Bioinformatics Analysis of Some Novel Potential Biomarkers Associated With Osteoarthritis and Nervous System

Zhongkui Guo  
Nankai University School of Medicine

Yong Qin  
Second Affiliated Hospital of Harbin Medical University

Yang Chen  
Chinese PLA General Hospital

Yi Li  
Chinese PLA General Hospital

Ya Gu  
Chinese PLA General Hospital

Ming Chen  
Chinese PLA General Hospital

Pengbin Yin  
Chinese PLA General Hospital

Yanpeng Zhao  
Chinese PLA General Hospital

Licheng Zhang  
Chinese PLA General Hospital

Peifu Tang (✉ pftang301@163.com)  
General Hospital of Chinese PLA  
https://orcid.org/0000-0003-4279-1704

Research article

Keywords: osteoarthritis, bioinformatics, pain, nervous system, DEGs, qRT-PCR

DOI: https://doi.org/10.21203/rs.3.rs-541295/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

Background

Osteoarthritis (OA) is an age-related chronic inflammatory and degenerative changes that carries heavy burden for individuals and the society. The specific mechanism of OA still remains unclear today, which requires new methods and technologies to achieve some new breakthrough. Bioinformatics technology is a novel method to extract genetic information from many diseases. In this study, we aims at screening out some key genes to help to illuminate the pathogenesis of OA to help to diagnosis and cure it.

Objective and Methods

Bioinformatics technology was used to screen some key target genes that were closely related to OA and nervous system, and by using qRT-PCR to preliminary verify the results.

Results

In this work, we analysis three gene expression profiles, GSE114007, GSE51588, and GSE55457, that downloaded from the Gene Expression Omnibus database (GEO). At last, a total of 878 DEGs were identified with dataset GSE114007 (P<0.05 and |logFC|>1.5), consisting of 495 up-regulated genes and 383 down-regulated genes between the osteoarthritis and normal cartilage tissues. And by combining with the screened results of GSE51588 and GSE55457, finally, three genes, HES1, JUN, and IRE2, which were closely correlated with the nervous system that may help to diagnosis and cure osteoarthritis in the future were identified, and the result of qRT-PCR preliminary confirmed our finding.

Conclusion

HES1, JUN, and IRE2 were three potential genes related to osteoarthritis and nervous system that may help to diagnosis and cure OA.

1. Introduction

Osteoarthritis (OA), mostly affects the knee joints, is an age-related chronic inflammatory and degenerative changes that usually accompanied by unbearable pain and dysfunction of joints, which brings great puzzles for individuals and the society [1]. Data shows that about 240 million people globally suffered this burdensome syndrome, which has been the 4th leading cause of years disability worldwide in 2020 [2]. Meanwhile, osteoarthritis can affect knees, hands, feet, hips, and spine, but knee osteoarthritis occupied approximately 85% part of all the osteoarthritis in the world [3]. Osteoarthritis is a complex and comprehensive disorder characterized by the degeneration of cartilage chondrocytes, subchondral bone remodeling, and synovium inflammation, and the loss of cartilage chondrocytes has been proved to be the primary pathological change [4].

Besides, a number of studies have illuminated that the nervous system, includes the peripheral nervous system and the central nervous system, also play vital parts in the ethiopathogenesis of osteoarthritis[5, 6]. And as we all know, pain, the major reason that drives patients to seek medical advice, is the dominant symptom of osteoarthritis and is an important manifestation of neuromodulation of osteoarthritis as well. Nervous system could not only innervate the nociceptive pain but also innervate synovium, joint capsule and subchondral bone [7, 8], and with the progression of osteoarthritis, the higher degree of damaged joints, the more nervous system were engaged in.
Therefore, there must be some factors that has the capacity of affecting the nervous system as well as osteoarthritis.

Nowadays, bioinformatics technology has been a novel method to extract genetic information from many diseases. In this work, we analysis three gene expression profiles, GSE114007, GSE51588, and GSE55457, that downloaded from the Gene Expression Omnibus database (GEO), by bioinformatics technology, and finally, three genes, HES1, JUN, and IER2, that may help to diagnosis and cure osteoarthritis in the future were identified, which were preliminary confirmed by using qRT-PCR.

2. Materials And Methods

2.1. Gene Expression Database

Gene expression profiles GSE114007, GSE51588, and GSE55457 were downloaded from the Gene Expression Omnibus (GEO, www.ncbi.nlm.nih.gov/geo/). Dataset GSE114007 includes 20 osteoarthritis and 18 normal mRNAs expression data of cartilage tissues. Dataset GSE51588 includes 20 osteoarthritis and 5 normal mRNA expression data of cartilage subchondral bone. Dataset GSE55457 includes 26 osteoarthritis and 20 normal mRNA expression data of synovial membrane.

2.2. Identification of Differentially Expressed Genes (DEGs)

The Limma package was used in R software to identify the DEGs. The corresponding P value of the gene symbols after t test was used, and genes with adjusted P < 0.05 and |logFC|>1.5 were defined as DEGs. Then the pheatmap package was used in R software to draw a heatmap and a volcano plot according to the DEGs.

2.3. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis of DEGs

Annotation, Visualization, and Integrated Discovery (DAVID) was used to analysis the genes. Target genes lists were submitted to the DAVID 6.8 (https://david.ncifcrf.gov/) to carry out the GO and KEGG enrichment analysis of the DEGs. Gene ontology (GO) was classified into three groups as follows: biological process (BP), molecular function (MF), and cellular component (CC). (P < 0.05)

2.4. Protein-protein Interaction (PPI)

Search Tool for the Retrieval of Interacting Genes (STRING; http://string-db.org) was used to illustrate the potential interactive relationships between DEGs. And then the Cytoscape software was used to draw the PPI network.

2.5. Key Genes Identification

To identify the key genes, firstly, biological process (BP) part of the GO analysis results was used to screen the neuro-related DEGs of the osteoarthritis. Then, the neuro-related DEGs list, together with the DEGs lists of GSE51588 and GSE55457 were submitted to Venn Diagrams (bioinformatics.psb.ugent.be/webtools/Venn/). Finally, genes in the intersection were regarded as our key genes.

2.6. Quantitative Real-time PCR (qRT-PCR)

Human chondrocyte cell line C28/I2 was chosen to confirm the results of bioinformatics analysis. Total RNA from normal group (normal C28/I2 human chondrocyte cells) and osteoarthritis group (IL-1beta induced C28/I2 human
chondrocyte cell) was isolated using RNA Extraction Kit (Solarbio, Beijing, China). Then, a Reverse Transcriptase Kit (Solarbio, Beijing, China) was used to generate cDNA. qRT-PCR was conducted on the Bio-Rad Real-Time PCR System with SYBR Green Master Mix (Yishan Biotechnology CO., LTD, Shanghai, China). The primer sequences are showed as follows:

| Gene Name | Primer Sequences                      |
|-----------|---------------------------------------|
| GAPDH     | Forward: AATGGGCAGCCGTTAGGAAA         |
|           | Reverse : GCGCCCAATACGACCAAAATC       |
| HES1      | Forward: ATGACAGTGAGACCTCCG           |
|           | Reverse : AAACACCTTAGCCGCTCTC         |
| JUN       | Forward: GTCCGAGAGCGGAGCTTATG         |
|           | Reverse : CTTTTTCGGGACTTTGGAGGC       |
| IER2      | Forward: GACTGGTCCCGAGCAAGAAA         |
|           | Reverse : AGGAGAGCGAAAGGTCACG         |

3. Results

3.1. Study Design

The study design was schematically depicted in Fig. 1. Dataset GSE114007 was used for DEGs screening (Step 1). Gene ontology (GO) enrichment analysis (Step 2) was used for screening the neuro-related DEGs (p < 0.05) of dataset GSE114007 (Step 3). Moreover, dataset GSE51588 and GSE55457 were used for further validation (Step 4). Then, the neuro-related genes of dataset GSE114007 together with the DEGs of dataset GSE51588 and GSE55457 were submitted to the Venn diagram to obtain the intersection to confirm the key genes (Step 5). Finally, qRT-PCR was used to further identify the accuracy of the key genes (Step 6).

3.2. Identification the DEGs in GSE114007

A total of 878 DEGs were identified with dataset GSE114007 (P < 0.05 and |logFC|>1.5), consisting of 495 up-regulated genes and 383 down-regulated genes between the osteoarthritis and normal cartilage tissues. The top 10 up-regulated genes and the top 10 down-regulated genes were listed in Table 1 and Table 2 respectively. Heatmap of the DEGs were obtained in Fig. 2A, and Volcano plot of the DEGs were obtained in Fig. 2B.
### Table 1
The top 10 up-regulated genes with P value < 0.05 and |logFC|>1.5

| Gene Name | logFC    | P value       | Adj. p value |
|-----------|----------|---------------|--------------|
| POSTN     | 6.516109 | 3.49E-08      | 1.43E-05     |
| TNFSF15   | 4.965648 | 1.58E-08      | 8.17E-06     |
| AMTN      | 4.842882 | 2.91E-06      | 0.000242     |
| HBB       | 4.799333 | 1.76E-05      | 0.000829     |
| LRRC15    | 4.790049 | 1.40E-08      | 8.01E-06     |
| ST6GALNAC5| 4.734142 | 2.60E-08      | 1.14E-05     |
| COL1A1    | 4.672018 | 6.28E-08      | 2.09E-05     |
| GRIA2     | 4.59511  | 1.14E-08      | 7.18E-06     |
| TMEM119   | 4.414562 | 4.03E-07      | 6.52E-05     |
| CDH2      | 4.31047  | 1.91E-08      | 9.23E-06     |

### Table 2
The top 10 down-regulated genes with P value < 0.05 and |logFC|>1.5

| Gene Name | logFC    | P value       | Adj. p value |
|-----------|----------|---------------|--------------|
| ADM       | -4.26835 | 3.09E-11      | 1.15E-07     |
| DUSP2     | -3.887   | 2.51E-06      | 0.000218     |
| DDIT4     | -3.76485 | 6.85E-09      | 5.07E-06     |
| ATF3      | -3.66345 | 1.50E-08      | 8.12E-06     |
| HILPDA    | -3.40776 | 8.35E-10      | 1.17E-06     |
| OPRK1     | -3.39783 | 8.65E-05      | 0.002532     |
| PCDH17    | -3.37392 | 5.63E-08      | 1.99E-05     |
| PLIN5     | -3.3294  | 7.10E-05      | 0.002209     |
| KIT       | -3.29465 | 5.91E-07      | 8.39E-05     |
| APOD      | -3.27313 | 2.17E-05      | 0.000947     |

### 3.3. GO function and KEGG pathway Enrichment Analysis of the DEGs in GSE114007

Gene ontology (GO) enrichment analysis, includes biological process (BP), cellular component (CC) and molecular function (MF), was used to explore the potential biological function of the 878 DEGs in dataset GSE114007. The top 5 functions for BP were extracellular matrix organization, collagen catabolic process, angiogenesis, collagen fibril organization, and cell adhesion (Fig. 3A), the top 5 functions for CC were proteinaceous extracellular matrix,
extracellular region, extracellular matrix, extracellular space, and plasma membrane (Fig. 3B), and the top 5 functions for MF were extracellular matrix structural constituent, transcription factor activity, RNA polymerase II core promoter proximal region sequence-specific binding, transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding, scavenger receptor activity, and metalloendopeptidase activity (Fig. 3C). KEGG pathway analysis revealed the mainly related pathway of the 878 DEGs in dataset GSE114007, which mainly were related with focal adhesion, PI3K-Akt signaling pathway, ECM-receptor interaction, staphylococcus aureus infection, protein digestion and absorption, and osteoclast differentiation (Fig. 3D).

3.4. PPI Network Construction of the DEGs in GSE114007 and Hub Gene Identification

In order to better understanding of the 878 DEGs in dataset GSE114007, STRING was used to analysis the potential interactive relationship between DEGs. Then, Cytoscape software was used to draw the PPI network (Fig. 4A). Besides, the top 10 hub genes of dataset GSE114007 were identified as well, included 7 up-regulated genes, MMP9, ITGAM, CCND1, CDK1, MMP2, COL11, and ITGB2, and 3 down-regulated genes, VEGFA, JUN, and FOS. (Fig. 4B).

3.5. Neuro-related Genes Identification

Previous studies have demonstrated that OA was correlated with the nervous system [2, 4], therefore, in order to obtain the neuro-related genes that have the capacity of regulating osteoarthritis, the biological process (BP) functions of GO enrichment analysis results was used for further identification. The result showed that the neuro-related genes significantly enriched in GO: 0007411 (axon guidance), GO: 0051930 (regulation of sensory perception of pain), GO: 0019233 (sensory perception of pain), GO: 0030182 (neuron differentiation), GO: 0010001 (glial cell differentiation), GO: 0014002 (astrocyte development), GO: 0022008 (neurogenesis), GO: 0043525 (positive regulation of neuron apoptotic process), GO: 0050767 (regulation of neurogenesis), GO: 0007399 (nervous system development), GO: 0002052 (positive regulation of neuroblast), GO: 0097150 (neuronal stem cell population maintenance). A total of 76 neuro-related differential expression genes (DEGs) were obtained as neuro-related genes with $p$ value < 0.05 (Table 3).
Table 3  
The neuro-related DEGs acquired in BP functions of GO enrichment analysis with p value < 0.05

| Ontology | ID       | Description                           | Count | P value      | Gene Name                                                                 |
|----------|----------|---------------------------------------|-------|--------------|---------------------------------------------------------------------------|
| BP       | GO:0007411 | axon guidance                         | 16    | 0.001846     | CSF1R/NRP1/NRP2/SEMA3C/WNT5A/ANK3/EFNA1/EFNA3/NR4A3/EFNB3/FLRT2/TUBB3/KIF5C/KIF5A/TNR/NCAM1 |
| BP       | GO:0051930 | regulation of sensory perception of pain | 6     | 0.003326     | GRIN2A/CCL3/OPRK1/ACPP/FAM19A4/GRIN2D                                      |
| BP       | GO:0019233 | sensory perception of pain             | 8     | 0.004488     | UCHL1/GRIN2A/MME/TRPA1/HOXD1/PENK/PROK2/OPRK1                              |
| BP       | GO:0030182 | neuron differentiation                  | 11    | 0.004713     | RTN1/FZD5/HOXD1/WNT7B/WNT5A/DDIT4/PCS9/SOX11/FZD10/PROX1/IER2             |
| BP       | GO:0010001 | glial cell differentiation             | 4     | 0.016762     | GAP43/CDH2/DNER/KLF15                                                      |
| BP       | GO:0014002 | astrocyte development                  | 4     | 0.024303     | CDK6/LAMC3/TSPAN2/S100A8                                                   |
| BP       | GO:0022008 | neurogenesis                           | 6     | 0.025485     | GRIN2A/PRDM16/SPOCK1/PCS9/CHAC1/KIF17                                     |
| BP       | GO:0043525 | positive regulation of neuron apoptotic process | 6 | 0.027919 | JUN/DDIT3/BPL3/PCS9/PAK3/RAPSN                                          |
| BP       | GO:0050767 | regulation of neurogenesis             | 4     | 0.038493     | DLL4/BCL6/TNR/ARNTL                                                      |
| BP       | GO:0007399 | nervous system development             | 19    | 0.041098     | FOS/PCDH18/GFRA2/ELAVL3/VEGFA/ NELL1/SLC7A5/MAFB/EFNB3/GDNF/GLRB/MAP1B/INSC/ATOH8/ENC1/ SPOCK1/HES1/INHA/SH2B2 |
| BP       | GO:0002052 | positive regulation of neuroblast proliferation | 4 | 0.043977 | ASPM/VEGFC/SOX10/VEGFA                                                  |
| BP       | GO:0097150 | neuronal stem cell population maintenance | 4 | 0.049826 | ASPM/CDH2/HES1/PROX1                      |

3.6. Key Genes Identification
In order to detect the genes that associated with nervous system and osteoarthritis, dataset GSE99662 and GSE51588 were used for further verification. Dataset GSE51588 contains the differentially expressed genes of subchondral bones in osteoarthritis (obtains 1010 DEGs) and dataset GSE55457 contains the differentially expressed genes of synovial tissues in osteoarthritis (obtains 704 DEGs). To obtain the most relevant neuro-related genes, the 76 neuro-related DEGs together with the DEGs of dataset GSE51588 and GSE55457 were submitted to Venn diagram to get the intersection, and the genes in the intersection were regarded as the key genes (Fig. 5). According to the Venn diagram, 3 key genes (HES1, JUN, and IER2) were identified at last, which may be associated with the nervous system as well as the pathophysiological process of OA.

3.7. qRT-PCR for Key Genes Validation

In order to validate the different expression levels of HES1, JUN, and IER2 in OA and normal chondrocytes, we chose the il-1 beta induced human chondrocytes cell line C28/I2 as OA group and the non-treated human chondrocytes cell line C28/I2 as normal group. By using qRT-PCR, we found that the expression of HES1, JUN, and IER2 genes were significantly changed in OA chondrocytes compared to normal condrocytes (Fig. 6), which were in full accord with our detection.

4. Discussion

Osteoarthritis is a leading cause of disability in the elderly with heavy burden on individuals, families and society. Today, for most non-terminally symptomatic patients, turning to medication for pain relief, but not a cure, is the main method for osteoarthritis treatment, and for terminal joint osteoarthritis patients, the only effective cure is the total joint replacement [2, 4]. Therefore, due to the unclear mechanism induced treatment resistant and high burden of osteoarthritis, illuminate the relationship between the nervous system and osteoarthritis may provide us a new method to solve the puzzle that osteoarthritis brings us.

Evidence from animal to clinical models have demonstrated that osteoarthritis-like joint damage is not only limited to the destruction of cartilage chondrocytes, synovial tissues, subchondral bone, but also associated with the disorder of the peripheral and central nervous system [9, 10].

It’s well known that joint pain evoking is mainly correlated with the sensitization of peripheral nociceptive neurons and hyperexcitability of nociceptive neurons in central nervous system, which is closely associated with the synovial inflammation. Lei et al. [11] suggested that SDIM1, whose overexpression exhibited a role of improving survival of neuro-progenitor cells after injury, showed an significantly increased expression in synovial tissues of osteoarthritis patients with high pain. Bullock et al. [12] revealed CGRP release might play an important role in the peripheral sensitization during joint degeneration in osteoarthritis. Brutus et al. [13] summarized four known functional genetic variants (SCN9A, COMT, TRPV1 and P2X7) in pain candidate genes that influence pain experiences in patients with osteoarthritis.

In this paper, bioinformatics technology was used to identify the DEGs of osteoarthritis, and at last, HES1, JUN, and IER2 were identified as the key genes that may take vital roles in the regulation of nervous system as well as osteoarthritis.

HES1, also known as Hes family bHLH transcription factor 1, is a downstream effector of Notch signaling. Activated Notch signaling can induce the activation of HES1 and hence inducing the expression of MMP13, which has the potential to promote the degradation of chondrogenic ECM, and resulted in the degeneration of cartilage [14]. Ni et
al. [15] revealed that OSM is up-regulated in the synovial tissue of knee osteoarthritis and OSM-treated MC3T3-E1 cells showed a down-regulated HES1 in a time-dependent manner. Sugita et al. [16] reported that Hes1 induced Adamts5 and Mmp13, which are catabolic enzymes that break down cartilage matrix. Additionally, Matsuzaki, et al. [17] identified that Hes1 expression in mature neurons in the adult mouse brain is required for normal behaviors. Harris, et al. [18] demonstrated that HES1 has the potential of promoting the quiescence and proliferation of adult neural stem cells.

JUN, one of the transcription factor for activator protein 1, can mediate catabolic transcription, cell apoptosis and cell death. Chen et al. [19] reported that JUN was down-regulated in osteoarthritis knee cartilage compared to normal knee cartilage. Cai, et al. [20] demonstrated that osteoarthritis synovial tissues exhibit a decreased expression of JUN. While, studies also showed that blocking the JUN could prevent the degradation and apoptosis of chondrocytes [21], and the inhibition of JUN transcriptional activity protects against osteoarthritis cartilage destruction [22]. Moreover, studies also showed that JUN could participate in the regulation of antioxidant responses in neurons [23] and may have the potential of regulating the peripheral myelin development [24] and neuronal polarization during brain development [25].

IER2, short for immediate early response 2, is a protein function as a potential transcriptional factor or transcriptional coactivator which seems to play a pivotal role in regulating tumor cells. Xu et al. [26] reported that IER2 promotes the migration and invasion of hepatocellular carcinoma cell adhesion and motility. Xu et al. [27] demonstrated that IER2 promotes the migration and invasion of hepatocellular carcinoma cells via regulating the activity of Rho GTPases. Moreover, Moriya et al. [28] reported that ier2 mRNA was distributed in the telencephalon, midbrain and the hypothalamus. Besides, previous study [20] also reported that IER2 was down-regulated in osteoarthritis knee cartilage compared to normal knee cartilage. While, there is no research about IER2 on the specific mechanism of osteoarthritis and nervous system by now.

In this study, we demonstrated that HES1, JUN, and IER2 may take vital parts in the regulation of nervous system and osteoarthritis, and the result was validated by using qRT-PCR finally. Meanwhile, we also found that there are still somewhat controversial between the previous studies. Considering of this, there must be still have some unknown mechanism between these three genes, osteoarthritis as well as nervous system. So, it’s necessary to undertake more research to confirm our speculation.

5. Conclusion

Bioinformatics analysis has been a novel tool to find new targets for diagnosis and cure of disease. In this paper, we screened out three genes that may offer new targets to understand osteoarthritis. While, at the same time, more studies are required to further confirm our results.

Abbreviations

OA: osteoarthritis; DEGs: Differentially Expressed Genes; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; GEO: Gene Expression Omnibus; BP: biological process; CC: cellular component; MF: molecular function; qRT-PCR: Quantitative Real-time PCR; PPI: Protein-protein Interaction.

Declarations

Acknowledgments
Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding: No funding.

Conflicts of interest/Competing interests:

All authors read and approved the final manuscript and has no conflict of interest.

Availability of data and materials:

All data and materials analyzed and used during this study are included in this published article and were the authors' original work.

Code availability: Not applicable

Authors Contributions:

The study was designed by Yanpeng Zhao and Peifu Tang. Experiments in vitro were done by Zhongkui Guo, and experiments in vivo were done by Zhongkui Guo and Shi Cheng. The manuscript was drafted by Zhongkui Guo, Ming Chen, Yang Chen and Licheng Zhang. Data were collected and analyzed by Yi Li and Ya Gu. The manuscript was written by Zhongkui Guo.

Ethical considerations: Not applicable

Consent to participate: Not applicable

Consent for publication: Not applicable

References

1. Prieto-Alhambra, D., et al., Incidence and risk factors for clinically diagnosed knee, hip and hand osteoarthritis: influences of age, gender and osteoarthritis affecting other joints. Annals of the rheumatic diseases, 2014. 73(9): p. 1659-64.

2. Hunter, D. and S. Bierma-Zeinstra, Osteoarthritis. Lancet (London, England), 2019. 393(10182): p. 1745-1759.

3. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet (London, England), 2016. 388(10053): p. 1545-1602.

4. Glyn-Jones, S., et al., Osteoarthritis. Lancet (London, England), 2015. 386(9991): p. 376-87.

5. Grässel, S. and D. Muschter, Do Neuroendocrine Peptides and Their Receptors Qualify as Novel Therapeutic Targets in Osteoarthritis? International journal of molecular sciences, 2018. 19(2).

6. Dimitroulas, T., et al., Neuropathic pain in osteoarthritis: a review of pathophysiological mechanisms and implications for treatment. Seminars in arthritis and rheumatism, 2014. 44(2): p. 145-54.
7. Grässel, S. and D. Muschter, *Peripheral Nerve Fibers and Their Neurotransmitters in Osteoarthritis Pathology*. International journal of molecular sciences, 2017. 18(5).

8. Grässel, S., *The role of peripheral nerve fibers and their neurotransmitters in cartilage and bone physiology and pathophysiology*. Arthritis research & therapy, 2014. 16(6): p. 485.

9. Courties, A., J. Sellam, and F. Berenbaum, *Role of the autonomic nervous system in osteoarthritis*. Best practice & research. Clinical rheumatology, 2017. 31(5): p. 661-675.

10. Bratus-Neuenschwander, A., et al., *Pain-Associated Transcriptome Changes in Synovium of Knee Osteoarthritis Patients*. Genes, 2018. 9(7).

11. Lei, J., et al., *A novel neuron-enriched protein SDIM1 is down regulated in Alzheimer’s brains and attenuates cell death induced by DNAJB4 over-expression in neuro-progenitor cells*. Molecular neurodegeneration, 2011. 6(1): p. 9.

12. Bullock, C., et al., *Peripheral calcitonin gene-related peptide receptor activation and mechanical sensitization of the joint in rat models of osteoarthritis pain*. Arthritis & rheumatology (Hoboken, N.J.), 2014. 66(8): p. 2188-200.

13. Bratus, A., et al., *Candidate gene approach in genetic epidemiological studies of osteoarthritis-related pain*. Pain, 2014. 155(2): p. 217-21.

14. Hosaka, Y., et al., *Notch signaling in chondrocytes modulates endochondral ossification and osteoarthritis development*. Proceedings of the National Academy of Sciences of the United States of America, 2013. 110(5): p. 1875-80.

15. Ni, J., et al., *OSM is overexpressed in knee osteoarthritis and Notch signaling is involved in the effects of OSM on MC3T3-E1 cell proliferation and differentiation*. International journal of molecular medicine, 2015. 35(6): p. 1755-60.

16. Sugita, S., et al., *Transcription factor Hes1 modulates osteoarthritis development in cooperation with calcium/calmodulin-dependent protein kinase 2*. Proceedings of the National Academy of Sciences of the United States of America, 2015. 112(10): p. 3080-5.

17. Matsuzaki, T., et al., *Hes1 expression in mature neurons in the adult mouse brain is required for normal behaviors*. Scientific reports, 2019. 9(1): p. 8251.

18. Harris, L. and F. Guillemot, *HES1, two programs: promoting the quiescence and proliferation of adult neural stem cells*. Genes & development, 2019. 33: p. 479-481.

19. Chen, H. and L. Chen, *An integrated analysis of the competing endogenous RNA network and co-expression network revealed seven hub long non-coding RNAs in osteoarthritis*. Bone & joint research, 2020. 9(3): p. 90-98.

20. Cai, W., et al., *Identification of key biomarkers and immune infiltration in the synovial tissue of osteoarthritis by bioinformatics analysis*. PeerJ, 2020. 8: p. e8390.

21. Xu, W. and A. Huang, *Comment on: Deletion of c-Jun N-terminal Kinase Enhances Senescence in Joint Tissues and Increases the Severity of Age-Related Osteoarthritis in Mice*. Arthritis & rheumatology (Hoboken, N.J.), 2020.

22. Rhee, J., et al., *Inhibition of BATF/JUN transcriptional activity protects against osteoarthritic cartilage destruction*. Annals of the rheumatic diseases, 2017. 76(2): p. 427-434.

23. Ugbo, C., et al., *JNK signalling regulates antioxidant responses in neurons*. Redox biology, 2020. 37: p. 101712.

24. Zhang, Y., et al., *FGF21 impedes peripheral myelin development by stimulating p38 MAPK/c-Jun axis*. Journal of cellular physiology, 2020.
25. Castro-Torres, R., et al., Involvement of JNK1 in Neuronal Polarization During Brain Development. Cells, 2020. 9(8).

26. Xu, X., et al., Immediate early response protein 2 promotes the migration and invasion of hepatocellular carcinoma cells via regulating the activity of Rho GTPases. Neoplasma, 2020. 67(3): p. 614-622.

27. Xu, Z., et al., Immediate early response protein 2 regulates hepatocellular carcinoma cell adhesion and motility via integrin β1-mediated signaling pathway. Oncology reports, 2017. 37(1): p. 259-272.

28. Moriya, S., et al., Cloning and localization of immediate early response 2 (ier2) gene in the brain of medaka. Journal of chemical neuroanatomy, 2016. 77: p. 24-29.

Figures

Figure 1

Schematic illustration of study design. A total of 878 DEGs were identified with dataset GSE114007 (Step 1), and then Gene ontology (GO) enrichment analysis was performed (Step 2). All the 878 DEGs were tested according to their potential as neuro-related genes with the biological process (BP) result of GO analysis, and 76 genes were screened out (Step 3). GSE51588 (includes 1010 DEGs) and GSE55457 (includes 704 DEGs) were chosen for further
verification (Step 4). The Venn diagram was performed and 3 genes were identified as our key genes (Step 5). Finally, qRT-PCR was used to identify the accuracy of the key genes (Step 6).

Figure 2

Heatmap and volcano plot of DEGs between the osteoarthritis and normal cartilage tissues. (A) Differentially expressed gene expression heatmap of cartilage tissues (includes all up-regulated and down-regulated genes). (B) Differentially expressed genes were selected by volcano plot filtering (P < 0.05 and |logFC| > 1.5).
Figure 3

GO and pathway analyses results of DEGs in GSE114007 between osteoarthritis and normal cartilage tissues. (A) (B)(C) GO analyses results of DEGs. (A) Biological process (BP) (B) Cellular component (CC) (C) Molecular function (MF) (D) Pathway analyses results of DEGs (P < 0.05 and |logFC| > 1.5).
Figure 4

PPI network and the top 10 significant module of DEGs in dataset GSE114007 between osteoarthritis and normal synovial tissues. (A) The PPI network of DEGs in dataset GSE114007. (B) The top 10 hub genes of DEGs in dataset GSE114007
Figure 5

Venn diagram of DEGs from three datasets for key genes identification. The genes in the intersection were regarded as our key genes, and at last, three genes were identified as our key genes.
Figure 6

Relative expression level of HES1, JUN and IER2 genes. (A) Gene expression level of HES1, (B) JUN, and (C) IER2 between the normal and osteoarthritis group. * p value <0.05