

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

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### 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.<sup>1</sup> 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.<sup>2,3</sup>

MicroRNA, a small noncoding RNA, induces degradation or translational repression of mRNA and regulates gene functions at the posttranscriptional level by pairing with 3'UTR of target mRNAs.<sup>4</sup> 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.<sup>5</sup>

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

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MiR-34a can inhibit osteoporosis and bone metastasis by downregulating osteoclastogenesis expression.<sup>15</sup>

GCF is a serum exudate found in the gingival crevice. The normal volume range of GCF is between 0.5 and 1.0  $\mu\text{L}$ .<sup>16</sup> The levels of cytokines and proteins in GCF were used as biomarkers in oral diseases, such as gingivitis, periodontitis, root resorption, and systemic diseases.<sup>17–19</sup> 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.<sup>20</sup> 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 (Oralflow, 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<sub>2</sub>-humidified chamber. The transfection was performed using 1  $\mu\text{M}$  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- $\Delta\Delta\text{Ct}$  method and normalized to RNA U6 controls.

## Statistical Analysis

The data were reported as mean  $\pm$  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/ $\beta$ -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<sup>a</sup>

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

<sup>a</sup> 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.<sup>21</sup> 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.<sup>22,23</sup> 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.<sup>24</sup>

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.<sup>25–30</sup> Previous studies also demonstrated that miR-34a was involved in tooth development, mechan-

ical force loading, and bone metabolism.<sup>31–34</sup> 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.<sup>35,36</sup> 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.

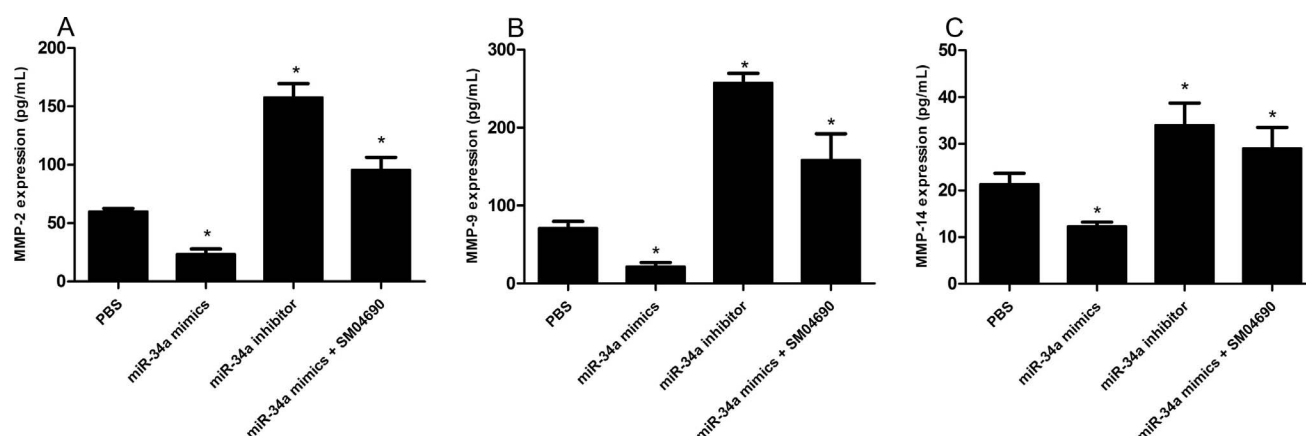
MMPs, widely distributed in mesenchymal tissues, are mainly produced by fibroblasts, synovial cells, macrophages, and neutrophils. The main function of MMP is degradation of extracellular matrix and, thus, closely correlated with bone formation. In humans, GCF MMP-1 and MMP-8, MMP-2 and MMP-9, and TIMP-1 have all been shown to increase at sites of compression and tension.<sup>37–39</sup> 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.<sup>40</sup> The miR-34a was also shown to inhibit ESCC cell migration and invasion by suppressing MMP-2 and MMP-9 expression levels.<sup>41</sup> Overexpression of miR-34a also inhibited migration and invasion in TSCC cells by downregulating expression of MMP-9 and MMP-14.<sup>42</sup> Therefore, to prove the effect of miR-34a on the regulation of OTM, in this study, hPDL cells were

**Table 2.** Correlation of miR-34a and MMPs in Gingival Crevicular Fluid of Study Tooth at Different Time Points During Orthodontic Tooth Movement<sup>a</sup>

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

<sup>a</sup> MMP indicates matrix metalloproteinase; NS, not significant; TS, tension side; PS, pressure side.



**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/ $\beta$ -catenin pathway was involved in the progression since Wnt/ $\beta$ -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 micro-RNA-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.

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