Transcriptomic analysis reveals distinct molecular signatures and regulatory networks of osteoarthritic chondrocytes versus mesenchymal stem cells during chondrogenesis

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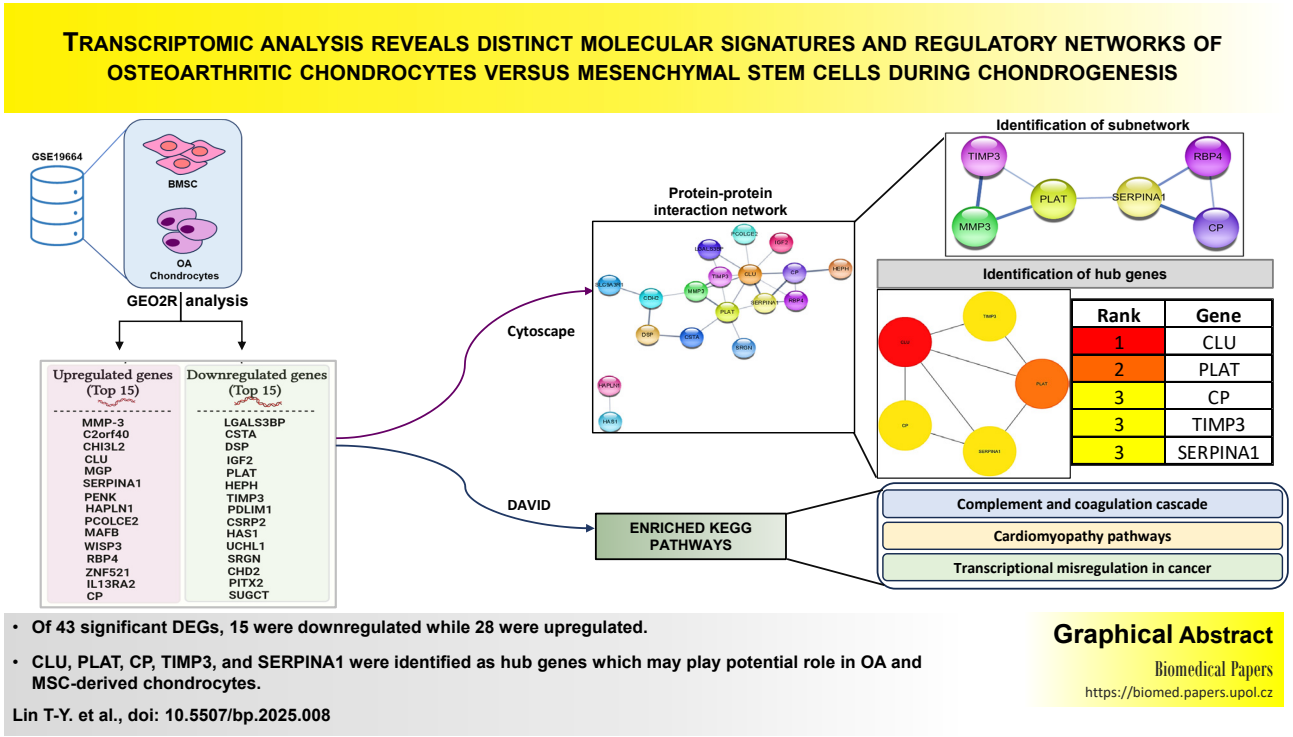
Background. Recent regenerative studies imply conflicting results on knee osteoarthritic (OA) chondrocytes and mesenchymal stem cells (MSC)-mediated cartilage constructs in terms of compressive properties and tensile strength. This could be attributed to different gene expression patterns between MSC and OA chondrocytes during chondrogenic differentiation. Therefore, we analyzed differentially expressed genes (DEGs) between OA and MSC-derived chondrocytes using bioinformatics tools.

Methods. We downloaded and analyzed the GSE19664 dataset from the Gene Expression Omnibus to identify DEGs. DAVID was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses, while a protein-protein interaction network of DEGs was constructed through the Search Tool for the Retrieval of Interacting Genes (STRING) and identified hub genes by CytoHubba.

Results. A total of 43 DEGs identified (15 downregulated and 28 upregulated) were found to be deregulated between OA and MSC-derived chondrocytes. KEGG analysis revealed the enrichment of complement and coagulation cascades and other pathways among the studied chondrocytes. The pathway enrichment identified top KEGG, gene ontology biological process, molecular function, and cellular component. The hub networks identified the top 5 hub genes involved in chondrogenesis, including *CLU*, *PLAT*, *CP*, *TIMP3*, and *SERPINA1*.

Conclusions. Our results identified significant genes involved in chondrogenesis. These findings provide new avenues for exploring the genetic mechanism underlying cartilage synthesis and novel targets for preclinical intervention and clinical treatment.



Key words: osteoarthritis, chondrocytes, mesenchymal stem cells, differentially expressed genes (DEGs), protein-protein interaction (PPI), hub genes, gene ontology (GO)

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INTRODUCTION

The globally high prevalence of knee osteoarthritis (OA) in aging populations translates as severely reduced quality of life (QoL) (ref.[1,2]). Knee OA is a progressive joint and cartilage degenerative disease. Factors such as aging, injury, subchondral bone remodeling, inflammatory factors, chondrocyte or synovial hypertrophy, along with systemic and chronic joint inflammation, induce pathophysiological changes leading to the progression of OA (ref.[2,3]), causing stiffness, pain, swelling, locking, and tenderness in the joint[4,5]. Notably, due to the lack of therapeutic agents to reverse the pathologic changes in the OA knee joint, only surgical intervention, like joint arthroplasties, remains a comprehensive treatment[6], and is not recommended in the early or mild stages.

In recent years, the progress in regenerative therapy, specifically using mesenchymal stem cells (MSC), has opened the way for reversing knee OA (ref.[7]). The use of mesenchymal stem cells (MSCs) results in pain alleviation and functional improvement through chondrocyte regeneration[7,8]. Additionally, the chondrocytes harvested from osteoarthritic patients could be used to regenerate cartilage. However, insufficient quantity and limited viability are major limiting factors[9,10]. Thus, the choice of MSCs seems the primary viable alternative owing to their chondrogenic differentiation. Various tissue sources like bone marrow, adipose tissue, amniotic fluid, periodontal ligaments, placenta, skeletal muscle tissue, synovial membranes, umbilical cord, and Wharton's jelly have been employed for harvesting stem cells to differentiate into chondrogenic lineage[11,12]. Studies have also demonstrat-

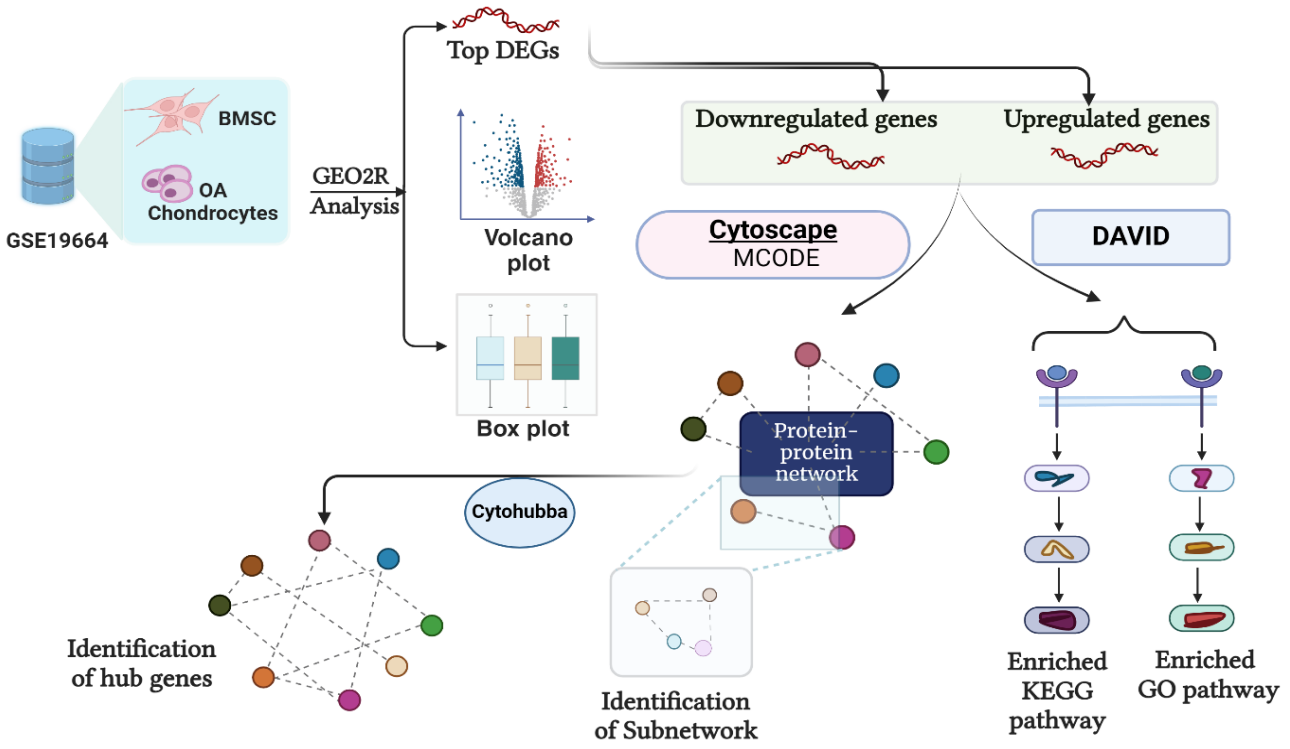

Fig. 1. Schematics of the proposed study. The dataset GSE 19664 was downloaded from gene expression omnibus (GEO) to identify downregulated and upregulated genes, protein-protein network, and hub genes. Created with BioRender.com.
BMSC, Bone marrow-derived stem cells; OA, osteoarthritis, DAVID, Database for Annotation, Visualization, and Integrated Discover, KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene ontology; GEO, Gene Expression Omnibus.

ed that chondroprogenitors harvested from moderately affected OA joints, may exhibit superior chondrogenic potential than bone marrow-derived MSC (BM-MSC) (ref.[13]). MSC and its secretome role in tissue repair is associated with its differentiation potential, immunomodulatory, and trophic effect[14]. Notably, though the MSCs' potential to regenerate cartilage is critical in OA therapy, the contradictory reports on the inferior quality of chondrocytes from differentiated MSCs limit its regenerative therapeutic potential.

MSC recruitment for the treatment of OA joints is a complex issue. The differential gene expression for OA chondrocytes and MSCs under chondrogenic conditions has been studied (GSE19664) using microarray technology[9]. This study indicates that MSCs produce inferior chondrogenic ECM and strong osteogenic lineage. For more details on the dataset used, please refer to Bernstein et al.[9]. Other recent studies too suggest that MSC-derived cartilage has poor compressive properties while their tensile strength is comparable[15-17]. On the other hand, OA-derived chondrocytes have been suggested for cartilage regeneration; however, the availability of therapeutically viable cells in sufficient quantity is still a challenge[9]. For these reasons, we analyzed GSE19664 using bioinformatics tools such as GEO2R, String, Cytoscape, and DAVID to shed light on the differential gene expression and identify important genes, pathways including the metabolic pathways between OA and MSC-derived chondrocytes. The schematic design of the study is represented in Fig. 1.

METHODS

Microarray data and screening of differentially expressed genes (DEGs)

Gene expression data of GSE 19664 were downloaded and analyzed using Gene Expression Omnibus 2R (GEO2R). The data were analyzed, including the control group to hBMSCs, which were isolated from iliac crest bone marrow from healthy female donors (n=5) (mean age: 45 years) and sample group as primary chondrocytes harvested from knee osteoarthritis (OA) joints of female patients (n=5) (mean age: 74 years) during alloarthroplastic procedures. A pellet culture system under chondrogenic conditions was employed to compare chondrocyte and MSC differentiation. Out of 8 dataset samples, 4 samples were grouped into hBMSCs, and 4 were placed into the OA cartilage chondrocytes group. The data were force normalized for analysis.

Functional and pathway enrichment analysis

Database for Annotation, Visualization, and Integrated Discovery (DAVID) provides a set of data-mining tools. Specifically, it provides exploratory visualization tools that promote discovery through functional classification, biochemical pathway maps, and conserved protein domain architectures, while simultaneously remaining linked to rich sources of biological annotation[18]. Hence, we used DAVID to enrich Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO). Analysis of GO is important to functionally study large-scale transcriptomic or genomic data, while the KEGG pathway database provides detailed information on the interactions and functions of molecules or genes. To investigate the biological functions of DEGs in tumor progression, GO functional enrichment analysis was conducted across the biological process (BP), molecular function (MF), and cellular component (CC) categories using the GO database. Additionally, pathway enrichment analysis was performed using the KEGG database. The significance threshold was set at $P<0.05$.

Construction of the protein-protein interaction (PPI), largest subnetwork, subnetwork, and hub genes

The STRING (Search Tool for the Retrieval of Interacting Genes) database offers uniquely comprehensive coverage and easy access to both experimental and predicted interaction information. To gain a better understanding of DEGs from an interactive perspective, we utilized the STRING database to construct a PPI network of the encoded products of all DEGs. Cytoscape, a standard tool for integrated analysis and visualization of biological networks, was used to visualize the PPI network using version 3.9.1 (http://www.cytoscape.org/). Subsequently, connectivity degree analysis was performed based on the scale-free properties of PPI networks, allowing for hub node identification. Molecular complex detection (MCODE) in Cytoscape, a graph-theoretic clustering algorithm designed to identify densely connected regions within large PPI networks that may represent molecular complexes, was used to extract modules from the PPI network with default parameter settings. The subnetwork was constructed using MCODE, version 2.0.2 plugin with criteria being degree cut-off = 2, MCODE score > 5, max depth=100, node score cut-off =0.2, and k-score=2. In addition, hub genes were identified using CytoHubba app. are used to construct PPI and sub-network, respectively. CytoHubba app is used to identify the top 10 ranked hub genes. Gene Cluster with Literature Profiles analyzes human genes with enriched keywords and molecular interactions to construct relevant molecular networks and modules associated with these terms.

RESULTS

Data retrieval and identification of DEGs

A total of 43 common genes were identified using GEO2R tools. Based on the criteria of log FC values i.e. >1.0 and <−1.0 (adj. $P<0.05$), 28 upregulated and 15 downregulated DEGs, respectively, were found, of those top 15 have been shown in Table 1. The volcano plot (Fig. 2) demonstrates up and downregulated genes. The normalized data revealed 43 common genes between hBMSCs and OA chondrocytes.

Functional enrichment analysis of DEGs

We used DAVID, an online tool, to enrich GO and KEGG pathways for 43 DEGs. Notably, GO enrichment analysis of 15 downregulated and 28 upregulated DEGs

Table 1. List of top 15 upregulated and downregulated DEGs.

Top 15 Upregulated Genes						
S. No.	ID	Gene symbol	Gene title	logFC	adj. *P* val	*P*
1	4314_at	*MMP3*	matrix metallopeptidase 3	5.147264	0.000981	0.000000076
2	84417_at	*C2orf40*	chromosome 2 open reading frame 40	4.795434	0.001843	0.000000385
3	1117_at	*CHI3L2*	chitinase 3 like 2	4.316284	0.005531	0.000005540
4	1191_at	*CLU*	Clusterin	3.384504	0.008679	0.000014800
5	4256_at	*MGP*	matrix Gla protein	3.236576	0.01934	0.000050600
6	5265_at	*SERPINA1*	serpin family A member 1	3.203134	0.014821	0.000034500
7	5179_at	*PENK*	Proenkephalin	3.089842	0.019371	0.000053600
8	1404_at	*HAPLN1*	hyaluronan and proteoglycan link protein 1	2.996599	0.013088	0.000027500
9	26577_at	*PCOLCE2*	procollagen C-endopeptidase enhancer 2	2.595092	0.01934	0.000051700
10	9935_at	*MAFB*	MAF bZIP transcription factor B	2.059865	0.043716	0.000211000
11	8838_at	*WISP3*	WNT1 inducible signaling pathway protein 3	1.925935	0.022453	0.000074000
12	5950_at	*RBP4*	retinol binding protein 4	1.904277	0.046285	0.000237000
13	25925_at	*ZNF521*	zinc finger protein 521	1.803847	0.00305	0.000001270
14	3598_at	*IL13RA2*	interleukin 13 receptor subunit alpha 2	1.624162	0.005709	0.000006530
15	1356_at	*CP*	ceruloplasmin (ferroxidase)	1.536566	0.002685	0.000000763
Top 15 Downregulated Genes						
S. No.	ID	Gene symbol	Gene title	logFC	adj. *P* val	*P*
1	3959_at	*LGALS3BP*	galectin 3 binding protein	−2.58588	0.01043	0.0000184
2	1475_at	*CSTA*	cystatin A	−1.89371	0.005709	0.0000068
3	1832_at	*DSP*	Desmoplakin	−1.85177	0.007181	0.0000114
4	3481_at	*IGF2*	insulin like growth factor 2	−1.64462	0.028512	0.0001150
5	5327_at	*PLAT*	plasminogen activator, tissue type	−1.62823	0.007099	0.0000109
6	9843_at	*HEPH*	Hephaestin	−1.58277	0.020901	0.0000630
7	7078_at	*TIMP3*	TIMP metallopeptidase inhibitor 3	−1.53527	0.003494	0.0000026
8	9124_at	*PDLIM1*	PDZ and LIM domain 1	−1.52084	0.005354	0.0000049
9	1466_at	*CSRP2*	cysteine and glycine rich protein 2	−1.41459	0.003494	0.0000024
10	3036_at	*HAS1*	hyaluronan synthase 1	−1.40659	0.04711	0.0002520
11	7345_at	*UCHL1*	ubiquitin C-terminal hydrolase L1	−1.3874	0.041799	0.0001950
12	5552_at	*SRGN*	Serglycin	−1.38213	0.006097	0.0000083
13	1000_at	*CDH2*	cadherin 2	−1.29876	0.036166	0.0001580
14	5308_at	*PITX2*	paired like homeodomain 2	−1.23249	0.017698	0.0000433
15	79783_at	*SUGCT*	succinyl-CoA:glutarate-CoA transferase	−1.03743	0.012166	0.0000249

The screening criteria for upregulated and downregulated genes were log FC>1.0 (P<0.05) and log FC<−1.0 (P<0.05), respectively.
DEGs, Differentially expressed genes; FC, Fold Change.

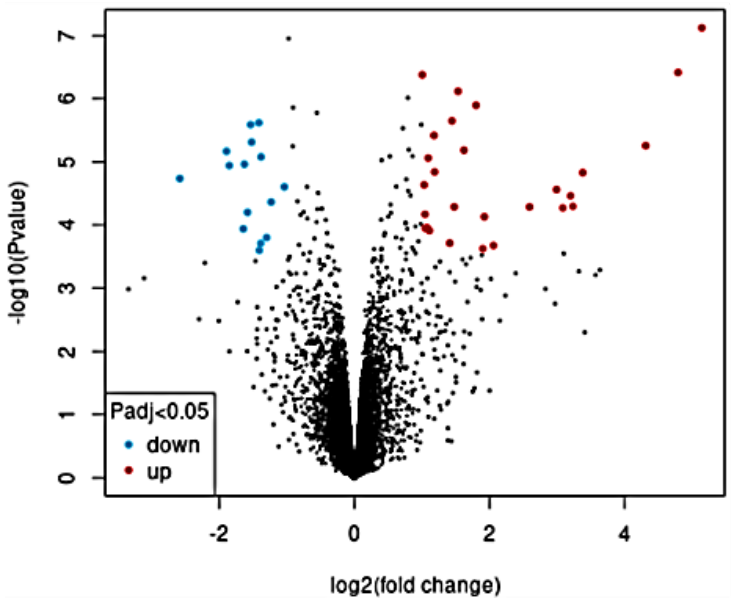

Fig. 2. Volcano Plot. Red dots and blue dots represented up-regulated and down-regulated DEGs, whereas gray dots represented genes that were not differentially expressed.
OA, Osteoarthritis; hBMSCs, Human bone marrow stem cells.

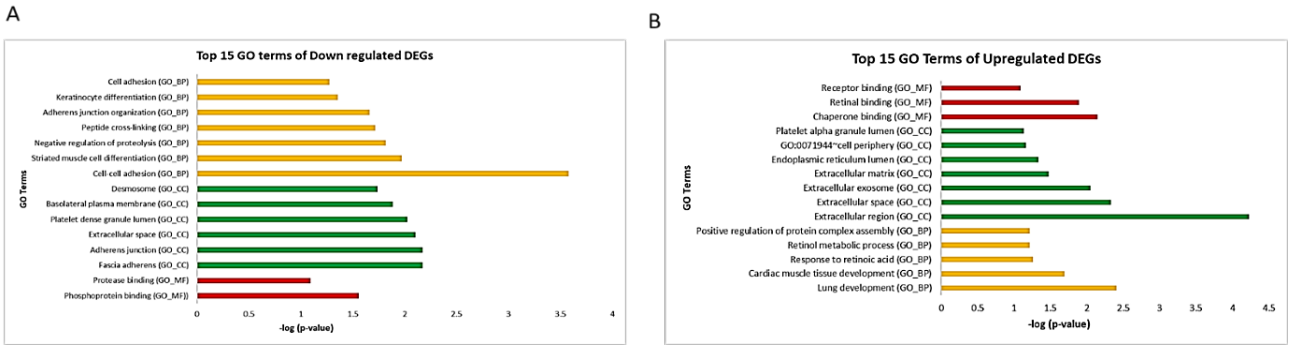

Fig. 3. Top 15 enriched GO terms associated with BP, CC, and MF of (**A**) Downregulated and (**B**) Upregulated genes. GO, Gene Ontology; GO_BP, Gene Ontology Biological Process; GO_CC, Gene_Ontology_Cellular Component; GO_MF, Gene Ontology Molecular Function.

Table 2. The top 15 enriched GO terms of downregulated DEGs.

Category	Term	Count	*P*	Genes
BP	Cell-cell adhesion	4	0.000263	*PDLIM1, DSP, CSTA, CDH2*
BP	Striated muscle cell differentiation	2	0.010643	*CDH2, IGF2*
BP	Negative regulation of proteolysis	2	0.015266	*CSTA, PLAT*
BP	Peptide cross-linking	2	0.019214	*DSP, CSTA*
BP	Adherens junction organization	2	0.021837	*DSP, CDH2*
BP	Keratinocyte differentiation	2	0.043874	*DSP, CSTA*
CC	Fascia adherens	2	0.006761	*DSP, CDH2*
CC	Adherens junction	3	0.00677	*PDLIM1, DSP, CDH2*
CC	Extracellular space	6	0.007879	*SRGN, LGALS3BP, CSTA, IGF2, TIMP3 PLAT*
CC	Platelet dense granule lumen	2	0.009454	*LGALS3BP, TIMP3*
CC	Basolateral plasma membrane	3	0.013105	*DSP, HEPH, CDH2*
CC	Desmosome	2	0.018159	*DSP, CDH2*
CC	Focal adhesion	3	0.033949	*PDLIM1, CSRP2, CDH2*
MF	Phosphoprotein binding	2	0.027696	*PLAT, PITX2*
MF	Protease binding	2	0.080280	*CSTA, TIMP3*

Table 3. The top 15 enriched GO terms of upregulated DEGs.

Category	Term	Count	*P*	Genes
BP	Lung development	3	0.003930766	*RBP4, ALDH1A2, PDPN*
BP	Cardiac muscle tissue development	2	0.020170976	*RBP4, ALDH1A2*
BP	Response to retinoic acid	2	0.055075233	*RBP4, ALDH1A2*
BP	Retinol metabolic process	2	0.060420641	*RBP4, ALDH1A2*
BP	Positive regulation of protein complex assembly	2	0.060420641	*MMP3, CLU*
CC	Extracellular region	11	0.000058919	*RBP4, SERPINA1, PCOLCE2, MMP3, PENK, CHI3L2, RSPO3, IL13RA2, CLU, CP, HAPLN1*
CC	Extracellular space	8	0.004681602	*RBP4, SERPINA1, MMP3, CHI3L2, IL13RA2, CLU, CP, HAPLN1*
CC	Extracellular exosome	8	0.008955750	*RBP4, SERPINA1, NEBL, CRTAC1, STK26, MGP, CLU, CP*
CC	Extracellular matrix	3	0.033352049	*MGP, MMP3, HAPLN1*
CC	Endoplasmic reticulum lumen	3	0.046476129	*SERPINA1, PENK, CP*
CC	Cell periphery	2	0.068950192	*STK26, CLU*
CC	Platelet alpha granule lumen	2	0.073105045	*SERPINA1, CLU*
MF	Chaperone binding	3	0.007137671	*PDPN, CLU, CP*
MF	Retinal binding	2	0.012688589	*RBP4, ALDH1A2*
MF	Receptor binding	3	0.081748100	*PDPN, RSPO3, CLU*

GO, gene ontology; BP, biological process; CC, cellular component; MF, molecular function.

Table 4. DAVID-based enrichment of KEGG pathways for up- and downregulated DEGs and subnetworks.

	Enriched KEGG PATHWAYS			
Category	Term	Count	*P*	Genes
Upregulated DEGs				
KEGG_PATHWAY	hsa04610: Complement and coagulation cascades	2	0.0952	*SERPINA1, CLU*
Downregulated DEGs				
KEGG_PATHWAY	hsa05412: Arrhythmogenic right ventricular cardiomyopathy	2	0.066095	*DSP, CDH2*
Subnetworks				
KEGG_PATHWAY	hsa04610: Complement and coagulation cascades	2	0.039133	*SERPINA1, PLAT*
KEGG_PATHWAY	hsa05215: Prostate cancer	2	0.044054	*MMP3, PLAT*
KEGG_PATHWAY	hsa05202: Transcriptional misregulation in cancer	2	0.086205	*MMP3, PLAT*

DAVID, Database for Annotation, Visualization, and Integrated Discovery; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, Differentially Expressed Genes.

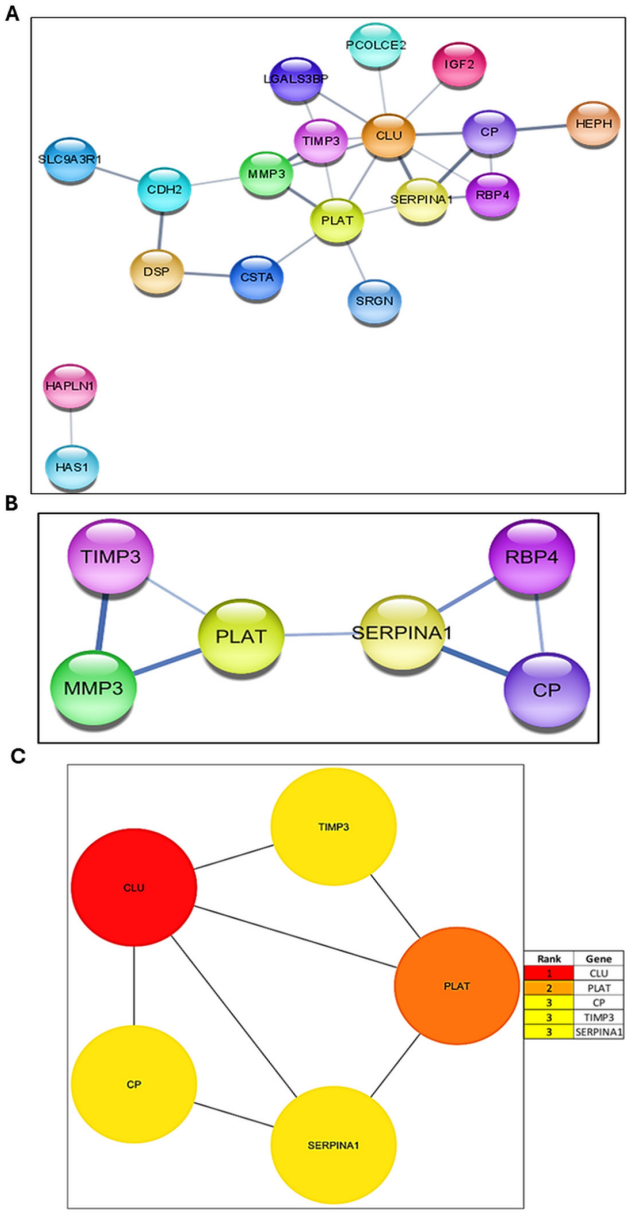

Fig. 4. STRING analysis of DEGs (A) PPI networks (B) Subnetwork (C) Top 5 ranked genes. STRING analysis was carried out by using Cytoscape version 3.9.1. In addition, the subnetwork construction and identification of the top five hub genes were done using MCODE and CytoHubba applications. DEGs, Differentially Expressed Genes; STRING, Search Tool for the Retrieval of Interacting Genes; PPI, Protein-protein interaction; MCODE, Molecular complex detection.

reveals enriched biological process (BP), cellular component (CC), and molecular function (MF) (Fig. 3A and B). The top 15 enriched GO terms (BP, CC, and MF) for downregulated DEGs are enlisted in Table 2, while the top 15 enriched GO terms of upregulated DEGs are summarized in Table 3.

The KEGG analysis revealed the enrichment of complement and coagulation cascades and arrhythmogenic right ventricular cardiomyopathy pathways for upregulated and downregulated DEGs, respectively (Table 4). Moreover, the subnetwork enrichment reveals enrichment of complement and coagulation cascades, prostate cancer, and transcriptional misregulation in cancer KEGG pathways (Table 4).

PPI, subnetwork, and hub genes

The Cytoscape string network analysis reveals that out of 43 DEGs, 18 interact with each other (Fig. 4A). The MCODE plugin is used to find subnetworks (Fig. 4B) consisting of the interaction of 6 genes (*TIMP3, MMP3, PLAT, SERPINA1, RBP4*, and *CP*). Finally, the top 5 identified hub genes (*CLU, PLAT, CP, TIMP3,* and *SERPINA1*) are represented in Fig. 4C.

DISCUSSION

The reduced chondrocyte viability leads to inhibited cartilage synthesis, which is a critical factor in OA therapy. Therefore, cartilage tissue regrowth for OA treatment is a practical approach that is feasible through regenerative therapeutic alternatives. In recent years, stem cells have been considered the primary source of material for regenerative knee OA therapy, which is attributed to their potential for chondrocyte differentiation and articular cartilage regeneration[19]. It has been reported that MSCs-derived chondrocytes have inferior mechanical properties and lower ECM proteins compared to primary chondrocytes[9].

We screened 43 DEGs, of which 28 genes were upregulated while 15 were downregulated. As observed in our study, the upregulated MMP3 indicates synovial inflammation and cartilage turnover contributing to OA progression[20] by accelerating vascular infiltration, suppressing MSC differentiation, elevating inflammatory cell levels, and enhancing cartilage degradation. *MMP3*

is transcribed and secreted by synovial cells, fibroblasts, cartilage cells, and osteoclasts, and it degrades proteoglycans, laminin, and fiber adhesive proteins in the ECM. Further, the upregulation of the *C2orf40* or esophageal cancer-related gene 4 (*ECRG4*) gene has been reported in OA chondrocytes[21]. The expression of *C2orf40* varies according to chondrocyte differentiation patterns, and its higher level is observed in mature chondrocytes when compared to MSCs (ref.[22]). We also observed an upregulated level of *CHI3L2* or *YKL-39* in OA chondrocytes, indicating the impact of OA on chondrocytes[23]. The increased level of *CHI3L2* genes in articular cartilage induces an inflammatory response resulting in cartilage injury[24]. The upregulated level of *CHI3L2* gene has also been reported in canine adipose stem cell (ASC)-derived chondrocytes compared to control (ASCs) (ref.[25]). The upregulation of *CHI3L2* indicates that the chondrocytes were from aged persons or OA patients[26]. Of note, the elevated level of *CHI3L2* is an important factor in the inflammatory response to articular chondrocytes resulting in the progression of OA (ref.[26,27]). Our study of top downregulated and upregulated genes of OA-derived and BMSCs derived chondrocytes implies that MSCs derived chondrocytes are a superior choice in comparison to OA-derived chondrocytes.

In our study, the *LGALS3BP* is downregulated in OA chondrocytes compared to hBMSCs. *LGALS3BP* is primarily expressed in early hypertrophic and mature chondrocytes[28,29]. The lower level of *LGALS3BP* has been observed in OA synovium[30] and plays a crucial role in inducing cell-to-cell adhesion and stimulating proinflammatory and pathological cascades[31]. The *LGALS3BP* binds to the galectin-3 (*Gal-3*) protein, and overexpressed Gal-3 in KOA leads to increased inflammation in synovium by inducing the PI3K/Akt pathway[32]. The low expression of *CSTA* suppresses the osteoclast synthesis and differentiation; however, it increases the bone volume in OA (ref.[33-35]). DSP is overexpressed in synovial fluid in OA (ref.[35,36]). Notably, our study shows that the DSP is downregulated in OA chondrocytes compared to BMSCs-derived chondrocytes, indicating that BMSCs-derived chondrocytes increase osteoclast density and promote bone remodeling. Osteoclasts adsorb mature bone and contribute to bone remodeling. *IGF2* level decreases in OA cartilage, specifically in degenerated regions, compared to normal tissues[37,38]. The *IGF2* participates in a cartilage matrix formation, suppressing subchondral bone sclerosis and osteophyte synthesis by lowering *MMP13* levels[39]. Our study revealed downregulated expression of *IGF2* in OA chondrocytes, indicating its lower efficiency in maintaining the structural integrity of cartilage compared to BMSCs-derived chondrocytes[40].

Further, our PPI network/subnetwork single out top DEGs and top 5 hub genes *CLU, PLAT, CP, TIMP3,* and S*ERPINA1*. The hub genes ranked in the top 15 downregulated and upregulated genes. The increased level of serum *CLU* in OA patients indicates the plausible role of the *CLU* gene in the progression of OA (ref.[41]). *CLU* expression is increased in early KOA patients compared to healthy counterparts. However, a low level of *CLU* expression is reported at advanced KOA. The increased level of *CLU* could be associated with its cytoprotective role[42]. The upregulation of the *CLU* gene represents the severity of OA, specifically synovial and systemic inflammation[43]. It also contributes to improved cell survivability by modulating lipid transport, cell differentiation, apoptosis, and removal of cellular debris[44,45]. The increased level of *CLU* is associated with synovial inflammation among OA patients. Similar to our finding, the hub gene *CLU* is upregulated in OA-derived chondrocytes and OA cartilage[46]. However, the role of *CLU* in OA progression is not well understood. Notably, *CLU* is secreted by chondrocytes and articular cartilage[47,48].

Plasminogen enoded by the *PLAT* gene effectively modulates joint inflammation and regulates arthritis progression[49]. In our study, the *PLAT* gene is downregulated and enriched in the negative regulation of proteolysis. Notably, the higher level of *PLAT* in OA cartilage induces subchondral osteopenia in mice[50], and the impaired influx of inflammatory cells into the synovial joints in the plasminogen-deficient mice further elaborates that the higher level of *PLAT* is prevalent in OA cartilage compared to normal cartilage[51]. A recent study in rats demonstrated that inhibition of plasminogen activator's (PA) activity of articular chondrocytes contributes to maintaining articular cartilage matrix[52]. The PA-mediated fibrinolysis decreases the fibrin accumulation, reducing the risk of inflammatory joint disorders[52]. The downregulated *PLAT* was enriched in phosphoprotein binding function. *MMP3* is highly upregulated in OA-derived chondrocytes, which indicates a greater risk of cartilage degradation[53]. The upregulation of *MMP3* in primary OA chondrocytes indicates injured chondrocytes, as *MMP3* plays a significant role in OA progression[20]. The increased levels of *MMP-3* accelerate vascular infiltration, suppress MSC differentiation, elevate inflammatory cell levels, and enhance cartilage degradation. The upregulated hub *SERPINA1* gene in OA-derived chondrocytes implicates higher dedifferentiation potential in chondrocytes[54]. Interestingly, a lower level of SERPINA1 is reported in OA cartilage compared to non-OA cartilage[55-57]. Notably, *SERPIN* is overexpressed after the induction of BMMSC to differentiate into chondrocytes, while it gets downregulated during dedifferentiation in monolayer cell growth[54,56]. Hub gene CP is upregulated in OA-derived chondrocytes, indicating improved copper transport and chondrogenic potential[58]. Like *CLU*, the S*ERPINA1* gene is also enriched in complement and coagulation cascades, indicating its potential role in inflammation.

Notably, *TIMP3* and *MMP3* are both associated with the progression of arthritis and other diseases, such as abdominal aortic aneurysm, prostate cancer, and ulcerative colitis[59]. Moreover, the interaction between *TIMP3* and *MMP3* and their polymorphism also contributes to the progression of OA. The expression of *TIMP3,* a primary chondroprotective protein, is found to be at a low level in the cartilage of OA patients compared to the cartilage of a normal individual[60]. *TIMP3* also upregulation inhibits the expression of osteopontin, osteonectin, and osteocalcin[59,61]. *TIMP3* protects cartilage by suppressing

matrix degradation in the articular cartilage[62]. Further, an elevated level of *CP* is observed in the cartilage of OA patients compared to healthy controls[63]. A recent study revealed that the overexpression of CP in rheumatoid arthritis and OA indicates increased inflammation[63].

The upregulation of CP in our study suggests the characteristics of OA-derived chondrocytes. The downregulated DSP gene and the arrhythmogenic right ventricular cardiomyopathy pathway are enriched in top BP. Similarly, *RBP4* and *ALDH1A2* were upregulated in our study and enriched in top biological processes. A recent study has confirmed the overexpression of *RBP4* and its association with MMPs and adipokines, resulting in the progression of OA (ref.[64,65]). However, the low level of *ALDH1A2* is linked with hand OA (ref.[66]). Thus, the DEG patterns of our study distinguish the chondrocytes derived from OA tissues and MSCs.

The subnetwork genes of our studies are also present in hub genes, and the subnetwork is enriched in complement and coagulation cascades, prostate cancer, and transcriptional dysregulation in cancer. Our study reveals that the OA-derived chondrocytes represent inflammatory markers more prominently, which could be associated with their origin. Further, our analysis identified distinct markers for OA-derived and stem cell-derived chondrocytes.

The downregulated DEGs were mainly enriched in "cell-cell division", "fascia adherens", and "phosphoprotein binding". However, the upregulated DEGs were primarily enriched in "lung development cardiac muscle tissue", "extracellular region", and "chaperone binding". Interestingly, subjects with knee OA had a declined lung function compared to those without OA (ref.[67]). Recently, in a Korean-based nationwide health and nutrition examination survey, a novel association between asthma and OA has been indicated[68], which might be mediated by endogenous and exogenous reactive oxygen and nitrogen species playing key roles in airway inflammation, which determines the severity of asthma and in the development of OA. The OA prevalence has also been recorded from 12% to 74% among chronic obstructive pulmonary disease (COPD) (ref.[69,70]). The lower lung function was evidenced among knee OA sufferers.

In addition, the upregulated and downregulated DEGs were enriched in KEGG pathways i.e., complement and coagulation cascades and arrhythmogenic right ventricular cardiomyopathy pathways. In line with our study, recent studies have demonstrated that the activation of the complement system significantly contributes to initiation and progression of OA development[71], leading to degradation of extracellular cartilage matrix (ECM), cell lysis, synovitis, stem cell recruitment, osteophyte formation, cartilage angiogenesis, and imbalanced bone remodeling[72]. Moreover, the knee injury triggers the activation of the complement system, resulting in inflammation and osteochondral fractures[73]. Furthermore, OA may increase the risk of prostate cancer, possibly mediated by increased levels of cartilage oligomeric matrix protein (COMP) (ref.[74]). In addition, the risk of cancer was reported to be significantly higher among OA patients, thus the KEGG enrichment of transcriptional misregulation in cancer is linked with OA (ref.[75]).

Limitations of the study

Notably, our study did not find any common top DEGs compared to Bernstein et al.[9], and only included normalized data and predefined criteria to identify DEGs. This could be attributed to various bioinformatics tools used in the analysis, such as GEO2R, DAVID, Cytoscape, and CytoHubba, to identify top DEGs, functional and pathway enrichment analysis, create a PPI network, and identify the top five hub genes, respectively. On the other hand, the Bernstein et al. study was based on microarray experimentation and immunohistochemical analysis. Further, the results of our research are based on dataset GSE19664, which provides biological insight and may support cases with existing expression data. Notwithstanding, this might not be the case for other conditions when such data does not exist, and statistical safety is important to the whole-genome dataset. Future experiments should explore the different metabolic behavior of chondrocytes and MSC and their biological relevance through wet-lab studies.

CONCLUSION

Our analysis identified hub genes, including *CLU, PLAT, CP, TIMP3*, and *SERPINA1,* that could be used as differential biomarker signatures for OA and MSC-derived chondrocytes. These findings offer novel avenues to explore the potential impact of identified genes underlying cartilage synthesis and degeneration.

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Availability of data and materials: Data is available at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE19664

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