Identification of crucial miRNAs and IncRNAs for ossification of ligamentum flavum

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Abstract. The present study aimed to screen crucial micro (mi)RNAs and long non-coding (Inc)RNAs involved in the development of ossification of ligamentum flavum (OLF) based on the miRNA-mRNA and IncRNA-miRNA-mRNA competing endogenous (ce)RNA regulatory network analyses, which are rarely reported. The differentially expressed genes (DEGs), differentially expressed IncRNAs (DEls) and differentially expressed miRNAs (DEMs) between 4 OLF and 4 healthy controls were identified using two microarray datasets GSE106253 and GSE106256 collected from the Gene Expression Omnibus database. A protein-protein interaction (PPI) network was constructed, followed by calculation of topological characteristics and sub-module analysis in order to obtain hub DEGs. The miRNA-mRNA and IncRNA-miRNA networks that were established based on their interaction pairs, obtained from miRwalk and starBase databases, respectively, were integrated to form the ceRNA network. The underlying functions of mRNAs were predicted using the database for annotation, Visualization and integrated discovery (DAVID). The present study screened 828 DEGs, 119 DEls and 81 DEMs between OLF and controls. PPI network and module analyses identified interleukin (IL)10, adenylate cyclase (ADCY)5, suppressor of cytokine signaling (SOCS)3, G protein subunit gamma (GNG) 4, collagen type II α 1 chain (COL2A1) and collagen type XIII α 1 chain (COL13A1) as hub genes. The miRNA-mRNA network analysis demonstrated IL10 could be regulated by miR-210-3p, while COL13A1 and COL2A1 could be modulated by miR-329-3p and miR-222-5p, respectively. IncRNA-miRNA-mRNA ceRNA network analysis identified that small nucleolar RNA host gene 16-hsa-miR-196a-5p-SOX3, ankyrin repeat and SOCS box containing 16-AS1-hsa-miR-379-5p-GNG4, nuclear enriched abundant transcript 1-hsa-miR-181b-5p-ADCY5, rhophilin 1-AS1-hsa-miR-299-3p-WNT7B interaction axes may be crucial. DAVID analysis predicted IL10, ADCY5, GNG4 and SOCS3 were involved in ‘adaptive immune response’, ‘Chemokine signaling pathway’ and ‘regulation of apoptosis’ processes, while COL2A1, COL13A1 and WNT7B may be ossification related. In conclusion, the identification of these crucial miRNAs and IncRNAs may be conducive for explaining the pathogenesis of OLF and provide certain natural, endogenous and nontoxic drug targets for the treatment of OLF.

Introduction

Ossification of ligamentum flavum (OLF) is a relatively common spinal disorder in Eastern Asian countries, with an estimated prevalence of 63.9% in Chinese (1), 36% in Japanese (2) and 16.9% in Korean (3) populations. OLF is characterized by ectopic bone formation in the spinal ligaments and ligamentous tissue hyperplasia (4) that cause spinal canal narrowing and result in the development of myelopathy and radiculopathy (5,6). Surgery is the predominant treatment option for OLF; however, the difficulty of surgery and a relatively high risk of complications have to be taken into consideration (7). Therefore, it is necessary to develop more effective, convenient and safe approaches for the treatment of OLF; an improved understanding of its molecular mechanisms may provide insight.

Although the pathogenesis of OLF remains to be elucidated, abnormal expression of osteogenic differentiation and cell proliferation related genes in LF cells may serve important roles (8). The mRNA levels of osteogenic markers [alkaline phosphatase (ALP), runt-related transcription factor 2, osterix and osteopontin] in addition to signaling pathway genes [bone morphogenetic proteins (BMPs), Wnt/β-catenin and Notch] (9,10), were identified to be higher in patients with OLF compared with non-OLF subjects. Recombinant BMP2 or BMP14 [also known as growth/differentiation factor (GDF) 5] modification induced the osteoblastic differentiation of LF cells and promoted bone nodule formation, finally triggering neurological impairment in rat models (11,12), while downregulation of Notch2 ameliorated the processes (10). In addition to accelerating osteoblast differentiation via osterix,
highly expressed pro-inflammatory cytokines [tumor necrosis factor (TNF)-α, interleukin (IL)-1α and IL-6] appear to stimulate cell proliferation and tissue hypertrophy by upregulating cyclin D1 and c-Myc in OLF (13-15). Therefore, targeted regulation of these genes may be potential strategies for the treatment of OLF.

A potential way to endogenously regulate the expression levels of target mRNAs is through microRNAs (miRNAs/miRs) that bind to the 3'-untranslated regions of target genes and subsequently mediate their degradation or translation inhibition (16). Therefore, researchers are exploring the crucial miRNAs that regulate the expression of osteogenic differentiation related genes in OLF. miR-132-3p and miR-615-3p have been demonstrated to be downregulated during osteogenic differentiation of LF cells (17,18). Overexpression of miR-615-3p by its mimics suppressed the osteogenic differentiation of LF cells by reducing the expression of GDF5 (17). miR-199b-5p and miR-487b-3p were reported to inhibit osteogenic differentiation in LF cells by downregulating Notch and Wnt signaling pathway genes, respectively (19,20). However, the OLF-related miRNAs have rarely been reported and the inflammation-associated miRNAs in OLF have not been identified.

In addition to miRNAs, long non-coding RNAs (lncRNAs) are considered to be crucial in regulating the expression of genes. IncRNAs can competitively bind to miRNAs through their miRNA response elements and influence the regulation of miRNAs for mRNAs, which is called the competing endogenous RNA (ceRNA) hypothesis (21). Therefore, IncRNAs may also be important targets for the treatment of OLF. However, OLF-related lncRNAs are rarely reported, with the exception of the previous study by Han et al (22).

The aim of the present study was to use the datasets uploaded by Han et al (22) to further identify novel miRNAs and crucial lncRNAs for OLF based on the miRNA-mRNA and lncRNA-miRNA-mRNA ceRNA regulatory networks. The findings may provide insight for underlying therapeutic strategies for OLF by changing the expression levels of miRNAs and lncRNAs, which could in turn regulate the target genes.

Materials and methods

Data sources. A total of two datasets under accession numbers GSE106253 and GSE106256 (22) were downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.
The GS e106253 dataset was analyzed to examine the mRNA and lncRNA expression profiles using the microarray technique (platform: GPL21827, Agilent-079487 Arraystar Human lncRNA microarray V4). Then, GS e106256 dataset was analyzed to detect the miRNA expression profile using high throughput sequencing (platform: GPL118573, illumina nextSeq 500). These two datasets contained the LF tissues from 4 patients with OLF and 4 healthy volunteers.

**Data preprocessing and differential analysis.** The raw TXT data were collected from the GEO database and preprocessed using the linear Models for Microarray data (limma) method (23) (version 3.34.0; http://www.bioconductor.org/packages/release/bioc/html/limma.html) in the Bioconductor R package (version 3.4.1; http://www.R-project.org/), including base-2 logarithmic (log2) transformation to normalize the skewed distribution, followed by quantile normalization. For the GSE106253 microarray data, all the probe sequences downloaded from the annotation platform GPL21827 were aligned and compared with the human genome using Clustal W program (version 2; http://www.clustal.org/) (24) to obtain the expression levels of lncRNA and mRNAs.

The differentially expressed genes (DEGs), differentially expressed lncRNAs (DEl) and differentially expressed miRNAs (DEM) between the patients with OLF and the healthy controls were identified using the LIMMA method (23). DEGs, DEl and DEM were screened based on the statistical threshold of \(|\log FC| > 1\) and false discovery rates (FDR) < 0.05. Two-way hierarchical clustering was performed using pheatmap R package (version: 1.0.8; https://cran.r-project.org/web/packages/pheatmap) based on Euclidean distance to render a heatmap of DEGs, DEl and DEM.

**Protein-protein interaction (PPI) network of DEGs.** The DEGs were mapped to the Search Tool for the Retrieval of Interacting Genes (STRING; version 10.0; http://stringdb.org/) database (25) to acquire PPI pairs. Then, the PPI network was constructed using these PPI pairs and visualized using Cytoscape software (version 3.6.1; www.cytoscape.org/) (26). Topological features of each node (protein) in the PPI network, including degree [the number of edges (interactions) of a node] and betweenness (BC; the number of shortest paths that run through a node), were used to screen hub candidate markers that serve crucial roles in OLF using the CytoNCA plugin in Cytoscape software (http://apps.

### Table I. Top 10 upregulated and downregulated differentially expressed genes, lncRNAs and miRNAs.

#### A. Upregulated

| miRNA        | FDR         | logFC  | lncRNA      | FDR         | logFC  | mRNA      | FDR         | logFC  |
|--------------|-------------|--------|-------------|-------------|--------|-----------|-------------|--------|
| hsa-miR-653-3p | 8.30x10^-4 | 4.00   | LINC01549    | 2.31x10^2   | 5.50   | PKIB      | 6.80x10^3   | 6.29   |
| hsa-miR-489-3p | 2.33x10^-2 | 3.81   | CLSTN2-AS1   | 1.45x10^2   | 4.678  | AMTN      | 2.95x10^2   | 5.47   |
| hsa-miR-508-3p | 4.51x10^-3 | 3.59   | LINC00347    | 2.04x10^3   | 4.09   | WISP3     | 1.77x10^2   | 5.34   |
| hsa-miR-4683  | 1.95x10^-3 | 3.46   | LINC02203    | 7.15x10^3   | 3.84   | ADCYAP1    | 1.70x10^2   | 5.32   |
| hsa-miR-138-5p | 1.70x10^-3 | 3.22   | LINC01508    | 4.07x10^3   | 3.75   | COL9A1     | 1.11x10^2   | 5.17   |
| hsa-miR-653-5p | 3.54x10^-3 | 3.14   | WASIR2       | 1.07x10^3   | 3.22   | SERPINA1   | 1.13e-02    | 5.13   |
| hsa-miR-4473  | 1.72x10^-2 | 2.88   | LINC01440    | 9.83x10^3   | 3.18   | CLEC3A     | 1.89x10^2   | 5.08   |
| hsa-miR-483-3p | 1.65x10^-3 | 2.86   | LINC02249    | 1.12x10^2   | 3.14   | SLITRK6    | 3.88x10^2   | 5.03   |
| hsa-miR-181b-3p | 2.28x10^-2 | 2.58   | DSG1-AS1     | 2.27x10^3   | 3.07   | ZMAT4      | 2.11x10^2   | 4.92   |

#### B. Downregulated

| miRNA        | FDR         | logFC  | lncRNA      | FDR         | logFC  | mRNA      | FDR         | logFC  |
|--------------|-------------|--------|-------------|-------------|--------|-----------|-------------|--------|
| hsa-miR-495-3p | 2.41x10^-6 | -3.16  | LINC01706    | 2.24x10^2   | 3.06   | ITIH6     | 1.11x10^2   | 4.73   |
| hsa-miR-495-5p | 2.41x10^-6 | -3.16  | LINC00601    | 3.88x10^2   | -1.839 | FAM3B     | 1.43x10^2   | -3.06  |
| hsa-miR-377-5p | 2.07x10^-2 | -3.18  | LINC01730    | 4.24x10^2   | -1.85  | ZIC3      | 1.79x10^2   | -3.22  |
| hsa-miR-551b-3p | 2.04x10^-3 | -3.21  | FAM230B      | 2.48x10^2   | -1.88  | ANGPTL4   | 4.87x10^2   | -3.31  |
| hsa-miR-369-5p | 3.61x10^-3 | -3.28  | FLG-AS1      | 1.63x10^3   | -1.93  | SOCS3     | 2.20x10^2   | -3.43  |
| hsa-miR-1185-1-3p | 2.11x10^-3 | 3.76   | SNHG16       | 2.56x10^2   | -2.06  | GPT       | 4.11x10^2   | -3.49  |
| hsa-miR-539-3p | 1.72x10^-2 | -3.91  | LINC01615    | 2.14x10^2   | -2.08  | ADAMTS4   | 3.43x10^2   | -3.73  |
| hsa-miR-222-5p | 1.02x10^-2 | -4.09  | HIPK1-AS1    | 1.98x10^2   | -2.30  | GPD1      | 4.02x10^2   | -3.79  |
| hsa-miR-412-5p | 1.02x10^-2 | -4.09  | LINC01485    | 3.95x10^2   | -2.53  | FAM71A    | 1.67x10^2   | -3.84  |
| hsa-miR-4443  | 3.53x10^-4 | -4.25  | MEG3         | 2.80x10^2   | -2.53  | SAA1      | 3.77x10^2   | -3.89  |
| hsa-miR-122-5p | 4.40x10^-10| -6.87  | VPS9D1-AS1   | 1.30x10^2   | -2.65  | CCL2      | 4.29x10^2   | -3.99  |

lncRNA, long non-coding RNA; miRNA/miR, microRNA; FDR, false discovery rate; FC, fold change.
cytoscape.org/apps/cytonca) (27). The Molecular Complex Detection (MCODE; version:1.4.2, http://apps.cytoscape.org/apps/mcode) (28) plugin of the Cytoscape software was applied to extract highly interconnected sub-modules from the overall PPI network.

**DEMs-regulated IncRNAs and genes.** The DEMs regulated target genes were predicted using the miRwalk database (version 2.0; http://www.zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2) (29). The DEMs regulated IncRNAs were predicted using the starBase database (version 2.0; http://starbase.sysu.edu.cn/index.php) (30). The target genes and IncRNAs of DEMs were respectively overlapped with the DEGs and DELs to obtain the DEM-DEG and DEM-DEL interaction networks, which were visualized using Cytoscape software (26). Based on the common miRNAs, the DEM-DEG and DEM-DEL networks were integrated to form a DEL-DEM-DEG ceRNA network, which was also visualized using Cytoscape software (26).

**Function enrichment analysis.** Gene Ontology (GO; release 2018-10-01; http://www.geneontology.org) term and The Kyoto Encyclopedia of Genes and Genomes (KEGG; release 88.0; https://www.kegg.jp) pathway enrichment analyses were conducted for genes in each sub-module network and all regulatory networks using the Biological Networks Gene Ontology (BINGO; version 3.0.3; https://www.psb.ugent.be/cbd/papers/BiNGO/Home.html) and the Database for

| Genes  | Degree | Genes       | Betweenness centrality | Overlap | LogFC |
|--------|--------|-------------|------------------------|---------|-------|
| AKT1   | 31     | TBCD10B     | 1.0000                 | VEGFA   | -1.55 |
| CTNNB1 | 30     | GPR153      | 1.0000                 | BMP4    | 2.26  |
| VEGFA  | 29     | PLK3        | 1.0000                 | CTNNB1  | -1.90 |
| GNG4   | 26     | POLQ        | 0.8333                 | GNG4    | 3.49  |
| ADCY5  | 26     | LMNA        | 0.6667                 | AKT1    | -1.27 |
| CCL5   | 19     | SUN2        | 0.6667                 | POTEJ   | -1.94 |
| IL10   | 19     | EXO1        | 0.5000                 | SH3GL1  | -1.62 |
| POTEJ  | 16     | CTNNB1      | 0.2625                 | IL10    | -2.29 |
| SAA1   | 16     | AKT1        | 0.1816                 | ICAM1   | 1.38  |
| SOCS3  | 15     | VEGFA       | 0.1401                 | MYC     | -1.19 |
| NMU    | 15     | GNA12       | 0.1294                 | ADCY5   | -1.46 |
| NMUR1  | 15     | ADCY5       | 0.1213                 | SOCS3   | -3.43 |
| ITGA4  | 15     | YWHAZ       | 0.0964                 | CCL5    | 1.38  |
| MYC    | 15     | POLR2D      | 0.0957                 | ITGA4   | 1.64  |
| COL4A2 | 14     | IL10        | 0.0833                 |         |       |
| COL9A2 | 14     | ITGA4       | 0.0756                 |         |       |
| SH3GL1 | 14     | POTEJ       | 0.0725                 |         |       |
| COL9A3 | 14     | GBF1        | 0.0718                 |         |       |
| CCL2   | 14     | MYC         | 0.0693                 |         |       |
| ICAM1  | 14     | PLA2G4F     | 0.0665                 |         |       |
| COL6A1 | 13     | SMG5        | 0.0639                 |         |       |
| COL10A1| 13     | CTTN        | 0.0580                 |         |       |
| BMP4   | 13     | PLD4        | 0.0550                 |         |       |
| COL6A2 | 13     | ICAM1       | 0.0545                 |         |       |
| COL9A1 | 13     | SOCS3       | 0.0539                 |         |       |
| CD40LG | 13     | RPS18       | 0.0536                 |         |       |
| COL2A1 | 13     | CCL5        | 0.0532                 |         |       |
| CRTAP  | 12     | RBBP4       | 0.0510                 |         |       |
| KBTBD7 | 12     | GNG4        | 0.0492                 |         |       |
| COL22A1| 12     | FZD9        | 0.0475                 |         |       |
| PPIB   | 12     | BMP4        | 0.0457                 |         |       |
| NT5E   | 12     | SH3GL1      | 0.0448                 |         |       |
| COL13A1| 12     | FOXO3       | 0.0426                 |         |       |
| PIK3R3 | 12     | TBL2        | 0.0424                 |         |       |

**Table II.** Top 30 genes ranked by topological characteristics.

FC, fold change.
Annotation, Visualization and Integrated Discovery (DAVID; version 6.8; http://david.abcc.ncifcrf.gov) (31) tools. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression pattern of mRNA, lncRNAs and miRNAs in OLF. Based on the cut-off criteria (FDR <0.05 and llogFC>1), a total of 828 DEGs (434 upregulated and 394 downregulated) and 119 DELs (94 upregulated and 25 downregulated) were identified in the gene chip GSE106253 (Fig. 1A); 81 DEMs, with 25 upregulated and 56 downregulated, were identified in the gene chip GSE106256 (Fig. 1A). The top 20 dys-regulated DEGs, DELs and DEMs are summarized in Table I. The hierarchically clustered heat map indicated that the DEGs and DELs in GSE106253 (Fig. 1B), and DEMs in GSE106256 (Fig. 1B) were well categorized into OLF and control groups.

DEGs interaction network construction. By searching the STRING database, 859 interaction pairs between DEGs were collected, which were used to create a PPI network, consisting of 372 nodes (168 upregulated and 204 downregulated; data not shown). A total of 14 nodes were identified and the top 30 genes were ranked following the calculation of the two topological features (the degree and BC), suggesting that 14 genes [vascular endothelial growth factor (VEGF), A, BMP4, catenin β (CTNNB) 1, G protein subunit gamma (GNG) 4, AKT serine/threonine kinase 1 (AKT1), POTE ankyrin domain family member (POTE) J, SH3 domain containing GRB2 like (SH3GL) 1, endophilin A2, IL10, intercellular adhesion molecule (ICAM) 1, MYC proto-oncogene (MYC), bHLH transcription factor, adenylate cyclase (ACD) Y 5, suppressor of cytokine signaling (SOCS) 3, C-C motif chemokine ligand (CCL) 5 and integrin subunit α (ITGA) 4] may be hub genes in the PPI network (Table II). In addition, several collagen genes, including collagen type II α 1 chain (COL2A1) and collagen type XIII α 1 chain (COL13A1) may be also important for OLF, according to the degree ranking. A total of seven highly interconnected sub-modules were extracted from the PPI network using the MCODE algorithm (Fig. 2). Among them,

Figure 2. Modules extracted from the protein-protein interaction network. (A) Module 1, (B) module 2, (C) module 3, (D) module 4, (E) module 5, (F) module 6 and (G) module 7 are presented. Red and green denoted upregulated and downregulated expression, respectively. The color depth is in proportion to differential levels. The size of the proteins is in proportion to the number of its interaction pairs.
### Table III. Function enrichment for genes in the different modules.

| A, M1 | GO-ID | P-value | Description | Genes in test set |
|-------|-------|---------|-------------|-------------------|
| 48731 | 4.98x10⁻³ | System development | COL2A1, COL13A1, COL9A1, COL10A1, COL9A3, COL9A2 |
| 48856 | 7.99x10⁻³ | Anatomical structure development | COL2A1, COL13A1, COL9A1, COL10A1, COL9A3, COL9A2 |
| 1501  | 2.69x10⁻⁴ | Skeletal system development | COL2A1, COL13A1, COL9A1, COL10A1, COL9A2 |
| 7155  | 1.08x10⁻⁴ | Cell adhesion | COL2A1, COL13A1, COL6A2, COL6A1, COL9A1 |
| 22610 | 1.08x10⁻⁴ | Biological adhesion | COL2A1, COL13A1, COL6A2, COL6A1, COL9A1 |
| 30198 | 5.74x10⁻⁴ | Extracellular matrix organization | COL2A1, COL4A2, COL6A2 |
| 43062 | 2.12x10⁻⁴ | Extracellular structure organization | COL2A1, COL4A2, COL6A2 |
| 16337 | 1.22x10⁻⁴ | Cell-cell adhesion | COL2A1, COL13A1, COL6A2 |

| B, M2 | GO-ID | P-value | Description | Genes in test set |
|-------|-------|---------|-------------|-------------------|
| 23052 | 6.96x10⁻⁷ | Signaling | GPR27, P2RY14, PTGER2, ADM, GIP, ADCYAP1, GPR176, CALCR, CCL5, NMU, CRH, OXER1, NMUR1, DRD1 |
| 7166  | 7.13x10⁻⁷ | Cell surface receptor linked signaling pathway | ADCYAP1, GPR176, CALCR, P2RY14, PTGER2, NMU, OXER1, NMUR1, DRD1, GIP |
| 23033 | 7.80x10⁻⁶ | Signaling pathway | ADCYAP1, GPR176, CALCR, P2RY14, PTGER2, NMU, OXER1, ADM, NMUR1, DRD1, GIP |
| 23046 | 8.09x10⁻⁵ | Signaling process | GPR27, GPR176, CALCR, CCL5, NMU, CRH, ADM, NMUR1, DRD1, GIP |
| 23060 | 8.09x10⁻⁵ | Signal transmission | GPR27, GPR176, CALCR, CCL5, NMU, CRH, ADM, NMUR1, DRD1, GIP |
| 50896 | 2.69x10⁻⁴ | Response to stimulus | CALCR, P2RY14, CCL5, NMU, SAA1, CRH, ADM, DRD1, CXCL3, CXCL2, CCL28, GIP |
| 65007 | 1.12x10⁻⁴ | Biological regulation | GPR27, PTGER2, ADM, GIP, ADCYAP1, CALCR, CCL5, NMU, SAA1, CRH, OXER1, NMUR1, DRD1, CCL28 |
| 50794 | 1.32x10⁻⁴ | Regulation of cellular process | GPR27, PTGER2, ADM, GIP, ADCYAP1, CALCR, CCL5, NMU, SAA1, CRH, OXER1, NMUR1, DRD1 |

| C, M3 | GO-ID | P-value | Description | Genes in test set |
|-------|-------|---------|-------------|-------------------|
| 7017  | 5.49x10⁻⁵ | Microtubule-based process | RNF19A, KIF5A, KIF1C, KIF1A |
| 46627 | 1.03x10⁻⁴ | Negative regulation of insulin receptor signaling pathway | SOCS3, SOCS1 |
| 7018  | 1.20x10⁻⁴ | Microtubule-based movement | KIF5A, KIF1C, KIF1A |
| 46626 | 1.59x10⁻⁴ | Regulation of insulin receptor signaling pathway | SOCS3, SOCS1 |
| 6890  | 1.59x10⁻⁴ | Retrograde vesicle-mediated transport, Golgi to ER | GBF1, KIF1C |
| 32570 | 2.08x10⁻⁴ | Response to progesterone stimulus | SOCS3, SOCS1 |
| 7259  | 4.46x10⁻⁴ | JAK-STAT cascade | SOCS3, SOCS1 |
| 31100 | 4.72x10⁻⁴ | Organ regeneration | SOCS3, SOCS1 |
| 32355 | 1.44x10⁻³ | Response to estradiol stimulus | SOCS3, SOCS1 |
| 16192 | 1.52x10⁻³ | Vesicle-mediated transport | KDELR1, GBF1, KIF1C, TMED2 |
| 51246 | 1.99x10⁻³ | Regulation of protein metabolic process | UBE2F, SOCS3, SOCS1, UBE2E2 |
| 31099 | 2.26x10⁻³ | Regeneration | SOCS3, SOCS1 |
| 46907 | 2.36x10⁻³ | Intracellular transport | KDELR1, GBF1, KIF1C, KIF1A |
### Table III. Continued.

#### D, M4

| GO-ID | P-value | Description                                | Genes in test set                      |
|-------|---------|--------------------------------------------|----------------------------------------|
| 16043 | 1.03x10^4 | Cellular component organization            | TFRC, ARPC4, EPS15, SH3GL1             |
| 16044 | 7.41x10^4 | Cellular membrane organization              | TFRC, EPS15, SH3GL1                    |
| 61024 | 7.47x10^4 | Membrane organization                       | TFRC, EPS15, SH3GL1                    |
| 16192 | 2.71x10^4 | Vesicle-mediated transport                  | TFRC, EPS15, SH3GL1                    |
| 43623 | 6.55x10^4 | Cellular protein complex assembly           | ARPC4, EPS15                           |
| 10324 | 1.41x10^4 | Membrane invagination                       | TFRC, SH3GL1                           |
| 6897  | 1.41x10^4 | Endocytosis                                 | TFRC, SH3GL1                           |
| 34622 | 2.83x10^3 | Cellular macromolecular complex assembly    | ARPC4, EPS15                           |
| 34621 | 3.58x10^3 | Cellular macromolecular complex subunit organization | ARPC4, EPS15         |

#### E, M5

| GO-ID | P-value | Description                                | Genes in test set                      |
|-------|---------|--------------------------------------------|----------------------------------------|
| 6139  | 6.85x10^9 | Nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | NT5E, PABPN1, ENTPD4, SRSF1, POLR2D, AMPD1, PPWD1, AKD1, CD2BP2, PRPF40A, CHERP |
| 34641 | 3.49x10^8 | Cellular nitrogen compound metabolic process | NT5E, PABPN1, ENTPD4, SRSF1, POLR2D, AMPD1, PPWD1, AKD1, CD2BP2, PRPF40A, CHERP |
| 6807  | 6.30x10^7 | Nitrogen compound metabolic process         | NT5E, PABPN1, ENTPD4, SRSF1, POLR2D, AMPD1, PPWD1, AKD1, CD2BP2, PRPF40A, CHERP |
| 44237 | 3.34x10^4 | Cellular metabolic process                  | NT5E, PABPN1, ENTPD4, SRSF1, POLR2D, AMPD1, PPWD1, AKD1, CD2BP2, PRPF40A, CHERP |
| 44238 | 5.98x10^4 | Primary metabolic process                   | NT5E, PABPN1, ENTPD4, SRSF1, POLR2D, AMPD1, PPWD1, AKD1, CD2BP2, PRPF40A, CHERP |
| 8152  | 1.95x10^2 | Metabolic process                           | NT5E, PABPN1, ENTPD4, SRSF1, POLR2D, AMPD1, PPWD1, AKD1, CD2BP2, PRPF40A, CHERP |
| 90304 | 9.29x10^6 | Nucleic acid metabolic process              | NT5E, PABPN1, SRSF1, POLR2D, PPWD1, CD2BP2, PRPF40A, CHERP |
| 44260 | 4.95x10^4 | Cellular macromolecule metabolic process    | NT5E, PABPN1, SRSF1, POLR2D, PPWD1, CD2BP2, PRPF40A, CHERP |
| 43170 | 1.20x10^2 | Macromolecule metabolic process             | NT5E, PABPN1, SRSF1, POLR2D, PPWD1, CD2BP2, PRPF40A, CHERP |
| 6396  | 2.09x10^7 | RNA processing                             | PABPN1, SRSF1, POLR2D, PPWD1, CD2BP2, PRPF40A, CHERP |
| 16070 | 7.49x10^6 | RNA metabolic process                       | PABPN1, SRSF1, POLR2D, PPWD1, CD2BP2, PRPF40A, CHERP |
| 10467 | 4.36x10^3 | Gene expression                             | PABPN1, SRSF1, POLR2D, PPWD1, CD2BP2, PRPF40A, CHERP |

#### F, M6

| GO-ID | P-value | Description                                | Genes in test set                      |
|-------|---------|--------------------------------------------|----------------------------------------|
| 48583 | 8.51x10^-9 | Regulation of response to stimulus         | IL10, CD40LG, SERPINE1, CCL2, AKT1, VEGFA, ICAM1 |
| 32879 | 8.51x10^-10 | Regulation of localization                 | IL10, CD40LG, SERPINE1, CCL2, AKT1, VEGFA, ICAM1 |
| 48522 | 1.05x10^-6 | Positive regulation of cellular process    | IL10, CD40LG, SERPINE1, CCL2, AKT1, VEGFA, ICAM1 |
eight of the hub genes were included in module 1 (COL2A1 and COL13A1; Fig. 2A), module 3 (SOCS3; Fig. 2C), module 6 (IL10, AKT1, ICAM1 and VEGFA; Fig. 2F) and module 7 (GN4; Fig. 2G), suggesting that these eight genes may be particularly crucial for OLF.

Subsequently, BINGO was used to predict the function of these genes in the sub-modules. The results demonstrated that COL2A1 and COL13A1 in module 1 were involved in ‘anatomical structure development’, ‘skeletal system development’ and ‘cell adhesion’; SOCS3 in module 3 was involved in ‘negative regulation of insulin receptor signaling pathway’, ‘JAK-STAT cascade’ and ‘regeneration’; IL10, AKT1, ICAM1 and VEGFA in module 6 were involved in ‘negative regulation of apoptosis’ or ‘regulation of immune system process’; and GN4 in module 7 participated in ‘signaling pathway’ (Table III).
and ‘Hsa04660: T cell receptor signaling pathway’ (IL10; Table IV; Fig. 4).

**Discussion**

Although the same datasets were used from the study by Han et al (22), the present study applied several different bioinformatics methods aiming to screen crucial molecular mechanisms for OLF: i) Hub genes were identified by constructing the PPI network, ranking the nodes according to the topological properties and extracting the sub-modules; ii) the target genes of miRNAs were predicted using the miRwalk database, which contained 12 prediction algorithms, not only three; and iii) the key lncRNAs were identified on the basis of the lncRNA-miRNA-mRNA ceRNA regulatory network, not the lncRNA-mRNA co-expression network. Accordingly, the present study may provide certain novel miRNAs and lncRNAs for explaining the pathogenesis of OLF, and developing novel therapeutic approaches for OLF.

As a result, it was identified, for the first time to the best of the authors’ knowledge, that mir-210-3p may be a key miRNA for OLF by regulating immune-related gene IL10. LncRNA SnHG16, ASB16-aS1 and NEAT1 may also be important by acting as ceRNAs for miR-196a-5p, miR-379-5p and miR-181b-5p to modulate the expression levels of miRNA target genes SOC3, GN4 and ADCY5, respectively. SOC3 was involved in ‘response to hypoxia’, ‘regulation of apoptosis’ and ‘regeneration’, while GN4 and ADCY5 participated in the ‘Chemokine signaling pathway’. All these miRNAs were hub genes in the PPI network.

Previous studies have demonstrated that inflammatory cytokines promote hypertrophy and ossification of LF cells, but only a number of them (TNF-α, IL-1α and IL-6) have been investigated (13-15). The present study predicted that IL10,
### Table IV. Function enrichment for genes in microRNA-mRNA network.

#### A. Biological process

| Term | P-value | Genes |
|------|---------|-------|
| GO:0042127—regulation of cell proliferation | 4.65x10^{-5} | FGF18, BAP1, EIF5A, GIA1, SESN1, GLI3, IL10, CDH5, MSX2, SERPINE1, SPN, IHH, PTGER2, ESRRB, RBBP4, TNNFRSF13C, LIFR, SKI, CDC25B, NCK2, PRKCQ, ATF3, ADM, VEGFA, MYO16, HBEGF, HGS, LAMC1, TGFB1I1, PLAU |
| GO:0002250—adaptive immune response | 2.87x10^{-4} | EXO1, C8B, CADM1, CD40LG, SLA2, VEGFA, IL10, RAB27A |
| GO:0010033—response to organic substance | 4.04x10^{-4} | CALCRI, IL1R1, DRD1, LEPR, ADCY5, CCL5, IL10, MSX2, COL6A2, SCARB1, PPP3CA, PIK3R3, GNG4, DAH2, IHH, MB, IAK1, ACADS, SOCS3, STRN3, LIFR, PPARGC1B, ERPP4, PRKCQ, ADM, TFRC, FGFI8, ESRRB, COL13A1, DMP1, COL2A1, FRZB, GLI3, CHAD, MSX2, COL9A2, TNFSF11, PHEX, CHRD, ADAMTS4, IHH |
| GO:0001501—skeletal system development | 9.07x10^{-4} | CALCRI, DRD1, ACADS, SOCS3, STRN3, LEPR, ADCY5, CCL5, IL10, PPARGC1B, PRKCQ, ADM, PIK3R3, GNG4, IHH, MB |
| GO:0009719—response to endogenous stimulus | 1.23x10^{-4} | CALCRI, DRD1, ACADS, SOCS3, STRN3, LEPR, ADCY5, CCL5, IL10, PPARGC1B, PRKCQ, ADM, PIK3R3, GNG4, IHH, MB |
| GO:0009725—response to hormone stimulus | 1.24x10^{-4} | CALCRI, DRD1, ACADS, SOCS3, STRN3, LEPR, ADCY5, CCL5, IL10, PPARGC1B, PRKCQ, ADM, PIK3R3, GNG4, IHH, MB |
| GO:0042060—wound healing | 1.36x10^{-3} | PRKCQ, FOX2A, CD40LG, SAA1, SERPINE1, GNA12, HBEGF, SCARB1, LMAN1, PLAU, RAB27A |
| GO:0009611—response to wounding | 1.37x10^{-3} | YWHAZ, FOX2A, GNA12, LYZ, LMAN1, CCL5, IL10, C8B, PRKCQ, ADM, TFRC, CD40LG, SAA1, SERPINE1, HBEGF, NFE2L1, SCARB1, CTSB, PLAU, RAB27A |
| GO:0007155—cell adhesion | 1.42x10^{-3} | PVRI, CLDN8, DCHS2, TYRO3, CADM1, COL13A1, CLDN3, COL22A1, CTNND2, COL2A1, ITGA4, CCL5, CDH5, CHAD, ISLR, CD40LG, COL6A2, COL6A1, LAMC2, SCARB1, CNTN3, LAMC1, TGFB1I1, PARVA |
| GO:0022610—biological adhesion | 1.45x10^{-3} | PVRI, CLDN8, DCHS2, TYRO3, CADM1, COL13A1, CLDN3, COL22A1, CTNND2, COL2A1, ITGA4, CCL5, CDH5, CHAD, ISLR, CD40LG, COL6A2, COL6A1, LAMC2, SCARB1, CNTN3, LAMC1, TGFB1I1, PARVA |
| GO:0002684—positive regulation of immune system process | 2.14x10^{-3} | PVRI, C8B, IAK1, PRKCQ, NCK2, CADM1, SLA2, VEGFA, CD247, TNNFRSF13C, AP3D1, SPN, FOX2A, CD40LG, SAA1, SERPINE1, GNA12, LMAN1, PLAU, RAB27A |
| GO:0007599—hemostasis | 2.16x10^{-3} | PVRI, CLDN8, DCHS2, TYRO3, CADM1, COL13A1, CLDN3, COL22A1, CTNND2, COL2A1, ITGA4, CCL5, CDH5, CHAD, ISLR, CD40LG, COL6A2, COL6A1, LAMC2, SCARB1, CNTN3, LAMC1, TGFB1I1, PARVA |
| GO:0016337—cell-cell adhesion | 2.28x10^{-3} | PVRI, CLDN8, DCHS2, TYRO3, CADM1, COL13A1, CLDN3, COL22A1, CTNND2, COL2A1, ITGA4, CCL5, CDH5, CHAD, ISLR, CD40LG, COL6A2, COL6A1, LAMC2, SCARB1, CNTN3, LAMC1, TGFB1I1, PARVA |
| GO:0008285—negative regulation of cell proliferation | 2.91x10^{-3} | RBBP4, BAP1, GIA1, SKI, SESN1, GLI3, IL10, CDH5, MSX2, NCK2, ADM, MYO16, HGS, TGFB1I1, PARVA |
| GO:0048545—response to steroid hormone stimulus | 4.86x10^{-3} | CALCRI, ADM, SOCS3, ACADS, STRN3, LEPR, CCL5, IL10, PPARGC1B, IHH |
| GO:0016477—cell migration | 6.55x10^{-3} | PVRI, NCK2, DRD1, ULK1, SAA1, HBEGF, SCARB1, LAMC1, ITGA4, CCL5, IL10, PLAU |
| GO:0016055—Wnt receptor signaling pathway | 6.81x10^{-3} | FZD9, WNT7B, FZD10, FRAT1, FRAT2, TGFB1I1, FRZB, FZD7 |
| Term                                      | P-value       | Genes                                                                 |
|-------------------------------------------|---------------|----------------------------------------------------------------------|
| GO:0001666~response to hypoxia            | 7.08x10^-3    | PRKQ, TFRC, ADM, SOCS3, CLDN3, VEGFA, PLAU, MB                       |
| GO:0042981~regulation of apoptosis        | 7.81x10^-3    | IRAK1, YWHAZ, CADM1, SOCS3, EIF5A, TRIO, COL2A1, FOXO3, GLI3, IL10, MSX2, CD40LG, BAG3, VEGFA, DIABLO, PSENN, DNAJC5, CTSB, DDHA2, ARHGIDIA, TRAF4, SPN, RAB27A, IHH |
| GO:0044271~nitrogen compound biosynthetic process | 8.29x10^-3    | FECH, ATP4A, ADCY5, AK5, CMPK2, ADM, CPOX, NFE2L1, THNSL1, FPGS, DDHA2, IMPDH1, NT5E |
| GO:0010648~negative regulation of cell communication | 8.62x10^-3    | DRD1, SOCS3, STRN3, SLA2, HGS, SKI, TGFB11, FRZB, CHRD, IHH, RGS13 |
| GO:0043067~regulation of programmed cell death | 8.74x10^-3    | IRAK1, YWHAZ, CADM1, SOCS3, EIF5A, TRIO, COL2A1, FOXO3, GLI3, IL10, MSX2, CD40LG, BAG3, VEGFA, DIABLO, PSENN, DNAJC5, CTSB, DDHA2, ARHGIDIA, TRAF4, SPN, RAB27A, IHH |
| GO:0010941~regulation of cell death       | 9.12x10^-3    | IRAK1, YWHAZ, CADM1, SOCS3, EIF5A, TRIO, COL2A1, FOXO3, GLI3, IL10, MSX2, CD40LG, BAG3, VEGFA, DIABLO, PSENN, DNAJC5, CTSB, DDHA2, ARHGIDIA, TRAF4, SPN, RAB27A, IHH |
| GO:0001503~ossification                   | 1.24x10^-2    | FGF18, TNFSF11, COL13A1, DMP1, COL2A1, CHRD, IHH                      |
| GO:0048870~cell motility                  | 1.38x10^-2    | PVR, NCK2, DRD1, ULK1, SAA1, HBEGF, SCARB1, LAMC1, ITGA4, CCL5, IL10, PLAU |
| GO:0051674~localization of cell           | 1.38x10^-2    | PVR, NCK2, DRD1, ULK1, SAA1, HBEGF, SCARB1, LAMC1, ITGA4, CCL5, IL10, PLAU |
| GO:0043066~negative regulation of apoptosis | 1.55x10^-2    | MSX2, IRAK1, YWHAZ, CD40LG, SOCS3, BAG3, VEGFA, DNAJC5, COL2A1, DDHA2, IHH |
| GO:0060348~bone development               | 1.68x10^-2    | FGF18, TNFSF11, COL13A1, DMP1, COL2A1, CHRD, IHH                      |

B, KEGG pathway

| Term                                      | P-value       | Genes                                                                 |
|-------------------------------------------|---------------|----------------------------------------------------------------------|
| hsa04512:ECM-receptor interaction         | 4.33x10^-3    | COL4A2, COL6A2, COL6A1, LAMC2, COL2A1, LAMC1, ITGA4, CHAD            |
| hsa04510:Focal adhesion                   | 1.00x10^-2    | COL4A2, VEGFA, COL6A2, COL6A1, LAMC2, COL2A1, LAMC1, ITGA4, PIK3R3, CRK, CHAD, PARVA |
| hsa00230: Purine metabolism               | 1.25x10^-2    | PDE7B, PDE2A, ADCY5, PDE4A, ENTPD6, AK5, ENTPD4, POLR2D, NT5E, IMPDH1 |
| hsa04150: mTOR signaling pathway          | 3.80x10^-2    | ULK1, VEGFA, ULK3, PKRAA2, PIK3R3                                     |
| hsa04514: Cell adhesion molecules (CAMs)   | 4.30x10^-2    | PVR, CLDN8, CADM1, CD40LG, CLDN3, ITGA4, CDH5, SPN                   |
| hsa04310: Wnt signaling pathway           | 4.77x10^-2    | FZD9, WNT7B, FZD10, PPP2R5B, FRAT1, FRAT2, PPP3CA, FZD7              |
| hsa04920: Adipocytokine signaling pathway  | 4.82x10^-2    | PRKQ, SOCS3, RXRB, LEPR, PKRAA2                                      |
| hsa04660: T cell receptor signaling pathway| 4.90x10^-2    | PRKQ, NCK2, CD40LG, CD247, PPP3CA, PIK3R3, IL10                     |

GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.
SOCS3 and ADCY5 may be anti-inflammatory due to their downregulation, while GNG4 may be pro-inflammatory due to its upregulation in OLF. The associations of the identified genes with inflammation can be indirectly confirmed. For example, IL10 is a known anti-inflammatory cytokine that was identified to have lower expression in subligamentous type of disc degeneration (8). SOCS3 may mediate the blockade of inflammation by inhibiting Janus kinase-STAT3 activity and to prevent the abnormal expression of il-6 (32,33). ADCY5 was also demonstrated to be significantly downregulated in cytokine-related hepatocellular carcinoma (34) and prostate cancer (35). Although GNG4 was previously demonstrated to be downregulated in glioblastoma cells and exogenous overexpression of GNG4 can inhibit stromal cell-derived factor 1/C-X-C motif chemokine receptor 4-dependent chemokine signaling (36), two recent studies observed that GNG4 was significantly upregulated in patients with colon cancer (37) and cardiovascular events (38), indicating its potential pro-inflammatory and pro-proliferation roles. In agreement with these two studies, the present study additionally identified that GNG4 was upregulated in LF cells.

Although there have been previous studies that examined the roles of miRNAs in OLF, all of these studies focused on miRNAs that regulate osteogenic differentiation related genes (17-20,39). miRNAs related with inflammation and cell proliferation in OLF have rarely been reported. Using

Figure 4. Function enrichment for the genes in the microRNA-mRNA interaction network. (A) GO analysis and (B) KEGG pathways. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; ECM, extracellular matrix.
The present study identified that miR-210-3p, miR-196a-5p, and miR-181b-5p targeting anti-inflammatory IL10, SOCS3, and ADCY5, respectively, were upregulated, but miR-379-5p, which targets pro-inflammatory GNG4, was downregulated in OLF. The interaction associations between miR-210 and miR-196 and their target genes has been demonstrated in other inflammatory diseases. For example, administration of agomir-210...
Table V. Function enrichment for genes in the long non-coding RNA-microRNA-mRNA competing endogenous RNA network.

### A. Biological process

| Term                                                                 | P-value       | Genes                                                                 |
|----------------------------------------------------------------------|---------------|------------------------------------------------------------------------|
| GO:0002684--positive regulation of immune system process             | 1.01x10^3    | PVR, C8B, PRKCQ, NCK2, CADM1, SLA2, VEGFA, CD247, SPN                  |
| GO:0010033--response to organic substance                            | 3.44x10^3    | IL1R1, SOCS3, STRN3, LEPR, ADCY5, PPARGC1B, MSX2, ERF44, PRKCQ, ADM, TFRC, COL6A2, PIK3R3, GNG4, IHH |
| GO:0007166--cell surface receptor linked signal transduction        | 3.57x10^3    | IL1R1, LEPR, ADCY5, GNA12, CD247, FST, CCL28, LG4, ADCYAP1, MSX2, NMUR1, OXER1, FRAT1, FRAT2, GNG4, PIK3R3, SPN, PTGER2, SLA2, ITGA4, FZD7, RGS13, EPS15, NCK2, WT7NB, ADM, P2RY14, VEGFA |
| GO:009725--response to hormone stimulus                              | 4.30x10^3    | PRKCQ, ADM, SOCS3, STRN3, ADCY5, LEPR, PIK3R3, GNG4, PPARGC1B, IHH    |
| GO:0042127--regulation of cell proliferation                         | 7.37x10^3    | PTGER2, RBPP4, BAP1, CDC25B, MSX2, PRKCQ, NCK2, ATF3, ADM, VEGFA, SERPINE1, MYO16, LAMC1, SPN, IHH |
| GO:009719--response to endogenous stimulus                           | 8.05x10^3    | PRKCQ, ADM, SOCS3, STRN3, ADCY5, LEPR, PIK3R3, GNG4, PPARGC1B, IHH    |
| GO:0043062--extracellular structure organization                      | 1.31x10^2    | WNT7B, CADM1, ANK3, DMP1, COL6A2, LAMC1                                |
| GO:0007568--aging                                                    | 1.48x10^2    | PRKCQ, TFRC, ADM, SOCS3, SERPINE1                                     |
| GO:0045137--development of primary sexual characteristics            | 2.37x10^2    | LEPR, FST, VEGFA, FOXO3, LG4                                           |
| GO:0048545--response to steroid hormone stimulus                     | 2.48x10^2    | ADM, SOCS3, STRN3, LEPR, PPARGC1B, IHH                                 |
| GO:0008284--positive regulation of cell proliferation               | 2.60x10^2    | PRKCQ, NCK2, ATF3, ADM, VEGFA, LAMC1, SPN, CDC25B, IHH                |
| GO:0001666--response to hypoxia                                      | 2.81x10^2    | PRKCQ, TFRC, ADM, SOCS3, VEGFA                                       |
| GO:0008202--steroid metabolic process                                | 2.99x10^2    | CYP3A4, SOAT1, SULT2A1, ADM, LEPR, PRKAA2                             |
| GO:0016337--cell-cell adhesion                                       | 3.15x10^2    | PVR, CADM1, COL13A1, CTNND2, COL6A2, ITGA4, CHAD                     |
| GO:0070482--response to oxygen levels                                | 3.31x10^2    | PRKCQ, TFRC, ADM, SOCS3, VEGFA                                       |
| GO:0050778--positive regulation of immune response                  | 3.61x10^2    | PVR, C8B, CADM1, SLA2, CD247                                         |
| GO:0007548--sex differentiation                                       | 4.09x10^2    | LEPR, FST, VEGFA, FOXO3, LG4                                           |

### B. KEGG pathway

| Term                                                                 | P-value       | Genes                                                                 |
|----------------------------------------------------------------------|---------------|------------------------------------------------------------------------|
| hsa00230:Purine metabolism                                           | 3.14x10^3    | PDE7B, ADCY5, PDE4A, ENTPD6, AK5, ENTPD4, POLR2D, NT5E                 |
| hsa04510:Focal adhesion                                              | 3.77x10^3    | VEGFA, COL6A2, COL6A1, LAMC2, LAMC1, ITGA4, PIK3R3, CRK, CHAD         |
| hsa04512:ECM-receptor interaction                                    | 4.15x10^3    | COL6A2, COL6A1, LAMC2, LAMC1, ITGA4, CHAD                             |
| hsa04920:Adipocytokine signaling pathway                             | 1.02x10^2    | PRKCQ, SOCS3, RXRB, LEPR, PRKAA2                                      |
| hsa04150:nTOR signaling pathway                                      | 2.82x10^2    | VEGFA, ULK3, PRKAA2, PIK3R3                                           |
| hsa04062:Chemokine signaling pathway                                | 2.90x10^2    | ADCY5, FOXO3, PIK3R3, GNG4, CRK, CCL28                                |
| hsa04310:Wnt signaling pathway                                       | 3.13x10^2    | WNT7B, PPP2RB, FRAT1, FRAT2, FZD7                                     |
| hsa04660:T cell receptor signaling pathway                          | 3.16x10^2    | PRKCQ, NCK2, CD247, PIK3R3                                           |
| hsa03320:PPAR signaling pathway                                      | 3.22x10^2    | RXRB, SCD, SLC27A                                                     |
| hsa04514:Cell adhesion molecules (CAMs)                              | 3.23x10^2    | PVR, CADM1, ITGA4, SPN                                               |
| hsa04810:Regulation of actin cytoskeleton                            | 3.29x10^2    | GNA12, RDX, ITGA4, PIK3R3, CRK                                       |

GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.
significantly upregulated IL-10 and attenuated cellular apoptosis and inflammation in an injured rat spinal cord, ultimately improving functional recovery (40). Ectopic expression of miR-196 promoted stemness and chemoresistance of colorectal cancer cells by targeting SOCS3, a negative regulator of the STAT3 signaling pathway (41). mir-181b has been reported to stimulate inflammation via the nuclear factor-κB signaling pathway (42), while miR-379 significantly suppresses the invasive capacity of cancer cells by inhibiting cytokine IL-18 (43). These findings may indirectly verify the important roles of these miRNAs in inflammatory OLF.

Furthermore, the present study also identified several crucial IncRNAs that regulated the mentioned inflammation and cell proliferation related genes based on the ceRNA hypothesis, including downregulated IncRNA SNHG16/NEAT1 and upregulated ASB16-AS1. Although their mechanisms in OLF require confirmation in further experiments, previous studies have indirectly identified their underlying associations. Zhao et al (44) demonstrated that NEAT1 was decreased in primary acute myeloid leukemia cells and THP-1 monocytes compared with normal cells; overexpression of NEAT1 inhibits cell proliferation,

Figure 7. Function enrichment for the genes in the IncRNA-miRNA-mRNA interaction network. (A) GO analysis and (B) KEGG pathways. IncRNA, long non-coding RNA; miRNA, microRNA; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.
promotes apoptosis and affects the cell cycle. Overexpressed ASB16-AS1 has been reported to increase the expression of osteoblastogenesis-related genes (BMP2 and ALP) (45) which were previously demonstrated to be induced by inflammatory cytokines (14). The roles of SNHG16 on cell proliferation may be controversial, although the majority of studies have demonstrated that SNHG16 may functions as an oncogene (46,47). However, the present study identified that expression of SNHG16 decreased in LF cells of patients with OLF compared with the controls and further investigation is necessary to elucidate the underlying biological associations between SNHG16 and OLF.

In addition to inflammation genes, the present study also identified the significant miRNAs and IncRNAs associated with osteogenic differentiation related genes. miR-329-3p and miR-222-5p were involved in ossification by regulating COL13A1 and COL2A1, respectively. RHPN1-AS1 functioned as a ceRNA for miR-299-5p to influence the Wnt signaling pathway through WNT7B. These results were in agreement with a previous study, in which inhibition of miR-222-3p in human bone mesenchymal stem cells promoted the expression of osteoblast-specific genes, ALP activity, and matrix mineralization, while overexpression of miR-222-3p inhibited osteoblast differentiation (48). The roles of other miRNAs and IncRNAs require further investigation.

There are certain limitations to the present study. Only two datasets were included to examine the molecular mechanisms of OLF due to limited previous studies. Also, the current sample size of these datasets was small. Therefore, further studies using high-throughput sequencing experiments with larger clinical samples would be valuable. Another limitation is that this is a preliminary study to identify the crucial miRNAs and IncRNAs for OLF. Further in vitro and in vivo experiments are necessary to confirm the expression levels of these identified miRNAs and IncRNAs in OLF, and to demonstrate the regulatory associations between them and the downstream DEGs.

In conclusion, the present study identified several inflammation and osteogenic differentiation related miRNA-miRNA interaction axes (SNHG16-hsa-miR-196a-5p-SOCS3, ASB16-AS1-hsa-miR-379-5p-GNG4, NEAT1-hsa-miR-181b-5p-ADCY5 and RHPN1-AS1-hsa-miR-299-3p-WNT7B), which may be involved in the pathogenesis of OLF. These miRNAs and IncRNAs may be natural, endogenous and nontoxic drug targets for the treatment of OLF.

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Availability of data and materials

The microarray data GSE106253 and GSE106256 were downloaded from The Gene Expression Omnibus database in National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/geo/).

Authors' contributions

DK and FW were involved in the conception and design of this study. DK and QZ collected the data and performed the bioinformatics analyses. WL prepared the figures and interpreted the data. DK drafted the manuscript. FW revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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