Treadmill exercise influences the microRNA profiles in the bone tissues of mice

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Abstract. As an important regulator involved in cell activity, microRNAs (miRNAs) are important in the process of exercise influencing bone metabolism. The present study aimed to detect and select differentially expressed miRNAs in the bone tissues of mice trained on a treadmill, predict the target genes of these differentially expressed miRNAs and lay a foundation for exploring the effect of treadmill training on bone metabolism through miRNAs. In this experiment, after the mice were trained on a treadmill for 8 weeks, the mechanical properties of mouse femur bone were assessed, and the alkaline phosphatase (ALP) activity and osteocalcin (OCN) protein levels of the bone were assayed. miRNA microarray and reverse transcription-quantitative (RT-q)PCR were performed to select and validate differentially expressed miRNAs in the bone, and the target genes of these miRNAs were predicted with bioinformatics methods. In addition, the differentially expressed miRNAs in the bone tissues were compared with those in mechanically strained osteocytes *in vitro*. Treadmill training improved the mechanical properties of the femur bones of mice, and elevated the ALP activity and OCN protein level in the bone. In addition, 122 differentially expressed miRNAs were detected in the bone, of which nine were validated via RT-qPCR. Among the target genes of these differentially expressed miRNAs, certain candidates were involved in bone metabolism. A total of eight miRNAs were differentially expressed in both bone tissue and osteocytes, exhibiting the same expression trends, and various target genes of these eight miRNAs were also involved in bone metabolism. Treadmill training resulted in altered miRNA expression profiles in the bones of mice (mainly in osteocytes) and the differentially expressed miRNAs may serve important roles in regulating bone metabolism and osteogenic differentiation.

Introduction

Bone tissue is a typical mechanoresponsive tissue. Lack of mechanical stimulation is the major cause of the loss of bone mass and osteoporosis (1,2). Exercise is able to apply mechanical stimulation to bone tissue, and the mechanical stress produced by exercise may increase the bone turnover rate and bone density (3), stimulate osteoblast activity, increase bone mass and promote bone reconstruction and metabolism (4,5). Thus, exercise is one of the primary modifiable factors associated with improved bone health outcomes (6).

Certain types of exercise may result in improved bone strength, even after menopause (7). An increasing number of trials have indicated that treadmill exercise may promote bone health, particularly by suppressing estrogen deficiency-induced osteoporosis, for instance, treadmill training could increase bone mineral density in specific parts of rats, such as cortical bone (8), and treadmill exercise could increase bone density, improve the bone trabecular microstructure of mice, and inhibit osteoporosis and osteoclast activation in bone tissues of ovariectomized mice (9,10). In addition, the mechanical loading of treadmill exercise substantially enhances the osteogenic response (11). However, these studies have not fully revealed the response of bone tissue to running motion stimulation at the molecular level, particularly in terms of regulation by microRNAs (miRNAs/miRs).

miRNAs, a class of small noncoding RNAs, are able to inhibit the expression of negative regulatory genes and protein-coding genes by binding to target mRNAs (12). MiRNAs participate in vital activities, including development, organ formation, tumorigenesis, cell proliferation, differentiation and apoptosis, by modulating gene expression (12,13). MiRNAs have multiple roles in osteoblasts and osteoclasts in the presence of mechanical cyclical stretch (14), fluid shear stress (15), compressive force (16), orthodontic force (17) and

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microgravity (18). In addition, miRNAs regulate osteogenesis, which may be translated into novel therapeutic approaches for orthodontic conditions and bone fractures, as well as for systemic diseases, such as osteoporosis (19).

Osteocytes account for >90% of the adult bone cell population and are critical sensors of mechanical loading in bone (20). In a previous study by our group, differentially expressed miRNAs [40 miRNAs, 10 of which were confirmed via reverse transcription-quantitative (RT-q)PCR] were identified in MLO-Y4 osteocytes mechanically stimulated *in vitro* (21). As osteocytes are dominant and mechanosensitive, it was speculated that the same miRNAs may be differentially expressed in mechanically stimulated osteocytes *in vitro* and in the bone tissue of mice subjected to exercise on a treadmill. These miRNAs are likely to be involved in the response to mechanical strain and bone metabolism.

In the present study, to explore the effect of treadmill exercise on the expression of miRNAs in the bone tissues of mice, the differentially expressed miRNAs in the bone tissues of mice trained on a treadmill were screened using a miRNA microarray and verified by RT-qPCR, and the target genes of these differentially expressed miRNAs were predicted. In addition, the differentially expressed miRNAs in mechanically stimulated osteocytes were compared with the differentially expressed miRNAs in the bone tissues of the mice. Specific differentially expressed miRNAs that exhibited the same expression trends were selected and their target genes were predicted.

Materials and methods

Treadmill running exercise. A total of 22 male BALB/c mice (age, 8 weeks old; weight, 25-30 g; purchased from Hunan Anshengmei Pharmaceutical Research Institute Co., Ltd.) were randomly divided into two groups. The treadmill training group (treadmill speed, 13 m/min; slope, 9°; training for 40 min per day at the same time each day, 6 days/week) and the control group (no treadmill training). All mice had free access to food and water under a relative humidity of 40-70%, temperature of 22-25°C and 12-h light/dark cycle. The training lasted for 8 weeks. All of the experimental protocols were performed according to the Guidelines for Animal Research of Guilin Medical University (Guide for the Care and Use of Laboratory Animals) and were approved by the Animal Ethics Committee of Guilin Medical University (approval no. 2019-0013; Guilin, China).

Bone tissue sampling. After 8 weeks of treadmill running exercise, the mice were all euthanized in transparent plastic boxes with CO_2 at a flow rate of 20% chamber air exchange/min (1.2 l/min) in December 2019. Death was confirmed by exposing the thorax to observe the lack of heartbeat, as well as observing pupil dilation and unresponsiveness to light. The femurs of the mice were collected and the soft tissue of the bone surface was removed. There were 11 mice in the treadmill training group and 11 rats in the control group, six of them were tested for bone mechanical properties. After testing the mechanical properties of the bone tissues of six mice, five of them were subsequently tested for alkaline phosphatase (ALP) activity and osteocalcin (OCN). The last five of the mice were used for

the miRNA microarray and RT-qPCR assays. The femur bone was used for each of the assays.

Application of mechanical stimulation to osteocytes. MLO-Y4 osteocytes (purchased from Guangzhou Jennio Biotech Co., Ltd.) were seeded in α-MEM (Invitrogen; Thermo Fisher Scientific, Inc.) containing 10% FBS (Invitrogen; Thermo Fisher Scientific, Inc.) and 1% penicillin (Invitrogen; Thermo Fisher Scientific, Inc.) in the loading culture chamber of a four-point bending loading device (Institute of Medical Equipment, Academy of Military Medical Sciences, Tianjin, China). The osteocytes were seeded at a density of $2.5x10^4$ cells/cm² in mechanical loading plates and cultivated until they reached confluence. Cyclic mechanical tensile strain (2,500 $\mu\varepsilon$, 0.5 Hz, 8 h) was applied to the cells, the cells were cultured in serum-free medium (Invitrogen; Thermo Fisher Scientific, Inc.) during the application of mechanical load. Cells not subjected to mechanical stimulation were used as the control group. These procedures were performed according to a previously outlined method (21).

Mechanical properties of bone tissue. Using a material testing machine (RGM6010; Anhui Regal Electronic Technology Co., Ltd.), the mechanical properties of the mouse femur bones were determined in a classical three-point bending experiment with the following parameters: Preload, 0.5 N; loading rate, 2 mm/min; and span, 10 mm (sufficient to break the bone tissue). The fracture (breaking) load, maximum elastic load, maximum bending stress and bending modulus of the mouse femurs were determined.

ALP and OCN measurements. The bone tissues (femurs) of the mice were weighed and fully ground with 1.5 ml PBS in a tissue grinder, and subsequently, the grinding solution was transferred to a 2-ml Eppendorf tube and centrifuged at 4°C and $2x10^3$ x g for 5 min. The supernatant was then discarded, and subsequently, 0.2 ml RIPA lysis buffer (Invitrogen; Thermo Fisher Scientific, Inc.) was added to the precipitate at the proportions of 1:10 (g:ml), and then the lysate was sonicated in an ultrasonic cell grinder (200 W, 30 cycles/6 sec). After centrifugation ($6x10^3$ x g, 20 min, 4°C), the protein content of the supernatant was determined by a BCA protein assay kit (Beyotime Institute of Biotechnology). The ALP activity of the supernatant was detected by using an ALP kit (cat. no. A059-2-1; Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's protocol. In addition, the OCN content of the bone tissue protein solution was detected using a mouse OCN ELISA kit (cat. no. H152; Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's protocol.

miRNA microarray and RT-qPCR. After the bone tissues of the mice were completely shredded and ground in liquid nitrogen, the total RNA was purified by using TRIzol® RNA extraction reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

Part of the total RNA was purified with the mirVana miRNA Isolation kit (Ambion; Thermo Fisher Scientific, Inc.) and labeled with Cyanine 3 using the ULS™ mirVana miRNA ArrayLabeling kit (Ambion; Thermo Fisher Scientific, Inc.). Target labeling, hybridization, imaging and data processing
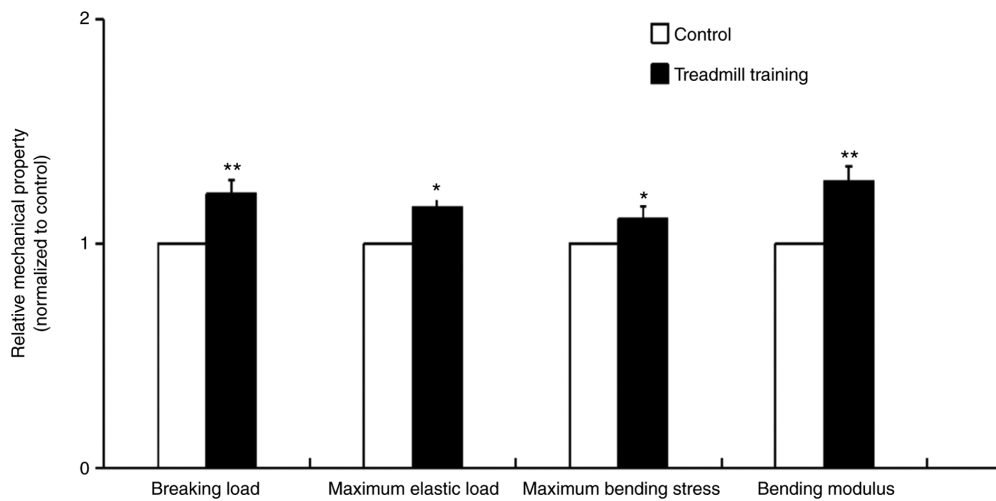
Figure 1. Mechanical properties of the femur bones in mice that were trained on a treadmill for 8 weeks. Training parameters: Speed, 13 m/min; slope, 9°; training for 40 min/day, 6 days/week. Treadmill training improved the mechanical properties of the femur bone. n=6. *P<0.05, **P<0.01 vs. Control.
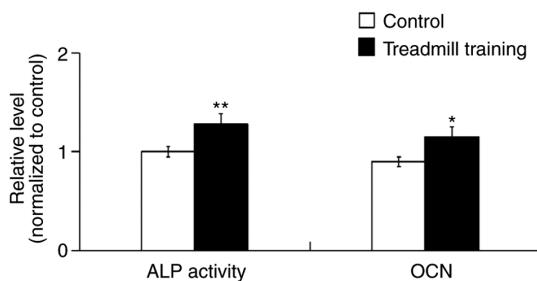

Figure 2. ALP activity and OCN content in the mouse femurs after treadmill training for 8 weeks. Treadmill training increased the ALP activity and OCN content in the femur tissues. n=5. *P<0.05, **P<0.01 vs. Control. ALP, alkaline phosphatase; OCN, osteocalcin.

were performed using a RiboArray miDETECT mouse array (Guangzhou RiboBio Co., Ltd.) that included all mouse miRNAs according to the manufacturer's protocol. miRNAs were considered to be differentially expressed if expression was >2-fold higher or lower than that in the control group (P<0.05).

Using the total RNA as a template, the miDETECT A Track™ miRNA qRT-PCR Start kit (Guangzhou RiboBio Co., Ltd.) was used to perform poly(A) tailing (to tail RNA) and Uni-RT (RT based on Uni-RT primers), followed by qPCR, according to the manufacturer's protocols of the 7900HT Fast Real Time PCR machine (Applied Biosystems; Thermo Fisher Scientific, Inc.). The miRNA-specific primers miDETECT A Track™ miRNA Forward Primers and qPCR primers for the miRNA mature chain were synthesized by Guangzhou RiboBio Co., Ltd. Following cDNA synthesis using Megaplex™ RNA RT mix (cat. no. 4444766; Applied Biosystems; Thermo Fisher Scientific, Inc.), qPCR was performed using Power SYBR-Green PCR Master Mix (cat. no. 4367659; Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions used were as follows: 10 min at 95°C, followed by 45 cycles of 15 sec at 95°C and 1 min at 60°C. miRNA microarray and RT-qPCR experiments were performed at Guangzhou RiboBio Co., Ltd.

The miRNA microarray and RT-qPCR analysis of MLO-Y4 mouse osteocytes that were stimulated with a mechanical tensile strain of 2500 $\mu\varepsilon$ at 0.5 Hz were performed as described in Zeng *et al* (21), this mechanical tensile strain has been demonstrated to promote the osteoblastic differentiation of osteoblasts *in vitro*.

miRNA target gene prediction. TargetScan (www.targetscan.org/), MicroRNA.org (www.microrna.org/) and miRDB (http://www.mirdb.org/2/) were used to predict the target genes of the miRNAs, and target genes related to osteogenic differentiation or bone metabolism were identified.

Statistical analysis. Values are expressed as the mean ± standard deviation from three separate experiments (n=5 or 6 mice/group). Data were tested for normality of distribution using the Shapiro-Wilk test and differences between groups were analyzed using one-way ANOVA. Statistical analysis was performed using SPSS software (version 18; SPSS, Inc.) and P<0.05 was considered to indicate a statistically significant difference.

Results

Evaluation of mechanical properties. After 8 weeks of treadmill training, the mechanical properties of the femur bone tissues of the mice were significantly improved. These improved properties included the breaking load, maximum elastic load, maximum bending stress and bending modulus, and the breaking load and bending modulus were particularly improved (Fig. 1). This result indicated that treadmill training was able to improve the mechanical strength and elasticity of bone tissue.

ALP activity and OCN expression. After 8 weeks of treadmill training, the ALP activity and OCN content of the femur bone tissues were significantly increased compared with in the control group (Fig. 2), which indicated that treadmill training was able to promote the osteogenic activity of cells in tissue.

Differential expression of miRNAs. After 8 weeks of treadmill training, the expression of all of the miRNAs in the femur bones was detected via miRNA microarray. A total of
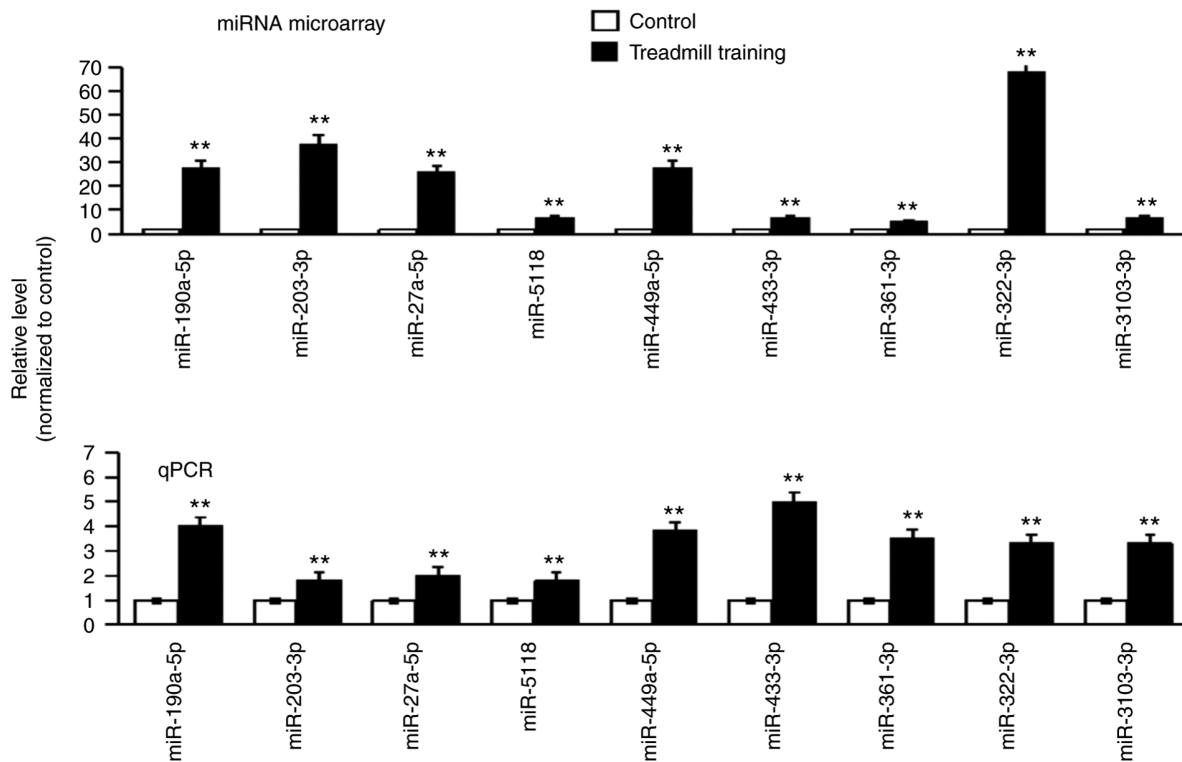
Figure 3. Microarray screening and qPCR verification of differentially expressed miRNAs in bone tissues of mice after treadmill training. The results of the chip screening are provided in the top panel and the results of qPCR are displayed below. n=5. **P<0.01 vs. Control. miR, microRNA; qPCR, quantitative PCR.

122 miRNAs were indicated to be differentially expressed. From these miRNAs, 19 miRNAs with P<0.01 were selected for further verification (data not shown). The RT-qPCR results suggested that the expression trend of each of the nine miRNAs was the same as that in the miRNA microarray; all of these miRNAs were upregulated (the expression level in the experimental group was higher compared with in the control group; Fig. 3). The nine differentially expressed miRNAs were as follows: miR-190a-5p, miR-203-5p, miR-27a-5p, miR-5118, miR-449a-5p, miR-433-3p, miR-361-3p, miR-322-3p and miR-3103-3p.

Prediction of target genes. Using bioinformatics techniques, certain osteogenic differentiation and bone metabolism-related target genes of the 9 aforementioned differentially expressed miRNAs in bone tissue were predicted and their details are presented in Table I.

Comparison of differentially expressed miRNAs. From the results of the miRNA microarray of the bone tissues and MLO-Y4 osteocytes, 19 miRNAs with the same expression trends in both bones and osteocytes were selected. These miRNAs were analyzed in bone tissues and MLO-Y4 osteocytes using RT-qPCR. The results indicated that each of the eight differentially expressed miRNAs in both the bone tissues and MLO-Y4 osteocytes had the same expression trend; four miRNAs were upregulated and four miRNAs were downregulated (Table II). The target genes of the eight differentially expressed miRNAs were predicted and Table III presents the osteogenic differentiation- and bone metabolism-related target genes, such as dickkopf homolog 2, wingless-related MMTV integration

site 2b (Wnt2b), frizzled homolog 5, transforming growth factor β receptor, cAMP responsive element binding protein 1.

Discussion

Bone is an important structure that bears mechanical loading and has a vital role in maintaining mineral homeostasis (22). Mechanical loading, particularly dynamic loading, is a major determinant in the regulation of the morphology and architecture of bone (13,23). Suitable mechanical loading prevents bone loss or promotes bone formation, and the absence of suitable mechanical loading results in a decline in bone mass (24). Exercise produces mechanical loading, which reduces bone loss, increases bone strength and prevents osteoporosis in aging individuals (25,26).

The effects of exercise on the structure and metabolism of bone tissue, such as cytokines and signaling pathways, have been studied at the cellular and molecular levels. However, only a limited number of studies have investigated the involvement of miRNAs induced by exercise in regulating bone metabolism and osteoblastic differentiation, such as mechanically-induced overexpression of miR-214 not only inhibited the expression of these osteogenic factors, but also attenuated mechanical strain-enhanced osteogenesis in osteoblasts (14).

Running is a normal form of exercise; it applies a dynamic mechanical loading to the bone tissues, particularly the femur and the tibia tissues, which is beneficial for bone health (27). In the present study, mice were forced to run on a treadmill. After 8 weeks of running exercise, it was determined that running training increased the mechanical properties of the femur, and increased the activity of

Table I. Predictive target genes of differentially expressed miRNAs in bone tissue.

miRNA	Target gene	Gene description	(Refs.)
miR-190a-5p	Smad2	SMAD family member 2	(39,47)
	Cacnb2	Calcium channel voltage-dependent β 2 subunit	(48,49)
miR-203-5p	Mmp13	Matrix metallopeptidase 13	(50)
	Wnt7b	Wingless-related MMTV integration site 7B	(51)
	Col3a1	Collagen type III α 1	(52,53)
miR-27a-5p	Ngfr	Nerve growth factor receptor	(54)
	Stk38	Serine/threonine kinase 38	(55)
	Ube2d2a	Ubiquitin-conjugating enzyme E2D 2A	(56)
miR-5118	Ngfr	Nerve growth factor receptor	(54)
	Sh3kbp1	SH3-domain kinase binding protein 1	(57)
	Ube2d2a	Ubiquitin-conjugating enzyme E2D 2A	(56)
miR-449a-5p	Arhgap26	Rho GTPase activating protein 26	(58)
	Atp2b4	ATPase Ca^{++} transporting plasma membrane 4	(36)
miR-361-3p	Rptor	Regulatory associated protein of MTOR complex 1	(59)
	Map3k9	Mitogen-activated protein kinase kinase kinase 9	(33,34)
miR-322-3p	Mettl9	Methyltransferase like 9	(60)
	Ptpre	Protein tyrosine phosphatase receptor type E	(37)
miR-3103-3p	Nfrkb	Nuclear factor related to κB binding protein	(35)
	Arhgap 30	Rho GTPase-activating protein 30	(58)
miR-433-3p	Creb1	cAMP responsive element binding protein 1	(61)
	Map2	Microtubule-associated protein 2	(62)

Target genes related to osteogenic differentiation and bone metabolism are listed. miR, microRNA.

Table II. Differentially expressed miRNAs in both bone tissue and in osteocytes with the same expression level trend, as indicated by miRNA microarray and quantitative PCR.

A, Upregulated

miRNA	miRbase accession no.
miR-5118	MIMAT0020626
miR-433-3p	MIMAT0001420
miR-190a-5p	MIMAT0000220
miR-470-5p	MIMAT0002111

B, Downregulated

miRNA	miRbase accession no.
miR-3082-5P	MIMAT0014872
miR-6348	MIMAT0025091
miR-669-5P	MIMAT0017346
miR-32-3p	MIMAT0017050

The osteocytes were stimulated with mechanical tensile strain (2,500 $\mu\varepsilon$, 0.5 Hz, 8 h). miRNA/miR, microRNA.

ALP and level of OCN in the bone tissues. ALP activity and OCN levels are important markers of osteogenic differentiation (28,29), and these markers are related to the deposition and mineralization of bone matrix (30). The present results indicated that treadmill training promoted the maturation and differentiation of bone tissue and was beneficial for bone health, which was consistent with the results of dynamic load countering ovariectomy-induced osteoporosis in rats (31) and mechanical stretch increasing osteogenesis-related protein expression in the bones of ovariectomized rats (32).

Subsequently, nine of the numerous differentially expressed miRNAs identified in the screening by miRNA microarray were selected for verification via RT-qPCR analysis, and the target genes of the nine miRNAs related to osteogenic differentiation were predicted. As an example, one target gene of miR-316-3p, Map3k9, is a MAPK signaling molecule (33). MAPKs function to regulate the key transcriptional mediators of osteoblast differentiation, with ERK and p38 MAPKs phosphorylating runt-related transcription factor 2, the master regulator of osteoblast differentiation. ERK also activates ribosomal S6 kinase 2, which in turn phosphorylates activating transcription factor 4, a transcriptional regulator of late-stage osteoblast synthetic functions (34). One target gene of miR-3103-3p, nuclear factor related to kB binding protein, is related to the NF-κB signaling pathway (35). The transcription factor NF-κB is a member of a family of proteins involved in signaling pathways that are essential for normal cellular functions and development (35). Deletion of various components of this pathway results in abnormal skeletal development, research in the last decade has indicated that NF-κB signaling

Table III. Predicted target genes of differentially expressed miRNAs both in bone tissue and osteocytes.

miRNA	Target gene	Gene description	(Refs.)
miR-5118	DKK2	Dickkopf homolog 2	(63)
	Wnt2b	Wingless related MMTV integration site 2b	(51)
	Fzd5	Frizzled homolog 5	(64)
	Tgfbr1	Transforming growth factor β receptor	(40,41)
miR-433-3p	Creb1	cAMP responsive element binding protein 1	(61)
	Map2	Microtubule-associated protein 2	(62)
miR-190a-5p	Smad2	SMAD family member 2	(39,47)
miR-470-5p	DKK2	Dickkopf homolog 2	(63)
	ATP2b1	Plasma membrane calcium ATPase	(59)
	Runx2	Runt related transcription factor 2	(65)
miR-3082-5p	Gria4	Glutamate receptor ionotropic AMPA4	(66,67)
	Creb1	cAMP responsive element binding protein 1	(61)
miR-6348	Map3k12	Activated protein kinase kinase kinase 12	(33,34)
	Dnm3	Dynamin 3	(68)
miR-669-5p	Creb1	cAMP responsive element binding protein 1	(61)
	Cask	Calcium/calmodulin-dependent serine protein kinase	(69)
miR-32-3p	Tgfbr1	Transforming growth factor β receptor	(40,41)
	ATP13a3	ATPase type 13A3	(70,71)
	Dnm3	Dynamin 3	(68)

Target genes related to osteogenic differentiation and bone metabolism are listed. miRNA/miR, microRNA.

mediates RANK ligand-induced osteoclastogenesis (35). Plasma membrane Ca^{2+}-transporting ATPase 4, a target gene of miR-449a-5p, has been proven to be overexpressed during the maturation or senescence of bone tissue cells (36). In addition, protein tyrosine phosphatase receptor type E, a target gene of miR-322-3p, belongs to the protein tyrosine kinase family and overexpression of this kinase may promote osteogenic differentiation (37). These target genes and their signaling pathways have been confirmed to be related to osteogenic differentiation or bone metabolism.

In addition, the differentially expressed miRNAs in the bones of the exercised mice were compared with the differentially expressed miRNAs in mechanically strained osteocytes *in vitro*. A total of eight differentially expressed miRNAs in both bone tissues and osteocytes were identified, and each of these miRNAs had the same expression trend in bone tissues and osteocytes. Of these, four miRNAs (miR-5118, miR-433-3p, miR-190a-5p and miR-470-5p) were upregulated and four miRNAs (miR-3082-5p, miR-6438, miR-669-5p and miR-32-3p) were downregulated, and these results were confirmed via miRNA microarray and RT-qPCR. Furthermore, the target genes of the eight differentially expressed miRNAs were predicted and certain target genes were confirmed to be related to osteogenic differentiation or bone metabolism. For instance, Wnt2b, one of the target genes of miR-5118, is a molecule of the Wnt/β-catenin signaling pathway and activation of Wnt/β-catenin signaling may promote osteogenic differentiation and increase mechanically induced bone formation (38). Smad2, a target gene of miR-190a-5p, is a Smad signaling molecule and

mechanical stress rapidly inhibits Smad3 phosphorylation, thereby inhibiting the activity of the key transforming growth factor-β (TGF-β) effectors Smad2 and Smad3 in osteocytes, and maintaining bone homeostasis via TGF-β signaling (39). In the present study, downregulation of miR-669m-5p expression was associated with one of its target genes, TGF-β receptor 1, and the reduction in TGF-β signaling, through its effector SMAD3, enhanced the mechanical properties and mineral concentration of the bone matrix as well as the bone mass (40,41).

The present results indicated that exercise-induced differentially expressed miRNAs in the bone most likely regulate bone metabolism and osteoblastic differentiation, and the same miRNAs that were differentially expressed in both mechanically stimulated osteocytes *in vitro* and in the bone tissues of mice subjected to exercise on a treadmill, also probably regulate bone metabolism and osteoblastic differentiation.

As terminally differentiated cells, osteocytes that are mechanically stimulated produce factors such as proteins, peptides and signaling molecules that regulate osteogenic differentiation or bone metabolism (42,43). Mechanical stimuli, such as fluid shear stress, increase the number of exosomes released by osteocytes, these exosomes contain factors such as sclerotinins, NF-κB receptor activators and osteoprotegerin, which regulate osteogenic differentiation (44,45). Certain osteocyte-derived miRNAs, such as miR-218, may influence osteoblastic differentiation (46). Therefore, in the present study, it was hypothesized that these differentially expressed miRNAs

in mechanically stimulated osteocytes *in vitro* and in bone tissue of treadmill trained mice likely regulate osteogenic differentiation or bone metabolism. It is hypothesized that this regulatory mechanism may be via exosomes, thus in the future, how these miRNAs regulate osteogenic differentiation or bone metabolism through exosomes will be studied.

In conclusion, treadmill training resulted in the differential expression of miRNAs in the bone tissues of mice. Certain differentially expressed miRNAs were also observed in osteocytes and these miRNAs probably have an important role in the regulation of bone metabolism and osteogenic differentiation.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The datasets generated and/or analyzed during the current study are available in the Gene Expression Omnibus repository, [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE179199 and https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE179201].

Authors' contributions

YG and JG designed the study, analyzed the data and participated in the bioinformatics analysis. HY and ZC performed all the RT-qPCR analyses at Guangzhou RiboBio Co., Ltd., (experimental equipment and reagents were provided by Guangzhou RiboBio Co., Ltd.)., and assays for ALP activity and OCN levels, and participated in the animal experiments. YW and JW performed the miRNA microarray at Guangzhou RiboBio Co., Ltd., (experimental equipment and reagents were provided by Guangzhou RiboBio Co., Ltd.), and performed the animal experiments. BH, FY and YQ performed the bioinformatics analysis and detected the mechanical properties of the mouse femur bones. YG, HY and ZC wrote and revised the manuscript. All authors have read and approved the final manuscript. YG, JG, HY, ZC, YW, JW, BH, FY and YQ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study involved animals and was approved by the Animal Ethics Committee of Guilin Medical University (Guilin, China; approval no. 2019-0013). All of the authors declare that the experiments complied with the current laws of China (Guangxi) where they were performed.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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