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Original Article

CD19, *ALDH18A1*, and *CACNA1G* as Significant Hub Genes in End-Stage Osteoarthritis

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Abstract

Background: Osteoarthritis is one of the principal causes of chronic joint disease and may progressively engender disability in elderly individuals. The present study aimed to identify differentially expressed genes and associated signaling pathways in end-stage osteoarthritis.

Methods: Differentially expressed messenger RNAs in the early and end stages of osteoarthritis were examined through gene expression omnibus 2R (GEO2R) in the GSE32317 dataset. Subsequently, gene ontology (GO) enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG), and protein-protein interaction (PPI) analyses were conducted. Furthermore, microRNAs targeting hub genes were investigated using the miRcode database. This study was conducted jointly at Bam University of Medical Sciences and Rajaie Cardiovascular, Medical and Research Center on October 2022.

Results: Differentially expressed data demonstrated downregulation in 134 genes and upregulation in 189 genes in end-stage knee osteoarthritis. The results of the enrichment and PPI analyses determined 4 end-stage knee osteoarthritis-related hub genes: *IL-1B, CD19, CACNA1G*, and *ALDH18A1*. The knee osteoarthritis-related key genes were involved in the Wnt signaling, B cell receptor signaling, calcium signaling, circadian entrainment, arginine and proline metabolism, axon guidance, and cytokine-cytokine receptor pathways. Additionally, the microRNAs targeting the 4 aforementioned genes were predicted.

Conclusion: The present study is the first to provide fresh insights into the potential therapeutic targets of key genes, namely *CD19, CACNA1G*, and *ALDH18A1,* differentially expressed in end-stage osteoarthritis and their relevant signaling pathways and interactive microRNAs.

Keywords: Osteoarthritis; *IL-1B Protein; CD19; CACNA1G;* Hub genes

Introduction

Osteoarthritis is the most prevalent degenerative joint disease that affects the knee, hip, and small finger joints, leading to chronic pain, limited joint movement, and joint deformity in the elderly (1-4). Treatment approaches to impede or reverse osteoarthritis are limited by its long-term nature, diagnosis time, and numerous associated mechanisms (1-3).

In osteoarthritis development, affected joints predominantly undergo articular cartilage degen-

eration, osteophyte formation, subchondral sclerosis, synovitis, and meniscus degeneration, reflecting the complication of osteoarthritis pathogenic mechanisms (2-4).

Factors contributing to osteoarthritis development and progression include trauma, aging, obesity, fracture, surgery, ligament tear, and genes (5-8). Nonetheless, the exact pathogenesis underlying osteoarthritis has yet to be determined, hence the need for fresh insights into the genes and pathways is of great importance.

Recent advanced approaches, such as genome-wide association, candidate gene, and global gene expression analyses via microarray and RNA-sequencing techniques, help understand the pathogenesis of musculoskeletal diseases, including osteoarthritis (9-13). Bioinformatics studies can provide comprehensive data on gene expression alterations in messenger RNAs (mRNAs) in early and end-stage knee osteoarthritis and introduce specific gene hubs in its pathophysiology.

Accordingly, we conducted the present study to 1) determine differentially expressed gene profiles and perturbed molecular functions and pathways in early and end-stage osteoarthritis via a functional enrichment analysis, 2) uncover differentially expressed protein-protein interaction (PPI) networks through a PPI analysis, 3) reveal hub genes in a network analysis, and 4) analyze microRNA (miRNA) regulatory binding sites using the miRcode database to provide a list for each hub gene.

Methods

Samples

This study was conducted jointly at Bam University of Medical Sciences and Rajaie Cardiovascular, Medical and Research Center on October 2022.The National Center for Biotechnology Information's Gene Expression Omnibus (GEO) database was employed to download GSE32317 datasets (https://www.ncbi.nlm.nih.gov/geo/). GSE32317 samples were from 10 patients with early and 9 patients with end-stage knee osteoarthritis of microarray experiments. Microarray analysis is a method that uses microchips containing probes for the extensive examination of gene expression. All the samples were from the synovial membrane and were obtained from the suprapatellar pouch of patients with osteoarthritis hospitalized for surgery.

Microarray Data Analysis

Gene expression data arising from microarray technologies were analyzed using the R program package. The limma package was utilized to identify differentially expressed mRNAs between the early and end-stage knee osteoarthritis samples. A *P*-value below 0.001 was considered statistically significant. The analyses were conducted using the R software.

Functional Enrichment, PPI Network, and miRNA Target Site Prediction Analyses for Significant Hub Genes

Functional enrichment analysis defines whether identified biological functions, ontologies, or pathways are over-represented in a selected list of genes. Gene ontology (GO), composed of biological processes, cellular components, and molecular functions, in conjunction with Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathways, was used for the functional enrichment analysis via the Enrichr database (https://maayanlab.cloud/Enrichr/).

The PPI network was constructed based on the STRING (https://string-db.org) database. Following important hub gene selection, miRNAs with target sites on these genes were examined using miRcode (http://www.mircode.org/).

Statistical Analysis

A *P*-value below 0.001 determined the significance of the differential expression of the selected genes between the early and end-stage osteoarthritis samples. A *P*-value below 0.001 was also considered statistically significant in the functional enrichment analysis.

Ethics approval and consent to participate

The study protocol was approved by Bam University of Medical Sciences

(IR.MUBAM.REC.1401.087). No human contributors were employed directly in the present study.

Results

Differentially Expressed Genes (DEGs)

Totally, 323 mRNAs were differentially expressed between the early and end-stage knee osteoarthritis samples (Supplementary Table 1). Downregulation was detected in 134 genes and upregulation in 189 genes in the end-stage knee osteoarthritis samples (Supplementary Table 2 and Table 3).

GO Enrichment Analysis

The GO enrichment analysis revealed several prominent roles for the differentially expressed mRNAs. The GO biological process illustrated the major assignment of the differentially expressed mRNAs to the modulation of B cell proliferation, the regulation of ATPase-coupled calcium transmembrane transporter activity, the negative regulation of osteoblast proliferation, the unsaturated fatty acid biosynthetic process, and the regulation of the muscle system process (Fig. 1A). The GO cellular component depicted the significant classification of the genes in the endocytic vesicle membrane and the collagen-containing extracellular matrix (Fig. 1B). The GO molecular function part showed that the differentially expressed mRNAs were enriched predominantly in phosphotyrosine residue binding, coreceptor activity involved in the Wnt signaling pathway, the planar cell polarity pathway, and protein phosphorylated amino acid binding (Fig. 1C).

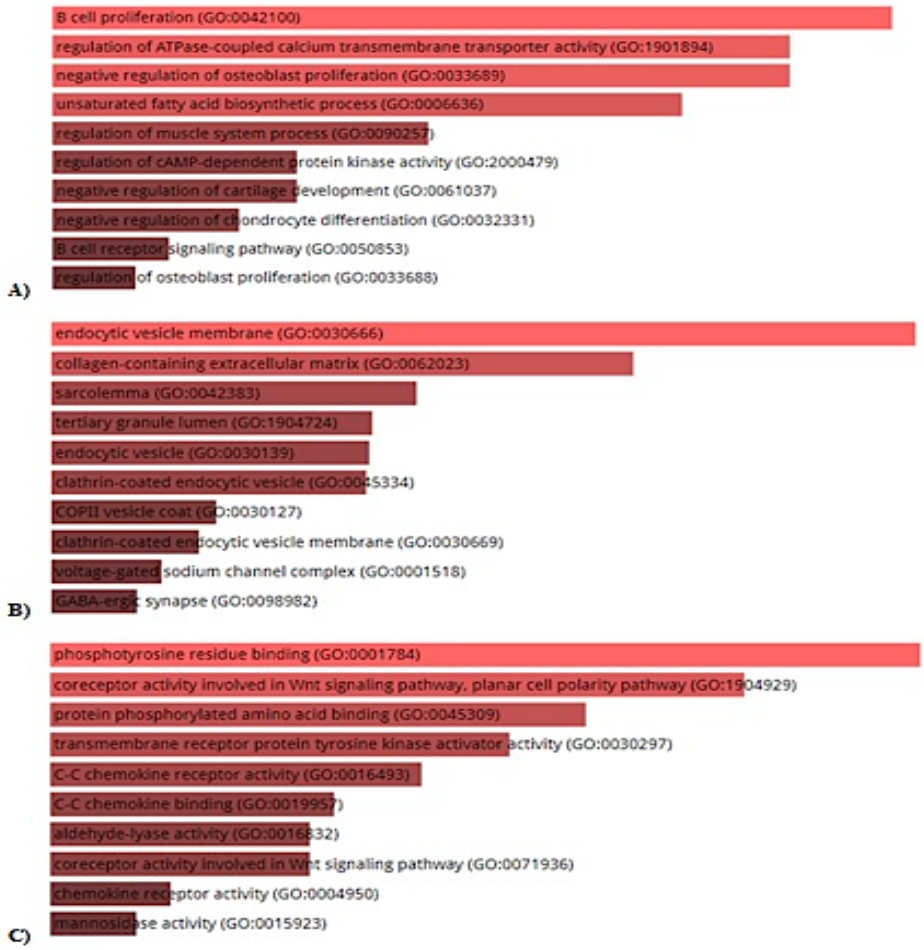

Fig. 1: The GO enrichment analysis of the top 10 differentially expressed mRNAs in end-stage knee osteoarthritis: A) biological process, B) cellular component, and C) molecular functions. GO, Gene ontology; mRNA, Messenger RNA

KEGG Pathway Analysis

The KEGG pathway analysis exhibited the remarkable attribution of the differentially expressed mRNAs to the pathways of primary immunodeficiency, hematopoietic cell lineage, arginine and proline metabolism, axon guidance, and cytokine-cytokine receptor interaction (Table 1).

Table 1: KEGG signaling pathway analysis of differentially expressed mRNAs in end-stage knee osteoarthritis (The top 20 KEGG terms are presented.)

Pathway	*P-value*	*Genes*
Primary immunodeficiency	0.0003551	*CD79A, CD8B, CD19, IGH, ADA*
Hematopoietic cell lineage	0.005566	*CD8B, CD19, IL1B, IL3RA, IGH, MS4A1*
Arginine and proline metabolism	0.008763	*ODC1, HOGA1, ALDH18A1, SRM*
Axon guidance	0.009990	*UNC5B, TRPC4, RAC2, NCK2, PTPN11, EFNA5, MYL9, CAMK2G*
GnRH secretion	0.02028	*TRPC4, GNA11, CGA, CACNA1G*
Circadian entrainment	0.02104	*GUCY1A2, GRIA2, RYR2, CAMK2G,CACNA1G*
Cortisol synthesis and secretion	0.02134	*GNA11, KCNK2, ATF4, CACNA1G*
Protein processing in endoplasmic reticulum	0.02198	*SEC24A, STT3A, SIL1, PDIA6, SEC23B, PDIA4, ATF4*
Cytokine-cytokine receptor interaction	0.02299	*CX3CR1, IL1B, INFE, CXCR2, IL3RA, CXCR5, TNFSF11, TNFRSF17, PPBP, BMP5*
Oxytocin signaling pathway	0.04019	*GUCY1A2, RYR2, MYL6, MYL9, PPP1R12C, CAMK2G*
Cushing syndrome	0.04126	*GNA11, FZD6, CAMK2G, KCNK2, ATF4, CACNA1G*
B cell receptor signaling pathway	0.04298	*CD79A, CD19, RAC2, IGH*
Calcium signaling pathway	0.04299	*RYR2, STIM2, GNA11, PPIF, IGH, ADRB2, CAMK2G, CACNA1G*
Focal adhesion	0.04645	*LAMA4, TNN, CAV2, RAC2, MYL9, PPP1R12C, ITGA9*
Cysteine and methionine metabolism	0.04759	*AHCY, SDSL, SRM*
Insulin secretion	0.05158	*RYR2, GNA11, CAMK2G, ATF4*
Wnt signaling pathway	0.05426	*DAAM2, FZD6, RAC2, RSPO2, ROR2, CAMK2G*
cGMP-PKG signaling pathway	0.05555	*GUCY1A2, GNA11, PPIF, ADRB2, MYL9, ATF4*

PPI Network Construction and Hub Gene Analysis

A PPI network of the differentially expressed mRNAs was constructed using the STRING database. PPIs are involved in almost all cellular processes, and a PPI analysis can provide unique insights into the human proteome and interactions between different proteins. The results of the PPI analysis pinpointed 4 prominent hub genes: Interleukin 1 beta (*IL-1B*), Cluster of Differentiation 19 (*CD19*), Calcium Voltage-Gated Channel Subunit Alpha1 G (*CACNA1G*), and Aldehyde Dehydrogenase 18 Family Member A1 (*ALDH18A1*), in terms of their gene interactions (Fig. 2). Among these hub genes, *IL-1B* had more gene interactions between the differentially expressed mRNAs, consisting of *IL-1B*, Interleukin 3 Receptor Subunit Alpha (*IL3RA*), *Rac Family Small GTPase 2* (*RAC2*), Interleukin Enhancer Binding Factor 2 (*ILF2*), Tachykinin Precursor 1 (*TAC1*), Adrenoceptor Beta 2 (*ADRB2*), Janus Kinase 1 (*JAK1*), NCK Adaptor Protein 2 (*NCK2*), Cytohesin 2 (*CYTH2*), Thyroid Hormone Receptor Interactor 10 (*TRIP10*), Dishevelled Associated Activator Of Morphogenesis 2 (*DAAM2*), Protein Kinase N3 (*PKN3*), TNF Superfamily Member 11 (*TNFSF11*), Calcium/Calmodulin Dependent Protein Kinase II

Gamma (*CAMK2G*), Ras Homolog Family Member C (*RHOC*), C-X-C Motif Chemokine Receptor 2 (*CXCR2*), Transforming Growth Factor Beta 1 Induced Transcript 1 (*TGFB1I1*), Pro-Platelet Basic Protein (*PPBP*), and TNF Receptor Associated Factor 5 (*TRAF5*) genes. Eight genes were upregulated, while 11 genes were downregulated.

The *CD19* hub gene was composed of 11 genes: *CD19*, CD79a Molecule (*CD79A*), Signal Transducing Adaptor Family Member 1 (*STAP1*), CD8b Molecule (*CD8B*), Immunoglobulin Lambda Like Polypeptide 5 (*IGLL5*), POU Class 2 Homeobox Associating Factor 1 (*POU2AF1*), Joining Chain Of Multimeric IgA And IgM (*IGJ*), TNF Receptor Superfamily Member 17 (*TNFRSF17*), C-X-C chemokine receptor type 5 (*CXCR5*), Immunoglobulin Heavy Variable 4-38-2 (*IGHV4-38-2*), and Membrane Spanning 4-Domains A1 (*MS4A1*), all of upregulated in the end-stage osteoarthritis samples.

The third significant hub gene comprised 11 genes: *ALDH18A*, Sphingosine-1-Phosphate Lyase 1 (*SGPL1*), 4-Hydroxy-2-Oxoglutarate Aldolase 1 (*HOGA1*), Protein Phosphatase, Mg2+/Mn2+ Dependent 1E (*PPM1E*), Pyrroline-5-carboxylate reductase (*PYCRL*), Spermidine Synthase (*SRM*), Adenosylhomocysteinase (*AHCY*), Ornithine Decarboxylase 1 (*ODC1*), Serine Dehydratase Like (*SDSL*), and Replication Factor C Subunit 3 (*RFC3*). Eight genes were upregulated, whereas 2 genes were downregulated.

The fourth hub gene interactions consisted of 7 genes: *CACNA1G*, Sodium Voltage-Gated Channel Alpha Subunit 9 (*SCN9A*), *Ryanodine Receptor 2* (*RYR2*), Potassium Voltage-Gated Channel Subfamily A Member 1 (*KCNA1*), Catsper Channel Auxiliary Subunit Epsilon (*C1orf101*), Caveolin 2 (*CAV2*), and Transient Receptor Potential Cation Channel Subfamily C Member 4 (*TRPC4*). Two genes were upregulated, while 5 genes were downregulated (Fig. 2).

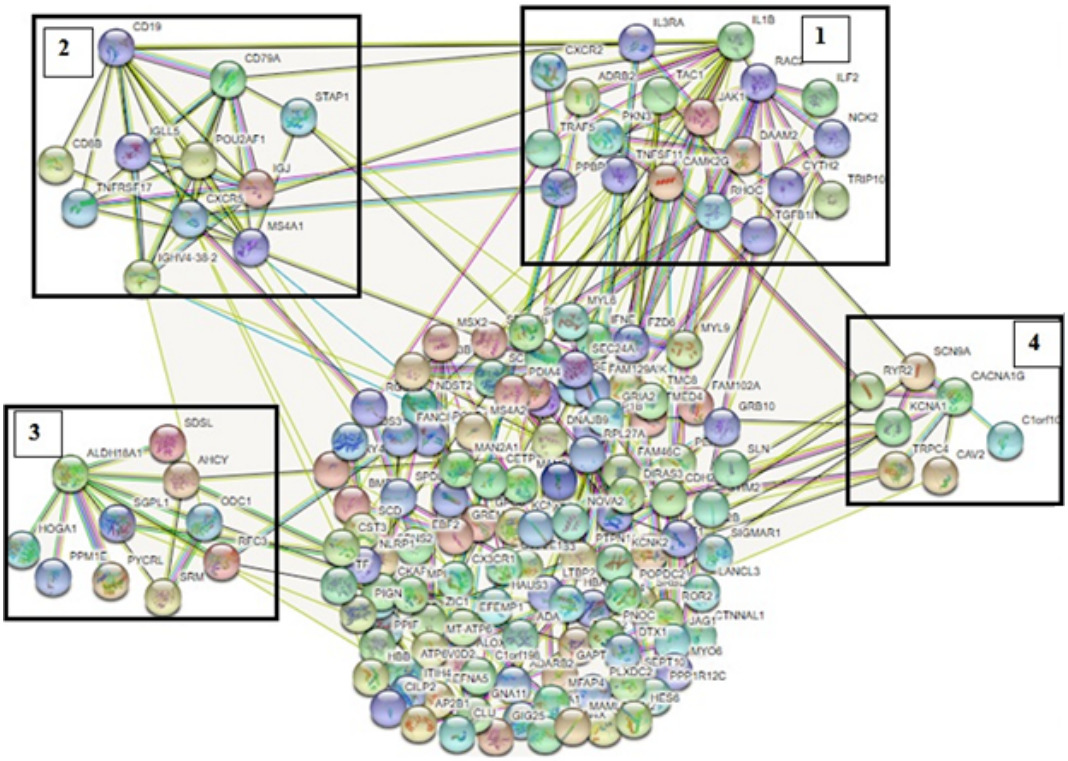

Fig. 2: The protein-protein network construction of differentially expressed mRNAs in end-stage knee osteoarthritis. Four important hub genes, namely *IL-1B, CD19, CACNA1G*, and *ALDH18A1*, and their interactions with other genes are demonstrated in a separate rectangle. mRNA, Messenger RNA

The pathway analysis for the first hub genes showed significant enrichment in the pathways of necroptosis (*P*=0.0004351), Wnt signaling (*P*=0.0004934), axon guidance (*P*=0.0006451), chemokine signaling (*P*=0.0007535), and cAMP signaling (*P*=0.001059) (Fig. 3A). The second hub gene was enriched in the pathways of primary immunodeficiency (*P*=0.000001033), hematopoietic cell lineage (*P*=0.00001886), and B cell receptor signaling (*P*=0.0008701) (Fig. 3B). The pathway analysis for the third hub genes showed enrichment in cysteine and methionine metabolism (*P*=0.000001742), arginine and proline metabolism (*P*=0.000001742), and glutathione metabolism (*P*=0.0003539) (Fig. 3C). In addition, the pathway enrichment analysis indicated the enrichment of the third hub genes in the pathways of gonadotropin-releasing hormone (GnRH) secretion (*P*=0.0002095), circadian entrainment (*P*=0.0004812), and calcium signaling (*P*=0.002894) (Fig. 3D).

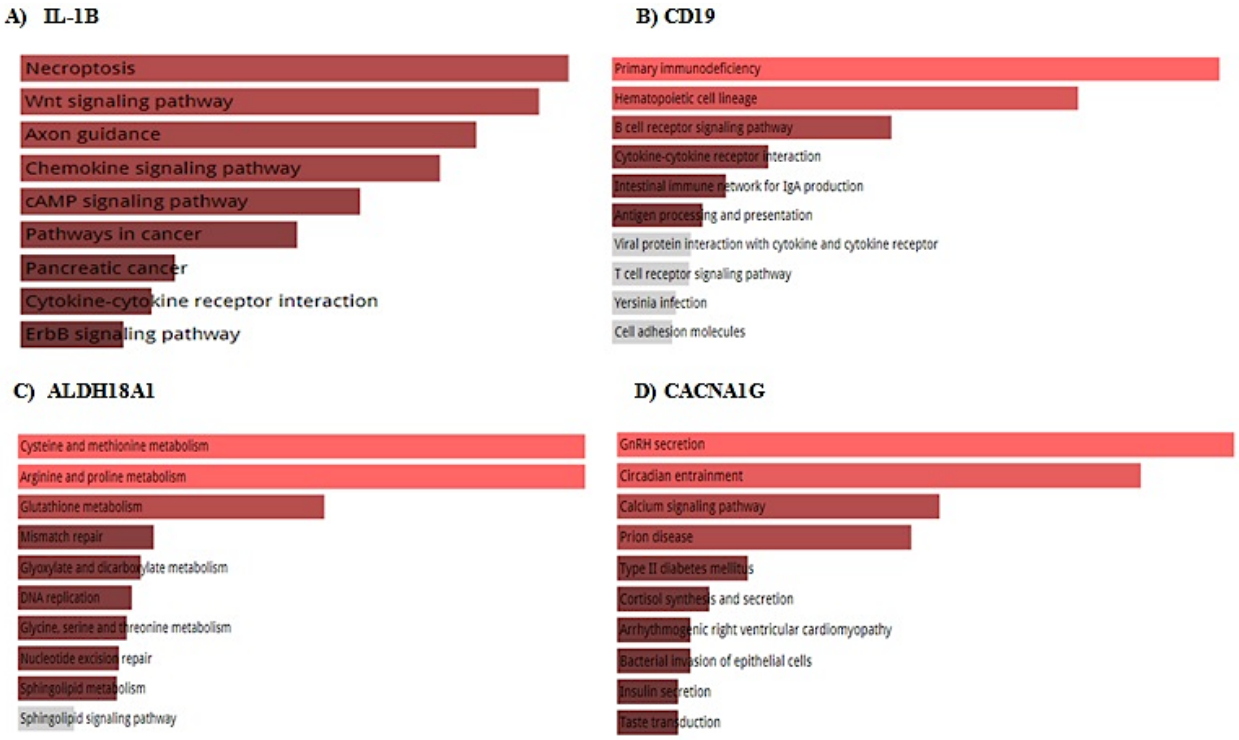

Fig. 3: The hub genes analysis. A) *IL-1B* hub genes are comprised of 19 genes enriched in the pathways of necroptosis, Wnt signaling, axon guidance, chemokine signaling, and cAMP signaling. B) *CD19* hub genes are composed of 11 genes enriched in the pathways of primary immunodeficiency, hematopoietic cell lineage, and B cell receptor signaling. C) *ALDH18A1* hub genes consist of 10 genes enriched in cysteine and methionine metabolism, arginine and proline metabolism, and glutathione metabolism. D) *CACNA1G* hub genes comprise 7 genes enriched in the pathways of GnRH secretion, circadian entrainment, and calcium signaling

MiRNAs Targeting IL-1B, CD19, CACNA1G, and ALDH18A1

The miRcode database was employed to identify the miRNAs targeting the 3´-UTR of the 4 hub genes (*IL-1B, CD19, CACNA1G*, and *ALDH18A1*). MiRcode provides whole transcriptome human miRNA target predictions corresponding to conserved sites in 46 species of vertebrates. The candidate miRNAs for each gene are presented in Table 2.

Table 2: MiRNA target sites of *IL-1B, CD19, CACNA1G*, and *ALDH18A1* based on the miRcode database

Gene name	*miRNA target sites (Highly conserved families)*	*miRNA target sites (Medium conserved families)*
IL-1B	miR-144, miR-21/590-5p, miR-24, miR-124/506, miR-204/211, miR-181/4262	miR-340-5p, miR-495/1192, miR-299/3563-3p, miR-149, miR-328, miR-488, miR-185, miR-544-3p, miR-326/330-5p
CD19	miR-7	miR-125a-3p/1554, miR-329/362-p,
CACNA1G	miR-96, miR-9, miR-150, miR-170, miR-132, miR-218, miR-129miR-216a, miR-190	miR-28-5p, miR-873, miR-544, miR-376, miR-758, miR-134, miR-154, miR-410, miR-450
ALDH18A1	miR-1ab/206/613, miR-182	miR-290-5p,miR-590-3p, miR-491-5p, miR-370, miR-758, miR-134, miR-149, miR-224, miR-873, miR-335, miR-149

Discussion

A complicated network encompassing numerous signaling pathways is implicated in osteoarthritis. Our differential expression gene analysis depicted 323 genes, of which 189 were upregulated and 134 were downregulated. Next, our GO enrichment analysis confirmed the prominent role of the DEGs in biological processes, cellular components, and molecular functions. To our knowledge, the current study is the first to compare DEGs and their associated pathways between early and end-stage osteoarthritis samples.

Our KEGG pathway analysis demonstrated that the DEGs were enriched in different pathways. Our results concerning these pathways are in line with the existing research outcomes (14, 15) In this regard, an abnormally activated Wnt signaling pathway plays a role in cartilage degradation, osteophyte development, and subchondral bone marrow osteoid islet formation and could, thus, be used to develop efficient therapeutic approaches to treat osteoarthritis (16, 17) Some clinical trial therapies have been run based on targeting Wnt signaling trials (ClinicalTrials.gov Identifier: NCT03928184).

Cytokines are manufactured in joint tissues and discharged into the synovial fluid. They are involved in various inflammatory processes, required for homeostasis, and implicated in osteoarthritis development (18, 19). Cytokine distribution is involved in osteoarthritis progression (19). Concordant with previous investigations (15, 19, 20), our results confirmed the role of the inflammatory process in end-stage osteoarthritis progression through the cytokine-cytokine receptor interaction and the B cell receptor signaling pathway, both of which might have the potential for use as targets for end-stage osteoarthritis diagnosis and treatment.

GnRH and pituitary gonadotropins undergo profound secretory alterations in rheumatoid arthritis development (21). Our results demonstrated GnRH secretion pathway involvement in end-stage osteoarthritis. The involvement of several inflammatory pathways is expected in this stage of osteoarthritis.

Evidence is scant vis-à-vis the involvement of the axon guidance pathway in osteoarthritis pathogenesis. Okubo et al (22) and Sumi et al (23) demonstrated the effects of Semaphorin 3A (Sema3A), an axon guidance molecule, on osteoarthritic chondrocytes. Our analysis, chiming in with Chang et al (11), indicated the possible role of this pathway in osteoarthritis progression.

Amino acid metabolism and profile changes in osteoarthritis are known (24, 25), yet no information is available regarding amino acid and nic-

otinamide metabolite profile alterations in early-stage osteoarthritis (26). A previous investigation demonstrated the benefit of functional amino acids in treating inflammation-associated diseases (24).

Our KEGG pathway analysis further confirmed the involvement of amino acid-associated pathways, composed of arginine and proline metabolism and cysteine and methionine metabolism, in end-stage osteoarthritis. It is, therefore, essential to study more in-depth analyses of amino acid profiles and associated metabolic alterations in end-stage osteoarthritis.

Accelerated articular cartilage degeneration and deficit constitute the fundamental characteristics of osteoarthritis and are considered the primary pathological change at the tissue level concerning osteoarthritis symptoms. Certain autophagy-enhancing drugs can diminish osteoarthritis cartilage degeneration (27). Chondrocytes play a pivotal role in cartilage homeostasis by secreting extracellular matrix components (28). The precise role of the calcium signaling pathway in loaded cartilage is not fully understood; nonetheless, the intracellular calcium signaling pathway is one of the earliest reactions of chondrocytes to physical stimuli (29, 30). Our analysis also confirmed the involvement of calcium signaling and focal adhesion pathways in the late stage of osteoarthritis. Further experimental analyses are needed to clarify the exact role of the aforementioned pathways in end-stage osteoarthritis.

Our PPI network analysis results demonstrated 4 prominent hub genes in end-stage osteoarthritis: *IL-1B, CD19, CACNA1G*, and *ALDH18A1*.

IL-1B, a major proinflammatory cytokine, is involved in the pathogenesis of numerous diseases and the activation of several signaling pathways, leading to osteoarthritis progression (31, 32). *IL-1B* prompts catabolic events, such as cartilage degradation, which is the most dominant process in osteoarthritis, reduces the production of the cartilage extracellular matrix (33, 34), and enhances the secretion of other cytokines (35). In our analysis, *IL-1B* was upregulated in end-stage osteoarthritis, in agreement with prior reports about *IL-1B* upregulation in osteoarthritis progression. Moreover, *IL-1B* predominantly interacts with *IL3RA, RAC2, ILF2, TAC1, ADRB2, JAK1, NCK2, CYTH2, TRIP10, DAAM2, PKN3, TNFSF11, CAMK2G, RHOC, CXCR2, TGFB1I1, PPBP*, and *TRAF5* and plays a significant role in the pathways of necroptosis, Wnt signaling, axon guidance, chemokine signaling, and cAMP signaling, all of involved in osteoarthritis progression.

In the current investigation, *CD19*, as the second hub, exhibited upregulation and interaction with *CD79A, STAP1, CD8B, IGLL5, POU2AF1, IGJ, TNFRSF17, CXCR5*, *IGHV4-38-2,* and *MS4A1*, enriched in the pathways of primary immunodeficiency, hematopoietic cell lineage, and B cell receptor signaling. Modulating CD19+ B cell status might provide novel therapeutic strategies for rheumatoid arthritis since these cells are associated with bone destruction in rheumatoid arthritis (36, 37). Thus far, no other investigations have demonstrated the involvement of *CD19* in osteoarthritis.

Our results showed *ALDH18A1* and *CACNA1G* as 2 other prominent hub genes. To our knowledge, the existing literature contains no information concerning the detection and function of these genes. According to our PPI network analysis, *ALDH18A1* exhibited upregulation and interaction with the differentially expressed *SGPL1*, *HOGA1*, *PPM1E*, *PYCRL*, *SRM*, *AHCY*, *ODC1*, *SDSL*, and *RFC3* genes in end-stage osteoarthritis. Further, *ALDH18A1* was predominantly enriched in amino acid metabolism signaling pathways such as cysteine and methionine metabolism, arginine and proline metabolism, and glutathione metabolism.

Altered profiles of amino acids and their metabolites are known in osteoarthritis. Our analysis also indicated the role of amino acid alterations in end-stage osteoarthritis and introduced a new marker, *ALDH18A1*, as a therapeutic target since it is probably involved in the metabolism and alteration of amino acids in end-stage osteoarthritis. Still, the precise role of *ALDH18A1* in osteoarthritis pathogenesis should be investigated through experimental analyses.

We found the downregulation of *CACNA1G*; its interaction with *SCN9A*, *RYR2*, *KCNA1*, *C1orf101*, *CAV2*, and *TRPC4*; and its enrichment in circadian entrainment and calcium signaling pathways in end-stage osteoarthritis.

The regulation of osteoarthritis progression by chondrocyte senescence has been demonstrated in several investigations (38, 39). Recent studies have shown the role of dysregulated circadian rhythms and the cellular clock in osteoarthritis pathogenesis (40). The involvement of calcium signaling pathways has been reported (29). In our analysis, the downregulation of *CACNA1G* suggested this gene as a new therapeutic target for osteoarthritis. However, the precise roles of *CACNA1G* and associated circadian entrainment and calcium signaling pathways need more in-depth investigations.

Prior investigations have demonstrated that miR-146a and miR-24-3p directly or indirectly target *IL-1B* and contribute to osteoarthritis pathogenesis by increasing vascular endothelial growth factor (VEGF) levels, impairing the TGF-β signaling pathway, and attenuating IL-1B-induced chondrocyte injury (41, 42). Notably, no report exists about the miRNAs that regulate the expression of *CD19, ALDH18A1*, and *CACNA1G* in osteoarthritis.

The expression of miR-138-5p, miR-146a-5p, miR-335-5p, and miR-9-5p is significantly upregulated, whereas miR-132 expression is meaningfully downregulated in osteoarthritis tissues (43, 44). Additionally, the downregulation of miR-24, miR-27b, miR-222, miR-370, miR-373, and miR-488 in osteoarthritis cartilage leads to matrix metalloproteinase production (45-49). miR-7-5p and miR-200c-3p expression levels in exosomes derived from synovial fluid were considerably higher in patients with osteoarthritis than in healthy subjects (50). According to our miRNA target analysis, miR-24 and miR-488 targeted *IL-1B*, miR-335 and miR-370 regulated *ALDH18A1*, miR-9 targeted the 3´-UTR of *CACNA1G* and *CD19*, and miR-7 targeted *CACNA1G* in end-stage osteoarthritis. Our findings should, however, be experimentally confirmed.

Conclusion

Prior studies have claimed the involvement of a convoluted network of signaling pathways in the development and progression of osteoarthritis, further validated by our pathway enrichment analysis. To our knowledge, the present investigation is the first to compare DEGs between early and end-stage osteoarthritis samples. We found DEGs in the synovial membrane of patients with end-stage osteoarthritis and, thus, added sets of genes and their related pathways to the list of associated genes and therapeutic agents for this group of patients. We also detected 3 novel therapeutic targets, namely *CD19, ALDH18A1*, and *CACNA1G*, for end-stage osteoarthritis; nevertheless, more in-depth investigations are needed to confirm their precise role.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interests.

Data Availability

The supplementary Table 1 (differentially expressed mRNAs between the early and end-stage knee osteoarthritis samples), supplementary Table 2 (downregulated genes in the end-stage knee osteoarthritis samples) and supplementary Table 3 (upregulated genes in the end-stage knee osteo-

arthritis samples) are accessible through request to corresponding author.

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