Research Article
RNA-Sequence Reveals the Regulatory Mechanism of miR-149 on Osteoblast Skeleton under Mechanical Tension

Yifan Wang,1,2 Guanyin Zhu,1,2 Fang Pei,1,2 Yigan Wang,1,2 Jun Liu,1,2 Caixia Lu,3 and Zhihe Zhao1,2

1State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases, West China Hospital of Stomatology, Sichuan University, No. 14, 3rd Section, South Renmin Road, Chengdu, 610041 Sichuan, China
2Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, Chengdu, China
3Center of Tree Shrew Germplasm Resources, Institute of Medical Biology, Chinese Academy of Medical Science and Peking Union Medical College, 650106 Kunming, Yunnan, China

Correspondence should be addressed to Caixia Lu; lcx@imbcams.com.cn and Zhihe Zhao; zhhao202@163.com

Received 28 July 2022; Accepted 25 August 2022; Published 23 September 2022

Academic Editor: Ibrahim Hakkı Cigerçi

Copyright © 2022 Yifan Wang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Objective. Based on RNA-sequencing (RNA-seq), the regulation of miRNAs differentially expressed in dental, periodontal, and alveolar bone tissue of orthodontic tree shrews on osteoblast skeleton under tension was investigated. Methods. Tree shrews were used to construct orthodontic models. We used RNA-seq to identify differentially expressed miRNAs in periodontal tissues of the treatment group and control group tree shrews. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were used for enrichment analysis. Human osteoblast MG63 was treated with 5000 U mechanical tension. Real-time quantitative polymerase chain reaction (RT-qPCR) detected the expression of miR-149 and ARFGAP with SH3 domain, Ankyrin repeat, and Ph domain 3 (ASAP3) mRNA. Western blot detected the protein levels of ASAP3, F-actin, osteogenic markers bone morphogenetic protein 2 (BMP2), and runt-related transcription factor 2 (RUNX2). Rhodamine phalloidin was used to observe the fluorescence intensity of F-actin. Validation of the targeting relationship between miR-149 and ASAP3 by dual luciferase reporter gene assay. Results. By performing miRNA-seq analysis on the dental and periodontal tissue of tree shrews in the treatment group and control group, we identified 51 upregulated miRNAs and 13 downregulated miRNAs. The expression of miR-149 in the dental and periodontal tissue of tree shrew and MG63 cells treated with mechanical tension was decreased, and miR-149 targeted ASAP3. Knockdown of ASAP3 inhibited the fluorescence intensity of F-actin in MG63 cells treated with 5000 U tension for 36 h, and overexpression of ASAP3 promoted the expression of F-actin and osteogenic markers BMP2 and RUNX2. Conclusions. These findings revealed that miR-149 could modulate osteoblast differentiation under orthodontics mechanical tension through targeting ASAP3.

1. Introduction
Orthodontics refers to the orthodontic treatment of malocclusion to achieve a new balance and coordination of the dental and craniofacial morphology. The biological process (BP) of orthodontic tooth movement is to trigger periodontal tissue and bone remodeling by mechanical force. Factors such as the anatomy of alveolar bone, pressure exerted by soft tissue, attachment level of periodontal tissue, and neuromuscular strength may affect the degree of orthodontic tooth movement [1, 2]. Osteoblast maturation from osteoblast progenitor cells regulates the dynamic balance of bone remodeling. Studies have found that mechanical stress could convert into chemical signals, which promoted the activity of osteoblasts, inhibited the activity of osteoclasts, regulated bone mass, and promoted bone formation [3, 4]. Therefore, osteoblasts play an important role in alveolar bone remodeling.
MicroRNAs (miRNAs) are endogenous short noncoding RNAs that bind to the 3′UTR of target genes and regulate the expression level of target genes, thereby inducing corresponding changes in cells or tissues [5]. Studies have shown
that miRNAs play an important role in bone metabolism by regulating the biological behavior of osteoblast, osteoclast, and chondrocytes [6–8]. For example, overexpression of miR-24-3p significantly relieved the injury of chondrocytes induced by interleukin-1beta (IL-1β) [9]. Yuan et al. [10] found inhibition of miR-142a-5p decreased the osteogenic differentiation of stromal cell ST2 and preosteoblastic cell MC3T3-E1 by targeting nuclear factor 1A. Recently, due to the regulatory role of miR-149 in a variety of diseases, more and more attention has been paid. Li et al. [11] found that miR-149-3p mimic reduced the osteogenic differentiation potential of bone marrow mesenchymal stem cells and increased their osteogenic differentiation potential. However, whether miR-149 regulates bone remodeling under mechanical forces during orthodontic treatment and the specific mechanism remains unclear.

Tree shrew is a small tropical and subtropical mammal that is similar to primates in physiology, biochemistry, and biology. In addition, tree shrews have the advantages of rapid production, low maintenance cost, small size, and so on. It is a new experimental disease animal model, and superior to other nonhuman primates. Tree shrews have been widely used in medical and biological research as a lower-level primate experimental animal [12]. In this study, we created a tree shrew model of orthodontic tooth movement (OTM) by means of a NiTi closed coil spring to induce orthodontic tooth movement and sequenced the small RNA of the dental and periodontal tissue of tree shrews to study the expression differences of miRNA in the dental and periodontal tissue of tree shrews under the mechanical tension and then explored the regulatory effects of the differentially expressed miRNA on osteoblasts under the mechanical tension.

2. Materials and Methods

2.1. Experimental Animals and Tissue Collection. The experimental protocol was reviewed and approved by the Ethics Committee of Kunming Medical University. Three tree shrews (18–20 months, 120–150 g) were obtained from the Kunming Institute of Biology, Chinese Academy of Medical Sciences.

The tree shrews were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital and fixed on the tree shrew operating panel. The maxillary bilateral canines of the first molar as measured by an orthodontic dynamometer (PAGE). Then, the 3' adapters were added and the 36-48 nt RNAs were enriched. The 5' adapters were then ligated to RNAs as well. The ligation products were reverse transcribed by PCR amplification, and the 140-160 bp size PCR products were enriched to generate a cDNA library and sequenced using Illumina HiSeq Xten by Gene Denovo Biotechnology Co. (Guangzhou, China).

2.3. miRNA-seq Analysis [14]. Reads obtained from the sequencing machines were filtered to get clean tags, and all the clean tags were aligned with small RNAs in GenBank database (Release 209.0) and Rfam database (Release 11.0) to identify and remove rRNA, scRNA, snRNA, snoRNA, and tRNA. Meanwhile, all of the clean tags were aligned with reference genome, and those mapped to exons or introns or repeat sequences were removed. All clean tags were then searched against miRBase database (Release 22) to identify known (species studies) miRNAs (exist miRNAs). So far, the miRNA sequences of some species were still not included in the miRBase database. For those species, the miRNA alignment with other species was a dependable way to identify the known miRNAs. The novel miRNA candidates were identified according to the unannotated tags’ genome positions and hairpin structures predicted by software miRDeep2. Total miRNA consists of exist miRNA, known miRNA, and novel miRNA; based on their expression in each sample, the miRNA expression levels were calculated and normalized to transcripts per million.

2.4. Differentially Expressed miRNA Analysis. Differential expressed analysis of miRNAs was performed by edgeR software between two different groups or samples. We identified miRNAs with fold change (FC) ≥ 2 and P value < 0.05 in a comparison as significant differentially expressed miRNAs.

2.5. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analyses [15]. All differentially expressed genes were mapped to GO terms in the Gene Ontology database (http://www.geneontology.org/), gene numbers were calculated for every term, and significantly enriched pathways or signal transduction pathways meeting this condition were de ned as significantly enriched pathways in differential expressed genes.

Pathway enrichment analysis identified significantly enriched metabolic pathways or signal transduction pathways in differential expressed genes compared with the whole genome background (http://www.genome.jp/kegg/). The calculation formula was the same as that in GO analysis. The calculated P value was gone through FDR Correction, taking FDR ≤ 0.05 as a threshold. GO terms meeting this condition were defined as significantly enriched GO terms in differential expressed genes. The functional enrichment of both target genes of miRNAs in single samples and
Table 1: Primer sequences of RT-qPCR.

| Target gene | Forward primer (F) 5′-3′ | Reversed primer (R) 5′-3′ |
|-------------|--------------------------|--------------------------|
| miR-149     | CTGGCTCCGTGTCTCCCT       | CAGCTGCCCCAGCACAG        |
| U6          | GGAAGAGGCTCTTTCTCCAT     | GTGTTCGTTCCTTTCCACAAG   |
| ASAP3       | ATTTGGAAGACCCAGAGCGG     | GTGGCTGTTGCCTAAGGAT     |
| GAPDH       | GGAGTCAACGGATTGTCG       | TTCCGGTTCTCGGCATGAG     |

Figure 1: miRNA expression profiles in tree shrew periodontal tissues. (a) PCA of miRNA in samples. (b) Heatmaps of stratified cluster analysis of differentially expressed miRNAs in the control (C-1, C-2, and C-3) and treatment (T-1, T-2, and T-3) groups. (c) The statistics of upregulated and downregulated miRNAs in the control and treatment groups.
differential expressed miRNAs in a compare group were carried in our analysis.

2.6. Cell Culture. The human osteoblast-like MG63 cells were purchased from Procell (Wuhan, China). The MG63 cells were cultured in Dulbecco’s modified Eagle medium (Procell, Wuhan, China) with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in a humidified incubator with 5% CO₂. The medium was refreshed every 3 days [16].

2.7. Mechanical Tension Application. MG63 cells were seeded into 6-well plates (Corning, NY, USA) at a density of 1 x 10⁵ cells per well and incubated for 24 h. Human osteoblasts MG63 were treated with mechanical tension of 5000 U using a four-point bending cell mechanical loading instrument for 12, 24, and 36 h, respectively.

2.8. Cell Transfection. Briefly, when approximately 70% confluency was reached, MG63 cells treated with 5000 U tension for 36 h were inoculated 24 h prior to transfection. si-NC, si-ARFGAP with SH3 domain, Ankyrin repeat, and Ph domain 3 (ASAP3) #1, si-ASAP3#2, si-ASAP3#3, pcDNA-NC, and pcDNA-ASAP3 were transfected into MG63 cells individually according to the Lipofectamine™ 2000 Kit (Invitrogen, China) transfection protocol. After 48 h, real-time quantitative polymerase chain reaction (RT-qPCR) and Western blot were used to detect the transfection efficiency.

2.9. RT-qPCR Assay. Total RNA from cultured cells was extracted using the TRIzol reagent. Then, total RNA of 1 μg was reverse transcribed to cDNA using a PrimeScript™ RT reagent Kit (RR037A, Takara, Japan). The cDNA was amplified using SYBR Green PCR Master Mix (4364344, Applied Biosystems, CA, USA) on an ABI 7500 Real-Time PCR System (4351105, Applied Biosystems). U6 served as the internal reference of miRNAs, and GAPDH was set as an internal control of mRNA. The 2-ΔΔCt method was used to analyze the relative gene expression [17]. The primer sequences were showed in table 1.

2.10. Rhodamine Phalloidin Assay [18]. The cells of each group were collected and fixed with 10% paraformaldehyde for 30 min. Then, it was washed with PBS, coated with precooled glacial acetic acid for 20 min, and blocked at 37°C. Primary antibody was added, incubated overnight at 4°C, rinsed with PBS for 10 min, and incubated with FITC-phalloidin stain (HY-K0903, MCE, Shanghai, China) for 4 h. The cytoskeleton of cytoskeleton was observed under a fluorescence microscope.

2.11. Western Blot. Cells were lysed using RIPA buffer to obtain total protein. Protein concentration was measured by a BCA Protein Assay Kit (P0012, Beyotime, Shanghai, China). Equivalent amounts of proteins were then electrophoresed on SDS-PAGE and transferred onto PVDF membranes (FP26, Beyotime, Shanghai, China). Membranes were blocked using 5% BCA for 2 h at room temperature and incubated with primary antibodies against protein ASAP3 (abs100894, Abisin, Shanghai, China), F-actin.

---

### Table 2: Top 10 downregulated and upregulated miRNAs in the treatment group.

| miRNA ID     | Sequence                  | Log2 (FC) | P value   |
|--------------|---------------------------|-----------|-----------|
| Downregulated|                           |           |           |
| novel-m0413-3p| AATGTATCATGACATTCCGTGACA | -5.82349  | 0.000635  |
| novel-m0797-3p| CAGAGCTCCTGTCTCTTGCC    | -5.34032  | 0.012099  |
| miR-4497-z   | GGCTCCGGGACGGCTGGG       | -3.95009  | 0.012736  |
| miR-8549-z   | GGAGGTAGTAGGTTGTAT       | -3.56081  | 0.000734  |
| miR-149-y    | GAGGGAGGGCCGGGGGCTGTGC   | -2.79196  | 0.004971  |
| miR-592-x    | TGTGTCATAATGGCGATGATGT   | -2.00797  | 0.012816  |
| novel-m0373-3p| AATGCGTCACTGACCTGTGAC   | -1.98382  | 0.020635  |
| miR-483-x    | AAGAGGAGGGCCGGGGGGCTGTGC| -1.67337  | 0.00366   |
| novel-m0352-3p| TGGATGCGGACTGAGTCTGTGTC  | -1.50881  | 0.030636  |
| novel-m0353-5p| TGGATGCGGACTGAGTCTGTGTC  | -1.50881  | 0.030552  |
| Upregulated  |                           |           |           |
| miR-7975-z   | ATCCCTAGTCACGGGACCA      | 4.82765   | 0.044497  |
| novel-m0392-3p| ATGAGCGTGACTCTCTCTGATGAC| 4.694058  | 0.000908  |
| novel-m0360-5p| CAGGAAAAATCGTAGACACATTCT| 4.440797  | 0.002222  |
| miR-375-z    | TTGTTTCCTTCGGCTCAGGGCA   | 4.099827  | 0.016099  |
| novel-m0482-3p| AATGCGATCATGACTTTTCTTA   | 3.966892  | 0.030562  |
| novel-m0454-3p| TCTGGCGCGGTCTGAGGGGA     | 3.918842  | 0.01347   |
| miR-9-x      | TTCTTGTATATCTATGCTGTATG  | 3.529339  | 0.002083  |
| novel-m0542-3p| AAACATATCGTCTGATTTTCCGA  | 3.325061  | 0.049574  |
| novel-m0543-3p| AAACATATCGTCTGATTTTCCGA  | 3.325061  | 0.04957   |
| novel-m0168-3p| AATGGATTTCGGGATAGGGGA    | 3.30548   | 0.000817  |

FC: fold change.
**Figure 2: Continued.**

(a) Level 2 GO terms of C-vs–T

(b) Top 20 of GO enrichment
Figure 2: Continued.
At 4°C overnight. After washing with PBST, membranes were incubated with secondary antibodies (Abcam, UK) at room temperature for 1 h. ECL solution (Thermo Fisher Scientific, China) was then prepared, and the bands on the membranes were scanned and imaged in a dark room. The results were quantified using ImageJ. GAPDH was used as the internal control [19].

2.12. Luciferase Reporter Assay. Cells \((1 \times 10^4)\) were seeded in 48-well plates and allowed to settle for 24 h. Then, the luciferase reporter plasmids or the control plasmids \(\text{100 ng}\) plus \(1 \text{ng}\) of the pGMLR-TK plasmid was transfected into cells using Lipofectamine™ 2000 Kit (Invitrogen, China) according to the manufacturer’s instruction. After 48 h, luciferase was measured using the Dual Luciferase Reporter Assay Kit (Beyotime, Shanghai, China) according to the manufacturer’s instruction.

2.13. Statistical Analysis. All data were expressed as the mean ± standard deviation from at least three biologically repeated experiments with three technical replicates per sample. GraphPad Prism8.0 was used to analyze the data and graph. Differences between parametric data of two groups were assessed through \(t\)-test. Differences among multiple groups were analyzed using one-way ANOVA test. Differences with \(P < 0.05\) were considered statistically significant.

3. Results

3.1. miRNA Expression Profiles in Tree Shrew Periodontal Tissues. We extracted total RNA from the dental and periodontal tissue of tree shrew in the normal control group and the orthodontic model group and performed small RNA sequencing. Principal component analysis (PCA) found that the differences of miRNA among all the groups were large, and there was no correlation (Figure 1(a)). The heatmap of hierarchical clustering analysis for differentially expressed miRNA in dental and periodontal tissue of tree shrews in the control (C) group and the orthodontic model (treatment, T) group are shown in Figure 1(b). Compared with the normal control group, there were 51 upregulated miRNAs and 13 downregulated miRNAs in the orthodontic model group \((\text{FC} > 2 \text{ and } P < 0.05\), Figure 1(c)). The top 10 upregulated genes were miR-7975-z, novel-m0392-3p, novel-m0360-5p, miR-375-z, novel-m0482-3p, novel-m0454-3p, miR-9-x, novel-m0542-3p, novel-m0543-3p, and novel-m0168-3p and top 10 downregulated genes were novel-m0413-3p, novel-m0797-3p, miR-4497-z, miR-8549-z, miR-149-y, miR-592-x, novel-m0373-3p, miR-483-x, novel-m0352-3p, and novel-m0353-5p, as shown in Table 2.
3.2. Enrichment of Differentially Expressed miRNA Target Genes. GO was used for BP, cellular component (CC), and molecular function (MF) enrichment analysis of differentially expressed miRNA target genes (Figure 2(a)). Target genes of differentially expressed genes in BP were mainly involved in cellular process, single-organic process, metabolic process, biological regulation, regulation of BP, response to stimulus, etc. Target genes of differentially expressed genes in CC were mainly involved in cell, cell part, organelle, organelle part, membrane, etc. Target genes of differentially expressed genes in MF were mainly involved in binding, catalytic activity, etc.

These differentially expressed genes significantly enriched the GO term GO: 0065007 Biological regulation, GO:0050789: regulation of the BP, GO: 0050794 regulation of cellular process and GO: 0050896 response to stimulus in BP GO ontologies (Figure 2(b)). The GO terms enriched in CC ontologies in the top 20 GO terms were GO: 0016020 membrane, GO: 0044425 part, and GO: 0031224 intrinsic component of membrane (Figure 2(c)). These differentially expressed genes significantly enriched the GO term GO: 0003824 catalytic activity, GO: 0043167: ion binding, and GO: 0043169 cation binding in MF GO ontologies (Figure 2(d)).

KEGG pathway enrichment analysis revealed that among the predicted target genes, several KEGG pathway were significantly enriched, including "metabolic pathways," "Herpes simplex infection," "Human papillomavirus infection," and "MAPK signaling pathway" (Figure 2(e)).

3.3. The Expression of miR-149 and ASAP3 in MG63 Cells under Mechanical Tension. miR-149 was chosen from the downregulated miRNAs in the treatment group for further investigation because miR-149 was reported to be involved in the regulation of osteogenic differentiation [11], while the mechanism of miR-149 in orthodontic model remains unclear. Therefore, we treated human osteoblast-like MG63...
with tension to construct orthodontic cell model and then explored the mechanism of miR-149.

Human osteoblast-like MG63 was treated with 5000 U of mechanical tension using a Four-point ben cell mechanical loading instrument for 12, 24, and 36 h, respectively. RT-qPCR detected the expression of miR-149 and ASAP3 mRNA. The results revealed that with the increase of mechanical tension time, the expression of miR-149 in MG63 cells decreased significantly (Figure 3(a)), and it was the lowest at 36 h. However, the expression of ASAP3 mRNA increased significantly with the increased of mechanical tension time (Figure 3(b)). And the results of Western blot analysis (Figures 3(c) and 3(d)) of ASAP3 protein showed the same results of RT-qPCR. Therefore, we chose 36 h as the best mechanical tension time for MG63.

Rhodamine-labeled phalloidin staining (Figure 3(e)) showed that in the ctrl group, the cells were disorderly arranged, and F-actin was arranged along the long axis of the cells, with a relatively slender shape. Under the action of mechanical stress, F-actin was polymerized to form stress fibers, which increased at the two levels of cells and around the nucleus. The stress fibers in the cytoplasm were dense and uniform, and the fluorescence intensity of F-actin was increased. In summary, under tension, the low expression of miR-149 and high expression of ASAP3 may participate in the regulation of osteogenesis during orthodontic treatment.

3.4. miR-149 Targeted ASAP3. We predicted that miR-149 and ASAP3 have targeted binding sites through the starBase database (Figure 4(a)). Dual luciferase report gene assay verified the targeting relationship between miR-149 and ASAP3. The miRNA expression of ASAP3 was detected by RT-qPCR. Compared with the NC mimic group, *P < 0.05. Compared with the NC inhibitor group, †P < 0.05.

Figure 4: miR-149 targeted ASAP3. (a) starBase database predicted the targeted binding site of miR-149 and ASAP3. (b) Dual luciferase reporter gene assay verified the targeting relationship between miR-149 and ASAP3. (c) The miRNA expression of ASAP3 was detected by RT-qPCR. Compared with the NC mimic group, *P < 0.05. Compared with the NC inhibitor group, †P < 0.05.

3.5. Regulation of Cytoskeleton and Osteogenesis of MG63 Cells by ASAP3. MG63 cells treated with 5000 U tension for 36 h were transfected with si-NC, si-ASAP3#1, si-ASAP3#2, and si-ASAP3#3, respectively. Transfection of si-ASAP3 inhibited the mRNA (Figure 5(a)) and protein (Figures 5(b) and 5(c)) levels of ASAP3 in MG63 cells, and the expression of ASAP3 protein was lower in the si-ASAP3#3 group. Therefore, the si-ASAP3#3 transfection group was selected for the research. Rhodamine-labeled phalloidin showed that the fluorescence intensity of F-actin in knocking down the ASAP3 group was lower than that in the si-NC group (Figures 5(d) and 5(e)). Then, we transfected pcDNA-NC and pcDNA-ASAP3 into MG63 cells treated with 5000 U tension for 36 h, respectively. Western blot results (Figures 5(f)–5(l)) found that overexpression of ASAP3 significantly upregulated the protein level of ASAP3, F-actin, and osteogenesis-related genes BMP2 and RUNX2. However, the phosphorylation of AKT was inhibited in the pcDNA-ASAP3 group compared with the pcDNA-NC group. In a word, downregulation of ASAP3 revised the effect of tension on MG63 cells and inhibited the phosphorylation of AKT. We speculated that ASAP3 may participate in the osteogenesis of MG63 cell during orthodontic treatment by regulating the AKT signaling pathway.
Figure 5: Continued.

(a) Relative expression of ASAP3 mRNA

(b) ASAP3 and GAPDH protein expression

(c) Relative expression of ASAP3 protein

(d) Relative fluorescence intensity

(e) Representative images of F-actin, DAPI, and Merge

(f) Representative images of ASAP3, F-actin, BMP2, RUNX2, AKT, p-AKT, and GAPDH
4. Discussion

Orthodontic treatment is based on the movement of teeth caused by the remodeling of alveolar bone induced by external forces. This movement is generally accomplished by highly coordinated and efficient bone remodeling, which requires bone formation coupling after bone resorption [20]. Studies have found that mechanical stress could convert into chemical signals that induce the formation of osteoclasts during orthodontic tooth movement [21, 22].

Figure 5: Regulation of cytoskeleton and osteogenesis of MG63 cells by ASAP3. (a) RT-qPCR detected the mRNA expression of ASAP3. (b, c) Western blot detected the levels of ASAP3 protein. (d, e) F-actin fluorescence intensity was detected by rhodamine-labeled phalloidin staining. Compared with the si-NC group, *P < 0.05, **P < 0.001. (f) Western blot detected the protein levels of ASAP3 (g), F-actin (h), BMP2 (i), RUNX2 (j), AKT (k), and p-AKT (l). Compared with the pcDNA-NC group, *P < 0.05, **P < 0.01, and ****P < 0.0001; ns: no significant difference.
miRNA plays an important regulatory role in epigenetics and is widely involved in the physiological processes of cells, while mechan-ical tension or fluid shear forces promote the proliferation of osteoblasts [23, 24]. In this study, we constructed the orthodontic tree shrew model through mechanical tension and performed miRNA sequencing on the periodontal membrane tissues of tree shrews in the treatment group and the control group. Compared to the control group, there were 51 upregulated miRNAs and 13 downregulated miRNAs in the treatment group. In addition, Gu et al. [25] performed transcriptome analysis on the alveolar bone around the left maxillary first molar in the tooth movement group and the tooth movement simultaneous removal cortex group through RNA-seq and found that there were 399 gene expression changes in the cortex removal group, of which 127 genes were upregulated and 272 genes were downregulated. Liu et al. [26] performed transcriptome sequencing of mouse cementoblasts exposed to 1.5 g/cm² pressure by RNA-seq and found that 70 lncRNAs and 521 mRNAs were differentially expressed in osteoblasts under compressive stress, among which 57 lncRNAs were upregulated and 13 were downregulated.

Epigenetic modifications play an important regulatory role in the biological function of cells. As noncoding RNA, miRNA plays an important regulatory role in epigenetics and is widely involved in the physiological processes of cell growth, proliferation, migration, and differentiation. In this study, we found that the expression of miR-149 in the periodontal ligament tissue of tree shrew in the mechanical tension treatment group was decreased. At the same time, the expression of miR-149 was decreased in osteoblasts MG63 treated with tension of 5000 U, and miR-149 targeted and negatively regulated ASAP3. This is consistent with the results reported by Li et al. [27]. In addition, the differentially expressed miR-149 has been reported to be involved in the development of various diseases including tumors [28, 29], atherosclerosis [30], myocardial ischemia-reperfusion [31], and psoriasis [32]. The expression of miR-149 in oxidized low-density lipoprotein-induced endothelial cells was decreased, and overexpression of miR-149 protected endothelial cells from the injury induced by oxidized low-density lipoprotein [33]. miR-149 alleviates Ox-LDL-induced endothelial cell injury by promoting autophagy through Akt/mTOR pathway [33].

ASAP3 located on the chromosome 1p36.13 is one of the members of the ADP ribosylation factor-GTPase activating protein family. As an important intracellular material transport regulatory molecule, ASAP3 plays an important role in cell vesicle transport [34], cytoskeletal assembly [35], cell growth [36], and so on. Zhang et al. [36] reported that ASAP3 overexpression promoted the migration and invasion of human lung adenocarcinoma cells and promoted the growth of lung adenocarcinoma in mice. ASAP3 could be used as the downstream target of miR-143-3p to reverse the inhibitory effect of miR-143-3p on colorectal cancer cell metastasis [37]. In this study, we found that ASAP3 expression was increased in mechanical tension-treated MG63 cells, and it was a target gene of miR-149. Knocking down ASAP3 inhibited the fluorescence intensity of F-actin. Over-expression of ASAP3 promoted the protein expression of F-actin as well as the expression of osteogenesis-related proteins BMP2 and RUNX2. F-actin, the main component of cytoskeletal microfilaments, is important for the remodeling of periodontal ligament under mechanical stress [38]. Wang et al. [38] stimulated the periodontal ligament cells with cyclic tension and found that the cyclic tension upregulated the expression of F-actin.

AKT is an important downstream regulator and effector of the messenger molecule PI3K, which can regulate cell proliferation and metastasis by phosphorylating downstream substrates. PI3K/AKT is one of the important signaling pathways in organisms. Under the stimulation of extracellular signals, the activation of intracellular PI3K leads to the binding of PI3P to the N-terminal PH domain of AKT, leading to the transfer of Akt to the plasma membrane and its activation, thus causing a cascade effect. Recent studies have found that the activation of the PI3K/AKT signaling pathway could improve the high glucose-induced pyroptosis and injury of podocytes [39]. The PI3K/AKT pathway was activated by Wnt3a and participated in Wnt3a-induced osteoblast proliferation and differentiation [40]. In this study, we found that the phosphorylation of AKT protein was decreased after overexpression of ASAP3 in MG63 cells under mechanical tension. We speculate that the AKT signaling pathway may be involved in the physiological changes of osteoblasts after mechanical tension, but the specific mechanism needs further investigation.

In summary, we found that the expression of miR-149 in the dental and periodontal tissue of tree shrews treated with mechanical tension was reduced by RNA-seq. At the same time, the mechanism of miR-149 was further explored in tension-treated MG63 cells, confirming that miR-149 targeted regulation of ASAP3. Furthermore, knocking down ASAP3 inhibited the expression of F-actin, and overexpression of ASAP3 promoted the expression of F-actin and tension-induced osteogenesis of MG63 cells.

**Data Availability**

All data that support the findings of this study are available from the corresponding authors upon reasonable request.

**Conflicts of Interest**

The authors declare no conflicts of interest.

**Authors’ Contributions**

CaiXia Lu and ZhiHe Zhao conceived and designed the experiments. YiFan Wang, GuanYin Zhu, Fang Pei, YiGan Wang, Jun Liu, and CaiXia Lu performed the experiments. YiFan Wang, GuanYin Zhu, and CaiXia Lu analyzed the data. YiFan Wang, Fang Pei, and YiGan Wang contributed reagents/materials/analysis tools. YiFan Wang wrote the original draft. YiFan Wang, GuanYin Zhu, CaiXia Lu, and ZhiHe Zhao did writing—review and editing.
Acknowledgments

This study was funded by the National Natural Science Foundation of China (81771048) and by the Science and Technology Department of Sichuan Province (grant no. 2020YFS0170).

References

[1] H. Huang, R. C. Williams, and S. Kyrkanides, “Accelerated orthodontic tooth movement: molecular mechanisms,” American Journal of Orthodontics and Dentofacial Orthopedics, vol. 146, no. 5, pp. 620–632, 2014.

[2] B. Li and Z. H. Zhao, “Adjunctive interventions to accelerate orthodontic tooth movement,” Hua Xi Kou Qiang Yi Xue Za Zhi= West China Journal of Stomatology, vol. 37, no. 6, pp. 648–655, 2019.

[3] T. N. Mcallister and J. A. Frangos, “Steady and transient fluid shear stress stimulate NO release in osteoblasts through distinct biochemical pathways,” Journal of Bone and Mineral Research, vol. 14, no. 6, pp. 930–936, 1999.

[4] A. Vatsa, D. Mizuno, T. H. Smit, C. F. Schmidt, F. C. MacKintosh, and J. Klein-Nulend, “Bio imaging of intracellular NO production in single bone cells after mechanical stimulation,” Journal of Bone and Mineral Research, vol. 21, no. 11, pp. 1722–1728, 2006.

[5] M. M. Weivoda, S. K. Lee, and D. G. Monroe, “miRNAs in osteoclast biology,” Bone, vol. 143, p. 115737, 2021.

[6] M. Wu, H. Wang, D. Kong et al., “mir-452-3p inhibited osteoblast differentiation by targeting Smad4,” PeerJ, vol. 9, article e12228, 2021.

[7] Q. Wang, X. Shen, Y. Chen, J. Chen, and Y. Li, “Osteoblast-derived exosomes regulate osteoclast differentiation through miR-503-3p/Hps4 axis,” Acta Histochemica, vol. 123, no. 7, p. 151790, 2021.

[8] C. Wang, L. Wang, X. Guan, and C. Yue, “miR-4303 relieves chondrocyte inflammation by targeting ASPN in osteoarthritis,” Journal of Orthopaedic Surgery and Research, vol. 16, no. 1, p. 618, 2021.

[9] J. Xu, X. Qian, and R. Ding, “miR-24-3p attenuates IL-1β-induced chondrocyte injury associated with osteoarthritis by targeting BCL2L12,” Journal of Orthopaedic Surgery and Research, vol. 16, no. 1, p. 371, 2021.

[10] H. Yuan, M. Li, X. Feng, E. Zhu, and B. Wang, “miR-142a-5p promoted osteoblast differentiation via targeting nuclear factor IA,” Journal of Cellular Physiology, vol. 236, no. 3, pp. 1810–1821, 2021.

[11] Y. Li, F. Yang, M. Gao et al., “miR-149-3p regulates the switch between adipogenic and osteogenic differentiation of BMSCs by targeting FTO,” Molecular Therapy-Nucleic Acids, vol. 17, pp. 590–600, 2019.

[12] J. Xiao, R. Liu, and C. S. Chen, “Tree shrew (Tupaia belangeri) as a novel laboratory disease animal model,” Zoological Research, vol. 38, no. 3, pp. 127–137, 2017.

[13] L. Brauchli, C. Senn, and J. Ball, “Force levels of 23 nickel-titanium open-coil springs in compression testing,” American Journal of Orthodontics and Dentofacial Orthopedics, vol. 139, no. 5, pp. 601–605, 2011.

[14] M. H. H. Withanage, H. Liang, and E. Zeng, “RNA-Seq experiment and data analysis,” Methods in Molecular Biology, vol. 2418, pp. 405–424, 2022.

[15] L. Chen, Y. H. Zhang, S. Wang, Y. Zhang, T. Huang, and Y. D. Cai, “Prediction and analysis of essential genes using the enrichments of gene ontology and KEGG pathways,” PLoS One, vol. 12, no. 9, article e0184129, 2017.

[16] H. E. Son and W. G. Jang, “Cip2A modulates osteogenic differentiation via the ERK-Runx 2 pathway in MG63 cells,” BioFactors, vol. 47, no. 4, pp. 658–664, 2021.

[17] A. Faridi, A. Afgar, S. M. Mousavi et al., “Intestinal expression of mir-130b, mir-410b, and mir-98a in experimental canine echinococcosis by stem-loop RT-qPCR,” Frontiers in Veterinary Science, vol. 7, p. 507, 2020.

[18] H. Amano, F. Iwaki, M. Oki, K. Aoki, and S. Ohba, “An osteogenic helioxanthin derivative suppresses the formation of bone-resorbing osteoclasts,” Regenerative Therapy, vol. 11, pp. 290–296, 2019.

[19] L. Pillai-Kastoori, A. R. Schutz-Geschwender, and J. A. Harford, “A systematic approach to quantitative Western blot analysis,” Analytical Biochemistry, vol. 593, p. 113608, 2020.

[20] Y. Li, Q. Zhan, M. Bao, J. Yi, and Y. Li, “Biomechanical and biological responses of periodontium in orthodontic tooth movement: up-date in a new decade,” International Journal of Oral Science, vol. 13, no. 1, p. 20, 2021.

[21] T. Miyazaki, R. Kurimoto, T. Chiba et al., “Mlx regulates the orthodontic tooth movement via osteoclast induction,” Journal of Bone and Mineral Metabolism, vol. 39, no. 5, pp. 780–786, 2021.

[22] H. Kondo, M. Kondo, K. Hayashi et al., “Orthodontic tooth movement-activated sensory neurons contribute to enhancing osteoclast activity and tooth movement through sympathetic nervous signalling,” European Journal of Orthodontics, vol. 44, no. 4, pp. 404–411, 2021.

[23] R. Holland, C. Bain, and A. Utreja, “Osteoblast differentiation during orthodontic tooth movement,” Orthodontics & Craniofacial Research, vol. 22, no. 3, pp. 177–182, 2019.

[24] H. H. Jeon, H. Teixeira, and A. Tsai, “Mechanistic insight into orthodontic tooth movement based on animal studies: a critical review,” Journal of Clinical Medicine, vol. 10, no. 8, 2021.

[25] Q. Gu, S. Guo, D. Wang et al., “Effect of corticision on orthodontic tooth movement in a rat model as assessed by RNA sequencing,” Journal of Molecular Histology, vol. 48, no. 3, pp. 199–208, 2017.

[26] H. Liu, Y. Huang, Y. Zhang et al., “Long noncoding RNA expression profile of mouse cementoblasts under compressive force,” The Angle Orthodontist, vol. 89, no. 3, pp. 455–463, 2019.

[27] G. Li, J. An, X. Han, X. Zhang, W. Wang, and S. Wang, “Hypermethylation of microRNA-149 activates SDF-1/CXCR4 to promote osteogenic differentiation of mesenchymal stem cells,” Journal of Cellular Physiology, vol. 234, no. 12, pp. 23485–23494, 2019.

[28] J. Ma, H. Wei, X. Li, and X. Qu, “Hsa-miR-149-5p suppresses prostate carcinoma malignancy by suppressing RGS17,” Cancer Management and Research, vol. 13, pp. 2773–2783, 2021.

[29] S. Ow, “miR-149 as a potential molecular target for cancer,” Current Medicinal Chemistry, vol. 25, no. 9, pp. 1046–1054, 2018.

[30] Z. M. Ye, S. Yang, Y. P. Xia et al., “LncRNA MIAT sponges miR-149-5p to inhibit efectorcytosis in advanced atherosclero- sis through CD47 upregulation,” Cell Death & Disease, vol. 10, no. 2, p. 138, 2019.
[31] J. Lin, H. Lin, C. Ma, F. Dong, Y. Hu, and H. Li, “miR-149 aggravates pyroptosis in myocardial ischemia-reperfusion damage via silencing Fox O3,” Med Sci Monit, vol. 25, pp. 8733–8743, 2019.

[32] A. Srivastava, L. Luo, W. Lohcharoenkal et al., "Cross-talk between IFN-γ and TWEAK through miR-149 amplifies skin inflammation in psoriasis,” The Journal of Allergy and Clinical Immunology, vol. 147, no. 6, pp. 2225–2235, 2021.

[33] Z. Zhu, J. Li, R. Tong, X. Zhang, and B. Yu, “miR-149 alleviates Ox-LDL-induced endothelial cell injury by promoting autophagy through Akt/mTOR pathway,” Cardiology Research and Practice, vol. 2021, Article ID 9963258, 9 pages, 2021.

[34] S. A. Ismail, I. R. Vetter, B. Sot, and A. Wittinghofer, “The structure of an Arf-ArfGAP complex reveals a Ca2+ regulatory mechanism,” Cell, vol. 141, no. 5, pp. 812–821, 2010.

[35] Y. Luo, F. Kong, Z. Wang et al., “Loss of ASAP3 destabilizes cytoskeletal protein ACTG1 to suppress cancer cell migration,” Molecular Medicine Reports, vol. 9, no. 2, pp. 387–394, 2014.

[36] P. Zhang, J. Sun, J. Kai et al., "ASAP3 is a downstream target of HIF-1α and is critical for progression of lung adenocarcinoma,” Oncotargets and Therapy, vol. Volume 12, pp. 5793–5803, 2019.

[37] L. Guo, J. Fu, S. Sun et al., "MicroRNA-143-3p inhibits colorectal cancer metastases by targeting ITGA6 and ASAP3," Cancer Science, vol. 110, no. 2, pp. 805–816, 2019.

[38] Y. F. Wang, Z. H. Zuo, P. Luo, F. S. Pang, and J. T. Hu, “The effect of cyclic tensile force on the actin cytoskeleton organization and morphology of human periodontal ligament cells,” Biochemical and Biophysical Research Communications, vol. 506, no. 4, pp. 950–955, 2018.

[39] B. H. Liu, Y. Tu, G. X. Ni et al., “Total flavones of Abelmoschus manihot ameliorates podocyte pyroptosis and injury in high glucose conditions by targeting METTL3-dependent m⁶A modification-mediated NLRP3-inflammasome activation and PTEN/PI3K/Akt signaling,” Frontiers in Pharmacology, vol. 12, p. 667644, 2021.

[40] J. Dong, X. Xu, Q. Zhang, Z. Yuan, and B. Tan, “The PI3K/AKT pathway promotes fracture healing through its crosstalk with Wnt/β-catenin,” Experimental Cell Research, vol. 394, no. 1, p. 112137, 2020.