Emerging roles of MicroRNAs in osteonecrosis of the femoral head

Zheng Li¹ | Bo Yang¹ | Xisheng Weng¹ | Gary Tse² | Matthew T. V. Chan³ | William Ka Kei Wu^{3,4}

¹Department of Orthopedics Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

²Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong

³Department of Anaesthesia and Intensive Care, The Chinese University of Hong Kong, Hong Kong

⁴State Key Laboratory of Digestive Disease and LKS Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong

Correspondence

Bo Yang and Xisheng Weng, Department of Orthopedics Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.
Emails: ybsury@sina.com and xshweng@medmail.com.cn

Funding information

National Natural Science Foundation of China (NSFC), Grant/Award Number: 81572143 and 81630064

Abstract

Osteonecrosis of the femoral head (ONFH) is one of the most common orthopaedic diseases. The exact pathogenic mechanism of ONFH is still unknown. MicroRNAs (miRNAs) are a class of non-coding RNAs that negatively modulate gene expression at post-transcriptional level. An increasing number of studies have shown that miRNAs play crucial roles in different physiological processes, including development, cell proliferation, differentiation and metabolism. Recently, multiple studies demonstrated that miRNAs are involved in the pathogenesis of ONFH. In this review, we summarize dysregulated miRNAs and their functions in ONFH. Furthermore, we discuss their potential clinical applications for diagnosis and treatment of ONFH.

1 | INTRODUCTION

Osteonecrosis of the femoral head (ONFH) is a refractory orthopaedic disease with progressive osteocyte and bone marrow necrosis as a result of interruption of blood supply to the femoral head, causing the associated structural changes and even collapse.¹⁻⁵ ONFH can be categorized as traumatic and non-traumatic.^{6,7} While the former occurs following physical trauma, the aetiology of non-traumatic ONFH is complicated and multifactorial.^{8,9} Alcohol addiction, pre-existing abnormal circulating function and glucocorticoid treatment all increase the risk of non-traumatic ONFH.^{1,10,11} ONFH is a devastating and progressive disease, which, if left untreated, will cause the collapse of the femoral head.^{12,13} Consequently, approximately 70% of patients need hip replacement.¹⁴⁻¹⁶ While existing theories have pointed to the roles of intravascular coagulation and fat embolism as well as extravascular fat accumulation-mediated vascular constriction in the pathogenesis of ONFH,¹⁷ the exact mechanism is still largely unknown.

MicroRNAs (miRNAs) are a group of small, single-stranded, endogenous non-coding RNAs that negatively modulate expression of target mRNAs through binding to their 3'-untranslated region (3'-UTR) to regulate their translation and/or stability.¹⁸⁻²¹ Previous studies have indicated that miRNAs play crucial roles in diverse physiological processes, including development, cell proliferation, differentiation, metabolism, migration and apoptosis.²²⁻²⁵ Increasing evidence also suggested that miRNAs can regulate bone development and regeneration and are directly involved in the pathogenesis of numerous of orthopaedic conditions, such as intervertebral disc degeneration, osteoarthritis, osteoporosis and osteosarcoma.²⁶⁻²⁹ Importantly, the roles of microRNAs in many of these orthopaedic conditions have been critically assessed.³⁰⁻³² Recently, several studies suggested that miRNAs play important roles in the development of ONFH.³³⁻³⁵ Nevertheless, an in-depth appraisal of the related literature is still lacking.

In this review, we summarize dysregulated miRNAs and their pathogenic roles in ONFH. Furthermore, we discuss the potential applications of miRNAs as diagnostic markers and druggable targets in ONFH.

2 | DYSREGULATED MICRORNAS IDENTIFIED BY GENOME-WIDE APPROACHES IN ONFH

Genome-wide miRNA profiling using microarray or RNA sequencing followed by validation of individual candidates with quantitative reverse transcription-polymerase chain reaction (qRT-PCR) is the most common approach for identifying differentially expressed miRNAs in specific disease state.³⁶⁻³⁸ Dysregulated miRNAs have been identified in both osteonecrotic tissues and mesenchymal stem cells (osteogenic precursors) of ONFH patients and experimental animals (Table 1).

2.1 | Human ONFH tissues

Yuan et al³⁹ compared the miRNA expression profiles in 9 patients with the ONFH and 6 patients with the fresh femoral neck fracture by microarray. A total of 12 upregulated miRNAs (miR-181c-3p, miR-34a-3p, miR-146a-5p, miR-187-3p, miR-181a-3p, miR-30c-1-3p, miR-650, miR-3652, miR-4444, miR-1273e, miR-99a-3p, miR-3064-5p) and 5 downregulated miRNAs (miR-132-3p, miR-212-3p, miR-212-5p, miR-6836-5p, miR-629-3p) were identified in ONFH tissues as compared with control tissues. The upregulation of miR-146a and miR-34a in the ONFH was confirmed by qRT-PCR. Following a similar approach, Wu et al⁴⁰ profiled miRNA expression in 4 cases of non-traumatic ONFH as compared with 4 cases of femoral neck fracture. A total of 22 upregulated and 17 downregulated miRNAs were identified in ONFH. Three upregulated miRNAs (miR-210-3p, miR-320e and let-7c) and three downregulated miRNAs (miR-133a-3p, miR-335-5p and miR-146b-5p) were validated by using qRT-PCR. Taken together, these two studies suggested that miRNA expression in non-traumatic ONFH and femoral neck fracture were significantly different.

2.2 | Mesenchymal stem cells

The use of glucocorticoids accounts for the majority of non-traumatic ONFH. Reduced proliferative capacity of the mesenchymal stem cells is implicated in the pathogenesis of glucocorticoid-induced ONFH. Based on the Illumina HiSeq 2000 sequencing platform, Bian et al⁴¹ profiled miRNA expression in human mesenchymal stem cells treated without or with dexamethasone (10^{-9} or 10^{-7} mol/L). The miRNA expression profile of mesenchymal stem cells was altered by the treatment, in which 16 miRNAs, including 11 upregulated (miR-16-5p, miR-103a-3p, miR-107, miR-196a/b-5p, miR-378d/f/g, miR-1268a/b, miR-4289) and 6 downregulated (miR-24-3p, miR-378a/h/l, miR-4448, miR-4634), were consistently altered by both concentrations of dexamethasone. The same group of researchers also profiled bone marrow mesenchymal stem cells from mice with steroid-induced ONFH.⁴² Affymetrix GeneChip (Affymetrix Inc., Santa Clara, CA, USA) was used to identify differentially expressed miRNAs in C57BL/6J mice subcutaneously injected with methylprednisolone (21 mg/kg) or normal saline (as control). A total of 23 miRNAs were upregulated and 16 miRNAs were downregulated in the experimental group.

Through bioinformatic analysis, the upregulation of miR-21-3p and miR-652-5p and downregulation of miR-34b-3p, miR-34c-5p, miR-148a-3p, miR-196a-5p, and miR-206-3p were predicted to be involved in osteogenic differentiation. These findings indicated that glucocorticosteroids could result in miRNA expression profile alterations in both human and murine mesenchymal stem cells.

3 | CIRCULATING MICRORNAS AS DIAGNOSTIC MARKERS FOR ONFH

Circulating miRNAs may be used as markers for ONFH diagnosis. Using deep sequencing technology, Wang et al⁴³ profiled miRNA levels in serums collected from 3 steroid-induced ONFH patients with systemic lupus erythematosus (SLE), 3 healthy controls and 3 SLE controls. A total of 27 differentially abundant miRNAs, including 15 upregulated (eg, miR-3960, miR-423-5p, miR-15b-3p, miR-1304-3p and miR-195-5p) and 12 downregulated (eg, miR-99a-5p, miR-100-5p, miR-140-5p, miR-532-5p, miR-181c-5p, miR-10b-5p, miR-433 and miR-10a-5p), were identified in ONFH serum as compared with SLE and healthy controls. In another study, Wei and Wei⁴⁴ used miRCURY™ (Qiagen, Valencia, CA, USA) locked nucleic acid (LNA) miRNA chip to delineate miRNA expression signatures in serum of patients with hormone-induced non-traumatic ONFH. As compared with serum collected from healthy volunteers, 9 upregulated and 3 downregulated miRNAs were identified in the hormone-induced non-traumatic ONFH group. By qRT-PCR, significant increase in miR-10a-5p and decrease in miR-423-5p were confirmed. These results suggested that genome-wide miRNA profiling may be used to identify potential miRNA markers for diagnosis of ONFH.

4 | MICRORNAS OF FUNCTIONAL SIGNIFICANCE IN ONFH

4.1 | miR-708

The imbalance between osteogenic and adipogenic differentiation in mesenchymal stem cells plays a crucial role in the pathogenesis of steroid-induced ONFH.⁴⁵⁻⁴⁷ Hao et al³³ found that the expression of miR-708 was significantly upregulated in glucocorticoid-treated and ONFH patients' mesenchymal stem cells. In addition, they found that Smad3 (small mothers against decapentaplegic 3) was a direct target of miR-708. Targeting miR-708 enhanced the osteogenic differentiation and inhibited adipogenesis differentiation capability of mesenchymal stem cells. Knockdown of miR-708 also rescued the suppressive effect of glucocorticoid on osteonecrosis.³³ These data suggested that glucocorticoid might result in miR-708 overexpression to mediate its ONFH-promoting effect. This miRNA may thus act as a new therapeutic target for the prevention and treatment of ONFH.

4.2 | miR-210

Damage of endothelial cells was suggested to be one of the underlying mechanisms of steroid-induced ONFH whereas angiogenesis is

TABLE 1 miRNAs expression profiles in osteonecrosis of the femoral head

Number	Method	Sample	Upregulated	Downregulated	References
1	Microarray RT-PCR	ONFH patients	12 miRNAs miR-181c-3p, miR-34a-3p, miR-146a-5p, miR-187-3p, miR-181a-3p, miR-30c-1-3p, miR-650, miR-3652, miR-4444, miR-1273e, miR-99a-3p, miR-3064-5p	5 miRNAs miR-132-3p, miR-212-3p, miR-212-5p, miR-6836-5p, miR-629-3p	36
2	Microarray RT-PCR	ONFH tissues	22 miRNAs miR-210-3p, miR-320e let-7c	17 miRNAs miR-133a-3p, miR-335-5p, miR-146b-5p	37
3	Microarray RT-PCR	Mesenchymal stem cell treated with dexamethasone	11 miRNAs miR-16-5p, miR-103a-3p, miR-107, miR-196a/b-5p, miR-378d/ f/g, miR-1268a/b, miR-4289	6 miRNAs miR-24-3p, miR-378a/h/l, miR-4448, miR-4634	38
4	Microarray RT-PCR	Mesenchymal stem cells from mice	23 miRNAs miR-21-3p, miR-652-5p	16 miRNAs miR-34b-3p, miR-34c-5p, miR-148a-3p, miR-196a-5p, miR-206-3p	39
5	Microarray RT-PCR	ONFH patients serum	15 miRNAs miR-3960, miR-423-5p, miR-15b-3p, miR-1304-3p, miR-195-5p	12 miRNAs miR-99a-5p, miR-100-5p, miR-140-5p, miR-532-5p, miR-181c-5p, miR-10b-5p, miR-433, miR-10a-5p	40
6	Microarray RT-PCR	ONFH patients serum	9 miRNAs miR-10a-5p	4 miRNAs miR-423-5p	41

ONFH: Osteonecrosis of the femoral head.

key to the repair process following both traumatic and non-traumatic ONFH.⁴⁸ Therefore, any changes in the migration, apoptosis, proliferation and inter-connection of endothelial cells could have significant impact on ONFH development. Yamasaki et al⁴⁹ demonstrated that expression of miR-210, an angiogenic miRNA, was higher in the non-traumatic ONFH (steroid-induced, alcohol-associated or idiopathic ONFH) than in the osteoarthritis of the hip. In addition, the expression of vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-7 and MMP-2 was upregulated. miR-210 was mainly expressed around the necrotic area with von Willebrand factor (vWF) and VEGF strongly expressed in miR-210-expressing cells.⁴⁹ Consistent with this finding, Yuan et al³⁴ showed that miR-210 expression was upregulated in steroid-associated ONFH as compared with normal tissues. Bisulphite sequencing also indicated that miR-210 upregulation was associated with demethylation of two CpG sites (regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence) in miR-210 gene. In particular, demethylating agents promoted miR-210 expression and increased viability and differentiation of endothelial cells. Several angiogenic factors, including basic fibroblast growth factor, VEGF and tumour necrosis factor- α , were also upregulated.³⁴ These results indicated that miR-210 is highly expressed in ONFH and may modulate angiogenesis in ONFH.

4.3 | miR-548d-5p

Sun et al⁵⁰ demonstrated that miR-548d-5p was downregulated during dexamethasone-induced adipogenic differentiation of human bone marrow mesenchymal stem cells. Functionally, reduced expression of miR-548d-5p was linked to upregulation of CCAAT-enhancer-binding protein (C/EBP) α and peroxisome proliferator-activated receptors (PPAR)- γ and increased cellular triglyceride content. In contrast, ectopic expression of miR-548d-5p enhanced expression of osteocalcin and Runx2 (an osteogenic transcription factor) as well as activity of alkaline phosphatase (ALP) (a marker for osteoblast differentiation). Furthermore, PPAR γ was identified as the direct target of miR-548d-5p. These findings suggested that restoring miR-548d-5p levels could promote osteogenic differentiation of mesenchymal stem cells and may serve as a therapeutic strategy to counteract glucocorticoid-induced ONFH.

4.4 | miR-17-5p

Jia et al⁵¹ reported that miR-17-5p expression level was lower in mesenchymal stem cells derived from non-traumatic ONFH patients (steroid-induced, alcohol-associated or idiopathic ONFH) than those from osteoarthritis. Ectopic expression of miR-17-5p increased β -catenin in nuclear translocation and promoted COL1A1 (collagen type I, alpha 1 chain) expression and induced mesenchymal stem cell proliferation and differentiation partly through targeting Smad7 expression. These data suggested that abnormal downregulation of miR-17-5p could contribute to the pathogenesis of ONFH. In another study, Wei et al⁵² demonstrated the role of HOTAIR (HOX

TABLE 2 Functional characterization of the lncRNAs in rheumatoid arthritis

lncRNAs	Expression	Functional role	Related gene	Role	References
miR-708	Up	Mesenchymal stem cell differentiation	Smad3	Damage	30
miR-210	Up	Angiogenesis		Damage	31,45
miR-548d-5p	Down	Mesenchymal stem cell differentiation	PPAR γ	Protect	46
miR-17-5p	Down	Mesenchymal stem cell differentiation	Smad7	Protect	47,48
miR-27a	Down	Mesenchymal stem cell differentiation	PPAR γ GREM1	Protect	32

Smad: small mothers against decapentaplegic; PPAR γ : peroxisome proliferator-activated receptors γ ; GREM1: gremlin 1.

transcript antisense RNA), a long non-coding RNA, in the regulation of miR-17-5p-induced osteogenic proliferation and differentiation in non-traumatic ONFH. The investigators showed that miR-17-5p expression was downregulated whereas the expression level of HOTAIR was upregulated in mesenchymal stem cells derived from non-traumatic ONFH as compared with those from osteoarthritis patients. Downregulation of HOTAIR increased miR-17-5p levels and suppressed the Smad7 expression, which was a direct target of miR-17-5p. Knockdown of HOTAIR promoted COL1A1 and Runx2 expression and ALP activity. These results suggested that HOTAIR could negatively regulate mesenchymal stem cell proliferation and osteogenic differentiation by regulating miR-17-5p and Smad7 expression and may serve as a therapeutic target in non-traumatic ONFH.

4.5 | miR-27a

Through miRNA microarray, Gu et al³⁵ found that miR-27a expression was significantly downregulated in a rat model of steroid-induced

ONFH. Besides, PPAR γ and gremlin 1 (GREM1), both of which are direct targets of miRNA-27a, were significantly upregulated. Functionally, downregulation of miR-27a promoted adipogenic differentiation whereas enforced expression of miR-27a suppressed adipogenesis and increased osteogenesis in the steroid-treated rat bone marrow mesenchymal stem cells. Knockdown of PPAR γ and GREM1 also enhanced the osteogenic effect of miR-27a. These results suggested that miR-27a suppressed adipogenesis and enhanced osteogenesis through regulating GREM1 and PPAR γ expression. Delivery of exogenous miR-27a might therefore be a novel therapeutic strategy in ONFH (Table 2).

5 | CONCLUSIONS AND FUTURE PERSPECTIVE

Osteonecrosis of the femoral head is a progressive disease with disabling outcomes.¹⁴ However, its unclear pathogenesis has hindered

FIGURE 1 Functional role of specific miRNAs in osteonecrosis of the femoral head

the development of mechanism-driven treatment and prevention strategies.^{53,54} miRNAs were found to play crucial pathogenic roles in ONFH,^{33,34} especially through interacting with transcription factors involved in adipogenesis, osteogenesis and angiogenesis (Figure 1). An increasing number of studies have also hinted at their potential therapeutic roles in ONFH. Nevertheless, one of the major unresolved issues is that osteonecrosis tissues, which is consisted of trabecular bone, bone marrow, blood vessels and cartilage, extracted from patients were used for microRNA profiling in most of the studies, in which the cellular source of the deregulated miRNAs remains undefined. It is noteworthy even the same miRNA could have different functions in different cell types. Future studies might have to resort to single-cell transcriptomics or other techniques that can address this issue. From a therapeutic point of view, tissue-specific delivery of miRNA mimics or inhibitors is still challenging and the exact roles of many dysregulated miRNAs in ONFH remain unclear. Further studies using tissue-specific knockout mice should be carried out to characterize the in vivo functions of specific miRNAs.

By genome-wide miRNA profiling, several studies identified differentially abundant miRNAs in serums of ONFH patients, suggesting their potential use as diagnostic markers. Nevertheless, whether these changes of miRNAs paralleled with the onset of ONFH or only reflected the progression to end-stage disease is unclear. Time-course analysis of circulating miRNAs in corticosteroid-treated patients that have not yet developed ONFH should clarify the temporal relationship between miRNA changes and ONFH development. In addition, most patients receiving corticosteroid treatment have some forms of systemic inflammatory diseases and appropriate control with patients with the same disease and drug treatment but without the development of ONFH should be included in all studies. Moreover, large-cohort validation in different populations is required to translate these findings into clinical benefits. Further basic and translational studies are therefore needed to maximize the clinical potentials of miRNA-based diagnostics and therapeutics in ONFH.

CONFLICTS OF INTEREST

The authors declare no competing financial interests.

ORCID

Zheng Li <http://orcid.org/0000-0001-6024-0194>

REFERENCES

- Kubo T, Ueshima K, Saito M, Ishida M, Arai Y, Fujiwara H. Clinical and basic research on steroid-induced osteonecrosis of the femoral head in Japan. *J Orthop Sci*. 2016;21:407-413.
- Villa JC, Husain S, van der List JP, Gianakos A, Lane JM. Treatment of pre-collapse stages of osteonecrosis of the femoral head: a systematic review of randomized control trials. *HSS J*. 2016;12:261-271.
- Du J, Liu W, Jin T, et al. A single-nucleotide polymorphism in MMP9 is associated with decreased risk of steroid-induced osteonecrosis of the femoral head. *Oncotarget*. 2016. <https://doi.org/10.18632/oncotarget.12034>.
- Zhang YL, Chen S, Ai ZS, Gao YS, Mei J, Zhang CQ. Osteonecrosis of the femoral head, nonunion and potential risk factors in Pauwels grade-3 femoral neck fractures: a retrospective cohort study. *Medicine*. 2016;95:e3706.
- Yang F, Zhu ZZ, Weng SY, et al. Morphological changes of the trabecular bone in osteonecrosis of the femoral head. *Int J Clin Exp Pathol*. 2016;9:4203-4211.
- Li DQ, Li M, Liu PL, Zhang YK, Ma L, Xu F. Core decompression or quadratus femoris muscle pedicle bone grafting for nontraumatic osteonecrosis of the femoral head: a randomized control study. *Indian J Orthop*. 2016; 50: 629-635.
- Kerimaa P, Vaananen M, Ojala R, et al. MRI-guidance in percutaneous core decompression of osteonecrosis of the femoral head. *Eur Radiol*. 2016;26:1180-1185.
- Adesina OO, Brunson AM, Gotlib JR, Keegan T, Wun T. Osteonecrosis of the femoral head in sickle cell disease: prevalence, comorbidities and surgical outcomes in california. *Blood*. 2016;128:2489.
- Du JL, Liu WL, Jin TB, et al. A single-nucleotide polymorphism in MMP9 is associated with decreased risk of steroid-induced osteonecrosis of the femoral head. *Oncotarget*. 2016;7:68434-68441.
- Saito M, Ueshima K, Ishida M, et al. Alcohol-associated osteonecrosis of the femoral head with subsequent development in the contralateral hip: a report of two cases. *J Orthop Sci*. 2016;21:870-874.
- Han N, Li ZC, Cai ZD, Yan ZQ, Hua YQ, Xu C. P-glycoprotein overexpression in bone marrow-derived multipotent stromal cells decreases the risk of steroid-induced osteonecrosis in the femoral head. *J Cell Mol Med*. 2016;20:2173-2182.
- Zhao DW, Huang SB, Lu FQ, et al. Vascularized bone grafting fixed by biodegradable magnesium screw for treating osteonecrosis of the femoral head. *Biomaterials*. 2016;81:84-92.
- Ikemura S, Yamashita A, Harada T, Watanabe T, Shirasawa K. Clinical and imaging features of a subchondral insufficiency fracture of the femoral head after internal fixation of a femoral neck fracture: a comparison with those of post-traumatic osteonecrosis of the femoral head. *Brit J Radiol*. 2016;89: <https://doi.org/10.1259/bjr.20150725>.
- van der Jagt D, Mokete L, Pietrzak J, Zalavras CG, Lieberman JR. Osteonecrosis of the femoral head: evaluation and treatment. *J Am Acad Orthop Sur*. 2015;23:69.
- Yang S, Halim AY, Werner BC, Gwathmey FW, Cui QJ. Does osteonecrosis of the femoral head increase surgical and medical complication rates after total hip arthroplasty? A comprehensive analysis in the United States. *Hip Int*. 2015;25:237-244.
- Swarup I, Lee YY, Movilla P, Figgie MP. Common factors associated with osteonecrosis of the femoral head in young patients requiring total hip arthroplasty. *Hip Int*. 2015;25:232-236.
- Tsai SW, Wu PK, Chen CF, et al. Etiologies and outcome of osteonecrosis of the femoral head: etiology and outcome study in a Taiwan population. *J Chin Med Assoc*. 2016;79:39-45.
- Zhao X, He W, Li J, et al. MiRNA-125b inhibits proliferation and migration by targeting SphK1 in bladder cancer. *Am J Transl Res*. 2015;7:2346-2354.
- Gao Y, Xue Q, Wang D, Du M, Zhang Y, Gao S. miR-873 induces lung adenocarcinoma cell proliferation and migration by targeting SRCIN1. *Am J Transl Res*. 2015;7:2519-2526.
- Jing W, Jiang W. MicroRNA-93 regulates collagen loss by targeting MMP3 in human nucleus pulposus cells. *Cell Prolif*. 2015;48: 284-292.
- Li Z, Yu X, Shen J, Liu Y, Chan MT, Wu WK. MicroRNA dysregulation in rhabdomyosarcoma: a new player enters the game. *Cell Prolif*. 2015;48:511-516.
- Liu F, Wang X, Li J, et al. miR-34c-3p functions as a tumour suppressor by inhibiting eIF4E expression in non-small cell lung cancer. *Cell Prolif*. 2015;48:582-592.
- Cirera-Salinas D, Pauta M, Allen RM, et al. Mir-33 regulates cell proliferation and cell cycle progression. *Cell Cycle*. 2012;11:922-933.

24. Lee SE, Kim SJ, Yoon HJ, et al. Genome-wide profiling in melatonin-exposed human breast cancer cell lines identifies differentially methylated genes involved in the anticancer effect of melatonin. *J Pineal Res.* 2013;54:80-88.
25. Lee SE, Kim SJ, Youn JP, Hwang SY, Park CS, Park YS. MicroRNA and gene expression analysis of melatonin-exposed human breast cancer cell lines indicating involvement of the anticancer effect. *J Pineal Res.* 2011;51:345-352.
26. Li Z, Shen JX, Chan MTV, Wu WKK. MicroRNA-379 suppresses osteosarcoma progression by targeting PDK1. *J Cell Mol Med.* 2017;21:315-323.
27. Yu X, Li Z, Shen J, et al. MicroRNA-10b promotes nucleus pulposus cell proliferation through RhoC-Akt pathway by targeting HOXD10 in intervertebral disc degeneration. *PLoS ONE.* 2013;8:e83080.
28. Liu G, Cao P, Chen H, Yuan W, Wang J, Tang X. MiR-27a regulates apoptosis in nucleus pulposus cells by targeting PI3K. *PLoS ONE.* 2013;8:e75251.
29. Xu JF, Zhang SJ, Zhao C, et al. Altered microRNA expression profile in synovial fluid from patients with knee osteoarthritis with treatment of hyaluronic acid. *Mol Diagn Ther.* 2015;19:299-308.
30. Li Z, Yu X, Shen J, Chan MT, Wu WK. MicroRNA in intervertebral disc degeneration. *Cell Prolif.* 2015;48:278-283.
31. Tang PF, Xiong Q, Ge W, Zhang LH. The role of MicroRNAs in osteoclasts and osteoporosis. *RNA Biol.* 2014;11:1355-1363.
32. Li Z, Yu X, Shen J, Wu WK, Chan MT. MicroRNA expression and its clinical implications in Ewing's sarcoma. *Cell Prolif.* 2015;48:1-6.
33. Hao C, Yang SH, Xu WH, et al. MiR-708 promotes steroid-induced osteonecrosis of femoral head, suppresses osteogenic differentiation by targeting SMAD3. *Sci Rep.* 2016;6. doi: Artn 2259910.1038/Srep22599.
34. Yuan HF, Christina V, Guo CA, Chu YW, Liu RH, Yan ZQ. Involvement of MicroRNA-210 demethylation in steroid-associated osteonecrosis of the femoral head. *Sci Rep.* 2016;6. doi: Artn 2004610.1038/Srep20046.
35. Gu CX, Xu Y, Zhang SF, et al. miR-27a attenuates adipogenesis and promotes osteogenesis in steroid-induced rat BMSCs by targeting PPAR gamma and GREM1. *Sci Rep.* 2016;6. doi: Artn 38491 <https://doi.org/10.1038/Srep38491>.
36. Nannini M, Ravegnini G, Angelini S, Astolfi A, Biasco G, Pantaleo MA. miRNA profiling in gastrointestinal stromal tumors: implication as diagnostic and prognostic markers. *Epigenomics.* 2015;7:1033-1049.
37. Ladeiro Y, Couchy G, Balabaud C, et al. MicroRNA profiling in hepatocellular tumors is associated with clinical features and oncogene/tumor suppressor gene mutations. *Hepatology.* 2008;47:1955-1963.
38. Gougelet A, Pissaloux D, Besse A, et al. Micro-RNA profiles in osteosarcoma as a predictive tool for ifosfamide response. *Int J Cancer.* 2011;129:680-690.
39. Yuan HF, Von Roemeling C, Gao HD, Zhang J, Guo CA, Yan ZQ. Analysis of altered microRNA expression profile in the reparative interface of the femoral head with osteonecrosis. *Exp Mol Pathol.* 2015;98:158-163.
40. Wu X, Zhang Y, Guo X, et al. Identification of differentially expressed microRNAs involved in non-traumatic osteonecrosis through microRNA expression profiling. *Gene.* 2015;565:22-29.
41. Bian Y, Qian W, Li H, Zhao RC, Shan WX, Weng X. Pathogenesis of glucocorticoid-induced avascular necrosis: a microarray analysis of gene expression in vitro. *Int J Mol Med.* 2015;36:678-684.
42. Wang BQ, Yu P, Li T, Bian YY, Weng XS. MicroRNA expression in bone marrow mesenchymal stem cells from mice with steroid-induced osteonecrosis of the femoral head. *Mol Med Rep.* 2015;12:7447-7454.
43. Wang X, Qian W, Wu Z, Bian Y, Weng X. Preliminary screening of differentially expressed circulating microRNAs in patients with steroid-induced osteonecrosis of the femoral head. *Mol Med Rep.* 2014;10:3118-3124.
44. Wei BF, Wei W. Identification of aberrantly expressed of serum microRNAs in patients with hormone-induced non-traumatic osteonecrosis of the femoral head. *Biomed Pharmacother.* 2015;75:191-195.
45. Aoyama T, Fujita Y, Madoba K, et al. Rehabilitation program after mesenchymal stromal cell transplantation augmented by vascularized bone grafts for idiopathic osteonecrosis of the femoral head: a preliminary study. *Arch Phys Med Rehab.* 2015;96:532-539.
46. Zhao DW, Liu BY, Wang BJ, et al. Autologous bone marrow mesenchymal stem cells associated with tantalum rod implantation and vascularized iliac grafting for the treatment of end-stage osteonecrosis of the femoral head. *Biomed Res Int.* 2015; <https://doi.org/Artn24050610.1155/2015/240506>.
47. Wang C, Wang Y, Meng HY, et al. Application of bone marrow mesenchymal stem cells to the treatment of osteonecrosis of the femoral head. *Int J Clin Exp Med.* 2015;8:3127-3135.
48. Kerachian MA, Seguin C, Harvey EJ. Glucocorticoids in osteonecrosis of the femoral head: a new understanding of the mechanisms of action. *J Steroid Biochem.* 2009;114:121-128.
49. Yamasaki K, Nakasa T, Miyaki S, Yamasaki T, Yasunaga Y, Ochi M. Angiogenic microRNA-210 is present in cells surrounding osteonecrosis. *J Orthop Res.* 2012;30:1263-1270.
50. Sun JK, Wang YS, Li YB, Zhao GQ. Downregulation of PPAR gamma by miR-548d-5p suppresses the adipogenic differentiation of human bone marrow mesenchymal stem cells and enhances their osteogenic potential. *J Transl Med.* 2014;12. doi: Artn 16810.1186/1479-5876-12-168.
51. Jia J, Feng XB, Xu WH, et al. MiR-17-5p modulates osteoblastic differentiation and cell proliferation by targeting SMAD7 in non-traumatic osteonecrosis. *Exp Mol Med.* 2014;46. doi: ARTN e10710.1038/emmm.2014.43.
52. Wei BF, Wei W, Zhao BX, Guo XX, Liu S. Long non-coding RNA HOTAIR inhibits miR-17-5p to regulate osteogenic differentiation and proliferation in nontraumatic osteonecrosis of femoral head. *PLoS ONE.* 2017;12. doi: ARTN e016909710.1371/journal.pone.0169097.
53. Qi XY, Zeng YR. Biomarkers and pharmaceutical strategies in steroid-induced osteonecrosis of the femoral head: a literature review. *J Int Med Res.* 2015;43:3-8.
54. Hu LB, Huang ZG, Wei HY, Wang W, Ren A, Xu YY. Osteonecrosis of the femoral head: using CT, MRI and gross specimen to characterize the location, shape and size of the lesion. *Brit J Radiol.* 2015;88. doi: Artn 2014050810.1259/Bjr.20140508.

How to cite this article: Li Z, Yang B, Weng X, Tse G, Chan MTV, Wu WKK. Emerging roles of MicroRNAs in osteonecrosis of the femoral head. *Cell Prolif.* 2018;51:e12405. <https://doi.org/10.1111/cpr.12405>