Abstract. – OBJECTIVE: Osteoarthritis (OA) is a chronic degenerative disease of the joints, adversely affecting the quality of life for the patients. To better understand the mechanisms underlying the pathological changes in osteoarthritis and identify the key genes associated with osteoarthritis pathogenesis, we utilized a comprehensive bioinformatics approach to analyze the transcriptome between osteoarthritis synovial and control samples with public microarray datasets.

MATERIALS AND METHODS: First, the GSE82107 microarray dataset containing ten osteoarthritis synovial and seven control samples were selected from the Gene Expression Omnibus (GEO) database.

RESULTS: A total of 52 overlapped differentially expressed genes (DEGs) in GSE82107 and OA-associated genes in the Comparative Toxicogenomics Database were identified. These OA-associated DEGs were further incorporated into a protein-protein interaction (PPI) network. Gene proopiomelanocortin (POMC) was identified in the largest cluster of PPI network with Cytoscape. GO and KEGG analyses suggested that these genes were associated with multiple functions. Other GEO datasets of osteoarthritis synovial tissues, including GSE55235 and GSE55457, were used to validate the expression level of POMC. Quantitative polymerase chain reaction and western blot analyses were also used to test the expression levels of POMC in our osteoarthritis samples. We found POMC was positively associated with transporter complex, ion channel activity, and G protein-coupled receptor signaling pathway.

CONCLUSIONS: This study highlighted the OA-associated gene POMC, and its related biological pathways, suggesting it served as a potential treatment target in osteoarthritis.

Key Words: Osteoarthritis, Synovial membrane, Pro-opiomelanocortin.

Introduction

Osteoarthritis (OA) is a chronic degenerative disease of the joints associated with multiple risk factors, including obesity, genetic predispositions, joint damage, age, and sex. OA is the second leading risk factor of disability among men aged > 50 years old after cardiovascular disease. Employment loss imposes an additional financial burden for OA patients and their families, making this disease a significant socioeconomic problem. According to the epidemiological data, over 300 million individuals were globally affected by arthritis until 2019, with a proportion of 10%-17% among individuals over 40 years old.

Although the degeneration of articular cartilage is the key pathogenesis of OA, the synovial tissue adjacent to the joint cavity plays an important role in maintaining joint homeostasis. In patients with OA, the degree of diffuse knee synovitis positively correlates with cartilage destruction. Moreover, the inflammatory level of synovial fluid can also reflect the prognosis of OA.

Articular cartilage, a form of hyaline cartilage, is made up of chondrocytes, and the surrounding extracellular matrix is partly produced by synovial cells. These matrix proteins serve as ligands for specific chondrocyte cell surface receptors, supporting the migration, differentiation, and proliferation of these cells. These cartilage matrix proteins also affect OA-related inflammatory processes by controlling signaling pathways in the synovium and cartilage. Furthermore, the synovium includes multiple functional cell types, such as synovial dendritic cells, macrophages, and fibroblasts. A report found that interleukin-6 (IL-6) and matrix metalloproteinase-3 (MMP-3) secreted by synovial fibroblasts could promote osteophyte formation and cartilage degeneration.
R-spondin 2 (Rspo2) protein secreted by synovial macrophage M1 cells activated the β-catenin pathway in chondrocytes, facilitating the differentiation of chondrocytes or osteoblasts, and leading to osteophyte formation. Currently, chronic synovial inflammation has been considered as a key driver of OA. Further studies focused on identifying novel targets for treating OA-related cartilage degeneration.

Functional changes in the synovium can be caused by pathologically altered gene expression or dysfunctional interactions between proteins, leading to reduced extracellular matrix synthesis, enhanced enzymatic degradation of this matrix, or abnormal rates of chondrocyte death/activation/differentiation. Consequently, it is greatly important to identify additional biomarkers associated with OA incidence to prevent or treat this condition. Therefore, comprehensive analyses are necessary and should be conducted in detail at the genetic level. Establishing a mechanistic basis for synovial lesion formation will aid the prognostic evaluation of patients with OA and may help define novel therapeutic biomarkers to improve the outcome of patients.

Proopiomelanocortin (POMC), a precursor of various neuropeptides, produces multiple peptides (e.g., melanocortins and β-endorphin) through enzymatic processes and exerts its pleiotropic effects through binding to their receptors [e.g., melanocortin receptors (MCR) or opioid receptors]. The functional POMC system in the osteoarticular system, including multiple cell types (e.g., synovium, cartilage, and bone), has been confirmed by previous studies. POMC-derived neuropeptides are potent inflammation inhibitors and immunosuppressants. In 2011, a study found that the overexpression of POMC in a rat model of OA could inhibit cartilage damage. Another review summarized the role of POMC-derived peptides in the osteoarticular system, indicating the pleiotropic effects of these peptides, such as bone mineralization, extracellular matrix synthesis, and immunomodulation. However, more studies focused on the alteration of POMC-derived peptides (e.g., melanocortin and opioid) or their receptors in OA. For example, α-melanocyte-stimulating hormone (α-MSH) can elicit an anti-inflammatory effect in the synovium of patients with OA. There is still a lack of focus on POMC itself in patients with OA.

We hypothesize that the altered expressions of specific hub genes in the synovium of patients with OA disrupt the normal function of synovial molecular networks and initiate pathological changes. The Comparative Toxicogenomics Database (CTD) defines OA-associated genes, whereas the public Gene Expression Omnibus (GEO) database provides OA-associated microarray data from both OA and healthy synovial tissues. Therefore, to identify OA-associated synovial hub genes, a comprehensive bioinformatic analysis is employed to explore OA-associated differentially expressed genes (DEGs) and their functional networks by analyzing data from both CTD and GEO. Then we explore the associated functions of the hub gene with some mechanism experiments. Together, these results will elucidate potential targets, including POMC, in the treatment of OA patients and may provide a potential strategy for designing novel pharmacological agents for this condition.

**Materials and Methods**

**Collection of Clinical Tissue Samples**

Synovial specimens with control subjects and OA patients were collected from the First Affiliated Hospital of Anhui Medical University. This study was approved by the Academic Committee of The First Affiliated Hospital of Anhui Medical University with No. PJ2019-06-06, and aligned with the Declaration of Helsinki. Informed consent was obtained from all participants.

Normal synovial specimens were obtained during the operation from the patients undergoing hip arthroplasty for femoral neck fracture without histories of hip pain, hip surgery, or rheumatoid arthritis. OA synovial specimens were obtained from patients receiving knee replacement treatment for knee osteoarthritis without histories of hip surgery and rheumatoid arthritis. Additionally, 5 normal synovial tissues and 5 OA synovial tissues were collected during the operation, respectively.

**Data Sourcing and Pre-Processing**

The GSE82107 dataset, containing gene expression data from synovial biopsy samples of 10 end-stage OA patients and 7 healthy controls, was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE82107). Data were downloaded using the “GEOquery” R package, processed, and normalized using the “affy” R package. Annotation files were used to guide the matching of probes to gene signals, with unmatched probes removed. In cases where multiple probes matched a given
gene, only the probe with the highest signal value was retained for the next analysis. The GSE55457 dataset was analyzed in the same manner with the exclusion of rheumatoid arthritis samples.

**Identification of OA-Associated Genes**

DEGs were identified by comparing OA and control samples in the GSE82107 dataset via a classical Bayes method using the limma package (v. 3.10.3, http://www.bioconductor.org/packages/2.9/bioc/html/limma.html)\(^{23}\) based on the following threshold criteria: \(\log_2|\text{fold change (FC)}| > 1\) and \(p < 0.05\).

Genes associated with OA in CTD database were identified using the search term “osteoarthritis”, with an inference score \(\geq 20\) serving as the threshold for OA-related gene identification. The overlapped OA-related DEGs were then identified via the VENN analysis.

**Enrichment and Clustering Analyses**

The Gene Ontology (GO) was performed to predict the functional annotations of biological processes (BPs), cellular components (CCs), and molecular functions (MFs) related to specific genes. GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted via “clusterProfiler” R package (v. 3.14.3)\(^{24}\). GSEA analysis was performed with c2.cp.all.v2022.1.Hs.symbols.gmt gene set from MsigDB\(^{25,26}\) via “clusterProfiler” R.

**Protein-Protein Interaction (PPI) Network Establishment**

Interactions among DEGs were performed using the STRING database (v 10.0, http://www.string-db.org/)\(^{27}\) with a median confidence threshold of \(> 0.4\). Cytoscape (v 3.2.0, http://www.cytoscape.org/)\(^{28}\) was then used for PPI network construction. Clusters in the PPI network were identified by the MCODE plug-in (v 1.4.2, http://apps.cytoscape.org/apps/MCODE). Hub genes in the PPI network were identified by the cytoHubba plug-in (v 0.1, http://apps.cytoscape.org/apps/cytoHubba).

**Quantitative Polymerase Chain Reaction (qPCR)**

Synovial tissues were homogenized in 1,000 μL TRIzol (Invitrogen, Waltham, MA, USA) to extract RNA, and 10 μL cDNA was synthesized from 500 ng of total RNA per sample using a PrimeScript™ RT Reagent Kit (Takara, Dalian, China). An SYBR® Premix Ex Taq™ II Kit (Takara, Dalian, China) was then used to conduct all qPCR reactions in a 10 μL volume containing SYBR (5 μL), F+R primers (0.4 μL each), Table I, template cDNA (1.0 μL), and ddH2O (3.0 μL). Thermocycler settings were as follows: 30 s at 95°C, 35 cycles of 5 s at 95°C, and 34 s at 60°C. β-actin expression was used to normalize gene expression results.

**Immunohistochemistry**

Tissue sections were deparaffinized, and Target Retrieval Solution (Dako, Glostrup, Denmark) was used for antigen retrieval. Tissue sections were incubated with anti-TRPC3 (#ab51560) antibodies overnight at 4°C. Immunoreactivity was detected using the Dako Envision HRP Detection system/DAB following the manufacturer’s instructions.

**Statistical Analysis**

The results of qPCR were statistically analyzed using GraphPad Prism 6.0 (La Jolla, CA, USA), with groups being compared using unpaired \(t\)-tests. Statistical significance was set at \(p < 0.05\). Correlations between the expression levels of POMC and other genes were assessed using Spearman’s correlation analysis (\(r > 0.4\) and \(p < 0.05\)).

**Results**

**OA-Associated DEG Identification**

To identify the OA-associated synovial hub genes that facilitated pathological changes in OA, we downloaded microarray data, including healthy and OA synovium, from the GEO database. First, we constructed a PPI network based on OA-associated DEGs. We then identified the

Table I. The primers for qPCR. All primers for qPCR experiments were listed here from 5' to 3'.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>POMC</td>
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<td>5′-CCCTCTGTTCTTCTTCTC-3′</td>
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<tr>
<td>β-actin</td>
<td>5′-CTGTCCTCTGTAGCCTGCT-3′</td>
<td>5′-ATGTCAGCAGATTTCCC-3′</td>
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<tr>
<td>β-actin</td>
<td>5′-CACCATGGCAAATGAGCGGTT-3′</td>
<td>5′-AGGTTCTTTGGGAGTGGCAG-3′</td>
</tr>
<tr>
<td>TRPC3</td>
<td>5′-TTGCTACTGGATGCGACCACCT-3′</td>
<td>5′-GGAGCATTGGAACACAGGACC-3′</td>
</tr>
<tr>
<td>ADORA1</td>
<td>5′-ATGCCAACTTTCTGCTCTC-3′</td>
<td>5′-TGAGGCAGGTGTGGAGCTAC-3′</td>
</tr>
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hub genes in the PPI network and explored the potential mechanism of the OA-related hub gene in synovium by gene correlation analysis and functional enrichment analysis.

After microarray data standardization, boxplots/histograms and principal component analyses suggested that the data were suitable for further analysis (Figure 1A-C). In total, 1,745

Figure 1. OA-associated DEGs identification. A-B, Data distribution of GSE82107 (A, Bar graph; B, Histogram). C, Sample clustering analysis. D, Volcano plot of DEGs; top three upregulated and downregulated OA-associated genes were labeled with gene names. E, Overlap between DEGs and CTD OA-associated genes. F, A heatmap showing the expressional levels of the 50 overlapping genes; top three upregulated and downregulated OA-associated genes were marked with red squares.
DEGs were identified in the GSE82107 dataset, of which 369 and 1,376 were significantly (log fold change (FC) > 1 and p < 0.05) upregulated and downregulated, respectively. The clustering results and volcano plot revealed clear differences between OA samples and controls concerning the expression of these genes (Figure 1C-D). For further analyses, 481 genes associated with OA were downloaded from the CTD database. Then, we used Venn diagram analyses to identify 50 genes overlapping between the OA-related genes and aforementioned DEGs, of which 26 and 24 were upregulated and downregulated, respectively (Figure 1E). Clustering analyses of these OA-related DEGs revealed a clear differentiation between OA and control samples in this dataset. The top three upregulated genes in patients with OA were FOS, MMP13, and SOCS3, whereas the three most downregulated genes were LEP, THRSP, and FABP4 (Figure 1D-F).

Functional Gene/PPI Networks of OA-Associated DEGs and the Hub Gene POMC

Metascape was used to construct a network incorporating the 50 OA-related DEGs based on their functional association. A subset of terms from the overall cluster was suggested and transformed into a network layout. Each term was represented by a circular node with a size proportional to the number of associated genes and colors indicating cluster identity (such that identically colored genes were part of the same cluster). Edges were used to link terms with similarity scores > 0.3, with edge thickness being proportional to similarity scores. GO and KEGG enrichment analyses were performed for these genes. Network diagram results (Figure 2A-B) and heatmaps (Figure 2C) helped predict the functions of these 50 OA-related DEGs. These results suggest that these genes play important roles in regulating inflammatory pathways, vitamin D receptor signaling, and responses to oxygen levels. Metascape was also used to generate a fully connected interaction network (Figure 2D), revealing three clusters (Figure 2E). The terms with the highest p-values were retained for the functional assessment of the components of these clusters. In this analysis, the IL-4 and IL-13 signaling pathways were the most enriched. POMC, CXCR4, PTGER3, and CXCL2 genes were clustered in MCODE_1, whereas FOS, MMP2, TIMP1, and IGF1 were enriched in MCODE_2, and LEP, PRKAA2, and PPARGC1A in MCODE_3.

Furthermore, based on the protein-protein interaction of the STRING database, 173 interacting protein-protein pairs were identified for these 50 OA-related DEG-encoded proteins (Figure 3A-B). GO enrichment analyses were performed to explore the functions of these genes, revealing 671 enriched GO terms (295 BP, 5 CC, and 44 MF), including regulation of inflammatory response on the top (Figure 3C). KEGG enrichment analyses were additionally conducted, revealing 19 enriched KEGG pathways, including the Tumor Necrosis Factor (TNF) signaling pathway on the top (Figure 3D). MCODE analyses revealed two sub-networks enriched within the constructed PPI network: Modules A and B having 11 (including POMC) and 6 nodes, respectively (Figure 3E-F). To further explore the function of the proteins involved in this network, GO and KEGG analyses were performed for the genes in these two modules. Genes in Module A were significantly enriched for multiple GO terms, including ossification, endoplasmic reticulum lumen, and receptor-ligand activity (Supplementary Figure 1A), and 19 KEGG pathways, including osteoclast differentiation, parathyroid hormone synthesis, secretion and action, and endocrine resistance (Supplementary Figure 1B). Similarly, genes in Module B revealed multiple enriched GO terms, including vitamin transport, triglyceride catabolic process, and fat cell differentiation (Supplementary Figure 1C), and 16 enriched KEGG pathways, including insulin signaling, insulin resistance, AMPK signaling, and PPAR signaling pathways (Supplementary Figure 1D).

To further validate the importance of POMC as a hub gene in the entire network, genes with more than five nodes were identified (Supplementary Figure 2A), and a gene network with gene sets including > 10 nodes was visualized using GeneMANIA (Supplementary Figure 2B). The identified hub genes were associated with the regulation of bone remodeling, collagen metabolism, and biosynthetic processes. POMC was also identified as a hub gene by ranking the number of nodes connected to it.

Correlated Genes and Potential Mechanism of POMC for OA Pathogenesis

According to the above analyses, POMC was not only included in both MCODE_1 of the Metascape network and Module A of the PPI network but was also one of the hub genes in the whole network.
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Figure 2. Enriched term network of the 50 genes and gene network clusters. A, Cluster IDs were used for coloring in the Metascope network, with nodes sharing a cluster ID typically being closer to one another. B, p-value-based coloration was conducted in the Metascope network. C, Metascape was used to visualize the top 20 clusters of 50 genes. D-E, Gene association network (D) and MCODE clusters: MCODE_1, MCODE_1, and MCODE_3 (E).
Figure 3. PPI network module identification and functional enrichment analysis on the 50 genes. A, Overlapping genes were used to construct a PPI network. B, A PPI network was constructed for proteins encoded by the 50 OA-related genes, with red and green corresponding to genes that were upregulated and downregulated in the OA group, respectively (A). GO enrichment analysis of BP (biological process), CC (cellular component), and MF (molecular function) terms associated with the PPI network (C). KEGG pathway associated with the PPI network (D). PPI network cluster Modules A (E) and B (F).
However, only a few literature have focused on POMC in patients with OA. Therefore, we focused on the alterations and functions of POMC in OA.

First, we validated the downregulation of POMC in the OA synovium using clinical samples. The protein and mRNA levels of POMC were downregulated in OA synovial specimens relative to control samples in the five OA samples (Figure 4A-B).

To explore the potential mechanism of downregulated POMC in OA, we performed a correlation analysis to obtain the positively and negatively correlated genes of POMC in the GSE82107 dataset ($r > 0.4$ and $p < 0.05$). We found that POMC positively correlated genes were enriched in “transmembrane transporter complex” and “ion channel activity”, including 31 genes in both annotations (Supplementary Figure 3 A-B), whereas the negatively correlated genes of POMC were enriched in multiple annotations, including “sphingolipid metabolic process” and “sphingolipid signaling pathway” with four genes overlapped, i.e., SPTLC1, SPTLC3, SGPI, and SGMS1 (Supplementary Figure 4).

*Figure 4.* The validation and potential biological processes of downregulated POMC in OA. A-B, The validation of POMC expression using western blot (A) and qPCR (B) on our clinical synovial samples [n (control) = 5; n (OA) = 5]. C, The expression level of SGPI in healthy control (n = 3) and OA (n = 3) samples. D, The expression level of TRPC3 in healthy control (n = 3) and OA (n = 3) samples. E, The representative IHC figure showing the protein level of TRPC3 in synovium tissues [n (control) = 3; n (OA) = 3, bar length: 25 μm]. F, The western blot result shows the protein level of TRPC3 in synovium tissues [n (control) = 3; n (OA) = 3]. Ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. 

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Figure 3 C-D) (only “sphingolipid metabolic process” showed under r > 0.4 and p < 0.01 threshold; hence, we focused on sphingolipid-associated annotations). We used PPI analysis and functional enrichment analysis to identify the known interaction between POMC and its correlated functional genes and found that POMC may impact the “transmembrane transporter complex” and “ion channel activity” by its most confident interaction with TRPC3 (Supplementary Figure 3B). However, there is no known interaction between POMC and the other four sphingolipid-associated proteins, SPTLC1, SPTLC3, SGPP1, and SGMS1 (Supplementary Figure 3D).

To validate the results in GSE82107 (Supplementary Figure 3E), we performed DEG analysis and found POMC and its correlated genes were similarly regulated in GSE55235 and GSE55457 (Supplementary Figure 3F-3G). SGPP1 was significantly upregulated in GSE55235 and GSE55457. However, its downregulation was not significant in OA from our clinical samples (Figure 4C). Furthermore, as TRPC3 was significantly downregulated in both GSE55457 and GSE82107, we validated its significant downregulation in OA from our clinical samples (Figure 4D). Then, we performed immunohistochemistry staining of POMC in synovial tissues from three healthy individuals and three patients with OA. The density of anti-TRPC3 staining in healthy controls was higher than that in OA synovial tissues (Figure 4F). TRPC3 protein level was also downregulated in OA synovial tissues from the western blot results (Figure 4E).

POMC Affected Signaling Pathways in the OA Synovium

To explore the altered signaling pathways of downregulated POMC in OA synovium, we performed a GSEA enrichment analysis and selected all signaling annotations from GO enrichment analysis on its positively correlated genes. We found that the G protein-coupled receptor (GPCR) signaling pathway was significantly enriched in both GSEA (Supplementary Figure 4A) and GO (Supplementary Figure 4B), with 36 genes overlapped (Supplementary Figure 4C), and their expression levels showed a consistent downregulating trend with POMC in OA synovial tissues (Supplementary Figure 4D). These results provide a potential explanation for the alteration in the GPCR signaling pathway of downregulated POMC in OA.

To validate the results, we chose three genes from the 36 GPCR genes with the filter setting of log2 (Fold Change) > 1 and p < 0.01 in GSE82107 (Supplementary Figure 4E-F). Then, we analyzed the differential expression of the three genes in other GEO datasets (GSE55235 and GSE55457, Supplementary Figure 4G-H) and our clinical synovial samples (Figure 5A-C). We found that ADORA1 was consistently downregulated in the OA group of all the datasets, and NPY2R was the most significantly downregulated. However, although the protein level of ADORA1 was downregulated in the OA synovium (Figure 5D), the protein level of NPY2R was not significantly different between OA and control groups (Figure 5E).

Discussion

The rate of OA incidence has continued to increase in the elderly population. However, there is still an absence of an effective cure method for OA, with pain relief being the primary treatment option for the affected individuals. Therefore, exploring the mechanisms of articular cartilage degeneration is a fundamental issue both in laboratory exploration and clinical practice. Recently, it has been suggested that inflammation, age, sex, obesity, and joint damage contributed to OA-related cartilage degeneration. In this study, we used the OA synovial microarray dataset to identify OA-associated hub genes in the synovium and POMC-associated regulatory pathways. Meanwhile, we performed gene correlation and signaling pathway enrichment analyses and found that POMC-correlated genes TRPC3 and ADORA1 were associated with the transporter complex/ion channel and GPCR signaling pathway, respectively. POMC, TRPC3, and ADORA1 were downregulated in the synovium of patients with OA.

First, we identified 1,745 DEGs in the GSE82107 dataset when comparing OA and control samples. Among that, 52 overlapped genes were found between OA-related DEGs and genes in the CTD database, suggesting that these 52 genes might represent viable OA-related biomarkers. In the functional prediction analysis, we found that these OA-related DEGs were enriched in the “response to extracellular stimulus”, “vesicle lumen”, “signaling receptor activator activity”, “Adipocytokine signaling pathway”, and “TNF signaling pathway”, which was partly consistent with previous reports9. Synovial tissues from OA patients exhibited pronounced infiltrations of macrophages and monocytes.
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Figure 5. The validation and potential biological processes of downregulated POMC-related proteins with clinical samples. A-B. The expression level of POMC-associated GPCR DEGs in our healthy control (n = 3) and OA (n = 3) samples: ADORA1 (A), CHRM4 (B), and NPY2R (C). D-E. The western blot results showing the protein level of ADORA1 (D) and NPY2R (E) in synovium tissues [n (control) = 3; n (OA) = 3]. Ns, not significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001.
Literature found that macrophage activation induced the NF-κB signaling pathway, resulting in enhanced production of IL-1β and IL-18 in OA synovium. Furthermore, TNF-α was subsequently produced and secreted into the synovium, synovial fluid, articular cartilage, and subchondral bone.

POMC, encoded by the hub gene POMC, can be enzymatically processed into active peptides in a tissue-specific manner. POMC presumably mediates the effects of leptin on blood pressure, obesity, blood glucose levels, and appetite. Age-related OA has also been linked to obesity. However, the influence of POMC on OA remains unclear. In addition, we identified POMC-correlated genes and predicted their enriched functions and signaling pathways that potentially underlie OA pathogenesis. Downregulation of transporter complex/ion channel-associated TRPC3 may lead to the downregulation of POMC and may be involved in the pathogenesis of OA. Among these, a few studies have been conducted on the relationship between transmembrane transporter complex/ion channel activity and OA previously, which was potentially connected by the interaction between TRPC3 and POMC. Sphingolipid is a bioactive molecule that regulates the cell cycle, apoptosis, and senescence and plays important roles in OA pathology. Sphingolipid is involved in multiple functional processes, including the promotion of apoptosis (ceramide), inhibition of apoptosis and promotion of cell proliferation (sphingosine-1-phosphate), and promotion of inflammation (S1P, ceramide-1-phosphate), which are associated with the pathogenesis of OA. POMC potentially influenced these functions by cooperating with sphingosine-1-phosphate phosphatase 1 (SGPPI), of which the upregulation in OA has been confirmed previously.

Furthermore, the pathogenesis of OA may be associated with the downregulation of the GPCR signaling pathway due to the low level of POMC in the OA synovium. ADORA1-enriched GPCR signaling has been identified as an important component in OA pathogenesis. However, the role of downregulated ADORA1 in OA remains unclear, in addition to the known role of adenosine receptors in rheumatoid arthritis. ADORA1, with the highest affinity for the anti-inflammatory molecule adenosine, is associated with adhesion to matrix and osteoclast differentiation. We speculated that the destruction or mutation of POMC-associated transporter complex/ion channel and GPCR signaling involving TRPC3 and ADORA1, respectively, led to a pathological alteration of OA. Therefore, we recommended research on the function and mechanism of POMC-associated genes and their associated signaling pathway in maintaining homeostasis of the joint synovium. Our results might guide further studies of the biological processes and regulatory pathways involved in OA pathogenesis.

**Limitations**

This study has some limitations. First, we analyzed only a limited number of GEO datasets containing relatively few clinical samples. A large-scale prospective validation study and mechanism research are essential to validate these results. Moreover, our study did not confirm the mechanism links between the hub gene POMC and OA pathogenesis with an intervention experiment. We will perform in-depth research in the near future.

**Conclusions**

In conclusion, this study highlighted the OA-associated signaling pathways and dysregulated POMC modules that might influence the pathogenesis of OA. We found that the hub gene POMC potentially regulated the transporter complex, ion channel, and sphingolipid signaling, as well as the GPCR signaling pathway, which maintained the homeostasis of the joint synovium. Therefore, these targets might offer promising targets for developing new diagnostic strategies or therapeutic medication.

**Data Availability**

All data relevant to the study are included in the article for figures and supplemental figures. Data are available from the corresponding author upon reasonable request.

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**Conflict of Interests**

The authors report no conflicts of interest in this work. None of the authors have any personal or financial involvement with organizations that have a financial interest in the content of this manuscript.

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Ethics Approval
This study was approved by the Academic Committee of The First Affiliated Hospital of Anhui Medical University with No. PJ2019-06-06 and aligned with the Declaration of Helsinki.

Informed Consent
Informed consents were obtained from all participants.

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