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Review

MicroRNAs: a critical regulator under mechanical force

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Summary Mechanical force is a kind of mechanical stimuli which actively participates in manipulating cellular activities in numerous types of cells. Progress in molecular and genetic research has uncovered various regulatory mechanisms underlying mechanical force-induced changes in cellular activities, which include both transcriptional regulation and post-transcriptional regulation. MicroRNAs (miRNAs) are 20-25 nucleotide (nt) non-coding RNAs which serve as post-transcriptional regulators of multiple physiological processes. To date, considerable research effort has focused on the expressions and functions of miRNAs in a wide range of biological and pathological processes, including but not limited to development, proliferation, metabolism and osteogenic differentiation. In this review, major emphasis is placed on the biogenesis, expressions and functions of miRNAs in a mechanical environment.

Key words: MicroRNAs, Mechanical Force, Post-transcriptional regulator, Osteogenic differentiation

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Introduction

In many cellular physiological activities, cells are in an extracellular mechanical environment which actively participates in the growth, differentiation and apoptosis of these cells. For example, blood vessels are routinely exposed to mechanical forces in the form of shear stress due to the effects of blood flow and blood pressure. During orthodontic treatment, cells respond to the applied mechanical stimuli and therefore result in remodeling changes in the periodontal ligament (PDL) and alveolar bone, which eventually facilitate tooth movement (Kawarizadeh et al., 2005). In addition, mechanical stress can stimulate bone formation and inhibit bone resorption, which in turn contribute to the development and regeneration of bone. When mechanical force is applied, biological mediators are expressed and secreted, leading to the activation of the stress sensing signal system. As a result of the following adjustment of cytoskeleton structure and the remodeling of the extracellular matrix, cells are able to adapt to the stress environment and avoid damage from the mechanical stimuli (Ho et al., 2007). There is no doubt that a thorough understanding of the mechanisms underlying mechanical force-induced effects on cellular activities may provide novel targets to develop new therapies. However, although it has been reported that mechanical stimuli-induced changes in cellular activities are closely coupled by several key signaling pathways (Miyazaki et al., 2006; Del Fattore et al., 2008), the underlying molecular mechanisms are complicated and remain largely unknown. To simplify the complicated *in vivo* mechanical environment, numerous *in vitro* loading

models have been developed to simulate the stress distribution *in vivo* as much as possible. In these *in vitro* models, the mechanical stimuli can be shear stress, centrifugation, compression/tension and mechanical vibration. Moreover, the magnitude and frequency of the mechanical force, as well as the exposure time of the cells to the mechanical stimuli, can be accurately controlled in these *in vitro* models.

In recent years, molecular and genetic research has unveiled various regulatory processes involved in the cellular function changes when the cells are subjected to mechanical stimuli, and most of these studies have been focused on the transcriptional level and the coding RNAs. However, non-coding RNAs account for 98% of all genomic output in humans (Mattick, 2001), which also actively participate in the regulation of cellular activities. As 20-25 nucleotide (nt) non-coding RNAs, microRNAs (miRNAs) regulate gene expression post-transcriptionally by binding to the 3'-UTR of messenger RNAs (mRNAs) (Erson and Petty, 2008). MiRNAs have been identified as major regulators in many aspects of cellular reactions and diverse biological processes including proliferation, differentiation, cell cycle and cell apoptosis (Hwang and Mendell, 2006; Bueno et al., 2008; Ameres and Zamore, 2013; Shi et al., 2014). To date, the function of miRNAs in bone remodeling and osteoblast differentiation has been extensively studied, and these molecules have been considered as potential therapeutic interventions. After exposure to a mechanical environment, miRNA expression profiles can be changed in many cell types by the mechanical stimuli, which in turn result in biological changes in osteogenic differentiation, inflammatory responses, cell proliferation, cell cycle, and cell apoptosis.

In this review, the biogenesis, structures and functions of miRNAs are briefly introduced, with the major emphasis being placed on the expression profiles and functions of miRNAs in a mechanical environment. The potential mechanisms underlying mechanical force-induced changes in miRNAs are also discussed. The functions of mechanical force-related miRNAs are listed in Table 1.

Biogenesis and functions of miRNAs

MiRNAs modulate mRNA stability and regulate protein levels post-transcriptionally. By promoting degradation of target mRNAs or inhibiting their translation, miRNAs decrease the protein levels of target genes (Erson and Petty, 2008). The biogenesis of miRNAs occurs through a series of steps. Firstly, the long primary miRNA (pri-miRNA) containing imperfectly base-paired stem loops is processed by RNA polymerase II to generate the precursor miRNA (pre-miRNA) (Lee et al., 2003, 2004; Zeng et al., 2005). The pre-miRNA is then cleaved by the ribonuclease III enzyme, Drosha, to form an approximately 70 nucleotide, stem-loop structure. After the cleavage, the pre-miRNA is actively translocated from the nucleus to the cytoplasm by exportin-5, a Ran-GTP-dependent nuclear export factor. There in the cytoplasm, the pre-miRNA is processed into a double-stranded structure by RNAse III endonuclease, Dicer and TRBP/Loquacious (Hutvagner et al., 2001; Yi et al., 2003; Lund et al., 2004). One strand of the double stranded structure is then selected as the mature miRNA, whereas the other one is degraded. Following Dicer cleavage, an Argonaute (Ago) protein incorporates into the short RNA duplexes, forming a ribonucleoprotein complex called the RNA-induced silencing complex (mRISC) (Bosse and Simard, 2010; Fabian and Sonenberg, 2012). The mature miRNA induces translational repression by guiding the RISC complex to bind to its target site in the 3'-untranslated region (3'-UTR) of the mRNA or degrades the mRNA depending on the extent of the complementarity of the mature miRNA with the target mRNA. Up to now, 2588 miRNAs have been identified in human cells and each is predicted to regulate several target genes (Kozomara and Griffiths-Jones, 2014). In addition, computational predictions indicate that miRNAs potentially regulate more than 50% of all human protein-coding genes (Lewis et al., 2005; Friedman et al., 2009). The abundance of mature miRNAs provides a high degree of regulation flexibility, which varies extensively from as few as ten to more than 80,000 copies in a single cell (Chen et al., 2009).

With respect to function, miRNAs have been demonstrated to play important roles in the regulation of many biological responses, including development, proliferation, differentiation, metabolism and apoptosis. There are studies suggesting that aberrant miRNA expressions are involved in the pathogenesis of a large number of human diseases including pulmonary hypertension, diabetes, heart failure, neurological disorders, and autoimmune diseases (Van Rooij et al., 2006; Stark et al., 2008; Caruso et al., 2010). In addition, miRNAs are essential for the development of murine teeth by regulating epithelial stem cell differentiation and mesenchymal odontogenic differentiation during early tooth development (Chen et al., 2009; Cao et al., 2010; Michon et al., 2010). Experiment results showed that conditional knockout of Dicer1 (mature miRNA) in Pitx2-Cre mice and K14 transgenic mice resulted in significant aberrations in tooth shape and enamel formation (Cao et al., 2010; Michon et al., 2010). By targeting major regulators of pathways involved in osteoblastogenesis and osteoclastogenesis, miRNAs play important roles at both early and late stages of the differentiation of both osteoblast and osteoclast (Kapinas et al., 2011; Lian et al., 2012). Other studies indicated that miR-146a promoted the differentiation of PDL cells through down-regulation of NF-kappaB signaling (Hung et al., 2010). Moreover, it has been demonstrated in the latest study that miR-218 occupied a critical position in osteogenic differentiation of periodontal ligament stem cells (PDLSCs) (Gay et al., 2014).

Functions of miRNAs under mechanical force

MiRNAs regulate osteogenic differentiation under mechanical force

Bone remodeling is a continuous process requiring physiological coupling of osteoclast with osteoblast activities. MiRNAs could be mechanosensitive and emerge as critical post-transcriptional regulators in osteogenic differentiation and bone remodeling processes. Through manipulating different signaling pathways, different miRNAs may play positive or

Table 1. The functions of related-miRNAs under mechanical force.

MicroRNA	Target	Expression	Type of mechanical force	Supporting observations	Cell type	Reference
miR-19a	cyclin D1	Increased	Laminar shear stress	Regulated endothelial proliferation and cell cycle arrest	HUVECs	Qin et al., 2010
miR-23b	Rb	Increased	Laminar shear stress	Cell cycle arrest	ECs	Wang et al., 2010
miR-155	RhoA, MYLK	Increased	Shear stress	Modulate phenotype and Cytoskeleton organization	ECs	Weber et al., 2014
miR-145	ERK1/2, ACE	Decreased	Mechanical stretch	Modulate the phenotype	VSMC	Hu et al., 2014
miR-21	PPAR-α	Increased	Oscillatory shear stress	Inflammatory response and modulated apoptosis	ECs	Weber et al., 2010; Zhou et al., 2011
	P27, PDCD4	Increased	Cyclic stretch	Regulated proliferation and apoptosis	HASMC	Song et al., 2012
	ACVR2B	Increased	Mechanical stretch	Induced osteogenic differentiation	PDLSCs	Wei et al., 2015
	PDCD4	Increased	Mechanical stretch	Regulated osteogenic differentiation	PDLSCs	Chen et al., 2016
miR-663	KLF-2	Increased	Oscillatory shear stress	Inflammatory response	HUVECs	Ni et al., 2011
miR-92a	KLF-2	Increased	Oscillatory shear stress	Inflammatory response	ECs	Wu et al., 2011
miR-195-5p	--	Down-regulated	tension force	osteogenesis and bone formation	PDLCs	Chang et al., 2015
miR-424-5p	--					
miR-1297	--					
miR-3607-5p	--					
miR-145-5p	--					
miR-4328	--					
miR-224-5p	--					
miR-34a	Foxj2	Up-regulated	Shear stress	Promote endothelial differentiation	Endothelial progenitor cells	Cheng et al., 2014
miR-146a	IRAK1, TRAF6	Increased	Oscillatory pressure	Induce inflammation	HSAEpCs	Huang et al., 2012; Comer et al., 2014
miR-26a	GSK-3	Increased	Cyclic strain	Induce Hypertrophy	VSMCs	Mohamed et al., 2010
miR-24	FURIN	Increased	Cyclic stress	Induction of TGFβ1	HTMCs	Luna et al., 2011
miR-29b	Collagen I, III,V	Up-regulated	Cyclic stretch	Modulated ECM homeostasis	hPDLCs	Chen et al., 2015
		Down-regulated	Compression forces			
miR-494-3p	FGFR2, ROCK1	Increased	Compressive force	Inhibit cell proliferation	MC3T3	Iwawaki et al., 2015
miR-146a-5p	-	Increased	Compressive force	Inhibit cell proliferation	MC3T3	Iwawaki et al., 2015
miR-210-3p	-	Increased	Compressive force	Inhibit cell proliferation	MC3T3	Chan et al., 2012; Iwawaki et al., 2015
miR-144/451	AMPK	Down-regulated	Mechanical stretch	Promoted contractile differentiation	VSMCs	Turczyńska et al., 2013
miR-153/223	IGF-1 receptor	Down-regulated	Mechanical stretch	Promoted proliferation	Venous smooth muscle cells	Song et al., 2012a,b
miR-1246	-	Increased	Mechanical stretch	A critical role in periodontal tissue homeostasis	PDLSCs	Stoecklin-Wasmer et al., 2012
miR-132	mTOR signaling pathway	Increased	Fluid shear stress	Regulated differentiation	PDLSCs	Qi and Zhang, 2014
miR-365	histone deacetylase 4	Increased	Mechanical loading	Chondrocyte proliferation and differentiation	Chondrocytes	Guan et al., 2011

ECs, endothelial cells; Rb, Retinoblastoma; MYLK, myosin light chain kinase; ERK1/2, extracellular signal-regulated kinase 1/2; ACE: angiotensin-converting enzyme; VSMC, Vascular Smooth Muscle Cell; PPAR-α, peroxisome proliferator-activated receptor-α; PTEN, Phosphatase and tensin homolog; HASMC, human aortic smooth muscle cell; HUVECs, human umbilical vein endothelial cells; ACVR2B, activin receptor type 2B; PDLCs, periodontal ligament cells; PDLSCs, periodontal ligament stem cells; PDCD4, programmed cell death protein 4; KLF-2, Kruppel-Like factor 2; Foxj2, Forkhead box j2; IRAK1, interleukin-1 receptor-associated kinase; TRAF6, tumor necrosis factor receptor-associated factor 6; HSAEpCs, human small airway epithelial cells; GSK-3, glycogen synthase kinase-3; HTMCs, Human Trabecular Meshwork Cells; ECM, Extracellular matrix; FGFR2, fibroblast growth factor receptor 2; ROCK1, Rho-associated coiled-coil kinase 1; AMPK, AMP-activated protein kinase; IGF-1, insulin-like growth factor-1.

negative roles in osteogenic differentiation.

In one of our previous studies, the genome-wide differential expressions of miRNAs were explored in normal and stretched PDLSCs. In 53 miRNAs investigated, 26 miRNAs were up-regulated and 27 were down-regulated in stretched PDLSCs when compared with those in normal PDLSCs (Wei et al., 2014). In another study, we investigated the function of miR-21 in the osteogenic differentiation of PDLSCs exposed to mechanical stretch. We found that miR-21 increased stretch-induced osteogenic differentiation of PDLSCs by targeting ACVR2B, which confirmed that mechanical force-induced osteogenic differentiation of PDLSCs is mediated by miR-21 (Wei et al., 2015). In addition, animal studies established that miR-21 responded to the orthodontic force in periodontal tissue in a dose- and time-dependent manner, and regulated the osteogenesis of PDLSCs following orthodontic tooth movement. Taken together, our results and others clearly indicate that miRNA 21 modulates orthodontic tooth movement and alveolar bone remodeling under both normal and mechanical environments *in vivo* (Chen et al., 2016).

MiR-132 was also reported to regulate the differentiation of periodontal ligament cells through activating the mTOR signaling pathway after fluid shear stress treatment (Qi and Zhang, 2014). As we know, periodontal ligament cells are pivotal for the regeneration of alveolar bone and dentin surfaces during the healing of periodontal wounds (Shimono et al., 2003). Therefore, maintaining the balance between miR-132 and the mTOR signaling pathway in periodontal ligament cells may serve as a novel target to develop new therapies to promote the healing of periodontitis and alveolar bone defects.

In another study, a number of miRNAs were identified which showed different expression patterns in cells stimulated with tension force for 72 hours when compared with those in the untreated cells. Among 3100 screened miRNAs, 17 miRNAs were upregulated and 15 miRNAs were downregulated in PDLCs after 72 h of mechanical loading (Chang et al., 2015). Among those down-regulated miRNAs, miR-195-5p, miR-424-5p, miR-1297, miR-3607-5p, miR-145-5p, miR-4328, and miR-224-5p were identified as core miRNAs and were shown to be down-regulated in tension force-induced osteogenesis and bone formation. Although the direct targets of these miRNAs still need to be investigated, this study further confirmed the role of miRNAs in mechanical force-induced osteogenic differentiation. These above-mentioned studies, which were all aimed at investigating the molecular mechanisms underlying mechanical environment-induced changes in osteogenic differentiation, bring deeper insights into the understanding of mechanical force-induced bone formation, and significantly contribute to the development of some breakthrough ideas which may lead to the invention of novel therapeutic approaches in promoting bone regeneration.

MiRNAs regulate proliferation under mechanical force

In response to mechanical force, several miRNAs have been shown to regulate proliferation. MiR-19a plays an important role in the flow regulation of cyclin D1 expression and endothelial proliferation in endothelial cells (ECs) under laminar shear stress (Qin et al., 2010). In MC3T3-E1 murine pre-osteoblastic cells subjected to compressive force, expression of miR-494-3p was increased, which specifically down-regulated protein levels of fibroblast growth factor receptor 2 (FGFR2) and Rho-associated coiled-coil kinase 1 (ROCK1), and therefore inhibited proliferation of these cells (Iwawaki et al., 2015). Similarly, compressive force up-regulated expressions of miR146a-5p and miR-210-3p in the MC3T3-E1 cells, which may also inhibit proliferation in osteoblasts (Chan et al., 2012; Iwawaki et al., 2015). These studies help us to better understand the molecular mechanisms underlying bone responses to mechanical stress. In addition, miRNAs as signaling molecules may be useful targets for maintaining bone health and treating bone diseases. By activating AMP-activated protein kinase (AMPK) signaling pathway, miR-144/451 levels can be reduced when exposed to mechanical stretch, which may play a role in promoting contractile differentiation of smooth muscle cells (Turczyńska et al., 2013). Song et al have demonstrated that down-regulation of miR-223 and miR-153 by mechanical stretch stimulated proliferation of venous smooth muscle cells via activation of insulin-like growth factor-1 (IGF-1) receptor (Song et al., 2012a,b). In another study, cyclic stretch was also shown to modulate miR-21 expression, suggesting that increased miR-21 expression may be involved in regulating stretch-mediated proliferation and apoptosis of human aortic smooth muscle cell (HASMC) (Song et al., 2012a,b). P27 and programmed cell death protein 4 (PDCD4), the potential downstream target genes of miR-21, participated in regulating apoptosis in stretched cells. These studies contribute to the better understanding of molecular mechanisms in the response of cellular differentiation subjected to mechanical stress. Furthermore, miRNA as the signaling molecule may be a potential useful target for maintenance of bone health and treatment of bone disorders related to mechanical stretch.

MiRNAs regulate inflammatory responses under mechanical force

In response to stretch stimuli, several miRNAs have been shown to regulate inflammatory responses. It was reported that by targeting the 3'-UTR of peroxisome proliferator-activated receptor-α (PPAR-α), miR-21 mediated the positive feedback loop that regulated oscillatory shear stress-induced inflammatory response in endothelial cells (ECs) (Zhou et al., 2011). In addition, decreased expression of PPAR-α promoted the

expressions of pro-atherogenic genes such as vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemotactic protein-1 (MCP-1), and enhanced the transcription of miR-21. These findings suggested that miR-21 could be a potential therapeutic target to treat vascular disorders associated with hemodynamic-induced EC inflammation or dysfunction such as atherosclerosis. In fact, there were 10 oscillatory shear stress-sensitive miRNAs, including miR-663 identified in human umbilical vein endothelial cells (HUVECs) and miR-92 (Ni et al., 2011; Wu et al., 2011). MiR-663 was involved in oscillatory shear stress-induced inflammatory responses through repression of Kruppel-Like factor 2 (KLF-2). Collectively, atheroprotective flow decreased the level of miR-92a in ECs, which in turn raised KLF2 expression to maintain endothelial homeostasis (Wu et al., 2011). These findings provided a new paradigm of mechanical transduction involving the shear regulation of miRNAs and vascular homeostasis. Furthermore, the shear-sensitive miRNAs could be potential therapeutic targets for the treatment of atherosclerosis.

Additionally, miR-146a was found to be a mechanically sensitive, early-response miRNA, and the expression of miR-146a was up-regulated in human small airway epithelial cells (HSAEpCs) exposed to oscillatory pressure (Huang et al., 2012; Comer et al., 2014). Besides, overexpression of miR-146a can regulate pressure-induced cytokine secretion by targeting key proteins in the toll-like receptor (TLR) signaling pathway such as interleukin-1 receptor-associated kinase (IRAK1) and tumor necrosis factor receptor-associated factor 6 (TRAF6). Therefore, miR-146a exerted an important role in mechanically induced inflammation in lung epithelia. These data indicated that miR-146a may be a potential therapeutic agent for reducing microbial and mechanically induced inflammation during ventilation. Moreover, TLR signaling pathway might be an effective way to reduce lung inflammation and ventilator-induced lung injury (VILI).

Researchers also found that through direct targeting of FURIN, miR-24 might contribute to modulating the induction of transforming growth factor beta 1 (TGFβ1) mediated by cyclic mechanical stress in human trabecular meshwork cells (Luna et al., 2011). Consequently, upregulation of miR-24 and its subsequent down-regulation of FURIN can serve as a homeostatic mechanism to limit the amount of TGFβ1 and prevent some of the potential pathogenic pathways of this cytokine in the outflow pathway. There may be more miRNAs which can regulate mechanically induced inflammatory response. A better understanding of how mechanical forces influence miRNA expressions and how miRNAs mediate the mechanotransduction processes responsible for inflammation may lead to the findings of novel biomarkers and/or the development of innovative treatments for inflammatory diseases.

MiRNAs regulate other cellular physiological activities under mechanical force

Besides mediating mechanical force-induced osteogenic differentiation, proliferation and inflammatory responses, miRNAs also play important roles in mediating mechanical environment-related changes in many other cellular physiological activities including cell cycle, cytoskeleton organization, modulating homeostasis, etc., in numerous cell types.

MiR-19a not only plays a role in endothelial proliferation, but also plays an important role in the cell cycle arrest in ECs under laminar shear stress (Qin et al., 2010). Laminar shear stress also increased the expression of miR-23b, which may lead to EC arrest in cell cycle and suppress EC proliferation (Wang et al., 2010). Besides, miR-155 was up-regulated in ECs and modulated actin cytoskeleton organization *via* targeting RhoA and myosin light chain kinase (MYLK) (Weber et al., 2014). The RhoA and MYLK pathways play a significant role in EC actin cytoskeleton organization and cellular responses (Dudek and García, 2001; Birukov et al., 2002; Birukova et al., 2004; Nazari-Jahantigh et al., 2012). Inhibition of MYLK expression in ECs has been shown to prevent stress fiber formation, cell contraction, and intracellular gaps (Dudek and García, 2001; Birukova et al., 2004). Especially, MYLK has a role in atherosclerotic plaque formation (Sun et al., 2011). The above research revealed unique insights into the role of miRNAs in mechanotransduction in ECs and the corresponding regulatory mechanisms of cardiovascular homeostasis in health and disease. In stretched PDLSCs, miR-1246 was reported to be the most highly expressed miRNA which plays an important role in periodontal tissue homeostasis (Stoecklin-Wasmer et al., 2012). In addition, miR-29b directly targets Col1a1, Col3a1 and Col5a1, and acts as a modulator for extracellular matrix (ECM) homeostasis in PDLCs during orthodontic tooth movement (Chen et al., 2015; Luna et al., 2009). Both studies suggested the possible roles of miRNAs in PDLCs as a modulator for ECM homeostasis in the periodontal ligament during orthodontic tooth movement.

On the other hand, shear stress forces regulate the expression of miRNAs in ECs, and miR-21 contributes to the endothelial biology by reducing apoptosis and activating the NO pathway (Weber et al., 2010). This improved our understanding of the mechanisms by which shear stress regulates the vascular environmental stability. However, mechanical stretch inhibited the expression of miR-145 by activating the extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway and promoting angiotensin-converting enzyme (ACE), and further to modulate the vascular smooth muscle cell (VSMC) phenotype (Hu et al., 2014). Thus, miR-145 exposed to hypervascularized blood vessels as occurs in hypertension may represent a potential therapy to inhibit pathological vascular remodeling. In another study, miR-

34a was identified to be up-regulated in response to shear stress, which promoted endothelial differentiation of endothelial progenitor cells by targeting Forkhead box j2 (Foxj2) (Cheng et al., 2014). In addition, miR-365 was found to be a critical mediator to stimulate chondrocyte proliferation and differentiation in response to mechanical stress by targeting histone deacetylase 4 (Guan et al., 2011). miR-26a was found to act as a hypertrophic gene under cyclic strain, which directly targets glycogen synthase kinase-3 to enhance hypertrophy in VSMCs (Mohamed et al., 2010). The above research indicated miRNAs also exert an important influence on regulating other cellular physiological activities under mechanical force.

In summary, there are plenty of miRNAs involved in the regulation of different cellular physiological activities mediated by mechanical stretch in multifarious kinds of cells. These findings not only provide new insights into mechanotransduction signaling pathways, but also suggest a potential target for tissue engineering in regenerative medicine and treating human pathological disorders related to elevated mechanical stretch.

Conclusions and future directions

Due to the complex mechanical environment and massive miRNAs gene library, how to best utilize previous findings to explore miRNAs function under mechanical force is a highly complex and dynamic process. Previous integrated analysis provided some important information that may inspire further experimental investigation into the behavior of miRNAs and their targets in response to mechanical force. Our data demonstrated the functions of miRNAs in osteogenic differentiation, inflammatory response and cellular physiological activity in different cells under mechanical environment, which may provide novel insights into therapeutic approaches in clinic.

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