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# Investigation of differentially expressed genes and protein network analysis in synovium tissue of osteoarthritis patients

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## Abstract

**Introduction:** Osteoarthritis is a degenerative joint disease that leads to chronic pain, inflammation, and cartilage degradation, primarily affecting the elderly. The molecular mechanisms underlying osteoarthritis remain poorly understood, necessitating a comprehensive study of gene expression and protein interactions to identify key pathways and therapeutic targets.

**Objectives:** The primary objective of this study was to identify differentially expressed genes (DEGs) in synovial tissue of osteoarthritis patients and to analyze their associated protein-protein interaction (PPI) networks. By uncovering key regulatory genes, biological pathways, and potential drug targets, this research aims to enhance the understanding of osteoarthritis pathogenesis and contribute to the development of novel therapeutic strategies.

**Materials and Methods:** In this cross-sectional study, DEGs were identified from synovial tissue samples of osteoarthritis patients and healthy controls. Gene ontology (GO) and pathway enrichment analysis were performed, followed by PPI network construction to identify hub genes. Thirteen hub genes were identified from synovial tissue samples of osteoarthritis patients through differential expression and PPI network analysis. Further analysis included drug-target prediction using DrugBank database, transcription factor analysis via ENCODE, and promoter motif exploration using Tomtom and GOMO databases.

**Results:** Key pathways involved in osteoarthritis included PPAR signaling, lipolysis regulation, focal adhesion, and iron homeostasis. Drug-target analysis identified multiple candidates for pharmacological intervention, such as EGFR, TFRC, and LIPE. Promoter analysis revealed motifs linked to transcription factor activity, cytoskeletal organization, and inflammatory regulation, providing insight into upstream regulatory control.

**Conclusion:** This study highlights key genes and pathways involved in osteoarthritis, particularly those related to lipid metabolism, inflammation, and transcriptional regulation. The identified hub genes and their druggable potential offer promising avenues for developing targeted therapies to manage or slow disease progression.

## Introduction

Osteoarthritis, recognized as the most common joint disease globally, is a degenerative joint disorder primarily affecting older adults. This disease is a leading cause of physical disability worldwide, impacting over 500 million people (1). This condition significantly affects individuals' ability to perform daily activities and greatly reduces their quality of life. Due to the need for long-term treatment and joint replacement surgeries in advanced cases, osteoarthritis imposes a substantial economic burden on healthcare systems and patients (2).

This disease gradually leads to the destruction of joint cartilage, changes in subchondral bone, and mild inflammation, ultimately resulting in pain, stiffness, and reduced joint function. Joints such as the knee, hip, and hands are commonly affected. Apart from age and gender, obesity, joint injuries, intense physical activities, and genetic factors are major risk factors for osteoarthritis (3).

Osteoarthritis is a multifaceted condition involving a combination of mechanical, genetic, and inflammatory processes. Articular cartilage is a soft and elastic that coats the ends of bones at the joints, preventing direct bone contact and friction. In this condition, processes such as the overproduction of extracellular matrix-degrading enzymes, like matrix metalloproteinases (MMPs), lead to the gradual breakdown of cartilage (4). Mild inflammation is observed in the synovium (the inner lining of the joint) in osteoarthritis. This inflammation is mediated by the

**Key point**

- To investigate differentially expressed genes and analyze protein-protein interaction (PPI) networks in synovial tissue of osteoarthritis patients, aiming to identify key regulatory genes and potential therapeutic targets.
- Thirteen hub genes [EGFR, CD36, PLIN1, PLIN4, PLIN5, TFRC, LEP, LIPE, GPAM, DGAT2, PRKACG, PRKACB, and stearyl coenzyme A desaturase (SCD)] were identified as central regulators involved in lipid metabolism, inflammation, oxidative stress, and joint remodeling in osteoarthritis.
- Significant pathways associated with osteoarthritis included PPAR signaling, regulation of lipolysis in adipocytes, focal adhesion, and oxidative stress regulation, highlighting both metabolic and inflammatory mechanisms.
- Key transcription factors such as PPARG, SUZ12, REST, and TP53 were found to regulate the expression of differentially expressed genes, suggesting their involvement in the disruption of transcriptional networks linked to osteoarthritis progression.
- Targeting identified hub genes and regulatory pathways could pave the way for novel therapeutic strategies to slow or reverse osteoarthritis progression.

release of inflammatory cytokines such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), which stimulate the production of degrading enzymes, accelerating cartilage breakdown. These cytokines can also exacerbate joint pain and further degradation (5).

Several cellular pathways contribute to the onset and progression of osteoarthritis. The nuclear factor-kappa B (NF-κB) pathway is one of the most critical inflammatory pathways associated with osteoarthritis (6). This pathway is activated by inflammatory cytokines, which in turn leads to the production of more inflammatory mediators. The mitogen-activated protein kinase (MAPK) pathway is activated in response to inflammatory factors, stimulating the production of MMPs, which play a direct role in cartilage matrix breakdown. Cytokines such as interleukin-17 (IL-17) and interleukin-6 (IL-6) are also involved in inflammatory pathways and joint deterioration associated with osteoarthritis and are associated with pain severity and joint damage. As osteoarthritis progresses, the synovium thickens, and new bony structures called osteophytes form around the joint edges, causing pain and limiting movement (7).

Protein networks include many connections between proteins and signaling pathways elaborate in the onset and progression of osteoarthritis (8). Network analysis of proteins, using expression data, helps identify hub proteins those playing central roles in cellular processes and regulating biological pathways. These proteins represent potential drug targets as modulating their activity may help control the degenerative and inflammatory processes of the disease (8). A major challenge in treating this disease is resistance to certain anti-inflammatory drugs. Protein network analysis helps to identify mechanisms of treatment resistance. For example, if the protein network reveals persistent activity of proteins like IL-1β or IL-6, then the alternative therapeutic approaches to inhibit these proteins could be considered (9).

## Objectives

The aim of this study is to identify differentially expressed genes (DEGs) and analyze protein interaction networks in the synovial tissue of osteoarthritis patients compared to healthy individuals to uncover key proteins involved in disease progression. Additionally, the study seeks to identify potential therapeutic compounds that target the identified hub proteins, offering new candidates for osteoarthritis treatment.

## Materials and Methods

### *Data collection*

The data conducted in this cross-sectional study were obtained from the Gene Expression Omnibus (GEO) database under the accession number GSE206848. This dataset includes gene expression profiles from synovial tissue samples, consisting of seven normal samples and seven samples from osteoarthritis patients. These samples provide a comparative foundation to analyze gene expression differences between healthy individuals and osteoarthritis patients. For the preliminary analysis of the gene expression data, the GEO2R tool was employed (DEGs). Genes with a fold change of 2 or greater (either upregulated or downregulated) and an adjusted *P* value of less than 0.05 were selected as significantly differentially expressed.

### *Analysis of gene ontology and biological pathway enrichment*

To explore the significant cellular and molecular pathways, functional enrichment analysis of Gene Ontology (GO) and KEGG pathway analysis were conducted. The next step involved performing GO and pathway enrichment analysis (KEGG) on all DEGs recognized from the initial data analysis. This analysis was conducted using the Enrichr database (10).

### *Analysis of protein-protein interaction networks and identification of key genes*

To further explore the relationships between hub DEGs and identify key regulatory genes, we conducted a protein-protein interaction (PPI) network analysis. This was performed using the STRING database (version 12.0, available at https://string-db.org), which provides a comprehensive resource of known and predicted protein interactions (11). The interactions obtained from STRING were then imported into Cytoscape (version 3.10.0, available at https://cytoscape.org/), an open-source software platform used for visualizing molecular interaction networks (12).

To identify hub proteins within the network, we utilized the CytoHubba plugin in Cytoscape, which offers various algorithms to rank nodes based on their position within the network. In this study, multiple ranking algorithms

including MCC, MNC, DMNC, and MNC were applied within the CytoHubba plugin to ensure robust identification of hub genes.

By focusing on hub genes, we aimed to pinpoint the most influential genes within the PPI network that play a central role in osteoarthritis pathology.

### *Network analysis and clustering*

For the analysis of PPI networks, the ClusterONE algorithm was applied within the CytoCluster plugin to detect overlapping protein complexes based on interaction density and cohesiveness. To identify meaningful clusters and focus on significant interaction groups, the following parameters were set: Thin Threshold: 0.3; this parameter was applied to filter out weaker connections, retaining only stronger edges in the network. Complex size threshold: 3; only clusters containing at least three nodes were considered, ensuring that identified clusters represent meaningful interaction complexes. Shortest path length: 3; this parameter was used to limit the clustering to nodes within a maximum distance of three, promoting the identification of closely connected groups (13).

These parameters allowed for the identification of clusters with significant protein interactions within the PPI network, enabling further analysis of key protein complexes involved in osteoarthritis pathology.

### *Drug target interaction analysis of key genes*

In the next step, a drug target analysis was conducted to detect candidate therapeutic targets for the hub genes obtained from the network analysis. We utilized the DrugBank database (https://www.drugbank.cam) to search for existing drugs or compounds that specifically target hub proteins (14).

This analysis was aimed at uncovering potential therapeutic options for modulating the expression or activity of these key proteins, which could play a noteworthy role in the pathology of osteoarthritis. DrugBank provided insights into the available drugs, their mechanism of action, and their approval status, allowing us to evaluate each gene's potential as a pharmacological target.

### *Transcription factor disruption and promoter analysis*

To investigate the transcriptional regulatory landscape in osteoarthritic synovium tissue, transcription factor enrichment analysis was performed using the "ENCODE and ChEA Consensus TFs from ChIP-X" panel in Enrichr. This dataset integrates experimental ChIP-seq evidence to identify transcription factors potentially regulating the input DEGs. DEGs identified in the initial analysis were used as input in Enrichr to identify significantly enriched transcription factors. This analysis provided a ranked list of transcription factors associated with the DEGs, highlighting key regulatory elements potentially involved in osteoarthritis progression (15).

In the next step, we conducted a promoter analysis to further explore specific motifs within the regulatory regions of these DEGs. Using Tomtom, we compared identified motifs from the promoter regions against known motif databases by HOCOMOCO, to identify potential transcription factor binding sites. To complement this, GOMO was conducted to associate these motifs with specific biological processes and functions, linking them to GO terms. This combined approach allowed us to characterize the transcriptional regulatory mechanisms in osteoarthritis, identifying key transcription factors and motifs that may play significant roles in disease progression (16).

### *Statistical analysis*

The statistical analysis was conducted to identify significant DEGs and validate the robustness of the findings. Gene expression data from the GEO database (GSE206848) were analyzed using the GEO2R tool, which applies the limma (linear models for microarray data) package for differential expression analysis. Genes with a |log2 fold change| ≥1 and an adjusted $P$ value <0.05 (using the Benjamini-Hochberg false discovery rate correction) were considered significantly differentially expressed. For pathway enrichment and GO analysis, the Enrichr database was conducted, and statistical significance was determined based on adjusted $P$ values < 0.05. PPI networks were analyzed using the STRING database, and network topologies were examined in Cytoscape using the CytoHubba and CytoCluster plugins. Hub genes were identified based on degree centrality and maximal clique centrality (MCC) scores. All statistical analysis were performed using appropriate bioinformatics tools and databases, ensuring results with a $P$ value < 0.05 were considered statistically significant (13,17).

## Results

### *Distinct gene expression profiles in osteoarthritis synovial tissue*

The analysis of gene expression profiles from synovial tissue samples revealed significant differences between osteoarthritis patients and healthy controls. DEGs were identified, highlighting key genes with upregulated and downregulated expression associated with osteoarthritis pathology. The clustering of samples based on gene expression patterns demonstrated a clear separation between the osteoarthritis and control groups, emphasizing distinct molecular characteristics in osteoarthritis-affected tissues. These findings suggest that specific gene expression changes are closely linked to the disease process in osteoarthritis, potentially offering insights into critical pathways and targets for therapeutic intervention (Figure 1).

### *Enrichment analysis of key pathways in osteoarthritis*

The analysis identified several pathways significantly

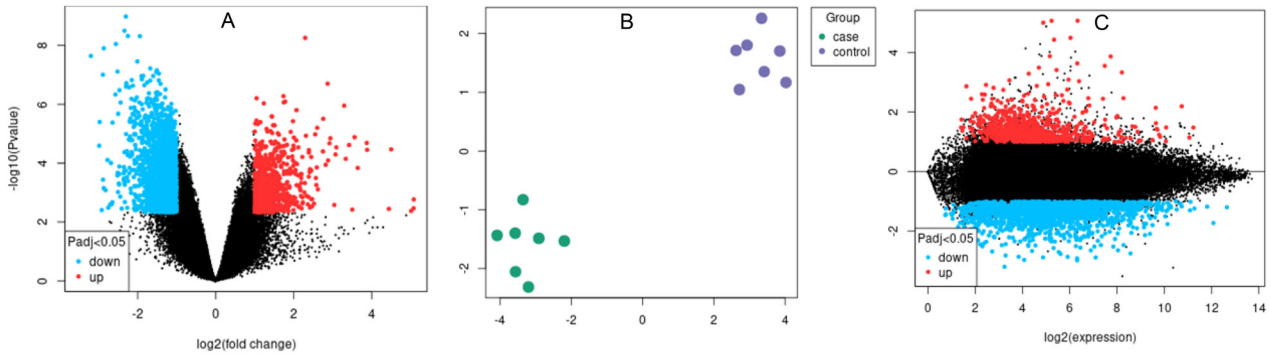



**Figure 1.** Differential gene expression analysis in synovium tissue of osteoarthritis patients. (A) Volcano plot showing gene expression changes based on logFC and -log10 (*P* value). Red and blue dots represent significantly upregulated and downregulated genes, respectively. (B) UMAP analysis: distribution of samples in the two groups (GSE206848). (C) Scatter plot of log2FC versus log2Exp to examine the distribution and expression changes of genes.

impacted in osteoarthritis, including "regulation of lipolysis in adipocytes," "PPAR signaling pathway," and "focal adhesion." In terms of cellular components, notable categories such as "lipid droplet," "myosin filament," and "adherens junction" were enriched. Furthermore, molecular functions like "aldehyde dehydrogenase (NAD+) activity," "protein kinase activator activity," and "voltage-gated sodium channel activity" were highlighted, reflecting processes involved in enzyme regulation, ion transport, and signal transduction (Figure 2). This enrichment analysis provides insights into key biological mechanisms and pathways that may contribute to the pathogenesis of osteoarthritis, potentially unveiling targets for therapeutic intervention.

### *Hub gene identification and protein-protein interaction network analysis*

In the analysis of the PPI network derived from DEGs in osteoarthritis synovial tissue, several key hub proteins were identified. These central proteins include EGFR, LPL, CDK1, TFRC, PLIN1, PLIN4, SPARC, SDC1, DIO2, FNDC4, and SPANXB1. Each of these genes demonstrated a high degree of interaction with other nodes in the network, suggesting pivotal roles in osteoarthritis pathogenesis. These genes exhibit significant connectivity within the network, suggesting that they are crucial regulators of molecular interactions associated with osteoarthritis (Figure 3). Identifying these hub genes and understanding their interactions offers valuable targets for therapeutic intervention. Modulating the activity or expression of these key genes could potentially disrupt the disease-promoting processes within osteoarthritic tissues, thus contributing to the development of targeted treatments for osteoarthritis.

### *Network clustering analysis of PPIs*

The clustering analysis of the PPI network was conducted using the CytoCluster plugin in Cytoscape, focusing on identifying significant sub-networks within the broader PPI network related to osteoarthritis. This analysis ranked the clusters based on node count and edge density, highlighting groups of proteins with potentially crucial interactions. As shown in Figure 4, a total of 10 significant

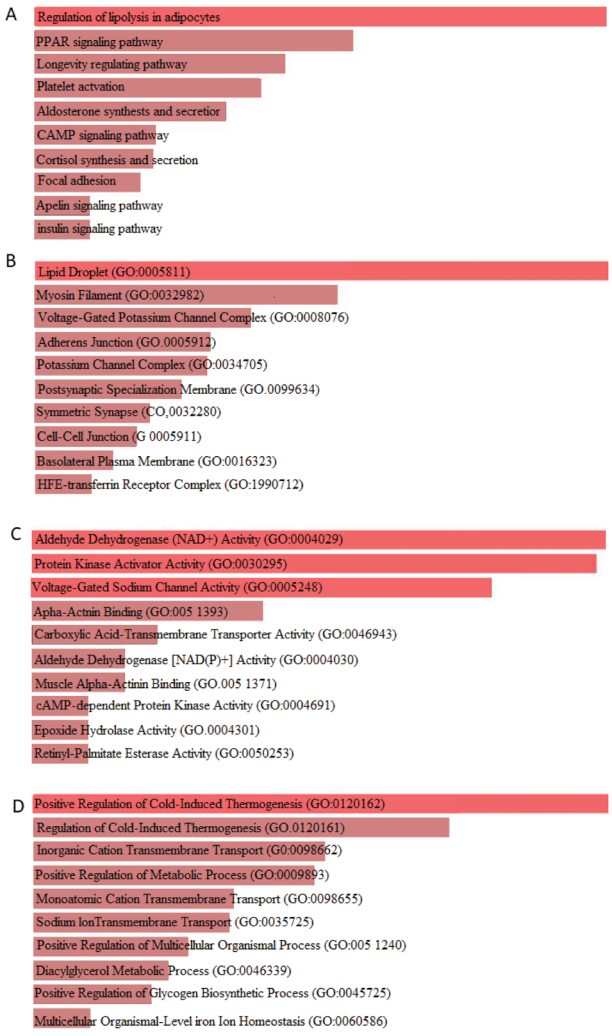



Figure 2; A: KEGG pathway enrichment alalysis, B: CC, C: MF, D: BP of DEGs

**Figure 2.** (A) KEGG pathway enrichment analysis of DEGs. (B) CC enrichment analysis of DEGs. (C) MF enrichment analysis of DEGs. (D) MP enrichment analysis.

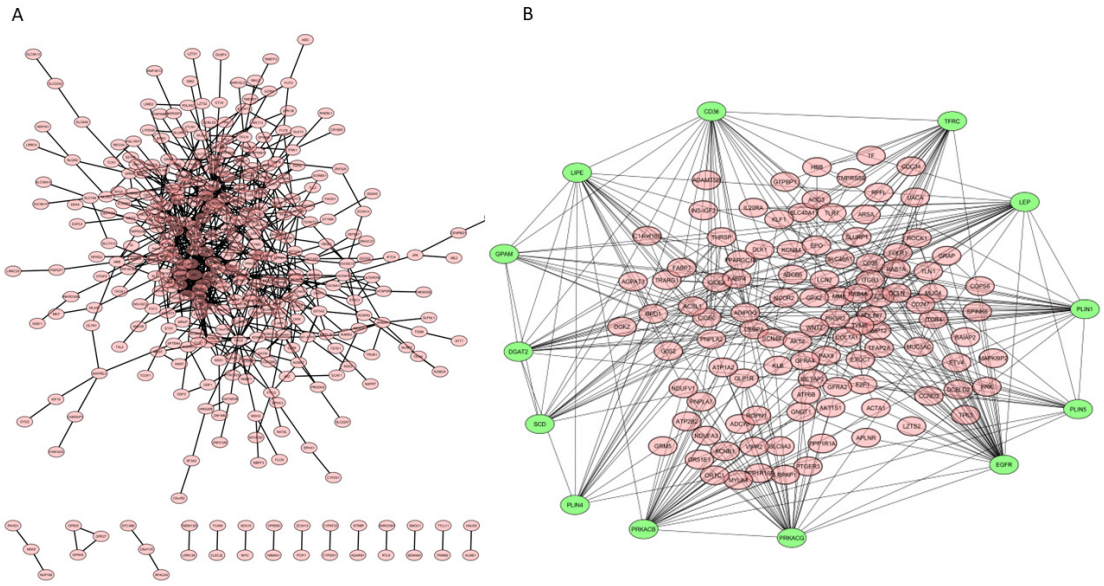



**Figure 3**. (A) Differentially expressed proteins network. (B) Hub proteins subnetwork.

clusters (A–J) were identified based on node connectivity, cluster density, quality score, and statistical significance (*P* value). Among them, cluster A was the largest and most interconnected module, consisting of 23 nodes with a density of 0.605 and a highly significant p-value of $2.82\times10^{-7}$. This cluster included central hub proteins such as EGFR, PLIN1, LPL, and SPARC, indicating its key role in essential biological processes such as lipid metabolism, extracellular matrix organization, and cellular signaling. Cluster B, with 7 nodes and a density of 0.810, included proteins like TYMP, CXCL12, and OSMR, which are associated with cytokine signaling and immune response—highlighting inflammatory mechanisms in osteoarthritis. Cluster C (6 nodes) comprised structural proteins such as ACTN1, TNS1, and FLNA, related to focal adhesion and cytoskeletal regulation. Similarly, cluster D contained actin-related proteins including ACTA1, MYH1, and MYL1, implicating muscular and mechanotransduction pathways in joint pathology. Cluster E, though smaller (3 nodes), showed perfect density and quality scores

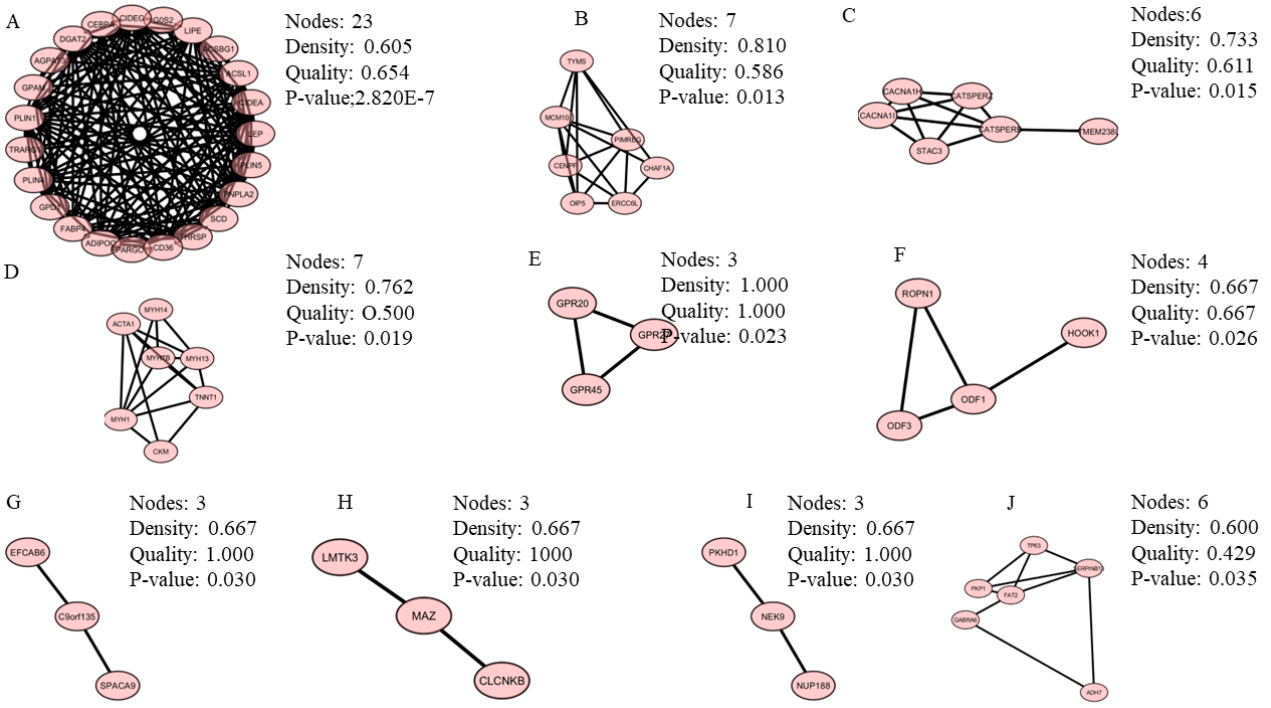



**Figure 4.** All significant clusters of proteins find by CytoCluster.

(1.000) and included G-protein coupled receptors GPR20, GPR45, and GPR85, suggesting potential signaling roles relevant to disease modulation. The remaining clusters—F to J—included diverse functional groups: for example, cluster F with proteins like HOOK1 and KPNB1 linked to nuclear transport; cluster G included calcium-binding and structural proteins such as EFCA8 and SPACA4; cluster H and I contained transcriptional regulators like MAZ and PHOT1; while cluster J featured genes such as PIM1 and ZNF207, potentially involved in signal transduction and transcriptional control.

Altogether, the identification of these distinct clusters underscores the modular organization of the osteoarthritis-related protein interaction network and highlights specific groups of functionally related proteins that may cooperatively drive disease progression. These modules represent potential multi-target therapeutic opportunities that go beyond single-gene interventions.

### *Drug target interaction analysis of hub genes*

To explore potential therapeutic strategies for osteoarthritis, a comprehensive drug target interaction analysis was performed on the identified hub genes using data from drug bank databases. Several drugs (both approved and investigational) were found to interact with key hub proteins involved in the osteoarthritis-associated network.

One of the most prominent druggable targets identified was EGFR, for which multiple inhibitors, antagonists, and antibodies are currently available or under investigation. These include afatinib, gefitinib, erlotinib, dacomitinib, lapatinib, osimertinib, brigatinib, and neratinib, among others. In addition, monoclonal antibodies such as cetuximab, panitumumab, necitumumab, and amivantamab were noted as EGFR down regulators or binders, highlighting EGFR as a highly actionable target in osteoarthritis-related pathways.

Fish oil, a nutraceutical compound, was identified as an agonist for DGAT3 and DGAT5, two lipid-associated genes implicated in metabolic regulation within the joint environment. Similarly, Niacin was reported to function as an inhibitor, though the specific gene target remains unspecified. Another druggable target was TFRC (transferrin receptor), which plays a role in iron transport and homeostasis. It was found to interact with multiple iron compounds such as ferrous fumarate, ferrous gluconate, ferrous glycine sulfate, ferrous succinate, and tetraferric tricitrate decahydrate, suggesting a potential link between iron metabolism and osteoarthritis progression. Fostamatinib, an inhibitor of PRKACB, a protein kinase potentially involved in intracellular signaling and inflammation. Lidocaine, noted as an antagonist, and lavendustin A and PD-168393, both experimental inhibitors, may also indirectly modulate key inflammatory signaling pathways. Benzyl benzoate was identified as a modulator of LIPE (lipase E), another gene related to lipid metabolism and joint tissue remodeling.

This drug-target profiling emphasizes that several hub genes are pharmacologically accessible through either direct inhibition, agonism, or modulation. Notably, EGFR, LIPE, TFRC, and DGAT family members emerged as promising candidates for therapeutic intervention in osteoarthritis, warranting further in vitro and in vivo validation studies (Table 1).

### *Transcription factor disruption and promoter motif analysis*

Using the Enrichr platform and the integrated ChEA 2022 and ENCODE ChIP-X databases, transcription factor enrichment analysis was performed to identify key regulators potentially involved in the observed gene expression changes. The analysis revealed that PPARG and SUZ12 exhibited the most significant enrichment (based on p-value ranking), suggesting a strong regulatory influence over the input gene set. Other transcription factors such as REST (from both ENCODE and ChEA), TP53, TP63, and ZC3H11A were also among the top candidates, indicating potential disruption in transcriptional regulation networks. These findings highlight the critical involvement of specific TFs, particularly PPARG and SUZ12, in modulating gene expression and possibly contributing to the underlying biological condition or phenotype (Figure 5).

Following transcription factor analysis, promoter analysis was conducted using Tomtom and GOMO to further explore specific motifs and functional associations of regulatory elements in the DEGs (16). Promoter analysis of selected hub genes revealed transcriptional regulatory motifs associated with diverse biological processes relevant to osteoarthritis. Notably, the promoter region of stearyl coenzyme A desaturase (SCD) and LEP was linked to GO terms such as negative regulation of signal transduction, protein dimerization, and transcription factor complex formation, suggesting their potential roles in modulating inflammatory and metabolic signaling pathways in joint tissues. The promoter of CD36 was enriched in motifs related to transcription initiation and regulation of organ growth, supporting its known involvement in chondrocyte differentiation and matrix remodeling. Similarly, PLIN4 showed associations with transcription factor activity and actin cytoskeleton organization, which may reflect its role in structural integrity and cellular response to mechanical stress. These findings offer mechanistic insights into the transcriptional regulation of osteoarthritis-relevant genes and suggest that upstream regulatory elements may serve as additional therapeutic targets (Table 2).

## Discussion

In this study, we conducted a comprehensive analysis of DEGs and PPI networks in synovial tissue samples from osteoarthritis patients compared to healthy controls. Our findings shed light on the molecular mechanisms underlying osteoarthritis and identify potential therapeutic

**Table 1.** Drug target interaction analysis of hub genes

| Drug | Drug group | Pharmacological action? | Actions | Target |
|---|---|---|---|---|
| Fish oil | Approved, investigational, nutraceutical | Yes | Agonist | DGAT3 DGAT5 |
| Niacin | | Unknown | Inhibitor | |
| Foreskin keratinocyte (neonatal) | Approved | | Agonist | |
| Afatinib | | | Inhibitor | |
| Amivantamab | | | Antibody down regulator | |
| Brigatinib | | | Inhibitor | |
| Cetuximab | | | Binder | |
| Dacomitinib | | | Inhibitor | |
| Erlotinib | | Yes | Antagonist | |
| Gefitinib | | | Antagonist | |
| Lapatinib | | | Antagonist | |
| Lazertinib | | | Inhibitor | |
| Necitumumab | | | Antagonist | |
| Osimertinib | Approved, investigational | | Inhibitor regulator | |
| Panitumumab | | | Suppressor | EGFR |
| Erlotinib | | | Inhibitor | |
| Fostamatinib | | | Inhibitor | |
| Mobocertinib | | | Inhibitor | |
| Neratinib | | | Inhibitor | |
| Nitroglycerin | | Unknown | Activator | |
| Sorafenib | | | Inhibitor | |
| Vandetanib | | | Inhibitor | |
| Zanubrutinib | | | Inhibitor | |
| Lidocaine | Approved, investigational | | Antagonist | |
| PD-168393 | Experimental | Yes | Inhibitor | |
| IGN311 | | | | |
| Lavendustin A | | | Inhibitor | |
| Fostamatinib | Approved, investigational | | Inhibitor | PRKACB |
| Phosphonothreonine | Experimental | | | |
| Benzyl benzoate | Approved | | Modulator | LIPE |
| Oxybenzone | Approved | Unknown | Inhibitor | |
| Ferrous fumarate | Approved, investigational | | | |
| Ferrous gluconate | Approved, investigational | | | |
| Ferrous glycine sulfate | Approved | | | |
| Ferrous succinate | Approved, investigational | | | |
| Iron | Approved, investigational | | | TFRC |
| Tetraferric tricitrate decahydrate | Approved, investigational | Yes | Ligand | |

targets that could contribute to the development of more effective treatments for this debilitating disease.

The initial gene expression analysis revealed significant differences between osteoarthritis patients and healthy individuals, highlighting specific genes that are upregulated or downregulated in osteoarthritis synovial tissue. These DEGs are likely contributors to the pathological processes of osteoarthritis, including cartilage degradation, inflammation, and joint remodeling (18,19). Pathways such as regulation of lipolysis in adipocytes, PPAR signaling, and focal adhesion, which are crucial for lipid metabolism, inflammation, and cell-matrix interaction in joint tissues. The regulation of lipolysis in adipocytes and the PPAR signaling pathway both play significant roles in the context of osteoarthritis, although the direct connection between "regulation of lipolysis in adipocytes" and osteoarthritis is less explicitly documented in the literatures. However, we can explore how PPAR signaling, which is closely related to adipocyte function, influences osteoarthritis (20,21). Cellular component enrichment (Figure 2B) revealed the significance of lipid droplets, adherens junctions, and potassium channel complexes, suggesting alterations in membrane structure and intercellular communication. In the molecular function category (Figure 2C), enriched terms such as aldehyde dehydrogenase (NAD+) activity, protein kinase activator activity, and voltage-gated sodium channel activity point to oxidative stress responses and ion channel dysregulation. Additionally, the biological

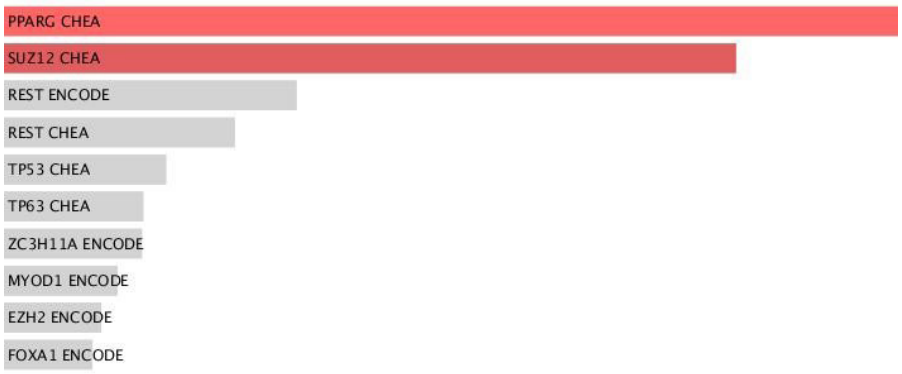



**Figure 5**. Top transcription factors identified from ENCODE database.

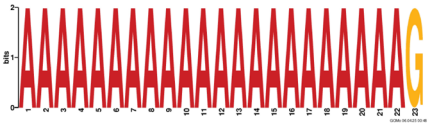
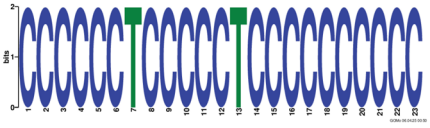
processes (Figure 2D) emphasized positive regulation of cold-induced thermogenesis, multicellular organismal homeostasis, and transmembrane ion transport, reflecting systemic metabolic adaptations and potential dysregulation of energy balance in osteoarthritis. Collectively, these enriched terms and pathways underline the multifactorial nature of osteoarthritis, involving inflammation, metabolism, and cellular structural alterations, and may offer novel directions for therapeutic intervention. Our PPI network analysis identified 13 hub genes: CD36, PRKACG, GPAM, PLIN5, SCD, EGFR, PLIN1, PLIN4, LEP, TFRC, DGAT2, LIPE, and PRKACB. A subset of the identified genes highlights a strong connection between lipid metabolism, inflammation, and cartilage degradation in osteoarthritis. CD36, a pattern recognition receptor, is involved in chondrocyte hypertrophy and cartilage repair, exerting anti-catabolic effects by modulating inflammatory pathways such as MKK3-p38 (20). Perilipins (PLIN1, PLIN4, PLIN5), along with GPAM, DGAT2, and LIPE, regulate lipid storage, lipolysis, and triglyceride synthesis processes that may influence chondrocyte energy balance and inflammatory lipid signaling (22). SCD, which alters fatty acid composition, may impact membrane fluidity and inflammation in joint tissues (23). Leptin (LEP), a key adipokine, promotes matrix degradation and cytokine production, especially in obesity-associated osteoarthritis (24). EGFR plays a dual role in cartilage homeostasis and repair but may also contribute to degradation when dysregulated. Furthermore, TFRC, through iron uptake, may increase oxidative stress, a known contributor to osteoarthritis progression (25). PRKACB and PRKACG, subunits of protein kinase A, participate in lipolysis and could influence chondrocyte signaling and inflammation (26). Collectively, these genes suggest that metabolic and signaling imbalances play a critical role in osteoarthritis pathophysiology and represent potential therapeutic targets.

The drug-target interaction analysis revealed several pharmacologically actionable hub genes, highlighting their therapeutic potential in osteoarthritis. EGFR emerged as the most druggable target, with a wide range of approved and investigational inhibitors, antagonists, and monoclonal antibodies such as afatinib, osimertinib, cetuximab, and panitumumab, capable of modulating it's signaling (27). In addition, TFRC, involved in iron metabolism, was targeted by multiple iron-based compounds, suggesting its role in oxidative stress regulation (28). Lipid metabolism-related genes such as LIPE and DGAT family members (DGAT3, DGAT5) were targeted by agents like fish oil, benzyl benzoate, and niacin, indicating a therapeutic avenue through modulation of lipid processing. Moreover, PRKACB, a component of cAMP signaling, was identified as a target of Fostamatinib, linking intracellular signaling pathways to potential pharmacological control. These findings support the idea that targeting metabolic and inflammatory components of osteoarthritis may provide novel and effective treatment strategies (Table 1).

The network clustering analysis further highlighted sub-networks of proteins that may act cooperatively in osteoarthritis progression. These clusters contain proteins involved in crucial cellular processes such as signal transduction, gene expression regulation, and cytoskeletal organization. Understanding these interactions provides a more integrated view of the molecular events in osteoarthritis and may identify combinatorial targets for therapy (Figure 4).

Promoter analysis of selected hub genes, in conjunction with transcription factor profiling from the ENCODE database, revealed key regulatory elements potentially involved in osteoarthritis progression (Figure 5). Genes such as SCD and LEP showed enrichment in motifs related to transcription factor complex formation and signal transduction regulation, highlighting their role in inflammatory and metabolic control. The presence of promoter motifs linked to transcription initiation and organ growth in CD36 supports its function in cartilage maintenance and response to injury. Additionally, PLIN4 exhibited associations with transcription factor activity and cytoskeleton organization, suggesting a role in maintaining chondrocyte structure and stress response. These findings underscore the importance of upstream regulatory elements in controlling gene expression networks in osteoarthritis and suggest novel targets for transcriptional modulation (Table 2).

**Table 2.** Promoter analysis of hub proteins

| Motif ID | Gene Symbol | Logo | Top 5 GO Predictions |
|---|---|---|---|
| ENSG00000099194 | SCD |  | CC transcription factor complex<br>BP negative regulation of signal transduction<br>MF protein heterodimerization activity<br>MF protein homodimerization activity<br>BP inner ear morphogenesis |
| ENSG00000135218 | CD36 |  | MF olfactory receptor activity<br>BP sensory perception of smell<br>BP regulation of organ growth<br>BP transcription initiation from RNA polymerase II promoter |
| ENSG00000167676 | PLIN4 |  | MF GTPase activity<br>MF transcription factor activity<br>BP actin cytoskeleton organization<br>MF potassium ion binding<br>MF protein homodimerization activity |
| ENSG00000174697 | LEP |  | CC transcription factor complex<br>BP negative regulation of signal transduction<br>MF protein heterodimerization activity<br>MF protein homodimerization activity<br>BP inner ear morphogenesis |

## Conclusion

This study enhances our understanding of the molecular mechanisms involved in osteoarthritis by identifying key DEGs, hub proteins, and transcription factors in synovial tissue. The identification of critical pathways and regulatory elements provides potential targets for therapeutic intervention. By focusing on these molecular targets, future therapies may more effectively halt or reverse the progression of osteoarthritis, improving the quality of life for millions affected by this condition.

## Limitations and future directions

Despite these significant findings, our study has limitations. The sample size is relatively small, which may affect the generalizability of the results. Future studies with larger cohorts are necessary to validate these findings. Additionally, our analysis is based on bioinformatic predictions and existing databases. Experimental validation through laboratory studies is essential to confirm the roles of the identified hub genes and transcription factors in osteoarthritis.

Moreover, while indirect drug targets were identified, the development of direct inhibitors for the hub proteins could provide more effective therapeutic options. Further research into the structural biology of these proteins may facilitate drug development efforts.

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### Authors' contribution

**Conceptualization:** Masoumeh Salari.
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### Ethical issues

The research was approved by the Ethics Committee of Mashhad University of Medical Sciences (Ethical code #IR.MUMS.REC.1403.375). The authors have fully complied with ethical considerations, including avoiding plagiarism, data fabrication, and duplicate publication.

### Conflicts of interest

The authors declare that they have no competing interests.

### Data availability statement

The data that support the conclusions of this study can be made available by the corresponding author upon a reasonable request.

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### References

1. Turkiewicz A, Petersson IF, Björk J, Hawker G, Dahlberg LE, Lohmander LS, et al. Current and future impact of osteoarthritis

on health care: a population-based study with projections to year 2032. Osteoarthritis Cartilage. 2014;22:1826-32. doi: 10.1016/j.joca.2014.07.015.
2. Gaigeard N, Cardon A, Le Goff B, Guicheux J, Boutet MA. Unveiling the macrophage dynamics in osteoarthritic joints: from inflammation to therapeutic strategies. Drug Discov Today. 2024;29:104187. doi: 10.1016/j.drudis.2024.104187.
3. Liu J, Jin H, Yon DK, Soysal P, Koyanagi A, Smith L, et al. Risk factors for depression in patients with knee osteoarthritis: a systematic review and meta-analysis. Orthopedics. 2024;47:e225-32. doi: 10.3928/01477447-20240821-10.
4. Maouche A, Boumediene K, Baugé C. Bioactive compounds in osteoarthritis: molecular mechanisms and therapeutic roles. Int J Mol Sci. 2024;25:11656. doi: 10.3390/ijms252111656.
5. Mukherjee A, Das B. The role of inflammatory mediators and matrix metalloproteinases (MMPs) in the progression of osteoarthritis. Biomater Biosyst. 2024;13:100090. doi: 10.1016/j.bbiosy.2024.100090.
6. Jeon Y, Kim J, Kwon H, Yeon YJ, Kim T, Ham J, et al. Cannabiorcol as a novel inhibitor of the p38/MSK-1/NF-κB signaling pathway, reducing matrix metalloproteinases in osteoarthritis. Phytomedicine. 2024;135:156141. doi: 10.1016/j.phymed.2024.156141.
7. Xu F, Li Z, Jiang Y, Liao T, Aschner M, Wei Q. Ononin delays the development of osteoarthritis by down-regulating MAPK and NF-κB pathways in rat models. PLoS One. 2024;19:e0310293. doi: 10.1371/journal.pone.0310293.
8. Rydén M, Sjögren A, Önnerfjord P, Turkiewicz A, Tjörnstrand J, Englund M, et al. Exploring the early molecular pathogenesis of osteoarthritis using differential network analysis of human synovial fluid. Mol Cell Proteomics. 2024;23:100785. doi: 10.1016/j.mcpro.2024.100785.
9. Apostu D, Lucaciu O, Mester A, Oltean-Dan D, Baciut M, Baciut G, et al. Systemic drugs with impact on osteoarthritis. Drug Metab Rev. 2019;51:498-523. doi: 10.1080/03602532.2019.1687511.
10. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016;44:W90-7. doi: 10.1093/nar/gkw377.
11. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023;51:D638-46. doi: 10.1093/nar/gkac1000.
12. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13:2498-504. doi: 10.1101/gr.1239303.
13. Li M, Li D, Tang Y, Wu F, Wang J. CytoCluster: a cytoscape plugin for cluster analysis and visualization of biological networks. Int J Mol Sci. 2017;18:1880. doi: 10.3390/ijms18091880.
14. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res. 2018;46:D1074-82. doi: 10.1093/nar/gkx1037.
15. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012;489:57-74. doi: 10.1038/nature11247.
16. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, et al. MEME SUITE: tools for motif discovery and searching. Nucleic Acids Res. 2009;37:W202-8. doi: 10.1093/nar/gkp335.
17. Wu B, Xi S. Bioinformatics analysis of differentially expressed genes and pathways in the development of cervical cancer. BMC Cancer. 2021;21:733. doi: 10.1186/s12885-021-08412-4.
18. Rai MF, Sandell LJ, Barrack TN, Cai L, Tycksen ED, Tang SY, et al. A microarray study of articular cartilage in relation to obesity and severity of knee osteoarthritis. Cartilage. 2020;11:458-72. doi: 10.1177/1947603518796122.
19. Li H, Bai B, Wang J, Xu Z, Yan S, Liu G. Identification of key mRNAs and microRNAs in the pathogenesis and progression of osteoarthritis using microarray analysis. Mol Med Rep. 2017;16:5659-66. doi: 10.3892/mmr.2017.7251.
20. Sheng W, Wang Q, Qin H, Cao S, Wei Y, Weng J, et al. Osteoarthritis: role of peroxisome proliferator-activated receptors. Int J Mol Sci. 2023;24:13137. doi: 10.3390/ijms241713137.
21. Zhang C, Lin Y, Yan CH, Zhang W. Adipokine signaling pathways in osteoarthritis. Front Bioeng Biotechnol. 2022;10:865370. doi: 10.3389/fbioe.2022.865370.
22. Sztalryd C, Brasaemle DL. The perilipin family of lipid droplet proteins: gatekeepers of intracellular lipolysis. Biochim Biophys Acta Mol Cell Biol Lipids. 2017;1862:1221-32. doi: 10.1016/j.bbalip.2017.07.009.
23. Ntambi JM, Miyazaki M, Stoehr JP, Lan H, Kendziorski CM, Yandell BS, et al. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. Proc Natl Acad Sci U S A. 2002;99:11482-6. doi: 10.1073/pnas.132384699.
24. Ait Eldjoudi D, Cordero Barreal A, Gonzalez-Rodríguez M, Ruiz-Fernández C, Farrag Y, Farrag M, et al. Leptin in osteoarthritis and rheumatoid arthritis: player or bystander? Int J Mol Sci. 2022;23:2859. doi: 10.3390/ijms23052859.
25. Jia H, Ma X, Tong W, Doyran B, Sun Z, Wang L, et al. EGFR signaling is critical for maintaining the superficial layer of articular cartilage and preventing osteoarthritis initiation. Proc Natl Acad Sci U S A. 2016;113:14360-5. doi: 10.1073/pnas.1608938113.
26. Espiard S, Drougat L, Settas N, Haydar S, Bathon K, London E, et al. PRKACB variants in skeletal disease or adrenocortical hyperplasia: effects on protein kinase A. Endocr Relat Cancer. 2020;27:647-56. doi: 10.1530/erc-20-0309.
27. Wei Y, Luo L, Gui T, Yu F, Yan L, Yao L, et al. Targeting cartilage EGFR pathway for osteoarthritis treatment. Sci Transl Med. 2021;13:eabb3946. doi: 10.1126/scitranslmed.abb3946.
28. Zhang L, Wang F, Li D, Yan Y, Wang H. Transferrin receptor-mediated reactive oxygen species promotes ferroptosis of KGN cells via regulating NADPH oxidase 1/PTEN induced kinase 1/acyl-CoA synthetase long chain family member 4 signaling. Bioengineered. 2021;12:4983-94. doi: 10.1080/21655979.2021.1956403.