Expression profiles of long non-coding RNAs in the cartilage of patients with knee osteoarthritis and normal individuals

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Abstract. Knee osteoarthritis is caused by a multifactorial imbalance in the synthesis and degradation of knee chondrocytes, subchondral bone and extracellular matrix. Abnormal expression of long non-coding RNAs (lncRNAs) affects the metabolism, synovitis, autophagy and apoptosis of chondrocytes, as well as the production of cartilage matrix. The aim of the present study was to identify novel targets for the treatment of osteoarthritis and to examine the pathogenesis of the disease. The lncRNA expression profiles of seven patients with knee osteoarthritis and six healthy controls were examined by RNA-sequencing. Differentially expressed lncRNAs were selected for bioinformatics analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. Reverse transcription-quantitative PCR (RT-qPCR) was used to further investigate the differential expression of the lncRNAs. A total of 23,583 lncRNAs were identified in osteoarthritis cartilage, including 5,255 upregulated and 5,690 downregulated lncRNAs, compared with normal cartilage. Although there were more downregulated lncRNAs compared with upregulated lncRNAs, among the changed lncRNAs (fold-change >6), there were more upregulated lncRNAs compared with downregulated lncRNAs. Several lncRNAs exhibiting differences were identified as potential therapeutic targets in knee osteoarthritis. GO and KEGG pathway analyses were performed for the target genes of the differentially expressed lncRNAs. RT-qPCR validation was performed on three randomly selected upregulated and downregulated lncRNAs. The results of RT-qPCR were consistent with the findings obtained by RNA-sequencing analysis. The findings from the present study may contribute to the diagnosis of osteoarthritis and may predict the development of osteoarthritis. Furthermore, the differentially expressed lncRNAs may aid in the identification of novel candidate targets for the treatment of knee osteoarthritis.

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Abbreviations: lncRNA, long non-coding RNA; NC, normal group; OA, knee osteoarthritis group; RT-qPCR, reverse transcription-quantitative PCR; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; ITGA5, integrin-α5; H MGB1, high mobility group protein B1; IFITM3; interferon induced transmembrane protein 3

Key words: knee osteoarthritis, long non-coding RNAs, osteoarthritis pathogenesis, RNA-sequencing

Introduction

Knee osteoarthritis is the most common chronic degenerative bone and joint disease in middle-aged and elderly individuals worldwide (1). Knee osteoarthritis is a heterogeneous disease with an incidence of ~3% (2,3), and results from a multifactorial imbalance in the synthesis and degradation of knee chondrocytes subchondral bone and extracellular matrix (4). Abnormal joint metabolism leads to degeneration of knee articular cartilage and subchondral bone, joint fiber hyperplasia, synovial non-specific inflammation and other lesions (5). Due to the continuous damage caused by chronic inflammation and the progressive structural changes of the knee joint tissue, the disease progresses continuously (6). An irreversible loss of knee function and pain are among the common causes of decreased quality of life and disability associated with knee osteoarthritis (7).

The main clinical manifestations of knee osteoarthritis are slow-developing knee pain, tenderness, stiffness, joint swelling, limited mobility and knee deformity (8). The occurrence and development of knee osteoarthritis are the result of the interaction between biomechanical and biological factors, including the mechanical environment around the knee joint, the metabolic rate of the individual, the apoptosis of chondrocytes and the levels of related cytokines, such as interleukin (IL) IL-4, IL-10 and tumor necrosis factor (TNF)-α (9-11). A number of risk factors for knee osteoarthritis have been identified, including age, sex, obesity, inflammation, strain, trauma and genetics (12-14). The development and progression of knee osteoarthritis is complex and at present, effective

treatment strategies and clear therapeutic targets have not been identified (15).

Long non-coding RNAs (lncRNAs) are RNA molecules >200 nucleotides in length that are the by-product of RNA polymerase II transcription, and were previously thought to have no biological function (16). lncRNAs do not encode proteins, but play a critical role in gene regulation and epigenetic modifications (17-19). lncRNA affect cartilage matrix production and metabolism, synovitis, autophagy and apoptosis of chondrocytes (20,21). Gaballah *et al* (18) reported that ZNF1 antisense RNA 1 can promote chondrocyte proliferation and inhibit apoptosis by regulating the expression level of Wnt family member 3A. A previous study by Wang *et al* (22) suggested that nuclear paraspeckle assembly transcript 1 can act on microRNA-181c to promote the proliferation of human synoviocytes and accelerate the progression of osteoarthritis (22). Although the specific function of lncRNAs has not been elucidated, previous studies have reported that the abnormal expression of lncRNAs is closely related to osteoarthritis (23,24).

The aim of the present study was to examine the lncRNA expression profiles of cartilage from young patients with amputations without joint lesions, and from patients with knee osteoarthritis who underwent total knee arthroplasty. The lncRNA expression profiles of 13 patients were compared to identify new potential regulatory targets involved in the pathogenesis of the disease. The results of the present study may aid in identifying the role of lncRNAs in the progression of knee osteoarthritis.

Materials and methods

Subjects. The present study recruited 13 patients from the Department of Orthopedic Surgery, The Second Affiliated Hospital of Anhui Medical University, between February 2016 and December 2018, including six males and seven females. The 13 patients were divided into two groups: Six patients aged 29-49 years in the normal group (NC), and seven patients aged 67-82 years in the knee osteoarthritis group (OA; average age 38.17±7.70 vs. 71.71±6.02 years). The NC group was made up of young patients with amputations without joint disease. Inclusion criteria for the NC group were as follows: i) No history of joint lesions; and ii) lower limb injury treated with amputation. All patients in the OA group were patients with knee osteoarthritis who underwent total knee arthroplasty. The inclusion criteria for the OA group were as follows: i) Diagnosed with knee osteoarthritis based on clinical manifestations, combined with X-ray examination, knee pain and severely affected quality of life, with failure of conventional oral drug therapy; ii) patients with primary total knee arthroplasty; iii) knee flexion malformation <15˚, varus deformity <15˚ or valgus deformity <15˚; and iv) Kellgren Lawrence III-IV level (25). Exclusion criteria for both groups were as follows: i) Severe internal and external valgus and flexion contracture deformity (flaw angle >15˚); ii) obese patients (body mass index >35); iii) patients with heart and lung dysfunction, cerebrovascular disease or diabetes; iv) history of open joint surgery; and v) active infection. The present study was approved by The Second Affiliated Hospital of Anhui Medical University Ethics Committee. Written informed consent was obtained from each patient or their relatives prior to surgery.

Sample collection and total RNA extraction. Approximately 5 g of cartilage tissue was collected from each patient and stored at -80˚C until further analysis. Total RNA was extracted from the tissue suspension using the TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The tissue was homogenized prior to RNA extraction. The amount and purity of the total RNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.), and RNA integrity was analyzed using an RNA Nano 6000 kit from the Agilent Bioanalyzer 2100 system (Agilent Technologies, Inc.).

Preparation for lncRNA-sequencing library. A lncRNA library was constructed using 3 μg of total RNA per sample. The Ribo-Zero™ Gold kit (Illumina, Inc.) was used to remove the rRNA from the sample and select the different index tags to build the library according to the manufacturer's instructions. The NEB Next Ultra™ Directional RNA Library Prep kit for Illumina (New England BioLabs, Inc.) was used to construct a sequencing library using rRNA-depleted RNA, according to the manufacturer's instructions.

High-throughput sequencing. Sequencing libraries were generated using the NEBNext Ultra™ Directional RNA LibraryPrep kit for Illumina (New England BioLabs, Inc.) according to the manufacturer's protocol. Raw reads were obtained by Illumina sequencing and high-quality sequences (clean reads) were obtained through a series of data processing procedures such as removing low-quality sequences, de-linking contamination and rRNA including removing reads of joint contamination (the base number of joint contamination in reads was greater than 5 bp, removing low-quality reads (reads intermediate value Q ≤19 accounted for >50% of the total base), removing reads with N ratio >5% and removing reads matched with rRNA.

All subsequent analyses were based on clean reads. Library sequencing was carried out on a HiSeq 4000 platform (Illumina, Inc.) according to the commercially available protocols from Chongqing Western Biotechnology, Inc.

Identification of differentially expressed lncRNAs. The analysis of differences in lncRNA expression between the two groups (NC and OA group) was performed using the DEGseq (2010) R package (http://dblp.uni-trier.de/db/journals/bioinformatics/bioinformatics26.html#WangFWWZ10). The P-value was adjusted using the q-value. P<0.05 and |log2 (fold-change)|>1 were set as the threshold for significant differential expression.

Reverse transcription-quantitative PCR (RT-qPCR). To validate the reliability of the RNA-sequencing data, 6 differentially expressed lncRNAs were randomly selected and RT-qPCR was used to examine the expression level of the lncRNAs. Total RNA was extracted from the cartilage using the TRIzol reagent (Invitrogen, Thermo Fisher

Scientific, Inc.) according to the manufacturer's instructions. RT-qPCR reactions were performed using the Luna Universal One-Step RT-qPCR kit (New England BioLabs, Inc.) according to the manufacturer's instructions. The following thermocycling conditions were used: 15 sec at 55˚C and 1 min at 95˚C, followed by 40 cycles of 10 sec at 95˚C and 30 sec at 60˚C, and 30 sec at 50˚C. GAPDH was used as an internal control and the relative expression levels of candidate lncRNAs were calculated using the $2^{-\Delta\Delta Cq}$ method (26). GAPDH forwards primer, AATGGG CAGCCGTTAGGAAA and reverse primer, GCCCAATAC GACCAAATCAGAG. Experiments were conducted in at least triplicate. The primer sequences that were used in the present study are listed in Table SI.

GO and KEGG enrichment analyses. Target mRNAs of the differentially expressed lncRNAs were classified according to the principles of GO classification. GO collects information from GO and NCBI databases, annotating and classifying genes based on biological processes, molecular functions and cellular components (http://www.geneontology.org/). KEGGis a comprehensive database for the systematic analysis of gene function, which is based on manually drawn metabolic pathways and is divided into metabolism, genetic information processing, cellular processes, environmental information processing, biological systems and human diseases (http://www.genome.jp/kegg/).

Statistical analysis. Data analyses were performed using SPSS software (version 19.0; IBM Corp.) and data are expressed as the mean ± SD. Comparisons of data between groups were performed by the Student's t-test. Categorical data were analyzed by the χ^2 test. $P<0.05$ was considered to indicate a statistically significant difference.

Table I. Baseline clinical characteristics.

Variable	NC, n=6	OA, n=7	P-value
Age, years (mean ± SD)	38.17±7.70	71.71±6.02	0.001
Sex (male/female)	3/3	3/4	0.817
BMI (mean ± SD)	20.82±2.11	22.16±3.09	0.389
Side (left/right)	2/4	4/3	0.751

NC, normal control group; OA, osteoarthritis group; BMI, body mass index.

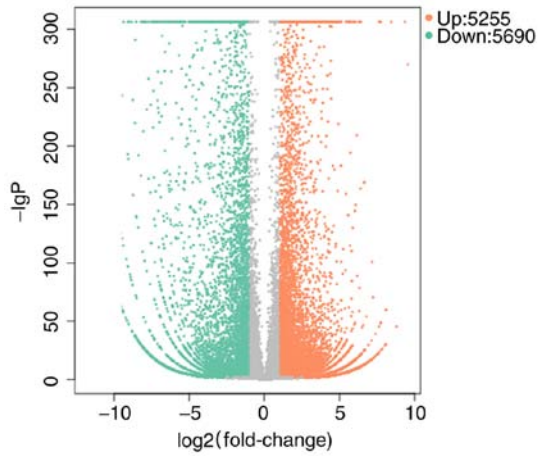

Figure 1. Volcano plot of differential lncRNA expression (OA vs. NC group). X-axis, log2 (fold-change). Y-axis, -1 x log10 (corrected P-value) for each probe. Grey dots represent no differential lncRNA expression. lncRNA, long non-coding RNA; OA, osteoarthritis; NC, normal control; up, upregulated; down, downregulated.

Results

Clinical characteristics. The clinical characteristics of the 13 patients are presented in Table I. The NC group consisted of six patients including three men and three women with a mean age of 38.17±7.70 years. The OA group consisted of seven patients including three women and three men with a mean age of 71.71±6.02. A significant difference was identified between the ages of the two groups (P=0.001). There were no significant differences between the two groups regarding sex, BMI and limb side (P=0.817, 0.389 and 0.751, respectively).

Differentially expressed lncRNAs. A total of 23,583 lncRNAs were identified in osteoarthritis cartilage, including 5,255 upregulated and 5,690 downregulated lncRNAs, compared with normal cartilage (log2 fold-change >1; P<0.05; Fig. 1). The most significantly upregulated lncRNA was MSTRG.95856 (log2 fold-change = 11.6488). The most significantly downregulated lncRNA was ENSG00000279725 (log2 fold-change = -9.6546). According to the microarray data, the number of downregulated lncRNAs was larger than that of the upregulated lncRNAs. However, among significantly differentially expressed lncRNAs (log2 fold-change >6), there were more upregulated than downregulated lncRNAs. Hierarchical clustering analysis displayed the differential expression of lncRNAs (Fig. 2), where red and blue represent expression values higher and lower than the median expression value, respectively. The results indicated that differences between osteoarthritis and normal cartilage could be identified by differences in the expression profiles of lncRNAs associated with osteoarthritis (Table II).

GO and KEGG pathway analyses. lncRNAs do not encode proteins and they function through cis- or trans-regulation of protein-coding genes (27). For the target genes of differentially expressed lncRNAs, the functions of these lncRNAs were indirectly predicted according to the target genes. GO and KEGG pathway analyses of the target genes of the differential lncRNAs were then performed.

Table III and Fig. 3 display the top 10 enriched GO entries with the most significant difference in target genes of lncRNAs in each group. In biological processes, target genes were mainly enriched in the metabolic process of substances, including 'organic substance metabolic process'. In cellular components, target genes were mainly enriched in 'intracellular part'. In molecular functions, target genes were mainly enriched in 'binding'. However, target genes do not appear to be mainly enriched in all of the terms listed here. Furthermore, Table IV and Fig. 4 display the KEGG pathways with the most significant

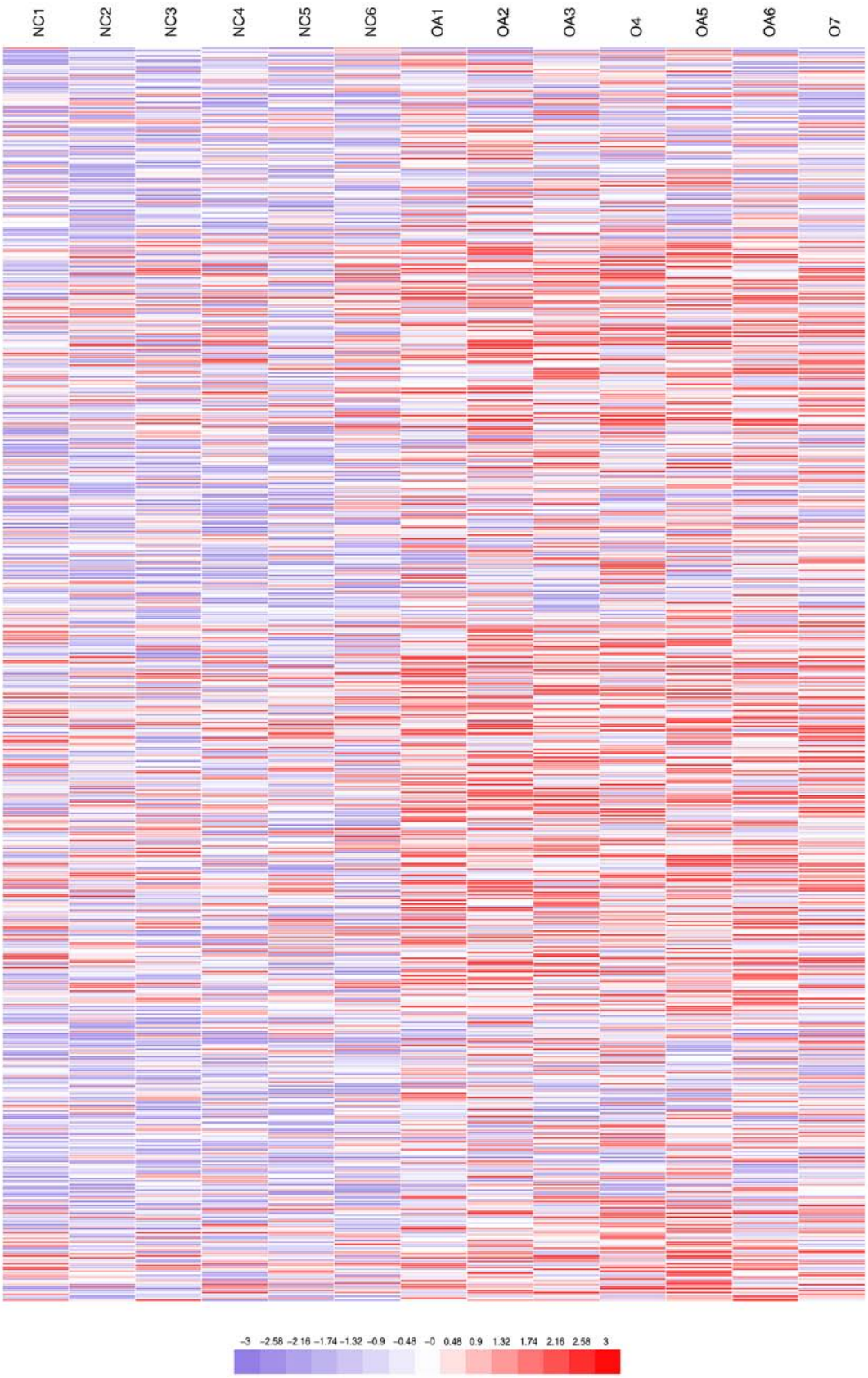

Figure 2. Hierarchical clustering of lncRNAs in the NC and OA groups. The red and blue shaded areas indicate increased and decreased expression levels, respectively, across all samples. The white areas indicate no differential lncRNA expression. lncRNA, long non-coding RNA; OA, osteoarthritis; NC, normal control.

differences in target genes for lncRNAs between the two groups. In the KEGG pathway analysis, the enrichment pathways associated with significant expression of lncRNAs were 'PI3K-Akt signaling pathway', 'endocytosis',

Table II. Most significantly expressed top 15 entries in upregulated and downregulated lncRNAs.

lncRNA ID	Position	Log2 (fold-change)	Regulation	P-value
MSTRG.95856	chr17:64319415-64413776	11.64880695	Up	$8.83x10^{-245}$
ENSG00000246430	chr8:56436674-56446734	11.12541347	Up	$3.69x10^{-122}$
ENSG00000253730	chr17:50183289-50201632	9.970804581	Up	$4.75x10^{-116}$
ENSG00000261959	chr17:50183289-50201632	9.970804581	Up	$4.35x10^{-241}$
ENSG00000214145	chr3:194355247-194369743	9.961630563	Up	$2.60x10^{-221}$
ENSG00000254855	chr11:66314487-66317044	9.259743264	Up	$4.07x10^{-232}$
ENSG00000255468	chr11:66314487-66317044	9.259743264	Up	$2.53x10^{-206}$
ENSG00000269053	chr19:17351448-17377350	8.885696373	Up	$2.93x10^{-190}$
ENSG00000282851	chr19:17351448-17377350	8.885696373	Up	$7.99x10^{-111}$
MSTRG.106332	chr19:17351448-17377350	8.885696373	Up	$3.09x10^{-214}$
MSTRG.143488	chr22:39223359-39244751	8.774787060	Up	$4.01x10^{-172}$
MSTRG.92410	chr17:31303766-31314112	8.727920455	Up	$6.30x10^{-233}$
ENSG00000227502	chr6:113857362-113863471	8.715226609	Up	$3.50x10^{-94}$
ENSG00000244161	chr3:58192257-58214697	8.640244936	Up	$7.63x10^{-237}$
ENSG00000258810	chr14:20801228-20803278	8.628141719	Up	$5.65x10^{-160}$
ENSG00000279725	chr10:127737235-127741186	-9.654636029	Down	$1.26x10^{-23}$
ENSG00000280302	chr18:12254319-12277595	-8.214319121	Down	$1.22x10^{-79}$
ENSG00000250012	chr3:126103562-126197994	-7.740118043	Down	$6.01x10^{-116}$
MSTRG.155259	chr3:126103562-126197994	-7.740118043	Down	$7.25x10^{-14}$
ENSG00000231966	chr1:179743163-179816198	-6.693486957	Down	$1.35x10^{-23}$
ENSG00000203288	chr1:151806071-151831872	-6.524023102	Down	$4.52x10^{-102}$
ENSG00000234614	chr1:151806071-151831872	-6.524023102	Down	$3.04x10^{-170}$
MSTRG.13443	chr1:151806071-151831872	-6.524023102	Down	$8.04x10^{-48}$
ENSG00000229178	chr3:195614947-195620233	-6.438791853	Down	$6.39x10^{-61}$
ENSG00000242086	chr3:195614947-195620233	-6.438791853	Down	$8.84x10^{-211}$
MSTRG.162045	chr3:195614947-195620233	-6.438791853	Down	$4.62x10^{-19}$
MSTRG.162046	chr3:195614947-195620233	-6.438791853	Down	$5.26x10^{-151}$
ENSG00000227388	chr9:35752990-35756613	-6.384897330	Down	$3.85x10^{-29}$
ENSG00000226133	chr1:48102068-48104707	-6.266786541	Down	$2.40x10^{-70}$
ENSG00000261026	chr8:22713251-23000000	-6.200762405	Down	$1.36x10^{-271}$

lncRNA, long non-coding RNA.

'cytokine-cytokine receptor interaction' and 'chemokine signaling pathway'.

RT-qPCR validation of lncRNA expression. To verify the sequencing results of the lncRNAs, three upregulated lncRNAs (ENSG00000261496, ENSG00000273447 and ENSG00000269971) and three downregulated lncRNAs (ENSG00000257477, ENSG00000254369 and MSTRG.119041) were randomly selected. The expression levels of the six lncRNAs were detected by RT-qPCR in 13 cartilage samples (six NC and seven OA). The results of the RT-qPCR analysis were consistent with the microarray data (Fig. 5). Each lncRNA displayed a similar trend of upregulation or downregulation.

Discussion

lncRNAs do not encode proteins, but function at the RNA level, regulating gene expression and epigenetic modifications (28). Abnormal expression of lncRNAs is involved in the pathogenesis of a number of diseases, such as glioma, allergic dermatitis and rheumatism (29-31). Previous studies have reported that the abnormal expression of lncRNAs in osteoarthritis cartilage *in vitro* is related to the degradation of the extracellular matrix of chondrocytes, suggesting that lncRNAs are involved in the pathogenesis of osteoarthritis (32,33). However, the potential targets and functions of lncRNAs associated with skeletal development and osteoarthritis are not fully understood. The present study systematically screened the lncRNA expression profiles of cartilage from normal control patients and patients with knee osteoarthritis to identify novel lncRNA targets involved in the mechanisms underlying osteoarthritis. The new lncRNA targets might aid in identifying the role of lncRNAs in the progression of knee osteoarthritis.

The results displayed differences in the expression levels of a number of lncRNAs between normal and osteoarthritis

Table III. Top 15 significantly enriched GO terms in biological processes, cellular components and molecular functions of the differentially expressed lncRNAs.

A, Upregulated

Category	GO accession no.	Description	Corrected P-value	Total number of genes
GOTERM_BP	GO:0008152	Metabolic process	$7.1x10^{-11}$	4,779
GOTERM_BP	GO:0071704	Organic substance metabolic process	$4.2x10^{-11}$	4,573
GOTERM_BP	GO:0044237	Cellular metabolic process	$2.7x10^{-14}$	4,486
GOTERM_BP	GO:0009058	Biosynthetic process	$2.6x10^{-09}$	2,858
GOTERM_BP	GO:1901576	Organic substance biosynthetic process	$2.7x10^{-09}$	2,820
GOTERM_CC	GO:0043226	Organelle	$2.5x10^{-17}$	5,787
GOTERM_CC	GO:0043229	Intracellular organelle	$6.0x10^{-20}$	5,414
GOTERM_CC	GO:0043227	Membrane-bounded organelle	$1.4x10^{-18}$	5,316
GOTERM_CC	GO:0043231	Intracellular membrane-bounded organelle	$3.9x10^{-22}$	4,902
GOTERM_CC	GO:0005737	Cytoplasm	$2.2x10^{-11}$	4,784
GOTERM_MF	GO:0097159	Organic cyclic compound binding	$2.9x10^{-6}$	2,861
GOTERM_MF	GO:1901363	Heterocyclic compound binding	$2.3x10^{-6}$	2,836
GOTERM_MF	GO:0043167	Ion binding	$3.4x10^{-6}$	2,639
GOTERM_MF	GO:0043169	Cation binding	$6.6x10^{-5}$	1,804
GOTERM_MF	GO:0046872	Metal ion binding	$2.5x10^{-5}$	1,779

B, Downregulated

Category	GO accession no.	Description	Corrected P-value	Total number of genes
GOTERM_BP	GO:0044238	Primary metabolic process	$6.6x10^{-11}$	4,424
GOTERM_BP	GO:0043170	Macromolecule metabolic process	$7.5x10^{-15}$	3,935
GOTERM_BP	GO:0044260	Cellular macromolecule metabolic process	$4.4x10^{-17}$	3,660
GOTERM_BP	GO:0006807	Nitrogen compound metabolic process	$7.8x10^{-09}$	2,947
GOTERM_BP	GO:0044249	Cellular biosynthetic process	$1.6x10^{-10}$	2,781
GOTERM_CC	GO:0005622	Intracellular	$1.0x10^{-29}$	6,263
GOTERM_CC	GO:0044424	Intracellular part	$3.0x10^{-27}$	6,127
GOTERM_CC	GO:0044422	Organelle part	$1.6x10^{-7}$	3,740
GOTERM_CC	GO:0044446	Intracellular organelle part	$8.1x10^{-8}$	3,675
GOTERM_CC	GO:0044444	Cytoplasmic part	$4.1x10^{-5}$	3,572
GOTERM_MF	GO:0005488	Binding	$2.6x10^{-6}$	4,991
GOTERM_MF	GO:0003824	Catalytic activity	$5.0x10^{-5}$	2,445
GOTERM_MF	GO:0003676	Nucleic acid binding	$1.9x10^{-4}$	1,994
GOTERM_MF	GO:0003677	DNA binding	$8.9x10^{-10}$	1,185
GOTERM_MF	GO:0003700	Transcription factor activity, sequence-specific DNA binding	$1.1x10^{-5}$	526

GO, gene ontology; lncRNA, long non-coding RNA.

cartilage, ranging from a few to several thousands. The variable differences may be attributed to the small sample size. However, RT-qPCR was performed to verify the functions of randomly selected lncRNAs. The results suggested that the trend was similar to that of the microarray data, which provided evidence of differential lncRNA expression levels between normal and osteoarthritis cartilage.

Bioinformatics methods were used to perform cis- and trans-target analyses to identify the target genes of differentially expressed lncRNAs, as it is difficult to predict the function of lncRNA based solely on nucleotide sequences (34,35). Then, the target genes were subjected to GO and KEGG enrichment analyses to explore the regulation of lncRNAs according to target gene function.

Target analysis identified a number of potential lncRNA regulatory targets. Certain targets were previously reported to play an important role in the pathogenesis of osteoarthritis, although their potential function as regulatory targets for lncRNA shad not previously been suggested (36,37). For

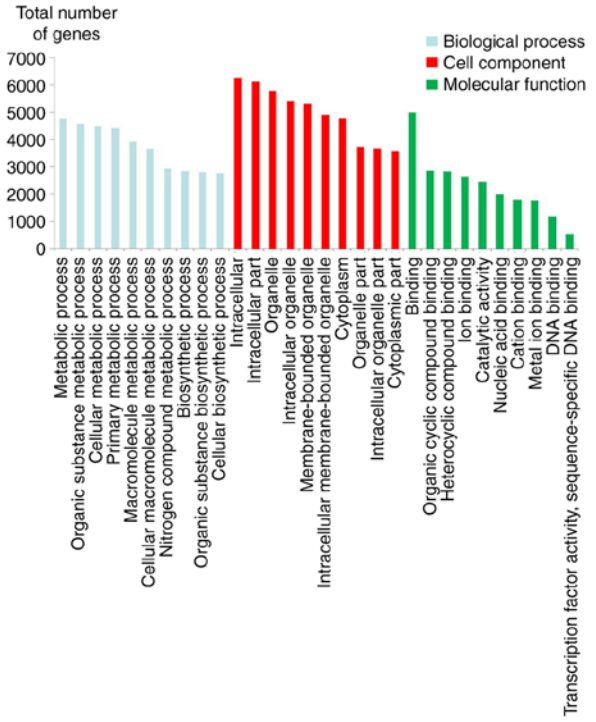

Figure 3. GO analysis of differentially expressed lncRNAs covering three domains: Biological processes, cellular components and molecular functions. The red columns indicate terms associated with cell components, the green columns indicate terms associated with molecular functions and the blue columns indicate terms associated with biological processes. GO, Gene Ontology; lncRNA, long non-coding RNA.

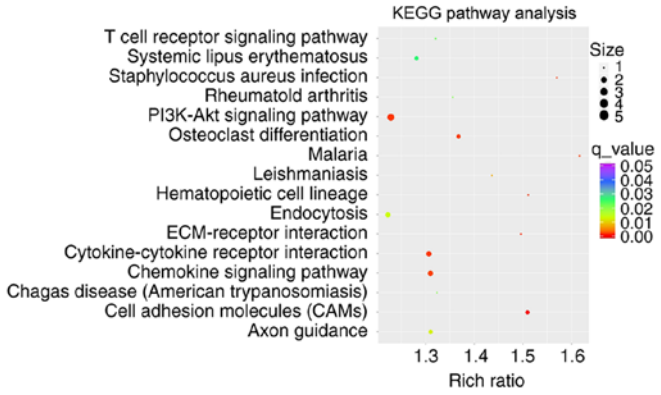

Figure 4. KEGG pathway analysis of differentially expressed lncRNAs. Each dot represents the degree of enrichment of the KEGG entry. The closer the color is to red, the higher the enrichment. The size of each dot represents the number of genes enriched in the KEGG entry; the larger the dot, the more genes are enriched in the KEGG entry. Rich ratio: (Differential genes of this pathway/all differential genes)/(genes annotated to this pathway/all can be annotated to Gene). KEGG, Kyoto Encyclopedia of Genes and Genomes; lncRNA, long non-coding RNA.

example, in the present study, integrin-α5 (ITGA5) was found to be a potential target of lncRNA ENSG00000257477, which is expressed at a 2.2787-fold higher level in normal cartilage than in osteoarthritis chondrocytes. ITGA5 can mediate mutual adhesion and two-way signal transduction between chondrocytes and the extracellular matrix (38). ITGA5 is also involved in physiological processes including inflammation, differentiation and migration (39), and the

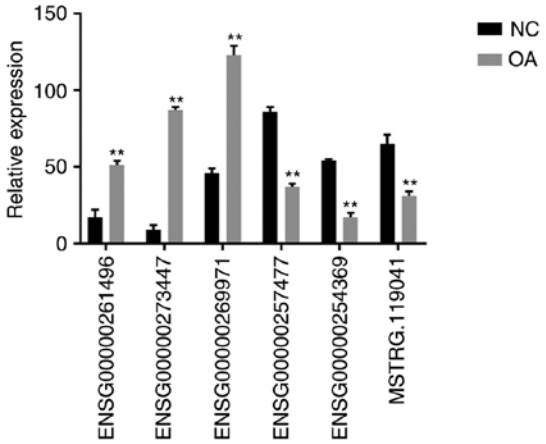

Figure 5. Validation of RNA-sequencing results by RT-qPCR. The data are expressed as the mean ± SD. **P<0.01 vs. NC. RT-qPCR, reverse transcription-quantitative PCR; OA, osteoarthritis group; NC, normal control group.

dedifferentiation of chondrocytes leads to a reduction in ITGA5 (40). Another example is JNK, a potential target of lncRNA ENSG00000261496, which was found in the present study to be expressed at a 4.4304-fold higher level in osteoarthritis than in normal chondrocytes. Elevated levels of JNK and activation of the JNK signaling pathway may aggravate chondrocyte inflammation and cause cartilage degradation (41). Mice overexpressing JNK are more susceptible to osteoarthritis compared to normal mice (42). In the present study, certain targets that have not previously been reported to play a role in osteoarthritis were identified as having a potential regulatory role in osteoarthritis. For example, high mobility group protein B1 (HMGB1), a late inflammatory regulator, has been studied for its role in coronary atherosclerosis and diabetes (43,44). However, results from the present study suggested that the expression level of HMGB1 was higher in articular cartilage tissue than in normal cartilage (~42.0320-fold), indicating that it may be a regulatory target of lncRNA ENSG00000272825, which plays a proinflammatory role (45). Interferon induced transmembrane protein 3 (IFITM3), a member of the IFITM gene family, is involved in cell adhesion, immune cell regulation and stem cell differentiation and maturation (46). IFITM3also plays a role in tumor proliferation and viral infection (47). Microarray data suggested that IFITM3 may be the regulatory target of lncRNA ENSG00000277310. The expression level of IFITM3 was lower in arthritic cartilage than in normal cartilage, which may be related to the continuous dedifferentiation of chondrocytes [(48), (Fig S1)].

The PI3K-Akt signaling pathway is involved in the release of the proinflammatory cytokines IL-1α, IL-1β, TNF-α and IL-6 and the regulation of the joint inflammatory microenvironment, based on significantly abundant KEGG signaling pathways. The present study suggested that the PI3K-Akt signaling pathway is related to the occurrence and progression of knee osteoarthritis. In addition, the present study identified several chemokine signaling pathways that are involved in the differentiation and metabolism of osteoclasts, which may also be related to the development of joint inflammation (49).

Table IV. KEGG pathway analysis of the differentially expressed lncRNAs.

A, Upregulated

KEGG pathway	Term	P-value	Total number of genes
hsa04320	PI3K-Akt signaling pathway	1.4458×10^{-5}	214
hsa05630	Hematopoietic cell lineage	6.3426×10^{-6}	60
hsa04722	Osteoclast differentiation	2.0712×10^{-5}	92
hsa05803	Staphylococcus aureus infection	2.3805×10^{-5}	44
hsa03638	Chemokine signaling pathway	3.8041×10^{-5}	115
hsa04327	Malaria	3.6349×10^{-5}	37
hsa02874	Leishmaniasis	2.4737×10^{-4}	51
hsa00381	Axon guidance	4.6875×10^{-4}	82

B, Downregulated

KEGG pathway	Term	P-value	Total number of genes
hsa00273	Cell adhesion molecules (CAMs)	8.5728×10^{-09}	98
hsa03256	Cytokine-cytokine receptor interaction	1.416×10^{-5}	130
hsa04438	ECM-receptor interaction	1.484×10^{-5}	58
hsa02931	Endocytosis	5.5678×10^{-4}	141
hsa01375	Chagas disease (American trypanosomias)	9.3938×10^{-4}	68
hsa02306	Rheumatoid arthritis	1.03056×10^{-3}	57
hsa00561	T cell receptor signaling pathway	1.20597×10^{-3}	66
hsa01934	Systemic lupus erythematosus	1.49979×10^{-3}	79

KEGG, Kyoto Encyclopedia of Genes and Genomes; lncRNA, long non-coding RNA.

The present study had several limitations. Firstly, the age of the patients in the NC group was lower than that of the patients in the OA group, therefore, a number of the detected transcripts may be age-related rather than disease-specific. Future studies should include a larger number of age-matched control cartilage samples. Secondly, as only a few patients agreed to donate knee cartilage, the present study consisted of a small sample size. In order to provide support for the conclusions made in the present study, further investigations should include larger cohorts. Thirdly, only indirect experimental evidence of the functional link between lncRNAs and their predicted target genes was included in the present study. Therefore, this also requires further investigation.

In summary, the present study used RNA microarray data to describe the expression profiles of lncRNAs in osteoarthritis and normal cartilage. Bioinformatics methods were used to predict the target genes and potential functions of differentially expressed lncRNAs. The results obtained by microarray analysis may contribute to the diagnosis of osteoarthritis and predict the development of osteoarthritis. Differentially expressed lncRNAs may also help to identify novel potential candidate targets for the treatment of knee osteoarthritis.

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Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Authors' contributions

YL and WC analyzed and interpreted the sequencing data regarding osteoarthritis and contributed to writing the manuscript. JJ designed the study. HY, JZ and QC performed the surgeries and collected tissue. XZ, JL and SZ performed secondary analysis and production of partial sequencing data and charts. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Second Affiliated Hospital of Anhui Medical University Ethics Committee (approval no. SL-YX2019-019). Written informed consent was obtained from each patient or their relatives prior to surgery.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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