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Original Article

Orthodontic tension promotes cementoblast mineralization by regulating autophagy

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Abstract *Background/purpose:* External root resorption is a main side effect of orthodontic treatment and is more likely to occur on the pression side than the tension side. To explore the potential protective mechanisms on the tension side, this study investigated the influence of mechanical tension on cementoblast mineralization and elucidated the role of autophagy in mediating this process.
Materials and methods: Mechanical tension was applied to cementoblasts using iStrain. The expression of mineralization-related and autophagy-related markers was detected by qRT-PCR, Western blot analysis, and immunofluorescence staining. RNA sequencing identified key regulators. Immunohistochemical staining assessed related markers expression in *in vivo* experiments.
Results: Applying tension to cementoblasts increased mineralization-related gene expression in a force-dependent and time-dependent manner. The immunohistochemical staining result of *in vivo* experiments supported these findings, demonstrating elevated expression of mineralization markers under tension. Mechanical tension also enhanced autophagic activity in cementoblasts, which was demonstrated by the results of qRT-PCR, Western blot analysis, immunofluorescence staining, and *in vivo* experiments. Suppression of autophagy with chloroquine attenuated the mineralization of cementoblasts induced by tension stimulus. RNA-seq identified Postn as a key regulator, and the knockdown of Postn impaired the mechanical tension-promoted mineralization of cementoblasts.
Conclusion: This study proposed the tension-induced promotion in mineralization of cementoblasts and emphasized the mediating role of autophagy in this process. Postn, a mediator

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connecting autophagy and mineralization, was identified as a key regulator. These discoveries helped elucidate orthodontic-related microprocesses on tooth roots and offer potential targets for therapeutic interventions to prevent and restore external root resorption clinically.

Introduction

External root resorption is the most common side effect during orthodontic treatment, which occurs significantly less on the tension side than the pressure side.[1] During external root resorption, cementoblasts play a crucial role in resisting root resorption, as these cells can repair root resorption pits by secreting extracellular matrix components and forming cementum.[2,3] Factors that regulate the differentiation and mineralization of cementoblasts determine the restorative capacity of the tooth root, thereby affecting the frequency and extent of external root resorption.[2,4]

During orthodontic treatment, various mechanical forces act on the layer of cementoblasts located on the surface of the tooth root, leading to multiple changes. Several reports have confirmed the mechanosensitivity of cementoblasts.[5,6] The application of mechanical pressure to cementoblasts has been shown to inhibit the mineralization-related genes expression and compromise the mineralization capacity of cementoblasts.[5,6] Conversely, mechanical tension has a multifaceted impact on cementoblast morphology, proliferative activity, and gene expression associated with mineral metabolism, although there are no consistent conclusions.[6–8]

Autophagy is a crucial endogenous protective mechanism which maintains cellular and tissue homeostasis in response to the changes of environment.[9] Under mechanical force stimuli, cells survive by utilizing autophagy to degrade misfolded proteins, dysfunctional organelles, and intracellular pathogens.[10] In a previous study, we found that mechanical pressure inhibited the autophagic flux of cementoblasts.[11] But the autophagy of cementoblasts under tension and whether it is related to mineralization is still unclear. The relationship between mechanical tension and cellular autophagic activity has been shown in several studies of other tissue cells.[12–15] In the osteocytes, mechanical tension was found to be able to trigger autophagy and then enhances osteogenesis.[13] In the periodontal ligament stem cells, researches demonstrated that the promoting effect of cyclic tension on the osteogenic differentiation of periodontal ligament stem cells was related to the activation of autophagy.[14] Mechanical tension was also validated to induce the degeneration of intervertebral disc through autophagy and induce chaperone-assisted selective autophagy, which was an essential pathway for mechanotransduction in muscle and immune cells.[12,15] Therefore, autophagy is a possible direction we explore the relationship between mechanical tension and mineralization of cementoblasts.

The present study aimed to investigate whether mechanical tension affected the mineralization of cementoblasts by modulating their autophagic activity, which would help us to further understand orthodontic-related microprocesses on the surface of tooth roots, and to explore the factors that play a protective or restorative role in orthodontic-related root resorption, so as to provide ideas for the prevention of root resorption, as well as the promotion of the restoration and regeneration of resorbed roots, which will be an effective clinical solution for root resorption.

Materials and methods

Cell culture and treatment

OCCM-30, the murine immortalized cementoblastic cell line, was generously provided by Dr. Martha J. Somerman (National Institutes of Health, Bethesda, MD, USA).[16] Cementoblasts were incubated at 37 °C and cultured with Dulbecco's modified Eagle's medium (Gibco, Waltham, MA, USA) containing 1% penicillin-streptomycin solution (Invitrogen, Waltham, MA, USA) and 10% fetal bovine serum (Gibco).

Chloroquine (CQ) was used at a concentration of 5 μg/mL to suppress autophagy in cementoblasts, and was purchased from ApexBio (Shanghai, China).

Tensile strain experiments

To apply mechanical tension to cementoblasts, a patent tension machine constructed by our research team iStrain system was used (Fig. 1A).[17] Cells were seeded on Bioflex plates (Flexcell International, Burlington, NC, USA), and then exposed to mechanical tension with different extension rates or different duration time through iStrain. The following assays were then conducted according to experimental design.

Quantitative real-time polymerase chain reaction

After cementoblasts were subjected to treatment, total cellular RNA was extracted as protocol with TRIzol reagent (Invitrogen) and reverse transcribed using PrimeScript RT Reagent Kit (TaKaRa Biotechnology, Dalian, China). After mixing samples with SYBR Green Master Mix (Roche, Mannheim, Germany), quantitative real-time polymerase chain reaction (qRT-PCR) analysis was performed and detected using ViiA 7 Real-Time PCR System (Thermo Fisher

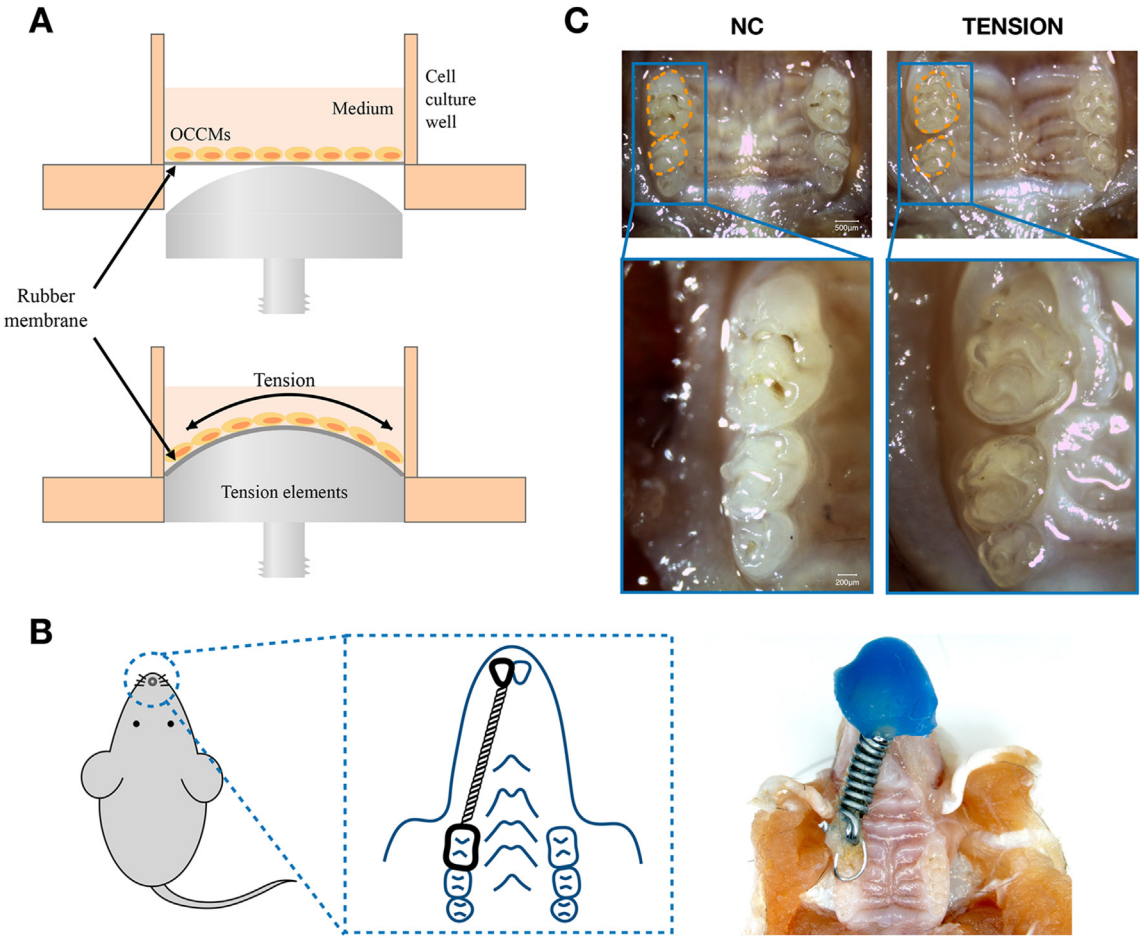

Figure 1 *In vitro* and *in vivo* mechanical tension models. (A) Diagram of the *in vitro* mechanical tension model conducted by iStrain. (B) Diagram of the *in vivo* mechanical force-induced orthodontic tooth movement (OTM) model. (C) The degree of tooth movement of the right maxillary first molar in the NC and TENSION group. Scale bars: 500 μm and 200 μm.

Scientific, Waltham, MA, USA). The primer sequences used were listed in Table 1.

Immunofluorescence staining

After exposure to cyclic tension and/or CQ, the cementoblasts were fixed with 4% paraformaldehyde and exposed to 0.1% Triton X-100. The cementoblasts were then incubated with anti-OCN antibody (Proteintech, Chicago, IL, USA), anti-LC3 antibody (Cell Signaling Technology, Danvers, MA, USA), or anti-Postn antibody (Abcam, Cambridge, MA, USA) diluted as manufacturer's protocols at 4 °C overnight after blockage with 5% goat serum (Zhongshan Golden Bridge Biotechnology, Beijing, China). Images were collected using LSM 5 EXCITER (Carl Zeiss, Jena, Germany) after the incubation and counterstaining of cells with secondary antibodies and DAPI (Solarbio, Beijing, China).

Western blotting

Cementoblasts were lysed using RIPA lysis buffer containing 1% protease inhibitor cocktail (Solarbio) after rinsed with pre-cold PBS. A BCA Protein Assay Kit (Solarbio) was utilized to measure and adjust protein concentrations to ensure uniformity. Samples were then mixed with 4 × loading buffer, heat-denatured, and 60 μg of protein per sample was loaded for Western blot analysis. Samples were blotted onto PVDF membranes (Millipore, Billerica, MA, USA) after electrophoresis on precast gels (Beyotime Institute of Biotechnology, Shanghai, China). The membranes were then blocked with 5% skim milk, followed by incubation with primary antibodies against RUNX2, Beclin1 (ABclonal, Woburn, MA, USA), BSP, COL-1, LC3, P62 (Cell Signaling Technology), OSX, Postn (Abcam), or GAPDH (Proteintech). The chemiluminescence signals were detected with the ECL Kit (CoWin Biotech, Jiangsu, China) and quantified using ImageJ software after the incubation of membranes with secondary antibodies (Zhongshan Golden Bridge Biotechnology).

High-throughput RNA sequencing

High-Throughput RNA Sequencing (RNA-seq) was applied to OCCMs after their exposure to cyclic tension for 12 h. After

Table 1 mRNA primer sequences and RNAi oligonucleotide sequences.

Gene	Forward sequence (5′→3′)	Reverse sequence (5′→3′)
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA
RUNX2	CCTGAACTCTGCACCAAGTCCT	TCATCTGGCTCAGATAGGAGGG
PTPLA	AGCCCAGGTATAGGAAGAATGT	CCGCATAACTAACCCAATAGCG
BSP	GAGACGGCGATAGTTCC	AGTGCCGCTAACTCAA
OSX	TTGAAAAAGGAGTTGGTGGC	TGCTGGTTCTGTAAGTTGGG
COL-1	GCAACATTGGATTCCCTGGACC	GTTCACCCTTTTCTCCCTTGCC
LC3B	GTCCTGGACAAGACCAAGTTCC	CCATTCACCAGGAGGAAGAAGG
BNIP3	GCTCCAAGAGTTCTCACTGTGAC	GTTTTTCTCGCCAAAGCTGTGGC
Beclin1	CAGCCTCTGAAACTGGACACGA	CTCTCCTGAGTTAGCCTCTTCC
ATG4b	ATGGGAGTTGGCGAAGGCAAGT	CAGCCAAGGAACTCCATGTGTC
Postn	TTTCACCGACCTGGTAGCC	TTGGTCCATGCTCAGAGTGTC
Ccn1	GGAAAAGGCAGCTCACTGAAGC	GGAGATACCAGTTCCACAGGTC
Isg15	CATCCTGGTGAGGAACGAAAGG	CTCAGCCAGAACTGGTCTTCGT
Phex	CTGGCTGTAAGGGAAGACTTCC	GCTCCTAAAAGCACAGCAGTGTC
Gene	**Sense (5′→3′)**	**Antisense (5′→3′)**
Postn-1	GGAGAACAAUGUCAAUGUUTT	AACAUUGACAUUGUUCUCCTT
Postn-2	GCAGAAGACGACCUUUCAUTT	AUGAAAGGUCGUCUUCUGCTT

Abbreviations: ATG4b, autophagy related 4b cysteine peptidase; BNIP3, BCL2 interacting protein 3; BSP, bone sialoprotein; Ccn1, cellular communication network factor 1; COL-1, collagen, type I, alpha 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Isg15, ISG15 ubiquitin-like modifier; LC3B, microtubule-associated proteins 1A/1B light chain 3B; OSX, osterix; Phex, phosphate regulating endopeptidase homolog, X-linked; Postn, periostin; PTPLA, protein tyrosine phosphatase-like protein, putative; RUNX2, runt-related transcription factor 2.

extraction of RNA with TRIzol reagent (Invitrogen), an Agilent 2200 instrument was used to assess the quality of RNA, and the RNA was stored at −80 °C. The TruSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, CA, USA) was then utilized to build a cDNA library according to the manufacturer's instructions. Paired-end sequencing was conducted on the Illumina NovaSeq 6000 platform (Illumina) by NovelBio Bio-Pharm Technology (Shanghai, China). Subsequently, through removing the low-quality reads and adaptor sequences, clean reads were generated from the raw reads after which the sequences were aligned to the mouse genome using HISAT2. HTseq was applied for gene counting, and the RPKM was calculated to assess the gene expression levels. Based on a fold change >1.5 or <0.667 and a false discovery rate (FDR) < 0.05, differentially expressed genes were identified. Gene Ontology (GO) analysis was performed by downloading the GO annotations from Gene Ontology, UniProt, and NCBI. And the top 15 significant GO categories were identified utilizing Fisher's exact test ($P < 0.05$).

Cell transfection

Small interfering RNAs (siRNAs) against Postn-1 and Postn-2 and a scrambled control (si-NC) were obtained from GenePharma (Shanghai, China). The sequences were listed in Table 1. Transfections were carried out when the cell confluence reached 70–80% and Lipofectamine 3000 (Invitrogen) was used according to the protocol.

In vivo tension experiments

The protocols of the *in vivo* tension experiments were approved by the Peking University Biomedical Ethics Committee (PUIRB-LA2023130). In the mechanical force-induced orthodontic tooth movement (OTM) model (Fig. 1B), five male C57BL/6 mice aged 6 weeks were used in each group and undergone general anesthesia with sodium pentobarbital, and the two ends of each nickel–titanium coil springs were extended to provide a nearly constant force of approximately 30 g and were respectively tied to the right maxillary first molar and maxillary incisors. Blue gel was used to retain the nickel–titanium coil springs on the incisors. The springs were dislodged after 0 days (indicated as NC) or 7 days (indicated as TENSION) of force application (Fig. 1C). Then the mice were euthanized and the maxillae were carefully removed from the surrounding tissues for the following immunostaining.

Immunohistochemical staining

For immunostaining analysis, a series of 5-μm sections were prepared. Primary antibodies, secondary antibodies (Zhongshan Golden Bridge Biotechnology), and DAB detection kit (Sigma–Aldrich, Saint Louis, MO, USA) were utilized according to the protocols from manufacturer. A microscope (Nikon, Tokyo, Japan) was used for images collection.

Statistical analysis

All the data were presented as means and standard deviation and were measured by independent t test or one-way analysis of variance (ANOVA) utilizing GraphPad Prism (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered to be statistically significant.

Results

Mechanical tension increases the mineralization of cementoblasts

With an iStrain, a patented tension machine constructed by our research team, mechanical tension at a frequency of 0.3 Hz was applied to the cementoblasts for 12 h, with elongation rates of 9%, 12%, and 15%, respectively. Cellular RNA was extracted immediately after the application of tension, and it was found that the expression levels of mineralization-related genes elevated in all tensioned groups compared to the control group, with all mineralization-related genes reaching their peak gene expression levels at 12% elongation; thus, we chose 12% elongation rate in the following duration-related experiments (Fig. 2A).

Subsequently, we applied mechanical tension at 12% elongation rate for 1 h, 6 h, 12 h, 24 h, and 48 h to observe the changes in the expression of mineralization-related genes in cementoblasts with respect to the duration of tension.

The mRNA expression levels of the mineralization-related gene PTPLA gradually increased with increasing force application time, while the mRNA expression levels of RUNX2, BSP and OSX tended to increase followed by a decrease, with the peak expression levels of RUNX2 and BSP occurring at 12 h and the peak expression level of OSX occurring at 6 h (Fig. 2B). The protein expression levels of mineralization-related genes showed a similar trend as the protein expression levels of RUNX2 and OSX were gradually increased with increasing duration of tension and peaked at 12 h, and the protein expression level of BSP was elevated in the tension groups and peaked at 24 h (Fig. 2C and D). In addition, we found significant upregulation of OCN expression after cementoblasts were subjected to 12 h of tension according to the immunofluorescence staining results (Fig. 2E).

These results suggested that both of lighter and heavier mechanical tension on cementoblasts can promote their mineralization activities. However, the increase in mineralization activity of the cementoblasts was most pronounced at appropriate force values, as force values that were either too low or too high affected the mineralization of the cementoblasts. The variation in mineralization activity with the duration of mechanical tension also showed that the increase in mineralization activity of the cementoblasts was most significant at a certain duration of mechanical tension and that too short or too long of a force application time would affect the mineralization activity of the cementoblasts.

Then, we used a mechanical force-induced OTM model to investigate the effect of mechanical tension on the mineralization activity of cementoblasts *in vivo*. The immunohistochemical staining results demonstrated that, compared to the control group, the cementoblasts showed increased expression of COL-1 and OSX in the site exposed to tension (Fig. 2F). These results corresponded to the *in vitro* results for the cementoblasts above.

Mechanical tension increases autophagy in cementoblasts

After applying mechanical tension to cementoblasts for 12 h with different elongation rates, we found that the mRNA expression levels of autophagy-related genes in each force group increased. The expression levels of the autophagy-related genes LC3B and BNIP3 were greatest at an elongation rate of 15%, while those of Beclin1 and ATG4b reached their peak at an elongation rate of 12%. We chose 12% elongation rate in the subsequent duration-related experiments (Fig. 3A).

After cementoblasts were subjected to mechanical tension at a 12% elongation rate with different duration of time, we observed that as the application time increased, the mRNA expression levels of the autophagy-related genes Beclin1 and ATG4b gradually increased. The mRNA expression levels of LC3B and BNIP3 first increased and then decreased, with the peak expression of LC3B occurring at 6 h and the peak expression of BNIP3 occurring at 24 h (Fig. 3B). Western blot analysis revealed that the LC3-II/I ratio and the expression level of Beclin1 first increased and then decreased with the extension of force time, with peak occurring at 12 h. The expression level of P62 showed a trend of first decreasing and then increasing with a trough occurring at 24 h (Fig. 3C and D). Moreover, the immunofluorescence staining results showed significant upregulation of LC3 expression in cementoblasts after exposure to tension (Fig. 3E).

These results suggested that applying either light or heavy mechanical tension to cementoblasts can promote their autophagic activity. In the light force stage, autophagic activity increased with increasing mechanical tension force, but when the mechanical tension reached a certain force value, the increase in autophagic activity would slow down, and the changes may be even reversed.

In the *in vivo* study, the immunohistochemical staining results demonstrated that, compared to the control group, the cementoblasts exposed to tension showed increased expression of Beclin1 and LC3 (Fig. 3F). These results corresponded to the *in vitro* results for the cementoblasts above.

Autophagy mediates the effect of mechanical tension on the mineralization of cementoblasts

To investigate the role of autophagy in the tension-induced increase in the mineralization of cementoblasts, we treated cementoblasts with 5 μg/mL CQ to suppress autophagy. The qRT-PCR results demonstrated that in the cementoblast groups that were not treated with CQ, the application of tension increased the mRNA expression levels of mineralization-related genes, including RUNX2, PTPLA, COL-1, and OSX. However, in the groups of cementoblasts treated with CQ, the application of tension did not induce a significant increase in the expression levels of mineralization-related genes (Fig. 4A). Moreover, compared with the group not treated with CQ, the increase in the protein expression levels of COL-1, RUNX2, and OSX was

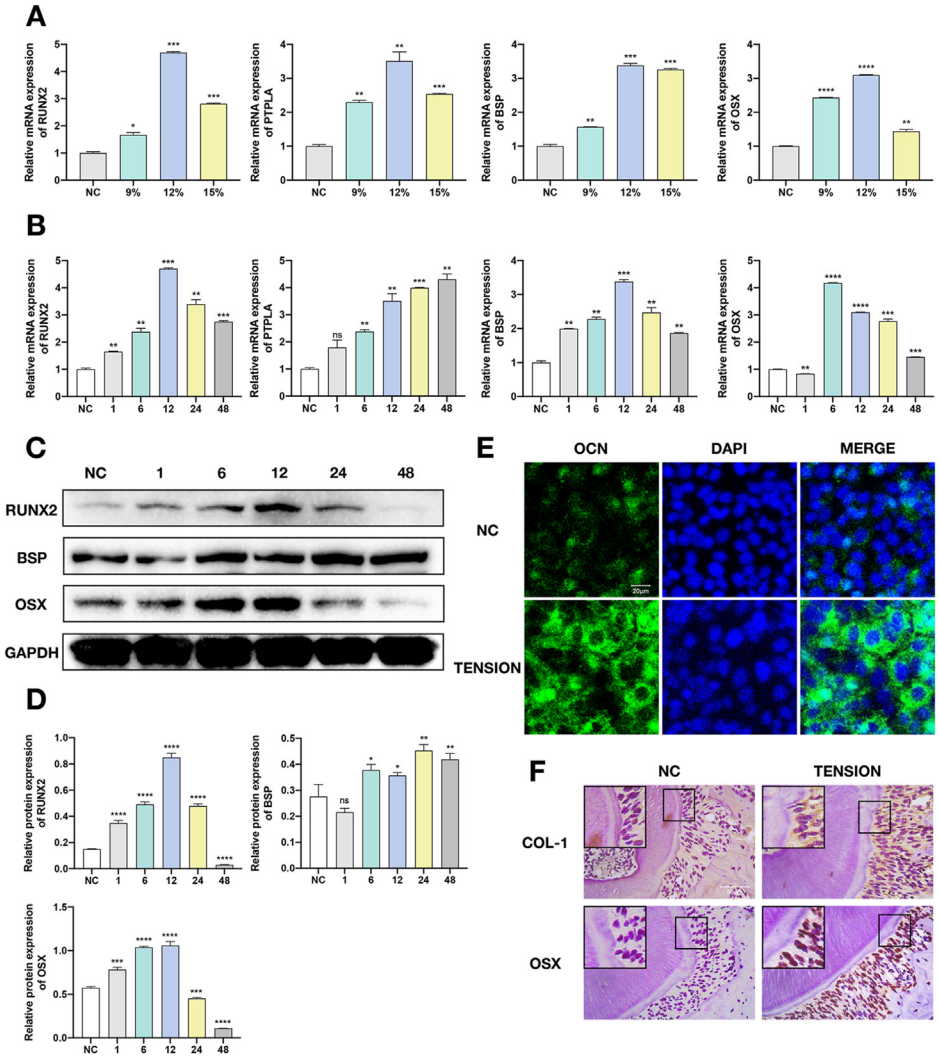

Figure 2 Mechanical tension increases the mineralization of cementoblasts. (A) qRT-PCR results of RUNX2, PTPLA, BSP, and OSX in the cementoblasts of control group and tension groups with different extension rates. GAPDH housekeeping gene was used for gene expression calibration. The data were presented as the means ± SDs; ns, no significant difference; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ vs. the control. (B) qRT-PCR results of RUNX2, PTPLA, BSP, and OSX in the cementoblasts of control group and tension groups with different duration of time. (C, D) Chemiluminescent images and Western bolt analysis of RUNX2, BSP and OSX in the cementoblasts of control group and tension groups with different duration of time. GAPDH was used for the gray values calibration. (E) Immunofluorescence images of OCN in cementoblasts in the control group or after 12 h of tension with a 12% elongation rate. Scale bars: 20 μm. (F) Immunohistochemical images of COL-1 and OSX in the roots of the right maxillary first molar in the NC (tension applied for 0 days) and TENSION (tension applied for 7 days) groups. Scale bars: 50 μm. Abbreviations: BSP, bone sialoprotein; COL-1, collagen, type I, alpha 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NC, negative control; OCN, osteocalcin; OSX, osterix; PTPLA, protein tyrosine phosphatase-like protein, putative; qRT-PCR, quantitative real-time polymerase chain reaction; RUNX2, runt-related transcription factor 2; SD, standard deviation.

lower and not as significant after exposure to mechanical tension in the group of cementoblasts treated with CQ, as shown by Western blot analysis (Fig. 4B and C). Immunofluorescence staining revealed similar results (Fig. 4D).

Identification and validation of key regulators of mechanical tension-induced mineralization of cementoblasts

To investigate the key regulators of mechanical tension-induced mineralization in cementoblasts, we performed RNA-seq on cementoblasts with or without exposure to mechanical tension. Differential expression analysis and GO analysis were subsequently performed. The results of the differential expression analysis were visualized in volcano plots (Fig. 5A). Compared to the negative control group, 48 genes were significantly downregulated, and 64 genes were significantly upregulated in the group of cementoblasts treated with mechanical tension. To further identify the genes related to mineralization of cementoblasts, we inspected the GO analysis results (Fig. 5B). Among the GO terms of bone regeneration (GO:1990523), bone

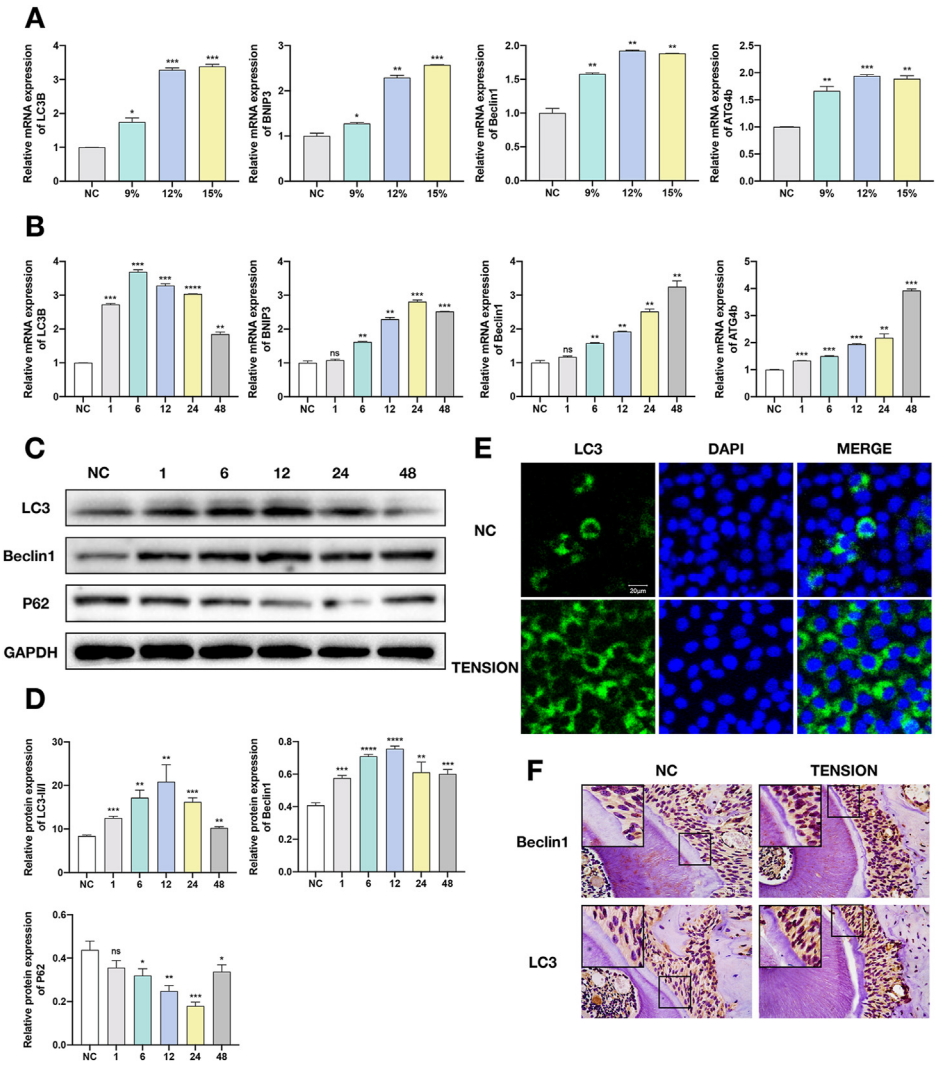

Figure 3 Mechanical tension increases autophagy in cementoblasts. (A) qRT-PCR results of LC3B, BNIP3, Beclin1, and ATG4b in the cementoblasts of control groups and tension group with different extension rates. (B) qRT-PCR results of LC3B, BNIP3, Beclin1, and ATG4b in the cementoblasts of control group and tension groups with different duration of time. (C, D) Chemiluminescent images and Western bolt analysis of LC3, Beclin1, and P62 in the cementoblasts of control group and tension groups with different duration of time. (E) Immunofluorescence images of LC3 in cementoblasts in the control group or after 12 h of tension with a 12% elongation rate. Scale bars: 20 μm. (F) Immunohistochemical images of Beclin1 and LC3 in the roots of the right maxillary first molar in the NC (tension applied for 0 days) and TENSION (tension applied for 7 days) groups. Scale bars: 50 μm. Abbreviations: ATG4b, autophagy related 4b cysteine peptidase; BNIP3, BCL2 interacting protein 3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LC3, microtubule-associated proteins 1A/1B light chain 3; LC3B, microtubule-associated proteins 1A/1B light chain 3B; NC, negative control; P62, ubiquitin-binding protein p62; qRT-PCR, quantitative real-time polymerase chain reaction.

mineralization (GO:0030282) and positive regulation of bone mineralization (GO:0030501), we validated four significantly differentially expressed genes: periostin (Postn), cellular communication network factor 1 (Ccn1), ISG15 ubiquitin-like modifier (Isg15), and phosphate regulating endopeptidase homolog, X-linked (Phex). The qRT-PCR results confirmed the RNA sequencing results (Fig. 5C).

Our previous research demonstrated that Postn is a key molecule in the mineralization of cementoblasts.[11] Therefore, we chose Postn for the following experiments to determine whether Postn also played a role in the mineralization of cementoblasts under tension stimulation. Therefore, we further examined the protein expression of Postn under mechanical tension. The Western blot and immunofluorescence staining results were consistent with the qRT-PCR results (Fig. 5D and E). The immunohistochemical staining results of the *in vivo* study also demonstrated that, compared to the control group, the cementoblasts exposed to tension showed increased expression of Postn (Fig. 5F).

Knockdown of Postn impedes tension-promoted mineralization of cementoblasts

For analysis of the function of Postn in the mechanical tension-induced promotion of mineralization in

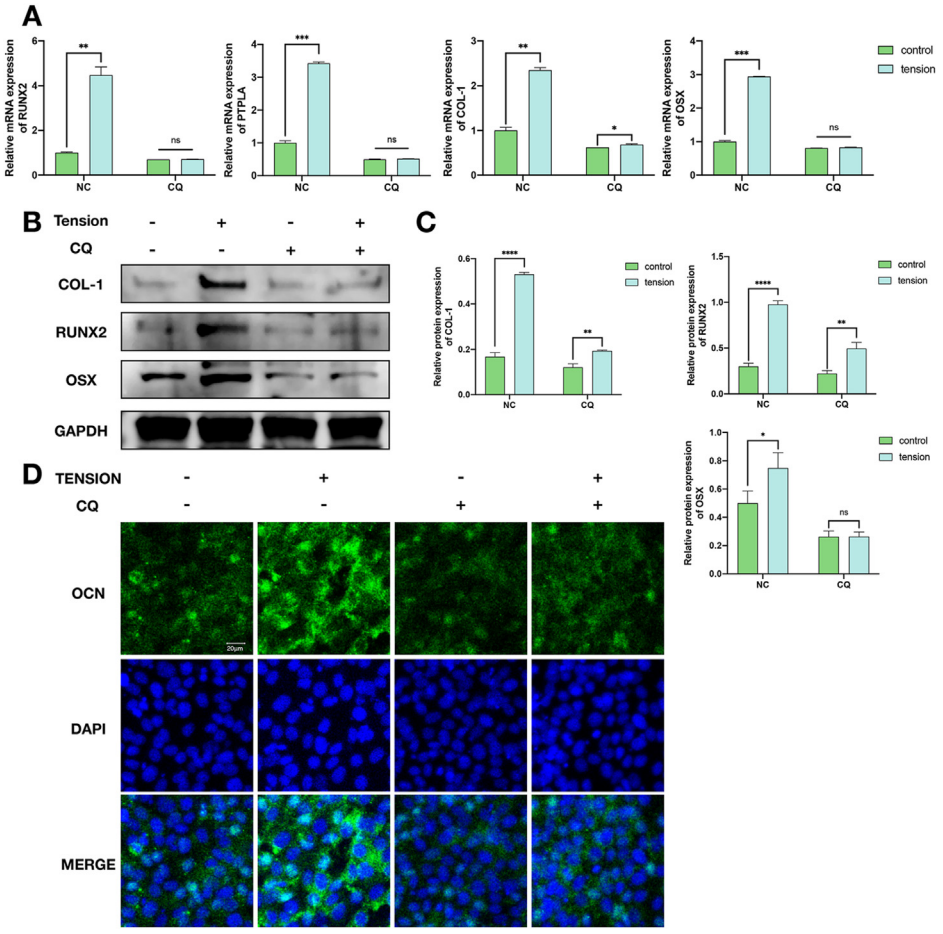

Figure 4 Autophagy mediates the effect of mechanical tension on the mineralization of cementoblasts. (A) qRT-PCR results of RUNX2, PTPLA, COL-1, and OSX in cementoblasts. Control: control group. CQ: pretreated with CQ. Tension: exposed to 12 h of tension with a 12% elongation rate. (B, C) Chemiluminescent images and Western bolt analysis of COL-1, RUNX2, and OSX in cementoblasts. (D) Immunofluorescence images of OCN in cementoblasts. Scale bars: 20 μm. Abbreviations: COL-1, collagen, type I, alpha 1; CQ, chloroquine; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NC, negative control; OCN, osteocalcin; OSX, osterix; PTPLA, protein tyrosine phosphatase-like protein, putative; qRT-PCR, quantitative real-time polymerase chain reaction; RUNX2, runt-related transcription factor 2.

cementoblasts, cells were transfected with siRNAs targeting Postn (indicated as si Postn-1/2) or a scrambled control (indicated as si NC), followed by exposure to mechanical tension. qRT-PCR and Western blot were conducted to assess the mineralization of the cementoblasts. According to the qRT-PCR and Western blot results, the increase in cementoblasts mineralization induced by mechanical tension was significantly inhibited with the existence of siRNAs against Postn, while no significant differences were shown in the cells transfected with the scrambled control (Fig. 5G, H, I). These results suggested that Postn played a key role in mechanical tension-induced mineralization of cementoblasts.

Discussion

Mechanical tension is an important type of mechanical stimulus involved in orthodontic treatment and has a substantial impact on the microenvironment of the root surface. In this research, we exposed cementoblasts to mechanical tension at different elongation rates and for different durations. We demonstrated that both lighter and heavier mechanical tension on cementoblasts promoted their mineralization activities, but the increase in the mineralization activity of cementoblasts was most pronounced at appropriate force values. The mineralization activity of cementoblasts was most significant at a certain duration of mechanical tension, as too short or too long of a force application time would affect the mineralization activity of cementoblasts. In our previous study, we found that during orthodontic tooth movement, M1/M2 ratio was relatively lower on the tension side and higher on the compression side,[16] suggesting the indirect effect of mechanical stimuli on the mineralization of cementoblasts. Combined with our previous finding that mechanical pressure attenuated mineralization of cementoblasts,[11] the direct effect and the indirect effect of mechanical force on the mineralization of cementoblasts may together serve as a possible explanation for the finding that orthodontic-induced root resorption was significantly more pronounced on the pressure side than on the tension side.[1]

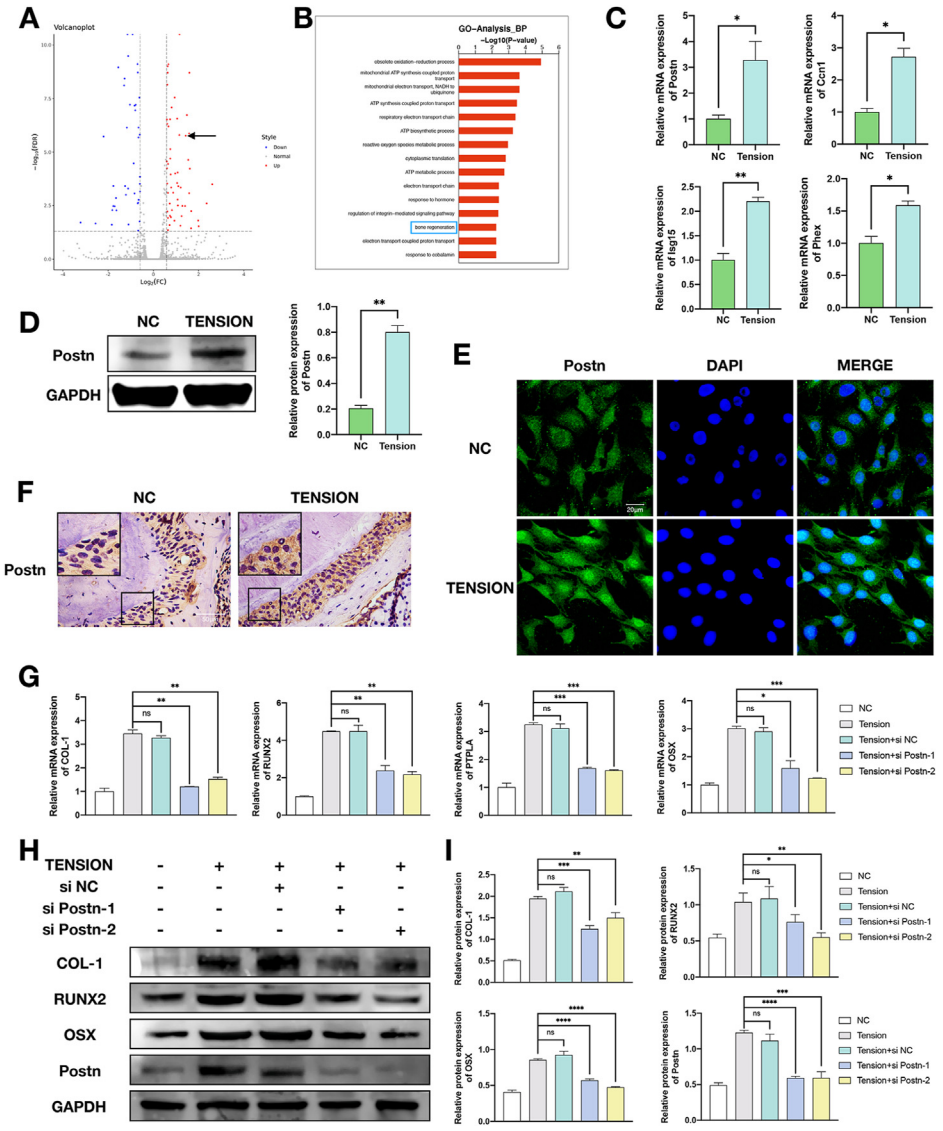

Figure 5 Identification and knockdown of Postn. (A) Differentially expressed mRNAs (fold change >1.5 or <0.667 and FDR <0.05) in cementoblasts between the control group and the group treated with tension force with a 12% elongation rate for 12 h were showed in volcano plots. The black arrow indicated Postn. (B) The top 15 enriched GO categories in the biological process term. (C) qRT-PCR results of Postn, Ccn1, Isg15, and Phex in cementoblasts in the control group or after 12 h of tension with a 12% elongation rate. (D) Chemiluminescent images and Western blot analysis of Postn in cementoblasts in the control group or after 12 h of tension with a 12% elongation rate. (E) Immunofluorescence images of Postn in cementoblasts in the control group or after 12 h of tension with a 12% elongation rate. Scale bars: 20 μm. (F) Immunohistochemical images of Postn in the roots of the right maxillary first molar in the NC (tension applied for 0 days) and TENSION (tension applied for 7 days) groups. Scale bars: 50 μm. (G) qRT-PCR results of COL-1, RUNX2, PTPLA, and OSX in cementoblasts in the control group or after 12 h of tension with a 12% elongation rate and pretreated with si NC, si Postn-1, or si Postn-2. (H, I) Chemiluminescent images and Western bolt analysis of COL-1, RUNX2, OSX, and Postn in cementoblasts in the control group or after 12 h of tension with a 12% elongation rate and pretreated with si NC, si Postn-1, or si Postn-2. Abbreviations: BP, biological process; Ccn1, cellular communication network factor 1; COL-1, collagen, type I, alpha 1; FC, fold change; FDR, false discovery rate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GO, Gene Ontology; Isg15, ISG15 ubiquitin-like modifier; NC, negative control; OSX, osterix; Phex, phosphate regulating endopeptidase homolog, X-linked; Postn, periostin; PTPLA, protein tyrosine phosphatase-like protein, putative; qRT-PCR, quantitative real-time polymerase chain reaction; RUNX2, runt-related transcription factor 2.

We further investigated the mechanism through which mechanical tension promoted the mineralization of cementoblasts. There is growing evidence on the role of autophagy in facilitating osteogenic differentiation and mineralization of other cell types, including bone marrow mesenchymal stem cells and osteoblasts,[18–21] which are similar to cementoblasts. The effect of mechanical tension on cellular autophagic activity has also been demonstrated in studies of other cell types.[12–15] In addition, we have also found that mechanical pressure can reduce autophagy of

cementoblasts in a previous research.[11] These findings gave rise to our hypothesis that autophagy may be involved in the process of mechanical tension regulating the mineralization of cementoblasts. By applying mechanical tension to cementoblasts by iStrain and *in vivo* mechanical force-induced OTM model, we found that mechanical tension can promote the autophagic activity of cementoblasts. Moreover, through inhibiting the autophagic activity of cementoblasts by CQ, we found that the autophagic activity of cementoblasts acted as a bridging intermediary between mechanical tension and the mineralization of cementoblasts, as the inhibition of autophagy would impair the promoting effect of mechanical tension on the mineralization of cementoblasts. The subsequent identification of Postn further confirmed our findings. The RNA-seq results showed that the expression level of Postn was elevated in cementoblasts after exposure to mechanical tension. The regulation axis of autophagy-Postn-mineralization genes has been verified before, as the expression of Postn decreased when autophagy was inhibited and the knockdown of Postn inhibited cementoblasts mineralization.[11] In this research, we confirmed that the knockdown of Postn attenuated the ability of mechanical tension to promote the mineralization of cementoblasts, suggesting the regulation axis of autophagy-Postn-mineralization genes also served as a possible pathway in the process of tension-induced mineralization of cementoblasts.

The mechanism we found involving mechanical tension, autophagy, and mineralization of cementoblasts would provide us with a more detailed and comprehensive understanding of the cellular microprocesses on the surface of tooth roots under tension during orthodontic tooth movement. New ideas for promoting cementum mineralization can be proposed, helping us explore new methods for promoting root repair and providing new ideas for alleviating external root resorption.

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.2024.03.009.

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