Expression profiling of LncRNA and miRNA in SDF-1-induced articular chondrocyte degeneration

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Abstract

Background

With the in-depth exploration of the gene regulation network associated with the pathogenesis of osteoarthritis (OA), lncRNA has been found to play a major role in regulating the development of osteoarthritis. In this study, the expressions of miRNAs and lncRNAs in chondrocytes (2 days) of SDF-1-induced articular chondrocyte degeneration model and in normal chondrocytes were detected and the difference between them was visualized. The bioinformatics analysis was performed in parallel to elucidate the interactions between miRNAs and protein molecules.

Results

It was found that 186 lncRNA changes had significant statistical differences, of which 88 lncRNA were up-regulated and 98 lncRNA were down-regulated. A total of 684 miRNA had significant statistical differences in their expression changes. Gene Ontology and Kyoto Encyclopedia of Genes were performed for the gene set enrichment analysis to determine the key biological processes and pathways. The protein-protein interaction (PPI) network indicated that CXCL10, ISG15, MYC, MX1, OASL, FIICT1, RSAD2, MX2, IFI44, and LBST2 are the ten core genes. The PPI network identified the most important functional modules to elucidate the differential expression of miRNA.

Conclusions

These data may provide new insights into the molecular mechanisms of osteoarthritis chondrocyte degeneration, and the identification of lncRNA and miRNA can provide potential therapeutic targets for the diagnosis and differential diagnosis of osteoarthritis.

Background

Osteoarthritis (OA) is a kind of chronic and progressive multifactorial disease characterized by subchondral bone destruction, reduced chondrocytes, and degradation of cartilage matrix(1–3). With the further study of the pathogenesis of OA at the gene level, the regulation pathway mediated by non-coding ribonucleic acid (RNA) has been finding to play an important role in the gene regulating process(4, 5). Researchers found that lncRNA plays a key regulatory role in the pathogenesis of OA and the expression of lncRNA is closely related to the development of OA(6). Compared with normal cartilage tissue, the lncRNAs with abnormal expression reach 125 to 4000 in OA articular chondrocytes(7). Besides, lncRNA plays an important regulatory role in the production of joints synovial inflammation, cartilage matrix synthesis and metabolism, angiogenesis and chondrocyte autophagy, apoptosis and other factors of OA (8–10).

Studies shows that Stromal Cell Derived Factor-1 (SDF-1) has strong effects in inducing cartilage matrix degradation. The SDF-1/CXCR4 signaling pathway plays a key role in the pathological process of cartilage degeneration in patients with OA(11–14). Synovial tissue of knee-joints in patients with OA can produce SDF-1 at a higher concentration than in normal people. The SDF1 can interact with CXCR4-specific receptors on the surface of the cartilage to form the SDF-1/CXCR4 signaling pathway, which activates extracellular signal-regulating enzymes (Erk) and related kinases (P38MAP Kinase) signaling pathways and promotes the release of matrix metalloproteinases from the cartilage matrix, and then degrades type II collagen and aggrecan of the cartilage matrix, and finally accelerates cartilage degeneration and induce OA(15–17).

In this research, we examined the expression of miRNA and lncRNAs after SDF-1 induced chondrocyte degeneration model. Subsequently, we conducted a visual analysis of expression differences of identified miRNAs and lncRNAs. We also analyzed the differential expression of lncRNAs in terms of transcript length distribution, classification, and exon number differences. Also, a bioinformatics analysis was performed to clarify the interaction between differentially expressed lncRNA and miRNA. This research would be beneficial to elucidate the molecular mechanism of osteochondrocyte degeneration and provide some reference for the diagnosis and treatment of OA.

Methods

Materials and OA cartilage modeling

All cartilage tissue obtained from patients diagnosed with knee OA and received total knee replacement at the First Affiliated Hospital of Kunming Medical University (January 2018-March 2019). The remaining cartilaginous tissue on the surface of the tibial plateau and femoral condyle after osteotomy were collected during the surgery. The cartilage tissue specimens are informed and consented before the material is taken. All patients gave informed consent and the experiments met the relevant requirements of the "Regulations for the Management of Medical Institutions." 10 patients (4 males and 6 females) underwent artificial knee arthroplasty due to OA (osteoarthritis in accordance with Altman et al.), aged from 55 to 75 years old who gross visual view as the cartilage tissue with a score of 0 or 1 points. 0 point, with the smooth articular surface, usual color; 1 point, with the rough articular surface, small cracks, and dark color. Liver and kidney diseases, connective tissue diseases, endocrine diseases, serious cardiovascular diseases, and tumors were excluded. The cartilage tissue was trimmed to a size of 2 mm × 2 mm × 1 mm under aseptic conditions. 10 pieces (100 pieces in total) of cartilage tissue were taken into a pre-prepared high-glucose DMEM medium for digestion and culture. The first generation of OA chondrocytes was randomly and averagely divided into two groups, the experimental group and the control group (N = 3). The cell culture medium in the two groups is a high-glucose DMEM medium containing 10% fetal bovine serum and penicillin-streptomycin double antibiotics. In the experimental group, 100 ng/mL SDF-1was added in the chondrocytes and there was no treatment in the control group. The chondrocytes in the two groups were cultured under the same conditions for 48 hours(16, 17).

RNA extraction

The total RNA samples were extracted using the (RNeasy Mini Kit (250) Qiagen#74106) Kit. The extraction was performed in accordance with the standard operating procedure manual provided by the kit manufacturer. The extracted total RNA was qualitatively examined using Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, US), and quantified with Qubit®3.0 Fluorometer and NanoDrop One spectrophotometer.

RNA amplification and labeling

Total RNA was amplified and labeled (Amp-WTLabeling kit, Cat.5190 – 2943; Agilent Technologies) according to the kit instructions; the labeled complementary RNA was purified with the RNeasy mini kit (Cat. # 74106; QIAGEN).

Hybridization

The hybridization system provided by NimbleGen for permutation hybridization, then the NimbleGen cleaning fluid kit was used for washing.

Data collection

The AXON4000B fluorescent scanner was used to scan the chip and convert the scanning signal into a digital signal and the bad and weak point data were struck out. Fold Change $\geq$ 2 times. The t-test was performed to calculate the scanning signal values of the two samples and to obtain the log2 (ratio) value and P value of each probe. The ratio of the intensity of the hybridization signal between the experimental group and the control group was greater than 2, the expression was defined as up-regulated, otherwise the expression was defined to be down-regulated. The miRNAs screening condition was fold change $\geq$ 2 and P $\leq$ 0.05 or $\left|\log2\ (\text{ratio}) \geq 0.8\right.$.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes analysis (KEGG)

Gene Ontology (GO) analysis was performed to describe the functional properties of differentially expressed miRNAs. GO analysis included Molecular Function (MF), Biological Process (BP) and Cellular Component (CC). The Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway analysis was performed to describe the biological pathways for differentially expressed miRNAs.

Analysis of protein-protein interaction network (PPI)

To elucidate the interactions between differentially expressed miRNAs, searching database of interacting genes was performed and Cytoscape visualization was used to integrate biological models with biological graphics visualization tools for molecular interaction networks(18). Differentially expressed miRNAs with fold change values > 4 and p < 0.05 in the study were identified and a string online tool was used to analyze differentially expressed miRNAs. Use string online tools to analyze differentially expressed miRNAs with a combined PPI score > 0.4 as the cut-off value.

Statistical analysis

The data was analyzed using SPSS 17.0 software package (SPSS). Differential expression levels of miRNAs and lncRNAs were compared by the paired sample t test. Student's t tests were used for comparisons between the groups. The DE lncRNAs and DE miRNAs with a threshold of fold change > 2 and $p \leq 0.05$ were regarded as statistically significant.

Results

Morphological changes in cell culture

Chondrocytes from the first-generation culture of osteoarthritis tissue were cultured with stromal cell-derived factor-1 for 48 h. The chondrocytes in the experimental group was irregular and long spindle-shaped, with a low refractive index, and fuzzy structure in living cells while the chondrocytes in the control group was spindle-shaped or oval, with an intact nucleus, high refractive index and clear structure in living cells (Fig. 1A-B).

Difference visualization

A, The heatmap for the DE miRNAs. B, The scatter plotfor the DE miRNAs. C, The volcano plot for the DE miRNAs. D, The heatmap for the DE lncRNAs. E, The scatter plot for the DE lncRNAs. F, The volcano plot for the DE lncRNAs. In the heatmap, red color represents upregulated miRNAs or lncRNAs and the green color represents downregulated miRNAs or lncRNAs. In the scatter plot, the X and Y values on the scatter plot are the average normalized signal values, shown in a log2 scale. The red and green lines were set as fold change lines with a default change of 2.0. Red points (fold change > 2) indicate upregulated miRNAs or lncRNAs, blue points (fold change < − 2) indicate downregulated miRNAs or lncRNAs. In the volcano plot, X-axis is fold change (log2) and Y-axis is P (− log10). Red points (fold change > 2) indicate upregulated miRNAs or lncRNAs, blue points (fold change < − 2) indicate downregulated miRNAs or lncRNAs. DE lncRNAs: differentially expressed lncRNAs; DE miRNAs: differentially expressed miRNAs; lncRNAs: long noncoding RNAs; miRNAs: messenger RNAs [Color figure can be viewed at wileyonlinelibrary.com].

LncRNA analysis revealed a total of 52,741 lncRNAs expression changed. Further analysis showed there were a total of 186 lncRNAs with significant statistical differences in their expressions, of which 88 lncRNAs were up-regulated and 98 lncRNAs were down-regulated. A total of 119,205 miRNAs were found to have changed expression, and there were 684 miRNAs with significant statistical differences in their expression. The heatmap, scatter plot, and volcano map of the differentially expressed miRNAs and lncRNAs are showed in Fig. 2 (miRNAs: A-C, lncRNAs: D-F).

Differential expression analysis of lncRNA

Table 1
The top 10 upregulated and top10 downregulated LncRNAs

Upregulated LncRNAs			Downregulated LncRNAs		
LncRNA ID	Pvalue	Fold change	LncRNA ID	Pvalue	Fold chage
NONHSAT094312.2	5.51E-05	10.274344	NONHSAT246243.1	4.01E-06	9.4392389
NONHSAT060379.2	3.42E-17	8.1940904	NONHSAT217441.1	3.97E-07	8.5671719
NONHSAT207507.1	1.60E-16	8.1151242	NONHSAT238505.1	6.22E-05	7.0696651
NONHSAT166467.1	5.44E-07	7.7672742	NONHSAT258030.1	5.57E-05	6.862237
NONHSAT198879.1	3.39E-06	7.7514829	NONHSAT176410.1	4.59E-06	6.80751
NONHSAT248596.1	1.21E-11	7.6149595	NONHSAT022132.2	0.0001381	6.6838074
NONHSAT152279.1	2.89E-06	7.4083673	NONHSAT119402.2	2.11E-06	6.5944295
NONHSAT000091.2	9.96E-05	7.2117072	NONHSAT022138.2	0.000169	6.5803383
ENST00000559458	4.66E-06	7.0494153	NONHSAT229871.1	5.25E-05	6.5343748
NONHSAT038052.2	1.00E-06	6.8709526	NONHSAT225394.1	4.07E-05	6.2272073

As showed in Table 1, there are the top 10 up- and down-regulated lncRNAs in the experimental group and the control group. The horizontal comparison based on transcript structure of the lncRNAs were performed, including the transcript length distribution, classification, and exon quantity differences, and so on. The length distribution showed that the length of lncRNA was mainly concentrated around 1000 bp, and lncRNA constitutes various RNA molecules (Figure 3A). The traditional classification method is based on the location of the transcript in the genome, including five major categories: (1) the sense class, (2) the antisense class, (3) the bidirectional class, (4) the intron class, and (5) the intergenic class (Figure 3B).

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes Analysis

The GO analysis found that the signaling pathways of miRNA and its target genes are enriched in receptor regulation activities (molecular functions); secondary lysosomes (cell composition); lipopolysaccharide regulatory signaling pathways (biological processes), and type I interferon signaling pathways, ionic transmembrane transporter activity regulation (Fig. 4). The Pathway analysis found that miRNA and its target genes were enriched in cytokine-cytokine receptor interaction, osteoclast differentiation, NF-κB signaling pathway, TGF-β signaling pathway, and ion signaling pathway, as showed in Fig. 5.

Protein – protein interaction (PPI) network construction

Table 2
the core genes and their corresponding degree.

Name	Degree	Eccentricity	EdgeCount	Name	Degree	Eccentricity	EdgeCount
CXCL10	16	4	16	IFIT1	10	5	10
ISG15	12	4	12	RSAD2	10	5	10
MYC	11	5	11	MX2	10	5	10
MX1	10	5	10	IFI44L	10	5	10
OASL	10	5	10	BST2	8	5	8

The PPI network showed 10 genes that have a higher possibility in involving the mechanism of chondrocyte degeneration, including CXCL10, ISG15, MYC, MX1, OASL, IFIT1, RSAD2, MX2, IFI44LBST2 (Table 2), and showed a network of up-regulated and down-regulated genes (Fig. 6).

Discussion

With the in-depth study of the gene regulation network related to the pathogenesis of OA, the regulatory role of non-coding ribonucleic acid (RNA) has been continuously revealed. And it has been found that a large number of lncRNAs play an important role in the regulation of osteoarticular cartilage degeneration(8–10) lncRNA can compete with endogenous RNA (ceRNA), miRNA sponge, to play a regulatory role(6) lncRNA participates in gene regulation as guide, signal, bait and scaffold. The specific regulation mechanism is mainly divided into four aspects, which regulates the degeneration of articular cartilage by regulating transcription factors, transcription process, mediating post-transcriptional regulation of miRNA and mRNA, and regulation of nuclear structure(19) At present, the most concerned research of lncRNA is that it has a "sponge" endogenous competition effect with miRNA that has the same binding site as itself, inhibiting the regulation of miRNA on mRNA, thereby affecting protein expression. The more binding sites there are, the stronger the "sponge effect" and the more obvious the inhibitory effect of lncRNA on miRNA. Under normal circumstances, different RNAs (such as lncRNA, miRNA, and mRNA) maintain a balanced state and when an RNA is abnormally expressed, the balance breaks and causes disease(20) Non-coding RNA with a common response element (microRNA response elements, MREs) can compete with mRNA endogenously to bind miRNA and inhibit miRNA's negative regulation of mRNA. Similarly, reducing ceRNA levels can lead to up-regulation of the target gene expression, which may ultimately affect cellular biological processes(21)

The expression differences of in lncRNA and miRNA in OA chondrocytes cultured with SDF-1 and those without SDF-1 were analyzed by high-throughput sequencing. A total of 52,741 lncRNAs were found to be changed in expression. Through analysis, a total of 186 lncRNA changes were found to have significant statistical differences, of which 88 lncRNAs were up-regulated and 98 lncRNAs were down-regulated. A total of 119,205 miRNAs were found to have differential expression and a total of 684 miRNAs had significant statistical differences in their expression, of which 323 miRNAs were up-regulated, and 361 miRNAs are down-regulated. Subsequently, through GO enrichment and KEGG enrichment analysis, we

analyzed the function of differential mRNAs and summarized the characteristics of differential lncRNAs. A series of key genes have been identified, which may be the pathological mechanism or biomarker of chondrocyte degeneration. Through GO analysis, we found that the signaling pathways of miRNA and its target genes are enriched in receptor regulation activities (molecular functions); secondary lysosomes (cell composition); signaling pathways regulatory by lipopolysaccharide (biological processes), type interferon signaling pathways, regulation of ionic transmembrane transporter activity.

Receptor regulatory factors such as Toll-like receptors (TLRs) are evolutionarily conserved molecules that promote immune responses by recognizing molecular patterns related to microorganism. During infection, TLR signaling is necessary for the proper activation of the body's immune response(22) TLRs produce a large amount of IL-1β and TNF-α inflammatory factors by activating the NK-κB inflammatory signaling pathway. Liu et al(23) found that the expression of TLR-2, NF-κB, MMP-13 and related inflammatory factors were significantly up-regulated with the severity of OA lesions, suggesting that TLR-2 / NF-κB signaling pathway may be involved in the occurrence of OA.

Lysosome is a complex intracellular organelle that positively interacts and involves in phagocytosis, autophagy, exocytosis, receptor circulation and regulation, intracellular signaling, immunity, chromatolysis, and bone biology. The accumulation of lysosomal storage materials not only changes its function, but also affects the function of the entire cell. Cells can digest proteins and organelles through phagocytosis by lysosomes. When intra-articular hemorrhage occurs, lysosomes release degrading enzymes and sugar Decreased protein (PG) concentration reduces chondrocyte synthesis activity and aggravates articular cartilage degeneration(24) The inducer SDF-1 used in this research is a degrading enzyme and a high concentration of SDF-1 can interact with the CXCR4 receptor on the surface of chondrocytes and accelerate the degradation of -collagen through the up-regulation of MMPS, leading to cartilage degeneration(25) Chondrocytes are non-excited cells. The multiple ion channels present on the cell membrane are the material basis for the cell to carry out various life activities, including transporting ions necessary for cell metabolism, regulating osmotic pressure inside and outside the cell, participating in the formation of electrical impulses, participate in signal transmission to adapt organisms to environmental conditions(26, 27)

Pathway analysis showed that miRNA and its target genes were enriched in cytokine-cytokine receptor interaction, osteoclast differentiation, NF-κB signaling pathway, TGF-b signaling pathway, and Ca^{2+} signaling pathway. Cytokines regulate the balance of anabolic and catabolic metabolism of cartilage matrix. And cytokines are divided into decomposing cytokines and synthetic cytokines according to the characteristics of cytokines regulating metabolism. The balance and imbalance between them are root causes of the degradation and destruction of the cartilage matrix in osteoarthritis. A large number of cytokines are involved in this pathway, such as tumor necrosis factorα (TNF-α), interleukin-1 (IL-1), IL-6, IL-2, and interferon-γ (IFN-γ). These cytokines penetrate into the synovium to produce an inflammatory response. Besides, these cytokines can activate synovial cells and stimulate the release of MMPs into the synovial fluid, leading to cartilage degradation(28, 29)

Currently, the most studied cytokines that promote chondrocyte catabolism are IL-1 and TNF-α. IL-1 not only inhibits the synthesis of characteristic matrix components type II collagen and aggrecan by articular chondrocytes, but also stimulates articular chondrocytes to secrete protease that degrades cartilage matrix components, induces articular chondrocytes to express type and type II collagen degradation, and promote the degeneration of articular chondrocytes(30) TNF-α also plays an important role in OA cartilage degeneration. The mechanism of action of TNF-α is similar to IL-1, including promoting the generation of MMP and inhibiting the synthesis of cartilage matrix, etc. Studies have shown that TNF inhibits the expression of type collagen and connexin genes through the pathway of MEK1 / 2 and nuclear factor (NF) -κB, which in turn interferes with the synthesis and reconstruction of articular cartilage(31) As a transcription factor regulating gene expression, studies have found that the NF-kB signaling pathway is activated in OA occurred articular cartilage and synovial cells(32) NF-kB regulates the response to joint injury and inflammation by regulating the cytokines IL-1β, TNF-α, etc.(33–37)

In addition, the PPI network revealed 10 genes that have a higher possibility in association with the pathological process of chondrocyte degeneration: CXCL10, ISG15, MYC, MX1, OASL, FIICT1, RSAD2, MX2, IFI44LBST2. Chemokines are a class of small molecule peptides with chemotactic effects that can attract immune cells to produce an immune response locally. Chemokines, mainly CXC and CC, and their receptors are expressed in human chondrocytes and their corresponding receptors while expression increased in OA articular cartilage(38) Chemokines are involved in cartilage destruction by inducing some related enzymes, mainly N-acetyl-β-D-glucosidase (NAG) and MMP. NAG is the main lysosomal glycosidase in OA synovial fluid and has the function of catalyzing the hydrolysis of glucosamine polysaccharides, and then causes the destruction of cartilage.

With the occurrence of OA, the cartilage surface is activated by a variety of chemokines, releasing enzymes that mediate the destruction of the cartilage matrix(39, 40) Kostopoulou et al(41) and Tardif et al(42) found that OA-related miRNA can inhibit matrix metalloproteinase 13 (MMP-13). MMP-13 is a class of human proteases with important biological functions in OA. It can degrade cartilage extracellular matrix, destroy articular cartilage, and cause and aggravate OA.

The human c-Myc gene is located on the eighth pair of chromosomes and consists of three exons. Its expression product is a protein containing 439 amino acids, which exists in the nucleus. c-Myc is not expressed in normal chondrocyte nucleus, but scattered in apoptotic chondrocyte nucleus. In the comparison of articular chondrocytes and OA articular chondrocytes in normal people, it was found that the degree of OA articular chondrocyte apoptosis was positively correlated with the degree of cartilage degeneration, and c-myc participated in the whole process of chondrocyte apoptosis. The mechanism of c-Myc causing apoptosis may be due to the imbalance of normal cell cycle, which leads to the inhibition of cell growth.(43)

Conclusions

In summary, this study discovered the differential expression of lncRNAs and miRNAs in SDF-1 induced chondrocyte degeneration model through high-throughput gene sequencing technology, but the biological functions of most of the lncRNAs and miRNAs are still unknown. In the future, in-depth studies on cytokine interaction, osteoarthritis signaling pathway and miRNA related to lncRNA will be conducted to investigate the relationship between lncRNA and miRNA to provide new basis and targets for the effective treatment of OA disease.

Declarations

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Competing interests

The authors declare that there is no conflict of interests.

Authors' contributions

Guoliang Wang and Yanlin Li participated in the study design. Lu He and Xiao Yang contributed to the Chondrocyte collection experiment. Guoliang Wang was in charge of interpreting the data analysis and drafting the manuscript. Yaoyu Xiang assisted in revising the manuscript. All authors read and agreed with the contents of the final manuscript.

Availability of data and materials

RNA-seq data has been uploaded to Sequence Read Archive (SRA) database and the BioProject ID is PRJNA638147.

Ethics approval and consent to participate

All experiments were performed in accordance with the ethical standards of the First Affiliated Hospital of Kunming Medical University.

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Figures

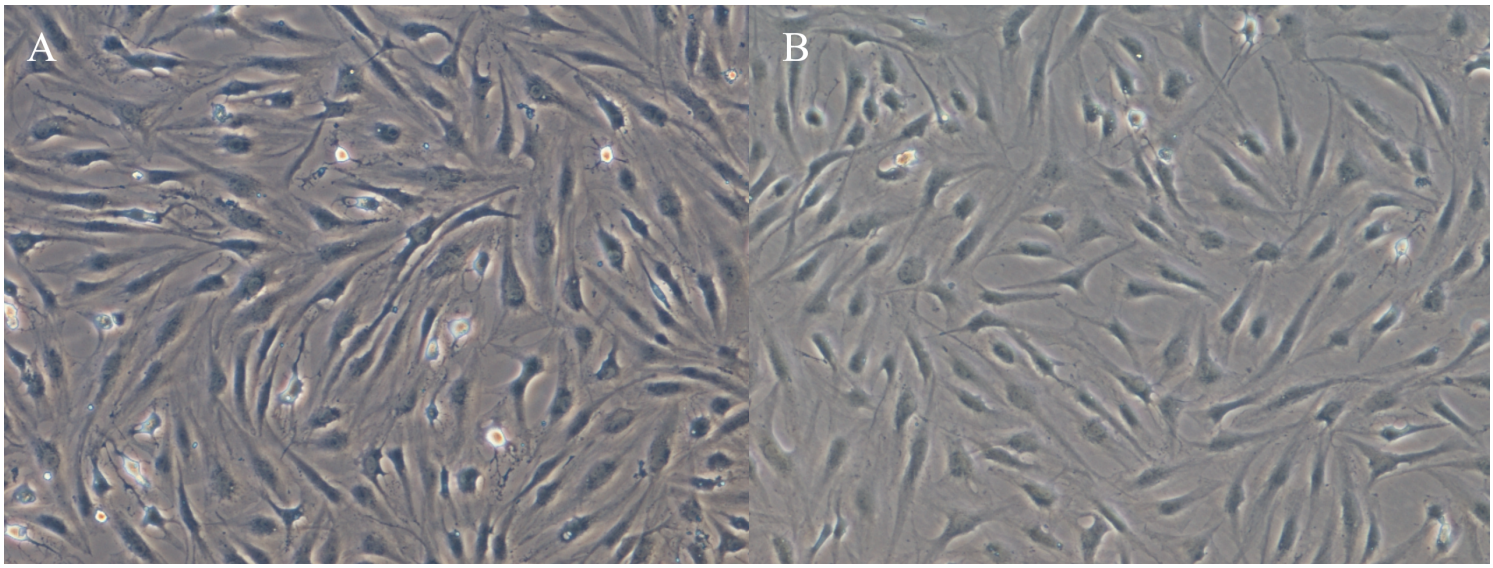

Figure 1

Morphology of chondrocytes in two groups (×100). A, Chondrocytes in the experimental group were cultured with stromal cell-derived factor 1 for 48h. B, Morphology of chondrocytes in the control group.

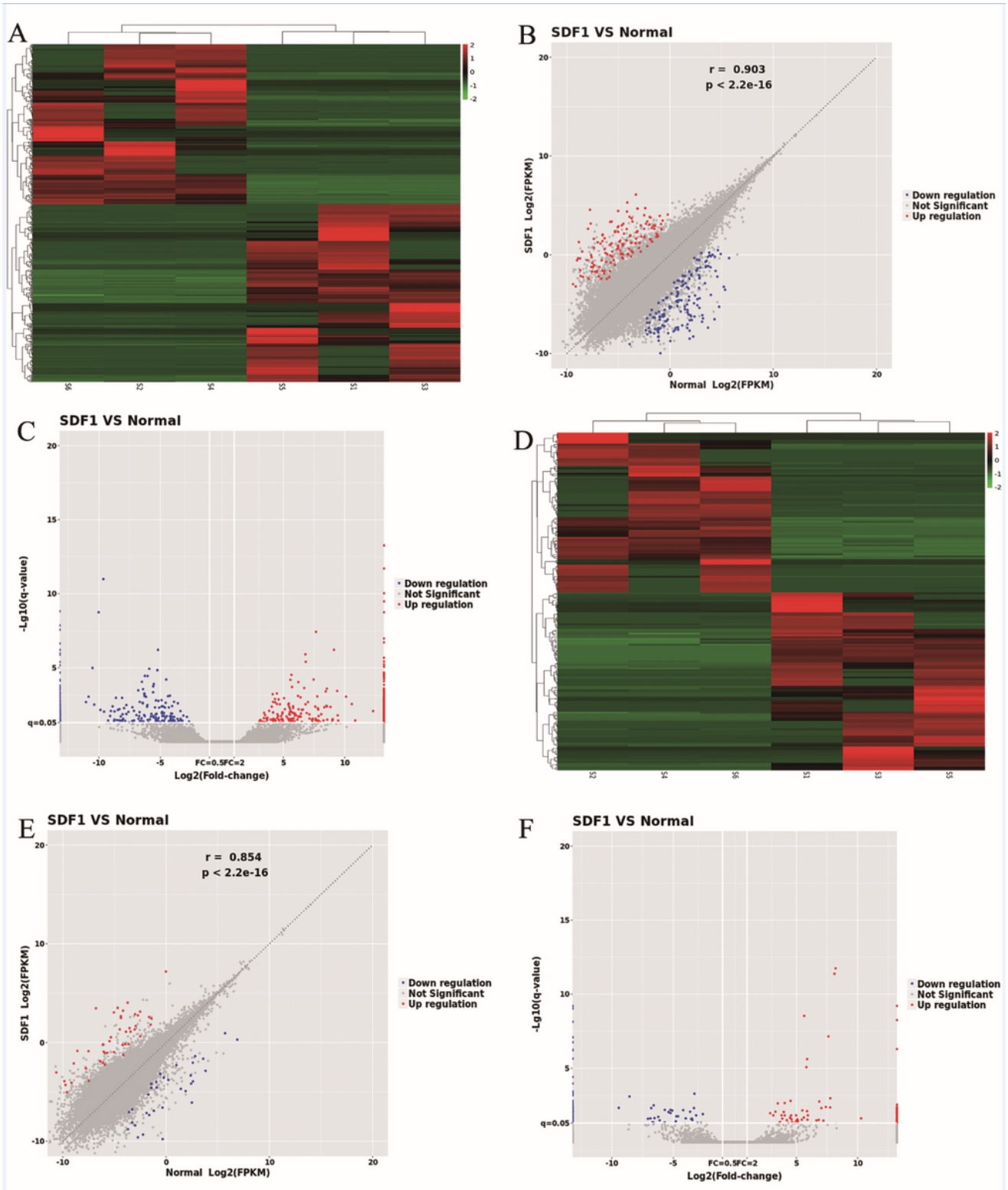

Figure 2

miRNAs and lncRNAs analysis A, The heatmap for the DE miRNAs. B, The scatter plotfor the DE miRNAs. C, The volcano plot for the DE miRNAs. D, The heatmap for the DE lncRNAs. E, The scatter plot for the DE lncRNAs. F, The volcano plot for the DE lncRNAs. In the heatmap, red color represents upregulated miRNAs or lncRNAs and the green color represents downregulated miRNAs or lncRNAs. In the scatter plot, the X and Y values on the scatter plot are the average normalized signal values, shown in a log2 scale.

The red and green lines were set as fold change lines with a default change of 2.0. Red points (fold change >2) indicate upregulated miRNAs or lncRNAs, blue points (fold change <−2) indicate downregulated miRNAs or lncRNAs. In the volcano plot, X-axis is fold change (log2) and Y-axis is P (−log10). Red points (fold change >2) indicate upregulated miRNAs or lncRNAs, blue points (fold change <−2) indicate downregulated miRNAs or lncRNAs. DE lncRNAs: differentially expressed lncRNAs; DE miRNAs: differentially expressed miRNAs; lncRNAs: long noncoding RNAs; miRNAs: messenger RNAs [Color figure can be viewed at wileyonlinelibrary.com].

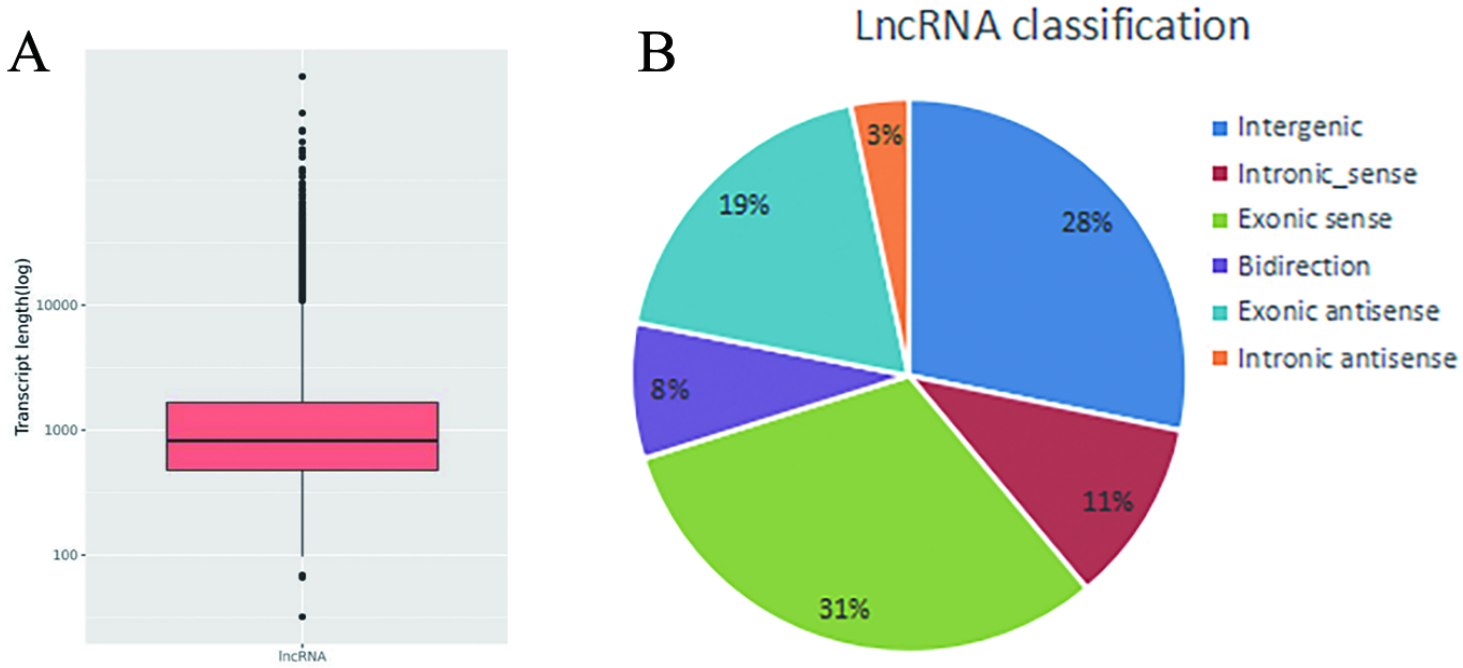

Figure 3

Expression signatures of dysregulated lncRNAs in SDF-1-induced articular chondrocyte degeneration. A, Length distribution showed that dysregulated lncRNAs were mainly concentrated between 700 and 3,000 bp in length. B, Differential lncRNAs were classified according their genomicarchitecture.

Figure 4

The GO enrichment results of differentially expressed genes. The circles represent biological process; the triangles represent cell component; the squares represent molecular function.

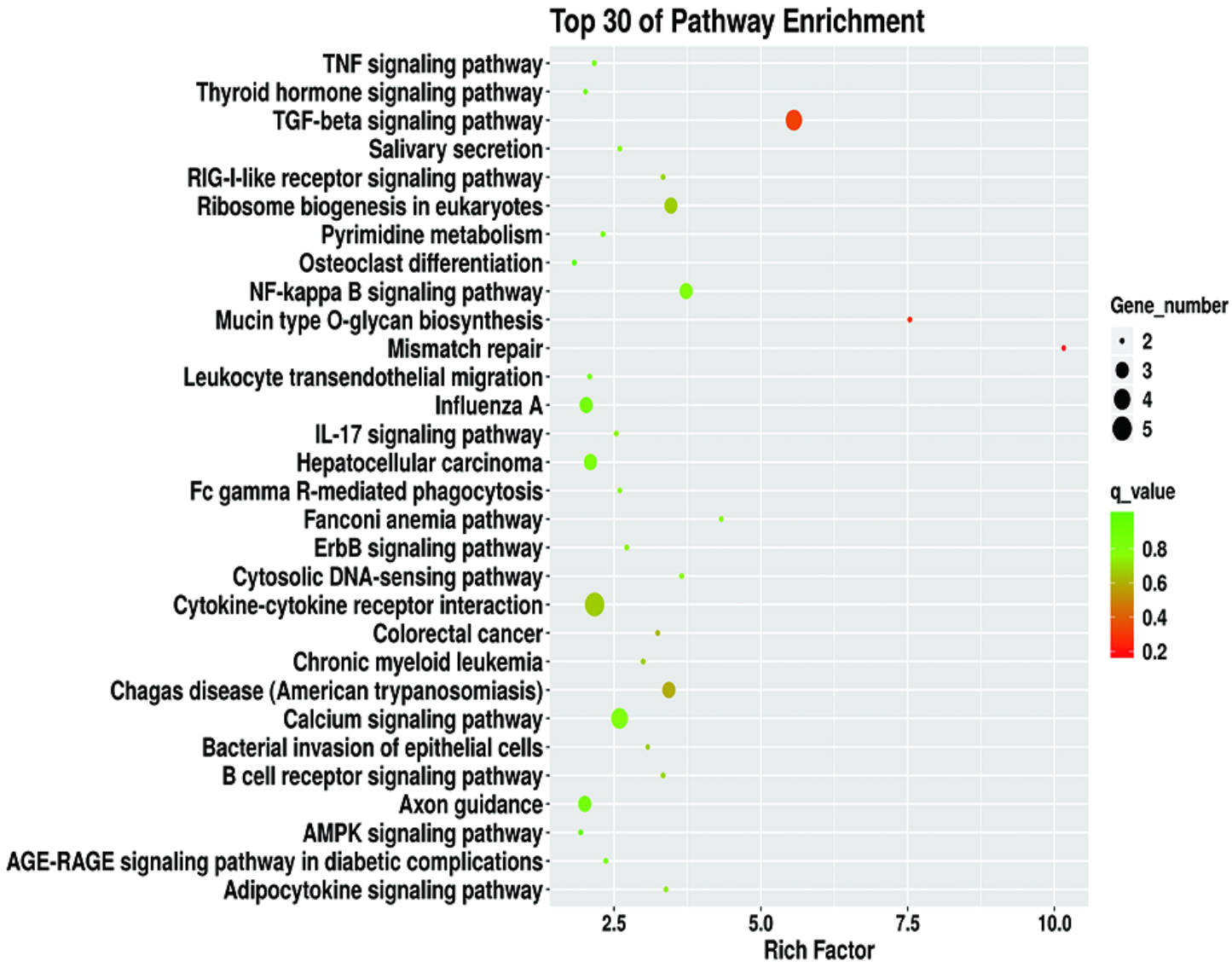

Figure 5

The KEGG enrichment results of differentially expressed genes.

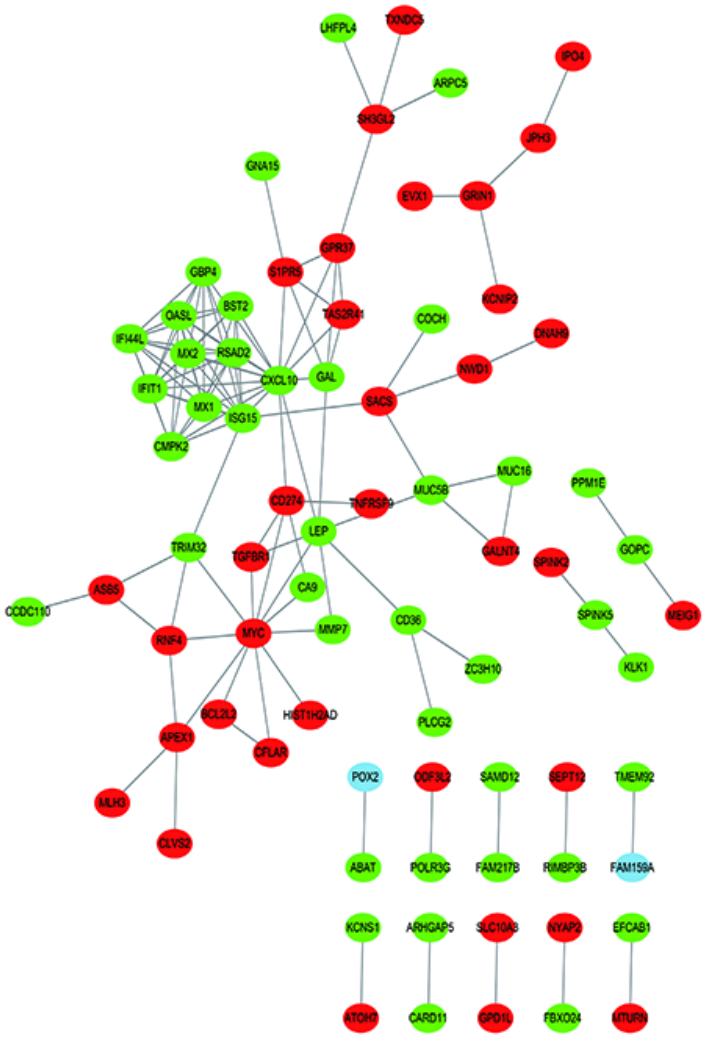

Figure 6

Interaction protein–protein network analysis of the top 10 differentially expressed genes. Red is up-regulated gene, green is down regulated gene.