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Original Article

Piezo1-mediated mechanotransduction in cementocytes via protein kinase B and p38 mitogen-activated protein kinase signaling

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Abstract *Background/purpose:* Cementocytes, terminally differentiated cells embedded within cellular cementum, are morphologically similar to osteocytes; however, their mechanosensory function remains poorly understood. This study aimed to investigate whether Piezo1, a mechanosensitive ion channel, contributes to the regulation of osteo/cementogenic gene expression in murine cementocyte-like IDG-CM6 cells.
Materials and methods: IDG-CM6 cells were subjected to cyclic stretch or treated with Piezo1-specific agonist Yoda1 or antagonist GsMTx4. Expression levels of osteo/cementogenic genes (*Wnt1*, *Sost*, *Opg*) and protein levels were analyzed. The involvement of intracellular signaling pathways was assessed using pharmacological inhibitors targeting mitogen-activated protein kinase and protein kinase B (PKB/AKT) pathways.
Results: Cyclic stretch upregulated *Wnt1* and *Opg*, and downregulated *Sost* expression, without altering *Piezo1* expression, suggesting an enhanced osteo/cementogenic potential.

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These effects were abolished by GsMTx4 and closely mimicked by Yoda1 stimulation. The Yoda1-induced gene expression changes were transient and diminished after withdrawal. Inhibitor experiments confirmed that Piezo1-mediated gene expression is modulated primarily through the AKT and p38 signaling pathways. Phosphorylation of AKT and p38 was rapidly induced by cyclic stretch.
Conclusion: Our findings demonstrate that Piezo1 functions as a mechanosensor in cementocytes, modulating the expression of osteo/cementogenic genes via the AKT and p38 pathways. This study provides new insight into the molecular mechanisms of cementocyte mechanotransduction and may inform strategies for periodontal regeneration and orthodontic treatment.
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Introduction

Mechanosensation—the ability of cells to detect and respond to mechanical stimuli—is essential for tissue development and remodeling.[1] Piezo1, a mechanosensitive cation channel identified in 2010,[2] converts mechanical forces such as stretch, shear stress, and compression into calcium influx,[3] activating downstream signaling that regulates gene expression and cell behavior. Accumulating evidence has underscored the critical role of Piezo1 in bone biology,[3] particularly in osteoblasts and osteocytes.[4] Piezo1 plays a pivotal role in matrix synthesis and mineralization in osteoblast-lineage cells, as evidenced by the reduced bone volume and mineral apposition observed in conditional Piezo1 knockout mice.[5]

Cementum is a mineralized tissue covering tooth roots, shares extracellular matrix features with bone but is avascular, non-innervated, and minimally remodeled.[6,7] Cementum is classified as acellular or cellular, depending on the presence of embedded cementocytes. During the development of cellular cementum, cementoblasts secrete an unmineralized extracellular matrix (cementoid), and some of these cells become entrapped within the matrix, differentiating into cementocytes. Cementocytes are terminally differentiated cells embedded in lacunae within cellular cementum. They extend dendritic processes through canaliculi, forming a lacuno-canalicular network similar to osteocytes.[8–11] This network likely facilitates intercellular communication, signaling with PDL surfaces, and fluid flow. In osteocytes, such fluid flow is known to generate shear stress and hydrostatic pressure, which serve as key mechanical cues.[4,12] Given the structural similarities, it is likely that cementocytes also participate in a mechanosensory feedback system. While the mechanosensory function of osteocytes is well established, the physiological role of cementocytes—particularly their capacity for mechanotransduction—remains largely unexplored. Whether Piezo1 contributes to this function has not yet been determined.

Although studies on Piezo1 in periodontal tissues are still limited, emerging research has begun to investigate its role in periodontal ligament cells[13,14] and cementoblasts,[15] suggesting a broader involvement in periodontal mechanoadaptation.[16] However, the expression and functional relevance of Piezo1 in cementocytes, the mature embedded cells of cellular cementum, remain unknown. Given their unique anatomical position and structural similarity to osteocytes, elucidating the role of Piezo1 in cementocytes could fill a critical knowledge gap in dental mechanobiology. Such insights may have important implications for periodontal regeneration and orthodontic therapy.

In this study, we investigated the expression and function of Piezo1 in IDG-CM6 cells, a murine cementocyte cell line. Using mechanical stretch and pharmacological modulation, we assessed the influence of Piezo1 on osteo/cementogenic gene expression and signaling pathways.

Materials and methods

Reagents

Yoda1, GsMTx4, ascorbic acid, and β-glycerophosphate were obtained from Sigma—Aldrich (St. Louis, MO, USA). Mouse interferon gamma (IFN-γ) was purchased from PeproTech (Rocky Hill, NJ, USA). PD98059 (ERK1/2 inhibitor), SB203580 (p38 mitogen-activated protein kinase (MAPK) inhibitor), and SP600125 (JNK inhibitor II) were purchased from Merck KGaA (Darmstadt, Germany); AKT inhibitor IV from Selleck Chemicals (Houston, TX, USA).

Tissue preparation and immunohistochemistry

Five male C57BL/6JJcl mice (9 weeks old, 19—21 g; Clea Japan, Inc., Tokyo, Japan) were used. Mice were specific pathogen-free and euthanized with isoflurane (Fujifilm Wako Chemicals, Tokyo, Japan). Mandibular bones including first molar roots were extracted, fixed in 4 % paraformaldehyde in phosphate-buffered saline (PBS) at 4 °C for 24 h. Sample was washed with water for 2 days and decalcified in 17.7 % EDTA (OSTEOSOFT, Merck Millipore, Tokyo, Japan) at 4 °C for 4 weeks before paraffin embedding. Paraffin-embedded serial sections (5 μm) were stained with hematoxylin and eosin (H&E) and analyzed under a light microscope. For Piezo1 detection, sections were stained with anti-Piezo1 antibody (1:400; NBP1-78537, Novus Biologicals, Centennial, CO, USA). All procedures were approved by the Institutional Animal Experiment Committee of Tohoku University (Approval No. 2024DnA-006-02).

Table 1 Primer sequences used for polymerase chain reaction amplifications.

Primer name	Direction	Sequence (5′-3′)
Gapdh	Forward	AATGTGTCCGTCGTGGATCTGA
	Reverse	GATGCCTGCTTCACCACCTTCT
Piezo1	Forward	AGCATCAACTTCCATCGCCA
	Reverse	CGGATGCGCTTCATCTGTCT
Wnt1	Forward	GCCTCCCGCGTCCTTTA
	Reverse	CACTGTACGTGCAGAAGTT
Opg	Forward	TGAATGCCGAGAGTGTAG
	Reverse	CTGCTCGCTCGATTTG
Sost	Forward	ACTCCTTCCACCCAAATG
	Reverse	CCAATCCTTGGAATCTCAGC
Wnt3a	Forward	AGGAGAGCTCCTAACACG
	Reverse	AACCGGGTCCTTAGGTAT
Wnt7a	Forward	TGCGCAGGCTATGTGGATT
	Reverse	CCGAAGAGAAGCCACCGAT

Abbreviations: *Glyceraldehyde-3-phosphate dehydrogenase (Gapdh), Osteoprotegerin (Opg).*

Cell line and culture

IDG-CM6 cementocyte-like cells[17] (Kerafast, Boston, MA, USA) were cultured in α-Minimum Essential Medium (α-MEM) (Gibco™, Life Technologies, Carlsbad, CA, USA) with 10 % heat-inactivated FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL) —referred to as complete α-MEM. Dishes were coated with 0.15 mg/mL rat tail type I collagen (Atelo Cell® KOKEN Co., Tokyo, Japan). For proliferation, cells were incubated at 33 °C in complete α-MEM with 50 U/mL IFN-γ. Differentiation was induced in IFN-γ-free complete α-MEM containing 50 μg/mL ascorbic acid and 4 mM β-glycerophosphate (differentiation medium) at 37 °C, with media changes every other day.

Cyclic stretch stimulation

Cyclic stretch was performed as previously described.[18–20] Collagen-coated silicon chambers (STB—CH—10.0; STREX Co., Osaka, Japan) were seeded with 1×10^6 cells and cultured in complete α-MEM containing IFN-γ at 33 °C. After reaching 80—90 % confluence, cells were differentiated for 2 weeks, then stretched uniaxially (10 % elongation, 10 cycles/min, 6 h) using the STB-140 STREX system. Controls were cultured without stretch. Supernatants and cells were collected for ELISA, RT-qPCR, or Western blotting.

Piezo1 agonist stimulation

Cells (1×10^5) in collagen-coated 24-well plates were treated with various Yoda1 concentrations for 6 or 24 h; while control received equal volumes of dimethyl sulfoxide (DMSO). To assess the sustained effects of Yoda1, Yoda1 was removed after 6 h, and cells were cultured in fresh medium for an additional 24 or 48 h. For pathway inhibition, cells were pretreated for 30 min with 10 μM of each inhibitor prior to Yoda1 exposure.

Real-time quantitative PCR (RT-qPCR)

Total RNA was extracted using Qiashredder and RNeasy® Kits (QIAGEN, Valencia, CA, USA), and reverse transcription was performed with the Transcriptor First Strand cDNA

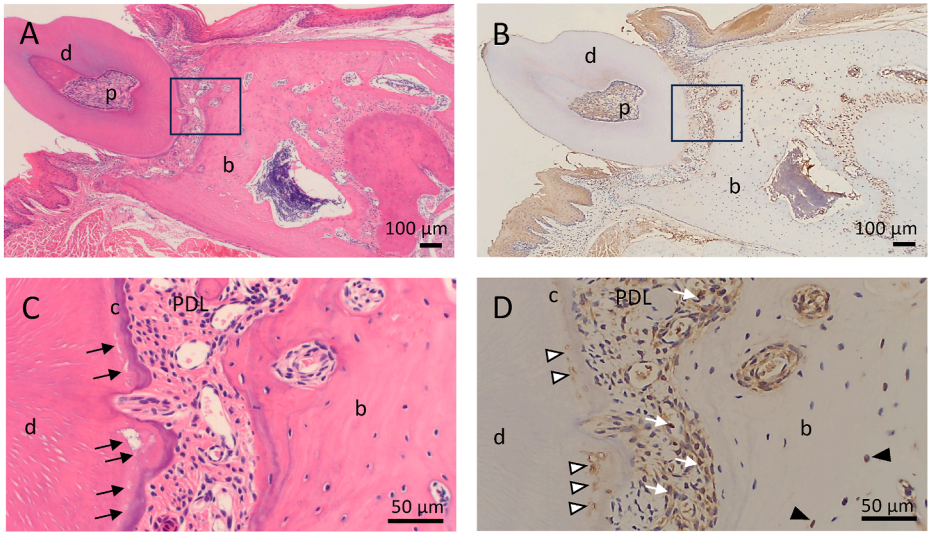

Figure 1 Piezo1 is expressed in cementocytes and surrounding periodontal tissues. Representative immunohistochemical staining for Piezo1 in the mandibular first molar region of 9-week-old C57BL/6JJcl mice. Paraffin-embedded sections were prepared from the furcation region, and hematoxylin and eosin (H&E) staining and Piezo1 immunostaining were performed on serial sections. Panel (A) shows H&E staining at 4 × magnification, while panel (B) displays Piezo1 immunostaining at the corresponding site. Panels (C) and (D) are 40 × magnification images corresponding to the boxed regions in (A) and (B), respectively. Black arrows indicate cemental lacunae; white arrowheads indicate Piezo1-positive cementocytes; white arrow indicate Piezo1-positive periodontal ligament cells; black arrowheads indicate Piezo1-positive osteocytes. Anatomical structures are labeled as follows: b, bone; c, cementum; d, dentin; p, dental pulp; PDL, periodontal ligament.

Synthesis Kit® (Roche Diagnostics, Indianapolis, IN, USA). RT-qPCR was performed using iQ SYBR Green Supermix® (Bio-Rad Laboratories, Hercules, CA, USA) as previously described,[18] with *Gapdh* as internal control. Primer sequences are listed in Table 1. Relative gene expression was calculated using the $2^{-\triangle\triangle CT}$ method.

Enzyme-linked immunosorbent assay (ELISA)

Osteoprotegerin (OPG) protein levels in supernatants were quantified using the Mouse SimpleStep ELISA Kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Absorbance was measured with SoftMax data analysis software (Molecular Devices, Menlo Park, CA, USA).

Western blotting

Cells were stretched for 10, 30, 60, or 180 min. Lysates were prepared with Cell Lysis Buffer® (Cell Signaling Technology, Beverly, MA, USA). Western blotting was performed as described.[21] Primary antibodies (Cell Signaling) included phospho/total p38 MAPK (1:1000), phospho-AKT (1:2000), and total AKT (1:1000). HRP-conjugated goat anti-rabbit IgG (1:2000) was used as the secondary antibody.

Statistical analysis

All statistical analyses and graph generation were conducted using GraphPad Prism 7 (GraphPad Software, La

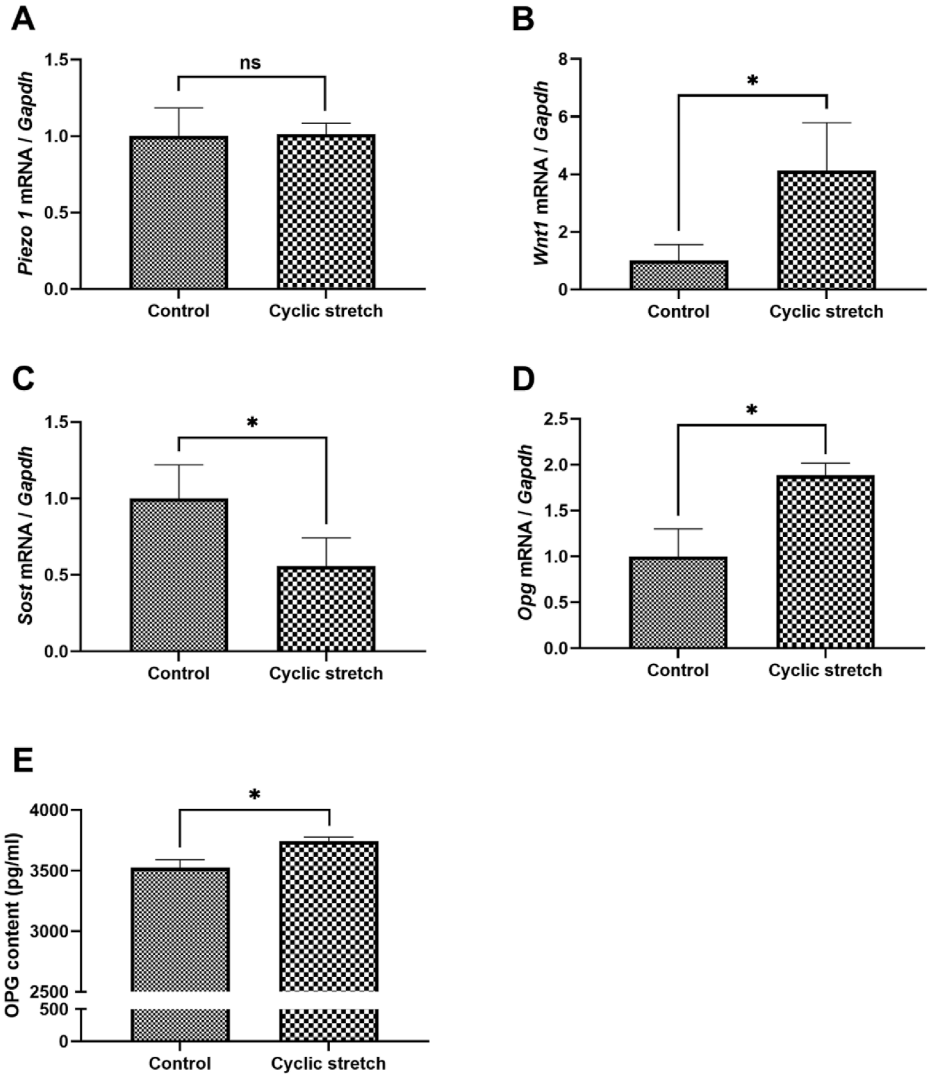

Figure 2 Cyclic stretch modulates osteo/cementogenic-related molecules in cementocytes. Cells were subjected to cyclic stretch at 10 % elongation (10 cycles/min) for 6 h. (A-D) Total RNA was extracted, and mRNA levels of *Piezo1*, *Wnt1*, *Sost*, and *Opg* were analyzed by real-time quantitative PCR. (E) OPG protein levels in the culture supernatant were measured by ELISA. Data are presented as the mean ± SD from triplicate samples in representative results of three independent experiments. $P < 0.05$ vs. control. ns, not significant. Abbreviation: OPG, osteoprotegerin.

Jolla, CA, USA). Comparisons between two groups were performed using Student's t-test, and multiple group comparisons were evaluated using one-way ANOVA. A *P*-value of <0.05 was considered statistically significant.

Results

Piezo1 is expressed in cementocytes

To assess whether Piezo1 is expressed in cementocytes *in vivo*, we performed immunohistochemical staining of mandibular first molars from 9-week-old C57BL/6JJcl mice, a stage corresponding to completed root and cellular cementum formation. Sections were taken from the furcation area, which undergoes high occlusal forces and stress compared to other root regions.[22] As shown in Fig. 1A–D, Piezo1 expression was clearly observed in cementocytes within the cellular cementum. Consistent with previous reports, Piezo1 expression was also observed in periodontal ligament cells and osteocytes within the alveolar bone.

Cyclic stretch regulates osteo/cementogenic-related genes in cementocytes

We used IDG-CM6 murine cementocyte cells expressing dentin matrix protein 1 by day 14 of differentiation.[21] Cells were subjected to 10 % cyclic stretch at 10 cycles/min for 6 h. The 10 % elongation approximates physiological strain based on prior studies. Previous studies have reported that physiological masticatory forces ranging from 3 to 20 N can cause tooth root displacement of up to 30 μm.[23,24] Given the periodontal ligament thickness of 0.15–0.38 mm,[25] this corresponds to 8–20 % strain. Thus, applying 10 % cyclic stretch *in vitro* approximates the tensile strain experienced by periodontal ligament-associated cells, including cementocytes. As shown in Fig. 2A–E, *Piezo1* expression remained unchanged, but *Wnt1*, a canonical Wnt ligand, was significantly upregulated, while *Sost* (Sclerostin), a canonical Wnt signaling antagonist,[26] was markedly downregulated. The expression of OPG, a decoy receptor for RANKL and an important regulator of osteoclastogenesis, was significantly increased at both the mRNA and protein levels.

Piezo1 antagonist GsMTx4 reverses the effects of cyclic stretch stimulation in cementocytes

To evaluate the involvement of Piezo1 in mediating these responses, we applied GsMTx4, a peptide inhibitor of stretch-activated ion channels including Piezo1, during cyclic stretch stimulation. As shown in Fig. 3A–D, the cyclic stretch–induced upregulation of *Wnt1* and *Opg* mRNA, as well as the downregulation of *Sost* mRNA, was abolished by GsMTx4 treatment. In addition, the stretch-induced

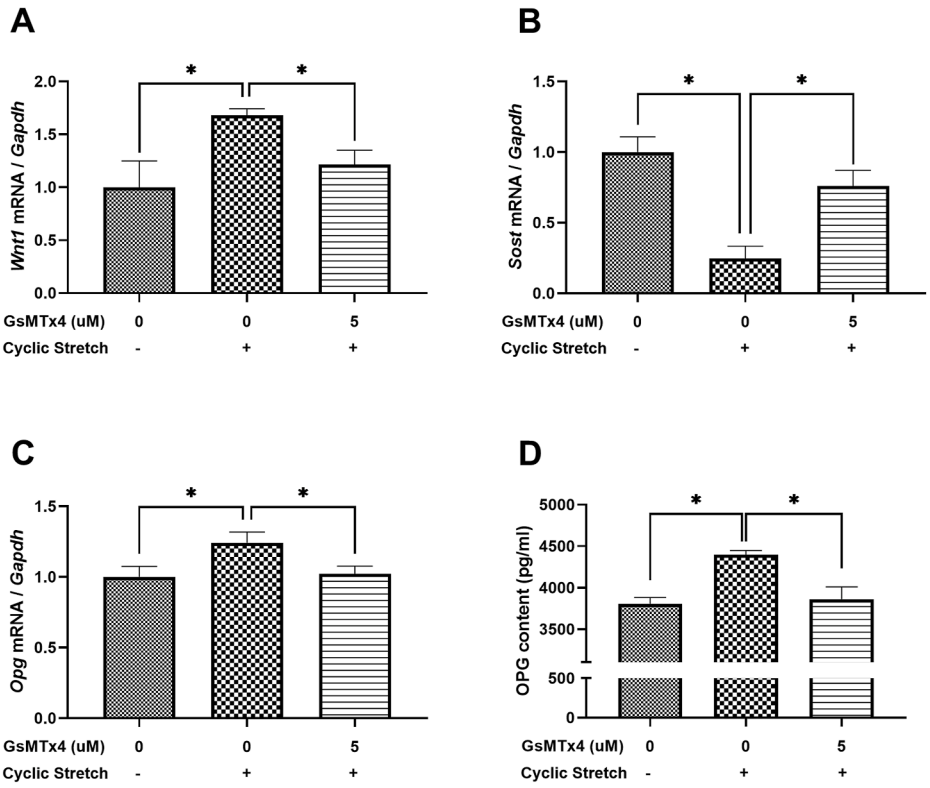

Figure 3 Piezo1 antagonist GsMTx4 reverses cyclic stretch–induced changes in osteo/cementogenic-related molecules in cementocytes. Cells were pretreated with 5 μM GsMTx4 for 30 min and then subjected to 10 % cyclic stretch (10 cycles/min) for 6 h in the continued presence of GsMTx4. Expression of *Wnt1*, *Sost*, and *Opg* were analyzed by real-time quantitative PCR, and OPG protein in the supernatant was quantified by ELISA. Data represent the mean ± SD of triplicate samples from three independent experiments. $P < 0.05$ vs. control. ns, not significant. Abbreviation: OPG, osteoprotegerin.

increase in OPG protein expression was also negated in the presence of GsMTx4, confirming Piezo1's role in mechanical gene regulation.

Piezo1 agonist Yoda1 induces osteo/cementogenic-related molecules in a similar manner to cyclic stretch stimulation in cementocytes

To further validate the involvement of Piezo1, cells were stimulated with Yoda1, a selective chemical agonist, without stretch. As shown in Fig. 4A—D, Stimulation with 1 μM Yoda1 caused a mild increase in *Opg*, while 5 μM Yoda1 robustly increased *Wnt1* and *Opg* and reduced *Sost* expression, closely mimicking stretch-induced gene patterns. These effects were evident at both 6 and 24 h, with more pronounced responses at 6 h, particularly for *Wnt1* and *Opg*. Correspondingly, OPG protein levels were also significantly elevated following 5 μM Yoda1 stimulation at 6 h. The expression of other canonical Wnt ligands such as *Wnt3a* and *Wnt7a* was not detectable (Ct values ~40), indicating negligible expression (Supplement Table). Collectively, these findings further support the involvement of Piezo1 in mediating stretch-induced regulation of osteo/cementogenic gene and protein expression in cementocytes. Based on these results, 5 μM Yoda1 with a 6-h stimulation was used in subsequent experiments.

Yoda1-induced Piezo1 activation is transient after removal of the agonist

To assess the temporal persistence of the Piezo1-mediated response, cells were stimulated with 5 μM Yoda1 for 6 h, followed by culture in Yoda1-free medium for 24 or 48 h. As shown in Fig. 5A—C, the initial Yoda1-induced gene expression changes in *Wnt1*, *Opg*, and *Sost* were partially retained at 24 h post-removal. However, by 48 h, *Wnt1* expression returned to baseline levels, indicating that the Yoda1 effect is transient and requires sustained Piezo1 activation to be maintained.

Piezo1 activation modulates osteo/cementogenic gene expression via AKT and p38 signaling

Given previous reports implicating MAPK (ERK1/2, JNK, p38) and PI3K/AKT pathways downstream of Piezo1 activation,[15,27] we investigated their involvement in Piezo1-mediated responses in cementocytes. Cells were pretreated with selective pharmacological inhibitors targeting ERK1/2 (PD98059), JNK (JNK inhibitor II), p38 (SB203580), and AKT (AKT inhibitor IV) prior to Yoda1 stimulation. As shown in Fig. 6A—C, inhibition of AKT almost completely abrogated the Yoda1-induced changes in *Wnt1*, *Opg*, and *Sost* expression. In contrast, p38 inhibition partially

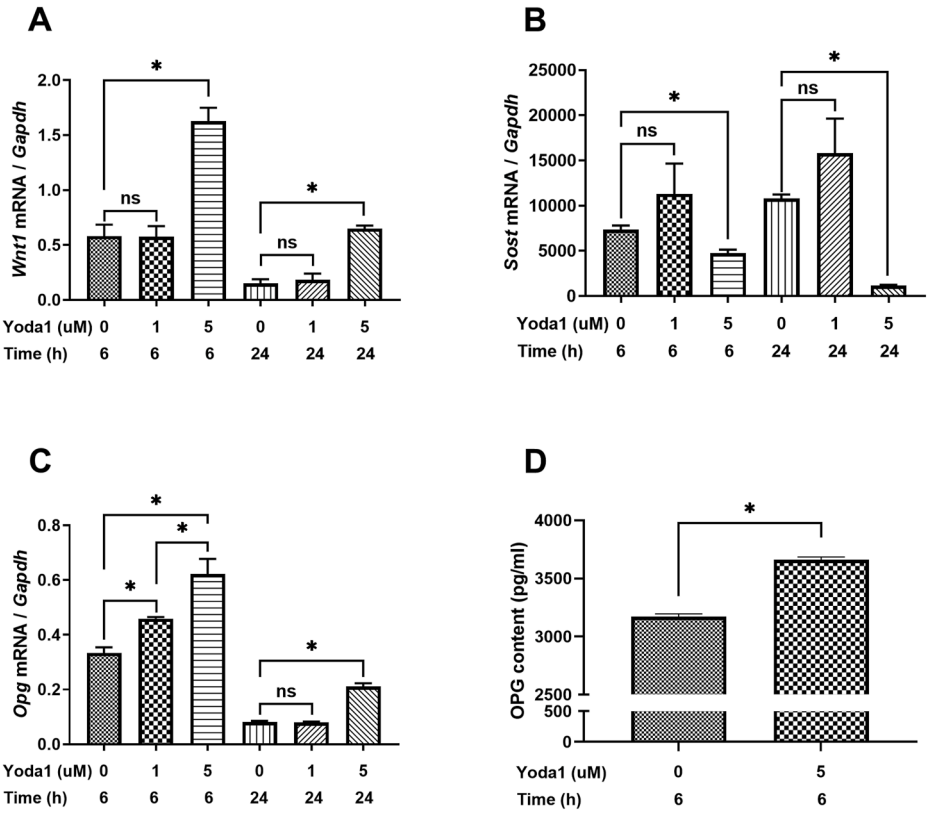

Figure 4 Piezo1 agonist Yoda1 mimics cyclic stretch—induced changes in osteo/cementogenic-related molecules in cementocytes. Cells were treated with the indicated concentrations of Yoda1 for 6 or 24 h without mechanical stimulation. Expression of *Wnt1*, *Sost*, and *Opg* were analyzed by real-time quantitative PCR, and OPG secretion was measured by ELISA. Data are shown as the mean ± SD from triplicate samples in representative results of three independent experiments. $P < 0.05$ vs. control. ns, not significant. Abbreviation: OPG, osteoprotegerin.

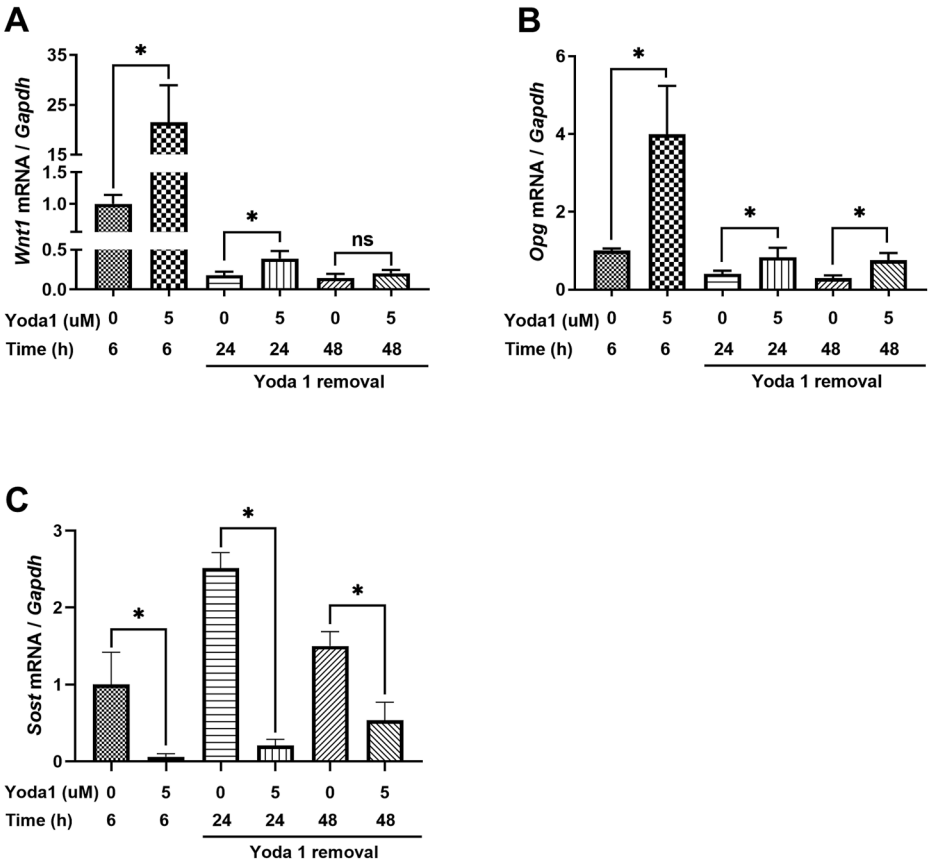

Figure 5 Yoda1-induced expression of osteo/cementogenic-related molecules in cementocytes diminishes after agonist removal. Cells were treated with 5 μM Yoda1 for 6 h, followed by three PBS washes and further incubation in Yoda1-free medium for 24 or 48 h. Expression of *Wnt1*, *Sost*, and *Opg* were assessed by real-time quantitative PCR. Data are shown as the mean ± SD of triplicate samples in representative results of three independent experiments. $P < 0.05$ vs. control. ns, not significant. Abbreviation: OPG, osteoprotegerin.

suppressed these effects, while ERK and JNK inhibition had minimal influence, indicating AKT as the primary mediator, with a contributory role for p38.

Cyclic stretch induces transient phosphorylation of AKT and p38 in cementocytes

To verify that Piezo1 activation by mechanical stimulation engages AKT and p38 signaling pathways, we assessed the phosphorylation status of these kinases following cyclic stretch. Western blot analysis demonstrated a transient increase in phosphorylated AKT (Fig. 7A, C) and p38 (Fig. 7B, D), with both reaching peak levels at 10 min post-stimulation before declining thereafter. These results suggest that cyclic stretch triggers Piezo1-dependent rapid and transient AKT and p38 activation in cementocytes.

Discussion

Immunohistochemistry confirmed Piezo1 expression in cementocytes, supporting their role in sensing mechanical cues. Piezo1 was also found in periodontal ligament cells and osteocytes, consistent with prior reports. We provide the first direct evidence that Piezo1 acts as a mechanosensor in cementocytes, regulating osteo/cementogenic gene expression via AKT and p38 pathways, indicating active mechanotransduction rather than a passive role.

Our findings align with osteocyte studies showing that mechanical loading or Piezo1 activation enhances bone formation by upregulating *Wnt1* and downregulating *Sost*.[5] Additionally, glucocorticoid-induced osteoporosis models have shown reduced Piezo1 expression in osteocytes, reversible by Yoda1, restoring bone mass and osteogenic activity.[28] Mechanistically, Piezo1 activation in IDG-SW3 osteocytes stimulates AKT phosphorylation and suppresses *Sost* expression,[27] consistent with our observations in cementocytes. Furthermore, in MLO-Y4 cells, Piezo1-mediated fluid shear stress promote OPG expression via NOTCH3 signaling,[29] supporting a conserved role in bone remodeling. While these osteocytic studies provide important context, our work is the first to directly demonstrate Piezo1 activation in cementocytes, embedded within cellular cementum. Notably, the anatomical location, extracellular matrix environment, and remodeling dynamics of cementocytes differ markedly from osteocytes. These differences suggest that, despite shared mechanistic features, the biological consequences of Piezo1 activation may be distinct and tissue-specific.

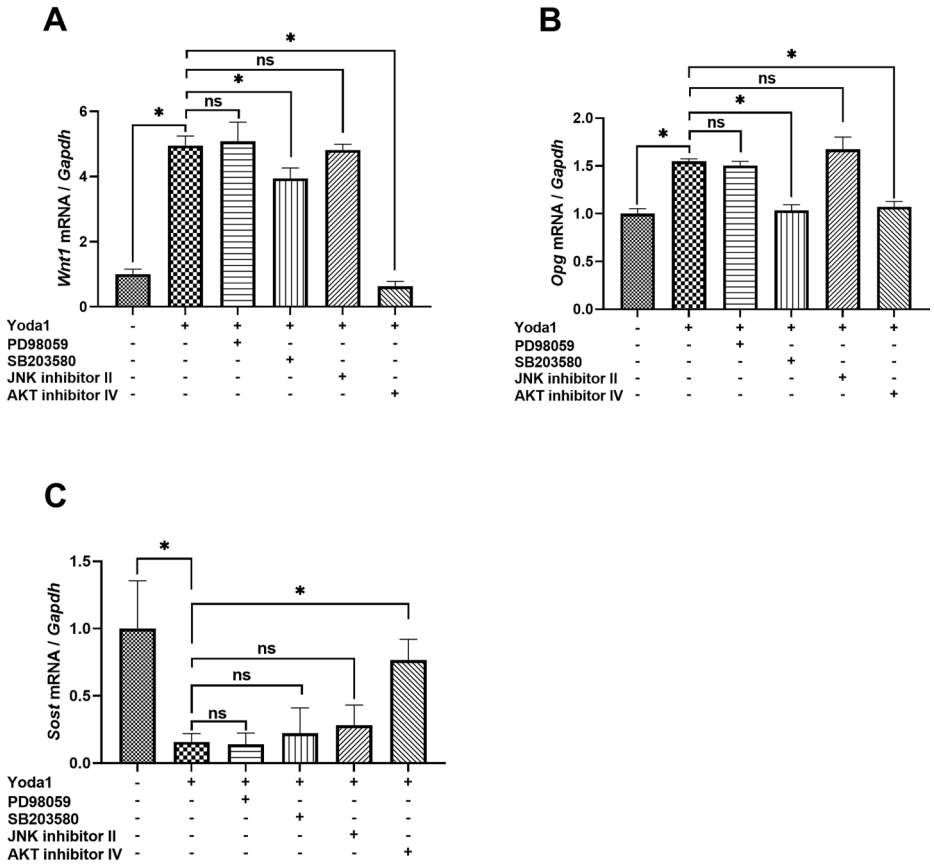

Figure 6 AKT and p38 signaling mediate Yoda1-induced regulation of osteo/cementogenic-related molecules in cementocytes. Cells were pretreated with selective inhibitors of ERK1/2 (PD98059), JNK (JNK inhibitor II), p38 (SB203580), or AKT (AKT inhibitor IV), followed by stimulation with 5 μM Yoda1 for 6 h in the presence of each inhibitor. Expression of *Wnt1*, *Sost*, and *Opg* were analyzed by real-time quantitative PCR. Data represent the mean ± SD of triplicate samples in representative results from three independent experiments. $P < 0.05$ vs. control. ns, not significant. Abbreviation: OPG, osteoprotegerin.

Piezo1 activation in cementocytes upregulates Wnt1 and Opg while suppressing Sost, creating a molecular environment favorable for Wnt signaling, which is critical for cementoblast differentiation,[30] cementum regeneration,[31] and periodontal homeostasis.[32] Reduced Wnt signaling is linked to pathological root resorption,[33] suggesting Piezo1 supports cementum maintenance and repair. Thus, the transcriptional effects observed in this study may reflect a mechanism by which Piezo1 mechanotransduction facilitates reparative or adaptive processes in cellular cementum. In this study, Wnt1 was notably upregulated by cyclic stretch and Piezo1 activation, while other canonical Wnt ligands were minimally expressed, suggesting Wnt1 plays a major role in cementocyte-mechanotransduction. However, other canonical and non-canonical Wnt pathways might also be involved depending on the mechanical stimuli or differentiation stage. Further studies are needed to clarify this. Furthermore, the observed increase in OPG—a key inhibitor of osteoclastogenesis—further supports a protective function for Piezo1 in the cementum—periodontal ligament complex under mechanical load.

Pharmacological activation of Piezo1 with Yoda1 mimicked mechanical stretch effects, reinforcing its role in mechanosensitive gene regulation. The transient Yoda1 effects highlight the need for continuous mechanical input, indicating Piezo1 acts as a dynamic modulator of cementogenic gene expression. Cementocytes are presumed to sense mechanical stimuli through dendritic processes and canaliculi that enable fluid movement and communication with surrounding tissues. Therefore, under dynamic loading conditions such as mastication or orthodontic tooth movement, intermittent yet frequent forces may induce repeated activation of Piezo1, contributing to the sustained transmission of mechanotransduction. Such recurrent Piezo1 activation is thought to play an important role in maintaining cementum homeostasis and promoting adaptive remodeling in response to mechanical loading.

Our identification of AKT and p38 as key downstream effectors of Piezo1 signaling aligns with osteocytic models and provides new insights into cementocyte signaling. Although the precise molecular link between Piezo1-induced calcium influx and AKT/p38 phosphorylation remains unclear, previous studies suggest a potential role for calcium/calmodulin-dependent pathways.[34] For example, calcium influx via TRPV5 channel activates AKT and p38 via Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) in rat chondrocytes.[35] Although TRPV5 differs from Piezo1, the shared Ca^{2+}/CaMKII signaling may operate in cementocytes.

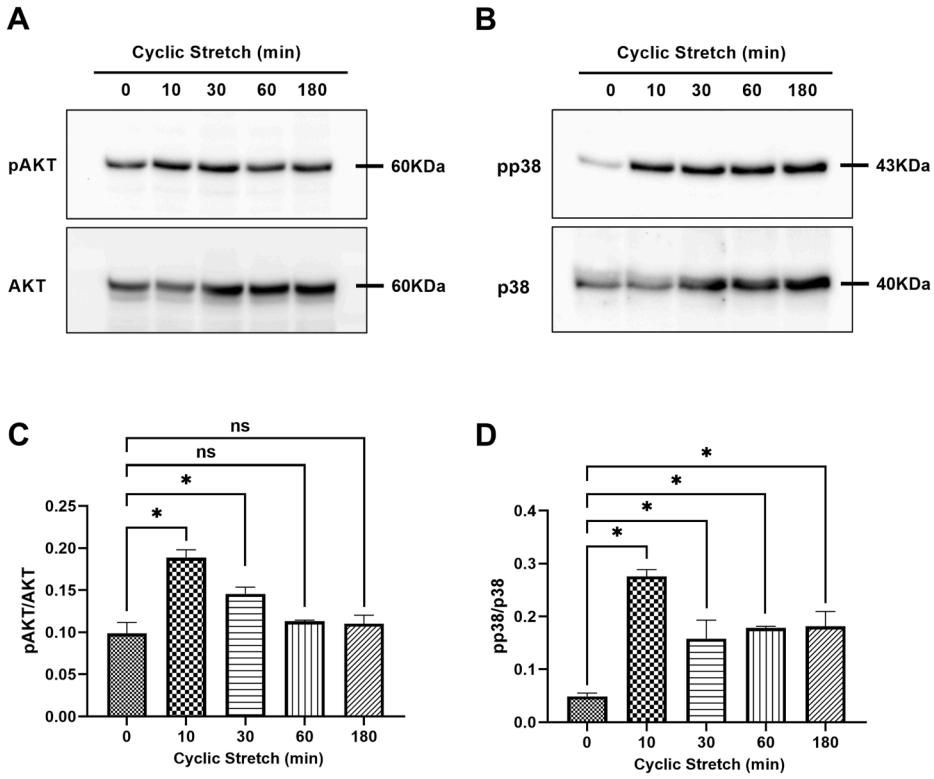

Figure 7 Cyclic stretch induces phosphorylation of AKT and p38 in cementocytes. Cells were subjected to cyclic stretch at 10 % elongation (10 cycles/min) for 10, 30, 60, or 180 min. Phosphorylation levels of AKT and p38 MAPK were analyzed by Western blotting using anti-phospho-AKT, anti-AKT, anti-phospho-p38 MAPK, and anti-p38 MAPK antibodies. Protein sizes: phospho-AKT and AKT (60 kDa); phospho-p38 MAPK (43 kDa) and p38 MAPK (40 kDa). Representative immunoblots and quantification graphs from three independent experiments are shown. $P < 0.05$ vs. control. ns, not significant.

In conclusion, our findings provide novel insights into cementocyte mechanobiology and highlight Piezo1 as a potential target for promoting cementum regeneration or preventing root resorption. Although mechanistic parallels with osteocytes support the validity of our results, this is the first study to directly demonstrate a mechano-transductive role for Piezo1 in cementocytes—marking a significant advancement in dental mechanobiology. Future work should validate these findings *in vivo* using cementocyte-specific genetic models and methods for localized delivery of Piezo1 modulators to the tooth root. Despite these limitations, our study lays important groundwork for future *in vivo* validation and therapeutic exploration.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2025.08.001.

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