SCIENTIFIC ARTICLE

Non-coding RNA Identification in Osteonecrosis of the Femoral Head Using Competitive Endogenous RNA Network Analysis

Ning Han, MD Zengchun Li, MD
Department of Emergency Trauma Surgery, Shanghai East Hospital of Tongji University, Shanghai, China

Objective: To investigate the regulatory network of long non-coding RNA (lncRNA) as competing endogenous RNAs (ceRNAs) in osteonecrosis of the femoral head (ONFH).

Methods: The gene expression profile GSE74089 of ONFH and microRNA (miRNA) expression profile of GSE89587 were obtained from the Gene Expression Omnibus (GEO) database. The GSE74089 contained four ONFH samples and four controls. The GSE89587 included 10 ONFH samples and 10 control samples. The differentially expressed lncRNAs (DE-lncRNAs) and DE-mRNAs between ONFH group and control group were identified from GSE74089 using the limma package based on criteria of adjusted P value <0.05 and |log fold change (FC)| ≥2. The DEmiRNAs between ONFH group and control group were screened from GSE89587 on the basis of adjusted P value <0.05. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for DE-mRNAs were analyzed using DAVID 6.7 and GSEA 3.0, respectively. Coexpressed lncRNA-mRNA pairs were identified by corr.test method in R based on the criteria of adjusted P value <0.01 and |r| ≥ 0.9. A ceRNA network was constructed and visualized using cytoscape 3.7.0 by integrating the DE-lncRNA, DE-miRNA, and DEMRNA data. The key mRNAs and IncRNAs in the ceRNA network were further validated in an independent dataset of GSE123568.

Results: Based on our analysis, a total of 28 DE-lncRNAs, 1403 DE-mRNAs, and 134 DE-miRNAs were identified, respectively. The DE-mRNAs were significantly enriched in the function of “skeletal system development,” “collagen fibril organization,” “blood vessel development,” and “regulation of nervous system development.” Besides, 72 KEGG pathways, including eight suppressed pathways and 64 upregulated pathways were identified, including which immune pathway was the most significantly activated one and which ribosome-related function was the most suppressed. A co-expression network including 161 DE-mRNAs and 16 DE-lncRNAs was built. Highly connected nodes were identified among IncRNAs such as H19, C20orf203, LINC00355, SFTA3, CRNDE, CASC2, LINC00494, C9orf163, C10orf91, and LINC00301. The ceRNA network indicated that IncRNA H19 functioned as a ceRNA of hsa-miR-519b-3p and hsa-miR-296-5p in ANKH and ECHDC1 regulation; IncRNA C9orf163 functioned as a ceRNA of hsa-miR-424-5p in CCNT1 regulation. The expression trends of ANKH, CCNT1, and C9orf163 were successfully validated in independent dataset of GSE123568.

Conclusion: The ceRNAs of IncRNA H19- hsa-miR-519b-3p/hsa-miR-296-5p-ANKH and IncRNA c9orf163- hsa-miR-424-5p-CCNT1 might play important roles in ONFH development. Our research provided an understanding of the important role of IncRNA-related ceRNAs in ONFH.

Key words: Competing endogenous RNAs; Long non-coding RNA; Osteonecrosis of the femoral head

Address for correspondence Zengchun Li, Chief Physician, Department of Emergency Trauma Surgery, Shanghai East Hospital of Tongji University, No. 150, Jinmo Road, Shanghai, China 200120 Tel: +86-13636692302; Fax: +86-21-38804518; Email: lizengchun113@163.com.

Disclosure: The authors declare no conflict of interest.
Received 13 January 2020; accepted 28 September 2020
**Introduction**

Osteonecrosis of the femoral head (ONFH) is a devastating condition that affects patients for about 30 to 50 years of life and usually progresses to femoral collapse\(^1\). About 20,000 to 30,000 new adults will be diagnosed in the United States annually\(^2\). Different etiologies participate in the ONFH. The femoral neck fracture or dislocation of the femoral head is one of the most common causes. Besides, autoimmune and chronic inflammatory disorders, long-term steroid treatment, and vascular damage were reported in the development of ONFH\(^3\). ONFH is a multifactorial disease that involves multiple factors. Several proteins have been identified in the progression of ONFH including lipoprotein, p-glycoprotein, zinc–α2-glycoprotein, glycoprotein-39, vascular endothelial growth factor (VEGF). There is research that demonstrated that lncRNA family with sequence similarity 20-member A (FAM201A) could promote osteogenic differentiation in ONFH patients\(^7\). Hua\ et al. demonstrated that lncRNA HOTAIR might function as a ceRNA to regulate expression of Family Member 7 (SMAD7) by sponging miRNA-17-5p in non-traumatic ONFH\(^21\). Several lncRNAs acting as ceRNAs have been reported in cancers\(^22\). However, the lncRNAs that function as ceRNAs in the ONFH has not been investigated in detail.

In recent years, more and more studies showed that the lncRNAs could target miRNA and regulate gene expression indirectly\(^20\). This kind of lncRNA was defined as acting as competing endogenous RNAs (ceRNAs), which indicated the lncRNAs competed for binding to miRNAs with mRNAs to regulate the target gene expression. Wei\ et al. demonstrated that lncRNA HOTAIr might function as a ceRNA to regulate expression of Family Member 7 (SMAD7) by sponging miRNA-17-5p in non-traumatic ONFH\(^21\). Several lncRNAs acting as ceRNAs have been reported in cancers\(^22\). However, the lncRNAs that function as ceRNAs in the ONFH has not been investigated in detail.

In this study, we aimed to: (i) analyze gene expression data of ONFH; (ii) identify critical lncRNAs and mRNAs involved in the development of ONFH; (iii) decipher the regulatory ceRNA network of lncRNAs–miRNAs–mRNAs in ONFH. The flow chart of this study is shown in Fig. 1. This study might be helpful to explore the molecular mechanisms of ONFH.

**Materials and Methods**

**Data Source**

The microarray data of expression profile of GSE74089 (GPL13497) was obtained from the National Center for Biotechnology Information (NCBI) GEO database\(^23\). The GSE74089 was obtained from GLP13974 (Agilent-026652 Whole Human Genome Microarray 4 × 44K v2) with samples from four ONFH patients and four controls\(^24\). miRNA microarray expression profile GSE89587 (GPL18402, Agilent-046064 Unrestricted_Human_miRNA_V19.0_Microarray) from 10 ONFH samples and 10 control samples was also obtained\(^25\). In these datasets, annotation "protein_coding" was defined as mRNA, "antisense, sense_intronic, lincRNA, sense_overlapping, processed_transcript, 3' overlapping_ncRNA, non_coding" were defined as ncRNA.

The dataset of GSE123568 was downloaded from GEO database as validation dataset. This dataset was deposited by Zhang\ et al. and included microarray data from 30 steroid-induced ONFH patients and 10 non-steroid-induced ONFH patients\(^26\). The platform of this dataset was GPL15207 ([PrimeView] Affymetrix Human Gene Expression Array).

**Differential Expression Analysis**

Differentially expressed lncRNAs (DE-lncRNAs) and DE-mRNAs were identified from the gene expression profile of GSE74089 using the limma package (version 3.10.3) in R\(^27\). Differentially expressed miRNAs (DE-miRNAs) were identified from the miRNA microarray expression profile GSE89587. The P value was adjusted using the Benjamini–Hochberg method. DE-lncRNAs and DE-mRNAs were filtered with the adjusted P value <0.05 and \(\log fold change\)
(FC) ≥2, while DE-miRNAs was screened based on the criterion of adjusted P value <0.05.

**Functional Enrichment Analysis**

The Gene Ontology analysis of DE-mRNAs was performed using DAVID version 6.7 and visualized by GOplot in R. KEGG pathway enrichment of DE-mRNAs was implemented using Gene set enrichment analysis version 3.0 (GSEA) with the adjusted P value <0.05. The pathways with Normalized Enrichment Score (NES) larger than 0 indicated the pathways were activated while NES smaller than 0 indicated the pathways were suppressed.

**CeRNA Network Construction**

Pearson’s correlation coefficients (PCC) between DE-lncRNAs and DE-mRNAs were calculated using psych package in R with corr.test method (ci = F, adjust=“BH”). Cytoscape (version 3.7.0) was used for visualization of the coexpressed lncRNA-mRNA pairs with adjusted P value <0.01 and |r| ≥ 0.9.

The ceRNA network was constructed under the following procedures: (i) the interactions between DE-lncRNAs and DE-miRNAs were predicted using starbase database; (ii) the interactions between DE-miRNAs and DE-miRNAs were predicted using miRtarBase, mirDB, and TargetScan and VENN diagram was used to filter out the miRNA-mRNA pairs localized in at least two of the three databases; (iii) the co-expressed DE-lncRNAs and DE-miRNAs that were regulated by same DE-miRNA and the expression patterns of DE-miRNA in ONFH was opposite (lncRNA and mRNA were upregulated in ONFH, while miRNA was downregulated in ONFH or lncRNA, and mRNA were downregulated in ONFH, while miRNA was upregulated in ONFH) with DE-lncRNAs and DE-miRNAs used for constructing ceRNA network by Cytoscape.

**Validation of Key Genes in the ceRNA Network**

The key mRNAs and lncRNAs in the ceRNA network were further validated in an independent dataset of GSE123568. The differential expression of these mRNAs and lncRNAs were compared using Welch’s non-paired t test. P < 0.05 was regarded as the statistical significant level.

**Results**

**DEGs Identification in ONFH Samples**

There were 17,372 mRNAs and 395 ncRNAs in GSE74089 dataset. There were 1153 miRNAs in the dataset GSE89587. A total of 1403 DE-mRNAs, 28 DE-lncRNAs, and 134 DE-miRNAs were identified, in which 378 mRNAs, 15 lncRNAs, and 59 miRNAs were up-regulated; 1025 mRNAs, 13 lncRNAs, and 75 miRNAs were down-regulated. Volcano plots revealed that mRNAs, lncRNAs, and miRNAs were differentially expressed between ONFH patients and control subjects (Fig. 2A,C,E) and the top 10 up-regulated and down-regulated genes were illustrated in heatmap (Fig. 2B,D,F).

**GO Enrichment Analysis**

To explore the biological function of these DE-miRNAs, GO enrichment analysis was performed. As a result, 18 GO terms including nine biological process (BP) functions and nine cellular component (CC) functions were significantly enriched in the ceRNA network.
Fig. 2 Differentially expressed genes (DEGs) identification between osteonecrosis of the femoral head (ONFH) and normal samples. The DE-IncRNAs and DE-mRNAs between ONFH and normal samples were identified from GSE74089 by limma package in R based on the criteria of with the adjusted P value <0.05 and |log fold change (FC)| ≥2. DE-miRNAs were identified from GSE89587 on the basis of adjusted P value <0.05. Volcano plots display the distribution of DE-mRNAs (A), DE-IncRNAs (C), and DE-miRNAs (E). The vertical dotted lines in A and C represent |log FC| = 2 and the horizontal dotted lines represent adjusted P value = 0.05. Red spots represent up-regulated genes and green spots represent down-regulated genes in ONFH samples compared with normal samples. Hierarchical clustering demonstrates the top 10 DE-mRNAs (B), DE-IncRNAs (D), and DE-miRNAs (F) in descending order by log FC.
enriched (Fig. 3). The significant GO-BPs included “skeletal system development” ($P$ value = 4.82E-07), “collagen fibril organization” ($P$ value = 2.25E-06), and “blood vessel development” ($P$ value = 1.51E-05). GO-CC analysis illustrated that the DE-mRNAs were mainly located in “extracellular region” ($P$ value = 1.20E-10), “proteinaceous extracellular matrix” ($P$ value = 3.86E-10), “collagen” ($P$ value = 8.81E-07), “fibrillar collagen” ($P$ value = 8.23E-06), and “Golgi membrane” ($P$ value = 1.06E-06). These results revealed that these genes functioned in skeletal development, collagen fibril organization, and blood vessel development.

**KEGG Enrichment**

Next, KEGG pathway enrichment was performed and 72 pathways were identified, in which eight pathways were activated (NES > 0) and 64 pathways were suppressed (NES < 0, Fig. 4A,B). Among the active pathways, “intestinal immune network for IgA production” ($P$ value = 0.009), “allograft rejection” ($P$ value = 0.007), and “Staphylococcus aureus infection” ($P$ value = 0.004) were the most significantly activated pathways (Fig. 4C). The suppressed pathways included “hippo signaling pathway” ($P$ value = 0.001), “TGF-beta signaling pathway” ($P$ value = 0.004), “p53 signaling pathway” ($P$ value = 0.008), “mTOR signaling pathway” ($P$ value = 0.008), and “PI3K-AKT signaling pathway” ($P$ value = 0.002). Among these, “ribosome” ($P$ value = 0.001) was the most significantly suppressed pathway (Fig. 4D). The top 10 significant suppressed pathways and the eight activated pathways are shown in the Supplementary Table S1.

![Gene Ontology (GO) enrichment analysis of DE-mRNAs.](image-url)

**Fig. 3** Gene Ontology (GO) enrichment analysis of DE-mRNAs. The GO enrichment analysis was performed using DAVID and visualized by GOplot in R. (A) The top five bubble plot of GO terms. X-axis represents the Z-score and Y-axis represents the negative log (adjusted $P$ value). The area of the bubble positively correlates with the gene numbers in the indicated term. The green represents the GO-biological process terms and the pink represents the GO-cellular component terms. (B) GO cluster of genes in the top eight GO terms grouped by their expression level.
LncRNA-mRNA Co-expressed Network and ceRNA Network Construction

Next, DE-mRNAs and DE-lncRNAs were used to construct a co-expression network (Fig. 5). There were 177 nodes and 175 edges in the co-expression network including 161 DE-mRNAs and 16 DE-lncRNAs. Highly connected nodes were identified among lncRNAs such as H19 Imprinted Maternally Expressed Transcript (H19), Chromosome 20 Open Reading Frame 203 (C20orf203), long intergenic non-protein coding RNA 355 (LINC00355), Surfactant Associated 3 (SFTA3), Colorectal Neoplasia Differentially Expressed (CRNDE), Cancer Susceptibility 2 (CASC2), long intergenic non-protein coding RNA 494 (LINC00494), Chromosome 9 Open Reading Frame 163 (C9orf163), Chromosome 10 Open Reading Frame 91 (C10orf91) and long intergenic non-protein coding RNA 301 (LINC00301).

CeRNA Network Construction

The miRNA-mRNA interactions were predicted in Targetscan, miRDB, and miRTarBase. The miRNA-mRNA interactions that were predicted in at least two databases were selected for further analysis. Based on the intersection elements, 240 miRNA-mRNA pairs were identified (Fig. 6A). Meanwhile, the lncRNA targeted by miRNA were predicted using starBase database and 10 lncRNAs were identified: H19, C20orf203, LINC00355, SFTA3, CRNDE, CASC2, LINC00494, C9orf163, C10orf91, and LINC00301. After integrating miRNA-lncRNA pairs and miRNA-mRNA pairs, a ceRNA network was constructed (Fig. 6B). This ceRNA network included three DE-mRNAs: Ethylmalonyl-CoA Decarboxylase 1 (ECHDC1), Ankylosis protein homolog human gene (ANKH), and Cyclin T1 (CCNT1); three DE-miRNAs: hsa-miR-519b-3p,
hsa-miR-424-5p, hsa-miR-296-5p; and two DE-lncRNAs: H19 and C9orf163. As shown in the ceRNA network, lncRNA H19 might function as a ceRNA of hsa-miR-519b-3p and hsa-miR-296-5p to enhance the expression of ECHDC1 and ANKH in ONFH. LncRNA C9orf163 might target hsa-miR-296-5p and then regulate the expression of CCNT1.

**Validation of Key Genes in the ceRNA Network**

The three key mRNAs (ECHDC1, ANKH, and CCNT1) and two lncRNAs (H19 and C9orf163) in the ceRNA network were further validated in an independent dataset of GSE123568. Because of the different platforms of the original dataset of GSE74089 and the validation dataset of
GSE123568, the lncRNA H19 was not detected in the dataset of GSE123568. The differential expression of ECHDC1, ANKH, CCNT1, and C9orf163 between ONFH samples and control samples is shown in Fig. 7. All of these four genes were significantly differentially expressed between ONFH samples and control samples. Among them, the expression trends of ANKH, CCNT1, and C9orf163 were consistent with the original dataset, while the expression trend of ECHDC1 was opposite the original dataset. The successful rate of validation is 75%, suggesting a relatively high reliability of the results.

Discussion

ONFH is usually induced by the destruction of the blood supply and the coagulation and fibrinolysis system disorder. However, the specific molecular mechanism of ONFH is unclear. In this study, we found DE-mRNAs were mainly functioned in skeletal system, blood vessel development. Further, we found the immune system might function in the ONFH development. Co-expression network analysis revealed several key lncRNAs played roles in ONFH development such as H19, C20orf203, LINC00355, SFTA3, CRNDE, CASC2, LINC00494, C9orf163, C10orf91, and LINC00301. Then, we first performed a miRNA-lncRNA-mRNA ceRNA network construction, in which two lncRNAs H19 and C9orf163 mainly targeted hsa-miR-519b-3p, hsa-miR-424-5p, and hsa-miR-296-5p and then regulated the expression of ECHDC1, ANKH, and CCNT1. Among them, the differential expression of ANKH, CCNT1, and C9orf163 was successfully validated in an independent dataset.

Previous research has illustrated multiple biological processes were involved in the development of ONFH. Circulation, steroid metabolism, immunity, and bone formation have been reported as the etiologies of ONFH. Immune disorder was associated with ONFH and several genes involved in immunity have shown correlation with ONFH. For example, the rs1800587 SNP of interleukin (IL)-1α has revealed increased risk of ONFH. Besides, IL-23 and IL-33 have shown their predictive value of ONFH. In this study, we found the DE-mRNAs were significantly enriched in immune-related pathways, such as intestinal immune network for IgA production and allo-graft rejection, and might function in the development of ONFH, which was consistent with previous research.

Recently, emerging evidence provided a novel regulatory mechanism between miRNA and lncRNA. LncRNAs could act as miRNA sponges to regulate the expression of miRNA target genes and participate in multiple biological processes. But the lncRNA-miRNA regulatory network in ONFH has not been
fully illustrated. ncRNAs have shown their incidence in the pathogenesis of ONFH. Several studies have suggested that miRNAs have been critically investigated and showed their important roles in the development of ONFH. MiR-146a and miR-34a were up-regulated in the ONFH patients. Circulating miRNAs were used as markers for ONFH diagnosis. Up-regulated miR-10a-5p and down-regulated miR-423-5p were confirmed in ONFH patients. Functional significance of up-regulated miR-708, miR-210 and down-regulated miR-548d-5p, miR-17-5p, miR-27a were found in ONFH patients. It has been reported that IncRNAs of CRNDE, CASC2, RP11-154D6, Myocardial Infarction Associated Transcript (MIAT), RP1-193H18.2, MALAT1, and HOTAIR have shown their correlations with ONFH.

In our research, we identified the 10 IncRNAs of H19, C20orf203, LINC00355, SFTA3, CRNDE, CASC2, LINC00494, C9orf163, C10orf91, and LINC00301 in the development of ONFH. Among these, CRNDE and CASC2 have been identified before in ONFH.

In the present study, we constructed a ceRNA network and several ceRNA relationships among IncRNAs, miRNAs, and mRNAs were identified. For example, we found the IncRNA H19 could bind to hsa-miR-519b-3p and hsa-miR-296-5p to regulate the expression of ECHDC1 and ANKH. Also, we found the IncRNA C9orf163 could up-regulate CCNT1 by sponging hsa-miR-424-5p.

It is well known that H19 is an imprinted gene, and H19 is re-expressed in a wide variety of tumor types and as tumor-suppressed genes. In non-small cell lung cancer, miR-296-5p suppresses cell viability. In muscle-wasting patients, rRNA synthesis is inhibited by miR-424-5p regulation in pol I pre-initiation complex formation. Decreased expression of miR-519b-3p was observed in colorectal cancer, which indicated the suppressed invasion and proliferation function in colorectal cancer cells. Ethylmalonyl-CoA decarboxylase (ECHDC1) has shown its function in bladder cancer, breast cancer, and ovarian cancer. Ankylosis protein homolog human gene (ANKH) mutations have been reported in inherited human disorders such as familial calcium pyrophosphate deposition disease (CPPD) and cranial metaphyseal dysplasia. But the roles of the following have not been revealed before: IncRNA of H19; miRNAs of hsa-miR-519b-3p, hsa-miR-296-5p and hsa-miR-424-5p; mRNAs of ECHDC1 and ANKH in ONFH.

Our research firstly investigated the function of identified RNAs in ONFH development through miRNAs-IncRNAs ceRNA network. Moreover, newly identified IncRNA C9orf163 was first identified and validated as a key IncRNA in the progress of ONFH.

Nevertheless, our study has several limitations. First, our ONFH sample size for analysis was small. Second, most of the differentially expressed IncRNAs and mRNAs still need validation. Finally, although the ceRNA network of IncRNAs and mRNAs were constructed, the mechanisms of these IncRNAs and mRNAs need to be further investigated.

**Conclusion**

Based on our analysis, a total of 28, 1403, and 134 DE-IncRNAs, DE-mRNAs, and DE-miRNAs were identified respectively. The differentially expressed miRNAs were enriched in “skeletal system development,” “collagen fibril organization,” “blood vessel development,” and “regulation of nervous system development.” Seventy-two KEGG pathways were identified including eight active pathways and 64 inactive pathways, including which immune pathway is significantly activated and which ribosome-related function was mostly inhibited. The ceRNA network indicated that IncRNA H19 might bind both hsa-miR-519b-3p and hsa-miR-296-5p, then ECHDC1 and ANKH were up-regulated. LncRNA C9orf163 could up-regulate CCNT1 by targeting hsa-miR-424-5p.

**An Authorship Declaration**

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

**Supporting Information**

Additional Supporting Information may be found in the online version of this article on the publisher’s web-site:

**Table S1.** The top 10 suppressed pathways and the eight activated pathways.

**References**

1. Mont MA, Cherian JJ, Sierra RJ, et al. Nontraumatic osteonecrosis of the femoral head: where do we stand today? A ten-year update. J Bone Joint Surg Am, 2015, 97: 1604–1627.
2. Choi HR, Steinberg ME. EYC. Osteonecrosis of the femoral head: diagnosis and classification systems. Curr rev. Musculoskeletal Med, 2015, 8: 210–220.
3. Goyal T, Singh A, Sharma R, et al. Osteonecrosis of femoral head in north Indian population: risk factors and clinicoradiological correlation. Clin Epidemiol Glob Health, 2019, 7: 446–449.
4. Wang G, Zhang C, Sun Y, et al. Changes in femoral head blood supply and vascular endothelial growth factor in rabbits with steroid-induced osteonecrosis. J Int Med Res, 2010, 38: 1060–1069.
5. Zheng L, Wang W, Ni J, et al. Plasma interleukin 33 level in patients with osteonecrosis of femoral head: an Alarmin for osteonecrosis of the femoral head? J Invest Med, 2014, 62: 635–637.
6. Wang Q, Yang Q, Chen G, et al. LncRNA expression profiling of BMSCs in osteonecrosis of the femoral head associated with increased adipogenic and decreased osteogenic differentiation. Sci Rep, 2018, 8: 9127.
7. Xiang S, Li Z, Weng X. The role of IncRNA RP11-154D6 in steroid-induced osteonecrosis of the femoral head through BMSC regulation. J Cell Biochem, 2019, 120: 18435–18445.
8. Huang G, Zhao G, Xia J, et al. FGF2 and FAM20A1 affect the development of osteonecrosis of the femoral head after femoral neck fracture. Gene, 2018, 652: 39–47.
9. Chen X, Li J, Liang D, et al. LncRNA AWPPH participates in the development of non-traumatic osteonecrosis of femoral head by upregulating Runx2. Exp Ther Med, 2020, 19: 153–159.

10. Kumar V, Westra HJ, Karjalainen J, et al. Human disease-associated genetic variation impacts large intergenic non-coding RNA expression. PLoS Genet, 2013, 9: e1003201.

11. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol, 2014, 15: 509–524.

12. Ji X, Chen Y, Xu Y. MicroRNAs in Osteoclastogenesis and function: potential therapeutic targets for osteoporosis. Int J Mol Sci, 2016, 17: 349.

13. Li Z, Shen J, Chan MT, et al. MicroRNA-379 suppresses osteosarcoma progression by targeting PDK1. J Cell Mol Biol, 2017, 21: 315–323.

14. 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.

15. Yu X, Li Z, Shen J, et al. MicroRNA-10b promotes nucleus pulposus cell proliferation through Rhô-Act pathway by targeting HOXD10 in intervertebral disc degeneration. PLoS One, 2015, 8: e083086.

16. Wu X, Zhang Y, Xiong G, et al. Identification of differentially expressed microRNAs involved in non-traumatic osteonecrosis through microRNA expression profiling. Gene, 2015, 565: 22–29.

17. Wei B, Wei W. Identification of aberrantly expressed serum microRNAs in patients with hormone-induced non-traumatic osteonecrosis of the femoral head. Biomed Pharmacother, 2015, 75: 191–195.

18. Yuan HF, Von Roenneling C, Gao HD, et al. Analysis of altered microRNA expression profile in the reparative interface of the femoral head with osteonecrosis. Exp Mol Pathol, 2015, 98: 158–163.

19. Bian Y, Qian W, Li H, et al. Pathogenesis of glucocorticoid-induced avascular necrosis: a microarray analysis of gene expression in vitro. Int J Mol Med, 2015, 36: 676–683.

20. Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet, 2014, 15: 7–12.

21. Wei B, Wei W, Zhao B, et al. Long non-coding RNA HOTAIR inhibits miR-17-5p to regulate osteoclast differentiation and proliferation in non-traumatic osteonecrosis of femoral head. PLoS One, 2017, 12: e0169097.

22. Xiong DD, Li ZY, Liang L, et al. The LncRNA NEAT1 accelerates lung adenocarcinoma deterioration and binds to Mir-193a-3p as a competitive endogenous RNA. Cell Physiol Biochem, 2018, 48: 905–918.

23. Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets—update. Nucleic Acids Res, 2013, 41: D991–D995.

24. Liu R, Liu Q, Wang K, et al. Comparative analysis of gene expression profiles in normal hip human cartilage and cartilage from patients with necrosis of the femoral head. Arthritis Res Ther, 2016, 18: 98.

25. Cavallini A, Rotelli MT, Lippolis C, et al. Human microRNA expression in sporadic and FAP-associated desmoid tumors and correlation with beta-catenin mutations. Oncotarget, 2017, 8: 41866–41875.

26. Li T, Zhang Y, Wang R, et al. Discovery and validation of an eight-biomarker serum gene signature for the diagnosis of steroid-induced osteonecrosis of the femoral head. Bone, 2019, 122: 199–208.

27. Ritchie ME, Phipson B, Wu D, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res, 2015, 43: e47.

28. Huang D, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc, 2009, 4: 44–57.

29. Kaneshisa M, Goto SKEGG. Kyoto encyclopedia of genes and genomes. Nucleic Acids Res, 2000, 28: 27–30.

30. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res, 2003, 13: 2498–2504.

31. Li JH, Liu S, Zhou H, et al. starBase v2.0: decoding miRNA-ceRNA, miRNA-nrRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res, 2014, 42: D92–D97.

32. Wang GJ, Sweet DE, Reger SI, et al. Fat-cell changes as a mechanism of avascular necrosis of the femoral head in cortisone-treated rabbits. J Bone Joint Surg Am, 1977, 59: 729–735.

33. Wang T, Azeddine B, Mah W, et al. Osteonecrosis of the femoral head: genetic basis. Int Orthop, 2019, 43: 519–530.

34. Samara S, Kollia P, Daliliana Z, et al. Predictive role of cytokine gene polymorphisms for the development of femoral head osteonecrosis. Dis Markers, 2012, 33: 215–221.

35. Oppmann B, Lesley R, Blom B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity, 2000, 13: 715–725.

36. Parham C, Chirica M, Timans J, et al. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. J Immunol, 2002, 168: 5699–5708.

37. El-Tahan RR, Ghoneim AM, El-Mashad N. TNF-alpha gene polymorphisms and expression. Springerplus, 2016, 5: 1508.

38. Hao C, Yang S, Xu W, et al. MiR-70B promotes steroid-induced osteonecrosis of femoral head, suppresses osteogenic differentiation by targeting SMAD3. Sci Rep, 2016, 6: 22599.

39. Yuan HF, Christina VR, Guo CA, et al. Involvement of MicroRNA-210 Demethylation in steroid-associated osteonecrosis of the femoral head. Sci Rep, 2016, 6: 20046.

40. Jia J, Feng X, Xu W, et al. MiR-17-5p modulates osteoblastic differentiation and cell proliferation by targeting SMAD7 in non-traumatic osteonecrosis. Exp Mol Med, 2014, 46: e107.

41. Sun J, Wang Y, Li Y, et al. Downregulation of PPARgamma 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: 168.

42. Fang B, Li Y, Chen C, et al. Hoxue Tong Luo capsule ameliorates osteonecrosis of the femoral head through inhibiting IncRNA-Miat. J Ethnopharmacol, 2019, 111826: 238.

43. Yamaguchi R, Yamamoto T, Motomura G, et al. Incidence of nontraumatic osteonecrosis of the femoral head in the Japanese population. Arthritis Rheum, 2011, 63: 3169–3173.

44. Igarashi M, Hayashi Y, Karube S, et al. An aspect of metabolic bone disease with idiopathic osteonecrosis of the femoral head. Nihon Seikeigeka Gakkai Zasshi, 1983, 57: 379–384.

45. Yoshimura H, Matsuda Y, Yamamoto M, et al. Expression and role of long non-coding RNA H19 in carcinogenesis. Front Biosci, 2018, 23: 614–625.

46. Xu C, Li S, Chen T, et al. miR-296-5p suppresses cell viability by directly targeting PLK1 in non-small cell lung cancer. Oncol Rep, 2016, 35: 497–503.

47. Connolly M, Paul R, Farre-Garros R, et al. miR-424-5p reduces ribosomal RNA and protein synthesis in muscle wasting. J Cachexia Sarcopenia Muscle, 2018, 9: 400–416.

48. Zhang Y, Sun M, Chen Y, et al. MiR-519b-3p inhibits the proliferation and invasion in colorectal cancer via modulating the uMtCK/Wnt signaling pathway. Front Pharmacol, 2019, 10: 741.

49. Zhuang Q, Ye B, Hui S, et al. Long noncoding RNA IncAIS downregulation in mesenchymal stem cells is implicated in the pathogenesis of adolescent idiopathic scoliosis. Cell Death Differ, 2019, 26: 1700–1715.

50. Abhishek A, Doherty M. Pathophysiology of articular chondrocalcinosis: role of ANKH. Nat Rev Rheumatol, 2011, 7: 96–104.