Original Article

MicroRNA-34 expression in gingival crevicular fluid correlated with orthodontic tooth movement

Bin Zhang[a]; Li Yang[a]; Weilong Zheng[b]; Ting Lin[a]

ABSTRACT

Objectives: To explore the expression of miR-34a and its effect on expression of matrix metalloproteinases (MMPs) during orthodontic tooth movement (OTM).

Materials and Methods: Twenty patients, age 12–18 years old, who underwent orthodontic treatment were enrolled. The expression of miR-34a and MMPs (MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, and MMP-14) were detected in gingival crevicular fluid by enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction at different time points. The miR-34a mimics or inhibitors were transfected into human periodontal ligament (hPDL) cells, and the MMP expression was measured by ELISA.

Results: The miR-34 expression in GCF on both the tension and pressure sides after orthodontic treatment were significantly downregulated, while the levels of MMPs were significantly upregulated compared with baseline level. The levels of miR-34 and MMPs returned to baseline level 3 months after orthodontic treatment. The expression of miR-34 was negatively correlated with the expression of MMP-2, MMP-9, and MMP-14. After transfection with miR-34, the MMP-2, MMP-9, and MMP-14 expression by hPDL cells were significantly downregulated compared with miR-control and miR-34 inhibitor.

Conclusions: Downregulated miR-34 expression was positively correlated with MMP-2, MMP-9, and MMP-14 expression. The miR-34a transfection into hPDL cells inhibited expression of MMPs. The results suggest that miR-34a is involved in expression of MMPs during OTM. (*Angle Orthod.* 2020;90:702–706.)

KEY WORDS: MiR-34; Matrix metalloproteinases; Orthodontic tooth movement; Human periodontal ligament

INTRODUCTION

Osteoclasts play an important role in orthodontic tooth movement (OTM). Increased activity of osteoclasts results in alveolar bone resorption and tooth movement.[1] Various molecules related to osteoclast activity, such as nuclear factor kappa-ligand (RANKL) receptor activator and osteoprotegerin (OPG) in gingival crevicular fluid (GCF), were used as potential biomarkers of OTM.[2,3]

MicroRNA, a small noncoding RNA, induces degradation or translational repression of mRNA and regulates gene functions at the posttranscriptional level by pairing with 30UTR of target mRNAs.[4] Secretory small RNA has been isolated and shown to be remarkably stable in body fluids. By controlling the differentiation and function of osteoblasts/osteoclasts, some small RNA play a key role in osteoblastogenesis and osteoclastogenesis.[5]

The changed expression of some microRNA was found in odontogenesis, tooth development, and tooth movement.[6–10] Studies have shown that some microRNAs were sensitive to the mechanical stimuli in human periodontal ligament (PDL) cells and PDL stem cells.[10,11] Of them, miR-34a can be found in different stages of tooth growth and are involved in bone remolding and dental stem cell differentiation.[12–14]

[a] Assistant Professor, Department of Orthodontics, Key Laboratory of Oral Medicine, Guangzhou Institute of Oral Disease, Stomatology Hospital of Guangzhou Medical University, Guangzhou, China.

[b] Lecturer, Department of Orthodontics, Key Laboratory of Oral Medicine, Guangzhou Institute of Oral Disease, Stomatology Hospital of Guangzhou Medical University, Guangzhou, China.

Corresponding author: Ting Lin, No. 39, Huangsha Avenue, Guangzhou 510140, China
(e-mail: linting610@sohu.com)

Accepted: January 2020. Submitted: September 2019.
Published Online: March 12, 2020

MiR-34a can inhibit osteoporosis and bone metastasis by downregulating osteoclastogenesis expression.[15]

GCF is a serum exudate found in the gingival crevice. The normal volume range of GCF is between 0.5 and 1.0 μL.[16] The levels of cytokines and proteins in GCF were used as biomarkers in oral diseases, such as gingivitis, periodontitis, root resorption, and systemic diseases.[17–19] However, the expression of microRNA in GCF was rarely reported. In this study, the expression of miR-34a during OTM was explored to investigate its role in OTM.

MATERIALS AND METHODS

Patients

This study was approved by the Guangzhou Medical University ethical committee. The patients signed informed consent.

Twenty patients, age 12–18 years old, who were undergoing orthodontic treatment were enrolled in this study. Patients with autoimmune diseases, evidence of type 1 or type 2 diabetes, or a history of drug use were excluded. The contralateral teeth of the same arch were selected as controls. Orthodontic treatment for study participants was performed as described by Iwasaki et al.[20] after using a rinse protocol with 0.12% chlorhexidine gluconate for 1 week. A canine tooth was randomly chosen as the study tooth, and the contralateral teeth of the same arch without orthodontic treatment were selected as controls.

GCF Collection

Before GCF collection, the plaque on the gingiva was removed and the gingival crevice was carefully isolated with cotton rolls to prevent saliva contamination. GCF was collected by gently placing Periopapers (Oraflow, Smithtown, NY) into the gingival crevice for 1 minute. The GCF was collected at seven time points: the day of application (T0), 1 hour (T1), 24 hours (T2), 1 weeks (T3), 4 weeks (T4), and 12 weeks (T5) after the application of orthodontic force.

Human PDL Cell Culture and Transfection by miR-34

Human PDL (hPDL) tissues were prepared as described previously. The hPDL cells were cultured in a-MEM medium (Wako, Osaka, Japan) supplemented with penicillin-G, gentamicin sulfate, amphotericin B, and 10% fetal calf serum (Cell Culture Laboratories, Cleveland, Ohio) at 37°C in a 5% CO_2-humidified chamber. The transfection was performed using 1 μM miR-34 mimics, inhibitors, or negative control for 1 hour at 37°C.

Quantification of Inflammatory Mediators

Per the manufacturer's instructions, the levels of the matrix metalloproteinases (MMPs) MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, and MMP-14 in the GCF sample were measured by multiplex Luminex (R&D system, Minneapolis, Minn).

RNA Isolation and Polymerase Chain Reaction Assay

The total RNA from GCF was reverse transcribed into cDNA using the Taqman microRNA reverse transcription kit and Taqman microRNA assays (Invitrogen, Carlsbad, Calif) as described by the manufacturer's instructions. The data were calculated using the 2-ΔΔCt method and normalized to RNA U6 controls.

Statistical Analysis

The data were reported as mean ± standard deviation and analyzed using the Friedman test followed by Bonferroni-corrected Wilcoxon paired signed-rank test when necessary. $P < .05$ was defined as the level of significance.

RESULTS

MiR-34 and Expression of MMPs in GCF at Different Time Points

The miR-34a expression in GCF on both the tension and pressure sides after orthodontic treatment was significantly downregulated at T2–T4, while the levels of MMPs were significantly upregulated at T2–T4 compared with T0 and T1. At T5, the levels of miR-34a and MMPs returned to baseline level (Table 1). For the control teeth, the miR-34a and expression of MMPs on both the tension and pressure sides were not significantly different. The expression of miR-34a was negatively correlated with the expression of MMP-2, MMP-9, and MMP-14 (Table 2).

MiR-34a Promotes the Expression of MMPs by hPDL Cells

When transfected with miR-34a, the MMP-2, MMP-9, and MMP-14 expression by hPDL cells was significantly downregulated compared with miR-control and miR-34a inhibitor (Figure 1). However, MMP-2, MMP-9, and MMP-14 expression were significantly downregulated when the Wnt/β-catenin pathway was inhibited (Figure 1).

DISCUSSION

MicroRNA has been reported to regulate its target gene by inducing the degradation of RNA or by

Table 1. Expression of miR-34a and MMPs in Gingival Crevicular Fluid of Study Tooth at Different Time Points During Orthodontic Tooth Movement[a]

Time Points	T0	T1	T2	T3	T4	T5
miR-34a TS	8.2 ± 3.6	7.3 ± 2.7	4.2 ± 1.8	2.1 ± 0.6	1.5 ± 0.4	6.4 ± 2.8
miR-34a PS	7.6 ± 2.9	8.1 ± 3.1	5.5 ± 2.3	3.6 ± 1.7	1.8 ± 0.5	6.9 ± 2.6
MMP-1 TS	194.5 ± 30.7	182.4 ± 27.6	217.3 ± 42.9	238.6 ± 48.3	285.2 ± 44.2	183.6 ± 30.7
MMP-1 PS	202.7 ± 34.8	186.5 ± 32.7	221.5 ± 37.2	246.1 ± 39.6	267.4 ± 31.8	191.6 ± 45.9
MMP-2 TS	72.4 ± 17.5	81.3 ± 18.5	98.5 ± 21.8	155.6 ± 27.2	174.1 ± 20.2	68.5 ± 20.3
MMP-2 PS	78.6 ± 14.1	76.3 ± 17.1	105.3 ± 23.8	167.8 ± 36.2	189.3 ± 31.8	71.2 ± 22.5
MMP-3 TS	17.3 ± 3.0	16.1 ± 2.8	41.5 ± 9.5	89.6 ± 14.8	115.7 ± 21.5	18.6 ± 4.9
MMP-3 PS	21.1 ± 3.6	23.4 ± 4.6	47.6 ± 10.3	76.5 ± 17.1	103.6 ± 15.9	18.6 ± 3.5
MMP-8 TS	9.6 ± 3.1	7.8 ± 3.5	15.9 ± 4.3	24.8 ± 6.1	37.9 ± 4.9	6.5 ± 2.1
MMP-8 PS	10.4 ± 2.6	9.1 ± 1.9	14.8 ± 3.8	23.9 ± 4.3	41.2 ± 5.7	7.9 ± 2.5
MMP-9 TS	184.6 ± 32.7	175.4 ± 26.4	218.4 ± 36.9	268.9 ± 41.2	295.3 ± 42.6	177.9 ± 29.3
MMP-9 PS	192.7 ± 43.8	181.3 ± 25.2	225.8 ± 31.8	271.3 ± 38.9	315.4 ± 45.7	189.2 ± 38.7
MMP-13 TS	29.6 ± 6.8	25.6 ± 7.1	63.8 ± 12.6	87.9 ± 19.8	119.2 ± 31.7	26.3 ± 5.4
MMP-13 PS	27.8 ± 5.9	23.4 ± 3.8	57.6 ± 11.7	91.3 ± 21.4	124.6 ± 41.5	25.4 ± 6.1
MMP-14 TS	11.5 ± 3.1	12.6 ± 4.1	19.8 ± 5.2	32.5 ± 6.7	45.7 ± 8.1	9.8 ± 3.4
MMP-14 PS	10.2 ± 2.8	10.7 ± 3.9	21.7 ± 4.6	34.6 ± 7.1	51.3 ± 9.2	10.7 ± 3.7

[a] MMP indicates matrix metalloproteinase; TS, tension side; PS, pressure side.

inhibiting translation. Previous studies showed that microRNA may be a key posttranscriptional regulator in bone remodeling.[21] MicroRNAs were reported to regulate the differentiation and activity of osteoblasts during OTM. For example, miR-21 promoted OTM by regulating the RANKL/OPG balance in T cells.[22,23] Additionally, anti-miR-503, anti-miR-103, and anti-miR-195 may be used to induce the expression of RUNX2 and promote osteoblast differentiation on the stretch side during OTM.[24]

The miR-34a, located in chromosome 1p36, is regulated by the p53 transcription factor. The expression of miR-34a is downregulated in multiple cancers, such as tongue squamous cell carcinoma, non–small cell lung cancer, colon cancer, pancreatic cancer, and others.[25–30] Previous studies also demonstrated that miR-34a was involved in tooth development, mechanical force loading, and bone metabolism.[31–34] For example, miR-34a was shown to promote osteogenic gene and protein expression and to enhance early osteogenic secretion function, and local alveolar bone anabolism both in vivo and in vitro.[35,36] Inconsistently, some studies showed that miR-34s and miR-34a inhibited osteogenesis under static conditions. The results of the current study showed that the miR-34a expression in GCF on both the tension and pressure sides after orthodontic treatment were significantly downregulated, but the miR-34a returned to baseline level after 3 months.

Table 2. Correlation of miR-34a and MMPs in Gingival Crevicular Fluid of Study Tooth at Different Time Points During Orthodontic Tooth Movement[a]

Time Points	T1	T2	T3	T4
MMP-1 TS	NS	NS	NS	NS
MMP-1 PS	NS	NS	NS	NS
MMP-2 TS	NS	–0.6	–0.49	–0.51
MMP-2 PS	NS	–0.52	–0.48	–0.52
MMP-3 TS	NS	NS	NS	NS
MMP-3 PS	NS	NS	NS	NS
MMP-8 TS	NS	NS	NS	NS
MMP-8 PS	NS	NS	NS	NS
MMP-9 TS	NS	–0.59	–0.47	–0.51
MMP-9 PS	NS	–0.44	–0.54	–0.59
MMP-13 TS	NS	NS	NS	NS
MMP-13 PS	NS	NS	NS	NS
MMP-14 TS	NS	–0.55	–0.46	–0.59
MMP-14 PS	NS	–0.51	–0.62	–0.57

[a] MMP indicates matrix metalloproteinase; NS, not significant; TS, tension side; PS, pressure side.

MMPs, widely distributed in mesenchymal tissues, are mainly produced by fibroblasts, synovial cells, macrophages, and neutrophils. The main function of MMP is degradation of extracellular matrix and, thus, closely correlated with bone formation. In humans, GCF MMP-1 and MMP-8, MMP-2 and MMP-9, and TIMP-1 have all been shown to increase at sites of compression and tension.[37–39] In the present study, it was confirmed that MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 on both the tension and pressure sides of study teeth were significantly increased and returned to baseline level 3 months later.

However, the regulation of MMPs in OTM is not yet fully understood. The miR-34a was reported to inhibit the growth and migration of glioma cells via MMP-9. Additionally, SB-3CT, an inhibitor of MMP-9, alleviated the action of miR-34a mimic on glioma cells.[40] The miR-34a was also shown to inhibit ESCC cell migration and invasion by suppressing MMP-2 and MMP-9 expression levels.[41] Overexpression of miR-34a also inhibited migration and invasion in TSCC cells by downregulating expression of MMP-9 and MMP-14.[42] Therefore, to prove the effect of miR-34a on the regulation of OTM, in this study, hPDL cells were

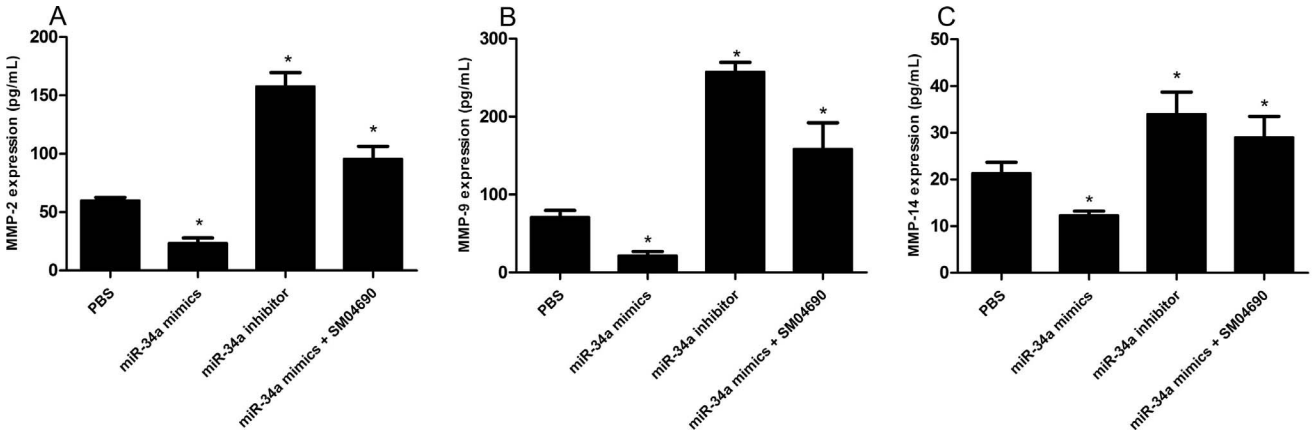

Figure 1. The expression of MMP-2, MMP-9, and MMP-14 by periodontal ligament (PDL) cells after being transfected with miR-34a mimics or inhibitor.

transfected with miR-34a. It was found that the MMP-2, MMP-9, and MMP-14 expression by hPDL cells was significantly downregulated compared with miR-control and miR-34 inhibitor, demonstrating that miR-34a promoted OTM by targeting MMPs. It was also found that the Wnt/β-catenin pathway was involved in the progression since Wnt/β-catenin inhibitor blocked the effect of miR-34a.

This study had some limitations. A sample-size calculation was not done, so further studies with a larger sample size will be needed to confirm the findings.

CONCLUSIONS

- Downregulated miR-34a expression was negatively correlated with MMP-2, MMP-9, and MMP-14.
- The effect of miR-34 on the expression of MMPs by PDL cells suggested that miR-34a plays a crucial role in the remodeling in OTM.
- The findings provided a baseline for future microRNA-based approaches to regulate OTM or prevent periodontitis caused by alveolar bone loss. However, more in-depth studies are needed to reveal the upstream molecular events regulating the miRNA expression and to set up a miRNA regulatory network in OTM.

DISCLOSURE

The authors report no conflicts of interest and no funding.

REFERENCES

1. Huang H, Williams RC, Kyrkanides S. Accelerated orthodontic tooth movement: molecular mechanisms. *Am J Orthod Dentofacial Orthop*. 2014;146:620–632.
2. Alhashimi N, Frithiof L, Brudvik P, Bakhiet M. Orthodontic tooth movement and de novo synthesis of proinflammatory cytokines. *Am J Orthod Dentofac Orthop*. 2001;119:307–312.
3. Kapoor P, Kharbanda OP, Monga N, Miglani R, Kapila S. Effect of orthodontic forces on cytokine and receptor levels in gingival crevicular fluid: a systematic review. *Prog Orthod*. 2014;15:65.
4. Ketting RF. MicroRNA biogenesis and function: an overview. *Adv Exp Med Biol*. 2011;700:1–4.
5. PI C, Li YP, Zhou X, Gao B. The expression and function of microRNAs in bone homeostasis. *Front Biosci*. 2015;20: 119–138.
6. Yin K, Hacia JG, Zhong Z, Paine M. Genome-wide analysis of miRNA and mRNA transcriptomes during amelogenesis. *BMC Genomics*. 2014;15:998.
7. Wang C, Liao H, Cao Z. Role of osterix and microRNAs in bone formation and tooth development. *Med Sci Monit*. 2016; 22:2934–2942.
8. Park MG, Kim JS, Park SY, et al. MicroRNA-27 promotes the differentiation of odontoblastic cell by targeting APC and activating Wnt/β-catenin signaling. *Gene*. 2014;538:266–272.
9. Sehic A, Tulek A, Khuu C, Nirvani M, Sand LP, Utheim TP. Regulatory roles of microRNAs in human dental tissues. *Gene*. 2017;596:9–18.
10. Chang M, Lin H, Luo M, Han G. Integrated miRNA and mRNA expression profiling of tension force-induced bone formation in periodontal ligament cells. *In Vitro Cell Dev Biol Anim*. 2015;51:797–807.
11. Wei FL, Wang JH, Ding G, et al. Mechanical force-induced specific MicroRNA expression in human periodontal ligament stem cells. *Cells Tissues Organs*. 2014;199:353–363.
12. Sun F, Wan M, Xu X, et al. Crosstalk between miR-34a and Notch signaling promotes differentiation in apical papilla stem cells (SCAPs). *J Dent Res*. 2014;93:589–595.
13. Grilli A, Sciandra M, Terracciano M, Picci P, Scotlandi K. Integrated approaches to miRNAs target definition: time-series analysis in an osteosarcoma differentiative model. *BMC Med Genomics*. 2015;8:34.
14. Boussif O, Lezoualc'h F, Zanta MA, et al. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. *Proc Natl Acad Sci U S A*. 1995; 92:7297–7301.

15. Tian H, Xiong W, Wei J, et al. Gene transfection of hyperbranched PEI grafted by hydrophobic amino acid segment PBLG. *Biomaterials*. 2007;28:2899–2907.
16. Lamster IB, Hartley LJ, Vogel RI. Development of a biochemical profile for gingival crevicular fluid. Methodological considerations and evaluation of collagen-degrading and ground substance-degrading enzyme activity during experimental gingivitis. *J Periodontol*. 1985;56(suppl):13–21.
17. George A, Evans CA. Detection of root resorption using dentin and bone markers. *Orthod Craniofac Res*. 2009;12:229–235.
18. Lamster IB, Ahlo JK. Analysis of gingival crevicular fluid as applied to the diagnosis of oral and systemic diseases. *Ann N Y Acad Sci*. 2007;1098:216–229.
19. Taba M Jr, Kinney J, Kim AS, Giannobile WV. Diagnostic biomarkers for oral and periodontal diseases. *Dent Clin North Am*. 2005;49:551–571.
20. Iwasaki L, Haack J, Nickel J, Reinhardt R, Petro T. Human interleukin-1 beta and interleukin-1 receptor antagonist secretion and velocity of tooth movement. *Arch Oral Biol*. 2001;46:185–189.
21. Ambros V. The functions of animal microRNAs. *Nature*. 2004;431:350–355.
22. Garlet TP, Coelho U, Repeke CE, Silva JS, Cunha Fde Q, Garlet GP. Differential expression of osteoblast and osteoclast chemmoatractants in compression and tension sides during orthodontic movement. *Cytokine*. 2008;42:330–335.
23. Ji X, Chen X, Yu X. MicroRNAs in osteoclastogenesis and function: potential therapeutic targets for osteoporosis. *Int J Mol Sci*. 2016;17:349.
24. Wang Y, Jia L, Zheng Y, Li W. Bone remodeling induced by mechanical forces is regulated by miRNAs. *Biosci Rep.* 2018;38(4).
25. Gallardo E, Navarro A, Viñolas N, et al. miR-34a as a prognostic marker of relapse in surgically resected non-small-cell lung cancer. *Carcinogenesis*. 2009;30:1903–1909.
26. Wu J, Wu G, Lv L, et al. MicroRNA-34a inhibits migration and invasion of colon cancer cells via targeting to Fra-1. *Carcinogenesis*. 2012;33:519–528.
27. Nalls D, Tang SN, Rodova M, Srivastava RK, Shankar S. Targeting epigenetic regulation of miR-34a for treatment of pancreatic cancer by inhibition of pancreatic cancer stem cells. *PLoS One*. 2011;6:e24099.
28. Sun H, Tian J, Xian W, Xie T, Yang X. miR-34a inhibits proliferation and invasion of bladder cancer cells by targeting orphan nuclear receptor HNF4G. *Dis Markers*. 2015;2015:879254.
29. Liu C, Kelnar K, Liu B, et al. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat Med*. 2011;17:211–215.
30. Corney DC, Hwang CI, Matoso A, et al. Frequent downregulation of miR-34 family in human ovarian cancers. *Clin Cancer Res*. 2010;16:1119–1128.
31. Wan M, Gao B, Sun F, et al. MicroRNA miR-34a regulates cytodifferentiation and targets multi-signaling pathways in human dental papilla cells. *PLoS One*. 2012;7:e50090.
32. Sun F, Wan M, Xu X, et al. Crosstalk between miR-34a and notch signaling promotes differentiation in apical papilla stem cells (SCAPs). *J Dent Res*. 2014;93:589–595.
33. Mai ZH, Peng ZL, Zhang JL, et al. MiRNA expression profile during fluid shear stress-induced osteogenic differentiation in MC3T3-E1 cells. *Chin Med J*. 2013;126:1544–1550.
34. Kang H, Chen H, Huang P, et al. Glucocorticoids impair bone formation of bone marrow stromal stem cells by reciprocally regulating microRNA-34a-5p. *Osteoporos Int*. 2016;27:1493–1505.
35. Chen L, Holmstrom K, Qiu W, et al. MicroRNA-34a inhibits osteoblast differentiation and in vivo bone formation of human stromal stem cells. *Stem Cells*. 2014;32:902–912.
36. Wei J, Shi Y, Zheng L, et al. miR-34s inhibit osteoblast proliferation and differentiation in the mouse by targeting SATB2. *J Cell Biol*. 2012;197:509–521.
37. Apajalahti S, Sorsa T, Railavo S, Ingman T. The in vivo levels of matrix metalloproteinase-1 and -8 in gingival crevicular fluid during initial orthodontic tooth movement. *J Dent Res*. 2003;82:1018–1022.
38. Cantarella G, Cantarella R, Caltabiano M, Risuglia N, Bernardini R, Leonardi R. Levels of matrix metalloproteinases 1 and 2 in human gingival crevicular fluid during initial tooth movement. *Am J Orthod Dentofacial Orthop*. 2006;130:568.e11–e16.
39. Takahashi I, Onodera K, Nishimura M, Mitnai H, Sasano Y, Mitani H. Expression of genes for gelatinases and tissue inhibitors of metalloproteinases in periodontal tissues during orthodontic tooth movement. *J Mol Histol*. 2006;37:333–342.
40. Wang X, Chen X, Sun L, et al. MicroRNA-34a inhibits cell growth and migration in human glioma cells via MMP-9. *Mol Med Rep*. 2019;20(1).
41. Nie J, Ge X, Geng Y, et al. miR-34a inhibits the migration and invasion of esophageal squamous cell carcinoma by targeting Yin Yang-1. *Oncol Rep*. 2015;34:311–317.
42. Jia LF, Wei SB, Mitchelson K, et al. miR-34a inhibits migration and invasion of tongue squamous cell carcinoma via targeting MMP9 and MMP14. *PLoS One*. 2014;9:e108435.