in mammalian sensory neurons. Nature Reviews. Neuroscience. 2011; 12: 139–153.

[44] Aragona M, Panciera T, Manfrin A, Giulitti S, Michielin F, Elvassore N, *et al*. A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. Cell. 2013; 154: 1047–1059.

[45] Byun MR, Jeong H, Bae SJ, Kim AR, Hwang ES, Hong JH. TAZ is required for the osteogenic and anti-adipogenic activities of kaempferol. Bone. 2012; 50: 364–372.

[46] Kanai F, Marignani PA, Sarbassova D, Yagi R, Hall RA, Donowitz M, *et al*. TAZ: a novel transcriptional co-activator regulated by interactions with 14-3-3 and PDZ domain proteins. The EMBO Journal. 2000; 19: 6778–6791.

[47] Ren L, Yang P, Wang Z, Zhang J, Ding C, Shang P. Biomechanical and biophysical environment of bone from the macroscopic to the pericellular and molecular level. Journal of the Mechanical Behavior of Biomedical Materials. 2015; 50: 104–122.

[48] Moriishi T, Fukuyama R, Ito M, Miyazaki T, Maeno T, Kawai Y, *et al*. Osteocyte network; a negative regulatory system for bone mass augmented by the induction of Rankl in osteoblasts and Sost in osteocytes at unloading. PloS One. 2012; 7: e40143.

[49] Li X, Kordsmeier J, Xiong J. New Advances in Osteocyte Mechanotransduction. Current Osteoporosis Reports. 2021; 19: 101–106.

[50] Volkers L, Mechioukhi Y, Coste B. Piezo channels: from structure to function. Pflugers Archiv: European Journal of Physiology. 2015; 467: 95–99.

[51] Cai X, Wang KC, Meng Z. Mechanoregulation of YAP and TAZ in Cellular Homeostasis and Disease Progression. Frontiers in Cell and Developmental Biology. 2021; 9: 673599.

[52] Zhang B, Hou R, Zou Z, Luo T, Zhang Y, Wang L, *et al*. Mechanically induced autophagy is associated with ATP metabolism and cellular viability in osteocytes in vitro. Redox Biology. 2018; 14: 492–498.

[53] Huang JC, Yue ZP, Yu HF, Yang ZQ, Wang YS, Guo B. TAZ ameliorates the microglia-mediated inflammatory response via the Nrf2-ROS-NF-κB pathway. Molecular Therapy. Nucleic Acids. 2022; 28: 435–449.

[54] Liu X, Sun Y, Shen J, Min HS, Xu J, Chai Y. Strontium doped mesoporous silica nanoparticles accelerate osteogenesis and angiogenesis in distraction osteogenesis by activation of Wnt pathway. Nanomedicine: Nanotechnology, Biology, and Medicine. 2022; 41: 102496.

[55] Lou T, Chen K, Luo Q, Liu C, Yuan Y, Fan C. Periosteum-inspired in situ CaP generated nanocomposite hydrogels with strong bone adhesion and superior stretchability for accelerated distraction osteogenesis. Biomaterials Research. 2022; 26: 91.