Identification of Genes and Pathways of Nonsteroidal Anti-inflammatory Drugs acting on Synovia from Women with Knee Osteoarthritis by Bioinformatics Analysis

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Research Article

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Abstract

Objective: Through the bioinformatics analysis, to identify the genes and pathways of nonsteroidal anti-inflammatory drugs(NSARDs) acting on synovia from women with knee osteoarthritis (KOA), and to provide reference for clinical application.

Methods: We downloaded the gene microarray datasets with the accession number of GSE55457 and GSE55584 from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) database, including 5 untreated KOA patients, 9 NSARDs treated KOA patients and 2 patients without KOA The samples in the untreated KOA group and the NSARDs treated KOA group were used for main analysis. The samples in the untreated KOA group and the normal control group were used for cooperative analysis. Then we performed robust multi-array (RMA) normalization with affy R programming package. After that, differential expression genes (DEGs) in main analysis and cooperative analysis were identified based on limma package separately. Screening the common DEGs from main analysis and cooperative analysis. Enriched gene ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of DEGs were obtained through the Database for Annotation, Visualization and Integrated Discovery (DAVID). What's more, protein-protein interaction (PPI) network was constructed, and we identified modules of PPI network through Cytoscape to screen valuable targets. The value of gene expression fold change (FC) $\geq$1.4 or $\leq$1/1.4, and P <0.05 were used as the screening conditions. P <0.05 and Associated genes count>5 were used as the screening conditions.

Results: There were 338 DEGs in main analysis. Among them, 211 genes were up-regulated and 127 genes were down-regulated. There were 7005 DEGs in cooperative analysis. Among them, 6952 genes were up-regulated and 53 genes were down-regulated. A total of 129 common DEGs were identified between main analysis and cooperative analysis. There are 2 biological processes, 3 cell components and 2 molecular functions for the enrichment of differentially expressed genes.

Conclusion: NSARDs may play a certain role in synovia from women with KOA by regulating the mRNA expressions of il-6, TNFRSF11A and CSF1R, which may become one of the indicators for monitoring the efficacy of NSAIDs.

Introduction

Knee osteoarthritis (KOA) is a degenerative disease characterized by degeneration of articular cartilage (1), which currently affects about 21.51% of the middle-aged and elderly (2), and is considered as one of the largest cause of disability (3). The genetic contribution of knee osteoarthritis surgery is higher in women than in men (4). KOA is often accompanied by synovitis, osteophyte formation and subchondral bone sclerosis (5). Clearly, all articular tissues, including the synovium, are involved in the overall pathologic process. In particular, synovitis, which plays a key role in the initiation and development of degenerative changes in cartilage, is associated with more severe pain and joint dysfunction (6). Synoviocytes produce pro-inflammatory mediators, which in turn attract immune cells, increase

angiogenesis and induce a phenotypic shift in chondrocytes. Chondrocytes produce additional cytokines and proteolytic enzymes, which eventually increase cartilage degradation and further induce synovial inflammation (7). Therefore, the study on synovial tissue of KOA patients will gradually attract extensive attention.

Nonsteroidal anti-inflammatory drugs (NSARDs) are a class of widely used drugs with antipyretic, analgesic and anti-inflammatory effects and it is often used in the clinical treatment of KOA, rheumatoid arthritis and other inflammatory diseases (8). It mainly inhibits the release and production of prostaglandin synthetase (PGs) by inhibiting the activity of cox-oxidase (COX) and other pathways, thus exerting the anti-inflammatory effect (9). In addition, NSARDs can also adjust the function of osteoblasts and osteoclasts by inhibiting PGs through the above pathways (10). However, there is little evidence that they can alter or stop the progression of KOA (11). Due to its general need for long-term oral, the resulting toxic side effects such as gastrointestinal reactions, cardiovascular side effects can not be ignored (12, 13). Therefore, the indications for its application are open to question, and many mechanisms are unknown. NSARDs last long in synovial fluid, and considerable concentrations of drugs can be achieved in synovial fluid (14). However, little research has been done on whether NSARDs play an intervention role in the expression of abnormal genes in synovial tissues of KOA patients and the details of its mechanism. Through the bioinformatics analysis, we tried to identify the genes and pathways of NSARDs acting on synovia from women with KOA. We downloaded the gene microarray datasets with the accession number of GSE55457 and GSE55584 from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) database, performed robust multi-array (RMA) normalization with affy R programming package, and differential expression genes (DEGs) in two groups were identified based on limma package separately for main analysis and cooperative analysis (15). Through our further analysis, to provide reference for clinical application.

Materials And Methods

Microarray data.

We downloaded the gene microarray datasets with the accession number of GSE55457 and GSE55584 from the GEO database, including 14 samples of synovia from women with KOA and 2 samples of synovia from women without KOA. Among them, there were 5 KOA patients in the untreated KOA group (untreated KOA) (GSM1337327, GSM1337330 from GSE55457 dataset, and GSM1339628, GSM1339629, GSM1339632 from GSE55584 dataset), 9 KOA patients in the NSARDs treated KOA group (NSARDs treated KOA) (GSM1337328, GSM1337329, GSM1337331, GSM1337334, GSM1337335, GSM1337336 from GSE55457 dataset, and GSM1339630, GSM1339631, GSM1339633 from GSE55584 dataset), and 2 patients without KOA in the normal control group (normal control) (GSM1337306 and GSM1337310 from GSE55457 dataset).The samples in the untreated KOA group and the NSARDs treated KOA group were used for main analysis. To repeat the grouping of BOBIN MI,the samples in the untreated KOA group and the normal control group were used for cooperative analysis.

Identification of DEGs.

To identify DEGs for main analysis and cooperative analysis, we did the following several times. We performed RMA normalization with affy R programming package. After that, DEGs in two groups were identified based on limma package separately for main analysis and cooperative analysis. The screening criteria for DEGs were: the value of gene expression fold change (FC) ≥ 1.4 or ≤ 1/1.4, and $P < 0.05$.

Screening for common DEGs.

Screening the common differentially expressed genes (DEGs) from main analysis and cooperative analysis.

Gene ontology(GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for common DEGs.

Enriched GO terms and KEGG pathways of common DEGs were obtained through the Database for Annotation, Visualization and Integrated Discovery (DAVID). For screening results, $P < 0.05$ and Associated genes count > 5 were used as the screening conditions.

PPI network analysis for common DEGs.

PPI network was constructed by using the web-based tool STRING (http://www.string-db.org). Subsequently, the PPI network was visualized by using Cytoscape software (http://www.cytoscape.org/).

Results

Identification of DEGs for main analysis

The untreated KOA group and the NSARDs treated KOA group had good clustering and no outlier samples, and all the 14 samples could be used for further analysis. FC ≥ 1.4 or ≤ 1/1.4,and $P < 0.05$ were used as the screening conditions. There were 338 DEGs in total between the untreated KOA group and the NSARDs treated KOA group. Among them, 211 genes were up-regulated and 127 genes were down-regulated. (Table 1 and Table 2).

Table 1
Upregulated top 10 DEGs for main analysis

Name	ad.j P value	log_2FC
ERAP2	0.039802988	2.204622561
EGFL6	0.010200799	1.639875579
CLEC5A	0.000370042	1.357457197
HYPM	1.18E-06	0.978032929
GPC5	0.046163148	0.975027997
METTL8	8.11E-07	0.89670674
METTL2B	0.000132549	0.883810499
RBM41	1.96E-05	0.866861208
RSBN1	0.000589657	0.863816353
KIAA1462	0.015328306	0.860426184

DEGs: differential expression genes; ERAP2: endoplasmic reticulum aminopeptidase 2; EGFL6: epidermal growth factor like domain multiple 6; CLEC5A: C-type lectin domain containing 5A; HYPM: huntingtin interacting protein M; GPC5: glypican 5; METTL8: methyltransferase like 8; METTL2B: methyltransferase like 2B; RBM41: RNA binding motif protein 41; RSBN1: round spermatid basic protein 1; KIAA1462: Junctional Protein Associated with Coronary Artery Disease.

Table 2
Down-regulated top 10 DEGs for main analysis

Name	ad.j P value	log_2FC
LOC100293211	0.008071821	-1.999464744
TNNC1	0.047915938	-1.936767919
IGK	0.03001768	-1.835423764
ACPP	1.06E-07	-1.738148309
TNNT3	2.75E-05	-1.658828783
STEAP4	0.017920088	-1.625012329
TNKS2	0.00318571	-1.622724424
IGLV1-44	0.020105589	-1.610300515
AMPD1	0.003600788	-1.583795204
PCSK1	0.01179745	-1.562040655

DEGs: differential expression genes; LOC100293211: This record has been withdrawn by NCBI because the model on which it was based was not predicted in a later annotation; TNNC1: troponin C1, slow skeletal and cardiac type; IGK: immunoglobulin kappa locus; ACPP: acid phosphatase, prostate; TNNT3: troponin T3, fast skeletal type; STEAP4: six transmembrane epithelial antigen of the prostate 4; TNKS2: tankyrase 2; IGLV1-44: immunoglobulin lambda variable 1–44; AMPD1: adenosine monophosphate deaminase 1; PCSK1: proprotein convertase subtilisin/kexin type 1.

Identification of DEGs for cooperative analysis.

The untreated KOA group and the normal control group had good clustering and no outlier samples, and all the 7 samples could be used for further analysis. $FC \geq 1.4$ or $\leq 1/1.4$,and $P < 0.05$ were used as the screening conditions. There were 7005 DEGs in total between the normal control group and untreated KOA. Among them, 6952 genes were up-regulated and 53 genes were down-regulated. (Table 3 and Table 4).

Table 3
Upregulated top 10 DEGs for cooperative analysis

Name	ad.j P value	log_2FC
CSN1S1	0.002915198	3.753268436
SCRG1	0.004842648	3.393259056
IGJ	0.037337558	2.98865458
CXCL13	0.029616323	2.951166838
FCER1A	0.020646921	2.911387621
LOC100293211	0.033474633	2.780834072
SFRP4	0.013146367	2.652018346
CXCL14	0.003753975	2.642478092
CPA3	0.003248469	2.53155903
AMPD1	0.012401312	2.490793598

DEGs: differential expression genes; CSN1S1: casein alpha s1; SCRG1: stimulator of chondrogenesis 1; IGJ: joining chain of multimeric IgA and IgM; CXCL13: C-X-C motif chemokine ligand 13; FCER1A: Fc fragment of IgE receptor Ia; LOC100293211: This record has been withdrawn by NCBI because the model on which it was based was not predicted in a later annotation; SFRP4: secreted frizzled related protein 4; CXCL14: C-X-C motif chemokine ligand 14; CPA3: carboxypeptidase A3; AMPD1: adenosine monophosphate deaminase 1.

Table 4
Down-regulated top 10 DEGs for cooperative analysis

Name	ad.j P value	log_2FC
DIP2A	0.003753975	-1.209337988
KATNBL1	0.006498532	-1.11132739
ANKRD36B	0.002108899	-1.083693789
RUFY2	0.027166392	-1.058688078
ADH1A	0.014392153	-1.053951323
WDR43	0.003117186	-1.044556176
HYMAI	0.016933768	-1.030648055
CD2AP	0.00500248	-1.029287464
SCN4A	0.046500125	-1.02868922
ACTL6A	0.007213949	-1.009298922

DEGs: differential expression genes; DIP2A: disco interacting protein 2 homolog A; KATNBL1: katanin regulatory subunit B1 like 1; ANKRD36B: ankyrin repeat domain 36B; RUFY2: RUN and FYVE domain containing 2; ADH1A: alcohol dehydrogenase 1A (class I), alpha polypeptide; WDR43: WD repeat domain 43; HYMAI: hydatidiform mole associated and imprinted; CD2AP: CD2 associated protein; SCN4A: sodium voltage-gated channel alpha subunit 4; ACTL6A: actin like 6A.

Screening for common DEGs.

Screening the common DEGs from main analysis and cooperative analysis. There were 129 common DEGs in total between main analysis and cooperative analysis. (Fig. 1).

GO and pathway enrichment analysis for common DEGs.

For screening results, $P < 0.05$ and Associated genes count > 5 were used as the screening conditions. There were 7 results that have been filtered out. Among them, there are 2 biological processes(BP), 3 cell components(CC) and 2 molecular functions(MF) for the enrichment of differentially expressed genes. (Table 5). There was 0 KEGG pathway enriched by differentially expressed genes.

Table 5
The result of Enriched GO terms and KEGG pathways of common DEGs

Term	ID	Function	Associated genes count	P Value
BP	GO:0008284	positive regulation of cell proliferation	9	0.012782
BP	GO:0043066	negative regulation of apoptotic process	8	0.032684
CC	GO:0009986	cell surface	15	1.33E-05
CC	GO:0005887	integral component of plasma membrane	20	0.002222
CC	GO:0005886	plasma membrane	37	0.036567
MF	GO:0019901	protein kinase binding	8	0.012663
MF	GO:0005524	ATP binding	17	0.037856

GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; BP: biological processes; CC: cell components; MF: molecular functions;

PPI network analysis for common DEGs.

Based on the information in the STRING and Cytoscape databases, the top 10 hub nodes with the highest degree of interaction were screened: CD24, CD70, KCNC1, BIRC3, TNFRSF11A, CSF1R, HP, CD69, CD40LG, and IL6. (Fig. 2).

Discussion

In this study, the discussion of DEGs, related pathways and PPIs in synovial tissue of female KOA patients after NSARDs treatment is helpful to strengthen the understanding of researchers on KOA and NSARDs. In this study, CD24, CD70, KCNC1, BIRC3, TNFRSF11A, CSF1R, HP, CD69, CD40LG, and IL6 may play an important role in the synovial tissue of female KOA patients after NSARDs treatment. In addition, NSARDs treatment will also affect a series of pathways: "positive regulation of cell proliferation", "negative regulation of apoptotic process", "cell surface", "integral component of plasma membrane", "plasma membrane", and "protein kinase Binding", "ATP binding". IL-6 is a soluble mediator that has multipotency effects on inflammation, immune response and hematopoiesis (16). It can not only stimulate the aggregation and activation of inflammatory cells, but also promote the formation of pain of the knee joint after trauma (17). IL-6 can mediate cartilage destruction (18). By stimulating synovial cells, IL-6 can produce PGs, which further aggravates joint inflammation, activates immature osteoclasts, and makes them participate in bone resorptive, causing progressive destruction of articular cartilage (18, 19).

TNFRSF11A is a member of the tumor necrosis factor receptor superfamily, which interacts with a variety of TRAF family proteins to induce the activation of NF-Kappa B and MAPK signaling pathway, and is also an important mediator of osteoclast development (20–23). CSF1 can stimulate CSF1R to promote the proliferation and differentiation of macrophages and the formation of osteoclasts, so in patients with KOA, inhibition of CSF1R is beneficial (24–26).

The limitation of this study is that only a small number of synovium samples were included, and the inclusion of other samples may change the current results. Therefore, it is necessary to collect more synovial samples from female patients with KOA to detect the expression levels of significant DEGs. In addition, the significance of PTGS1 and PTGS2 changes was relatively low, which was surprising.

In conclusion, NSAIDs may play a certain role in synovium of female KOA by regulating the mRNA expression of IL-6, TNFRSF11A and CSF1R, which may become one of the indicators for monitoring the efficacy of NSAIDs. Given their reported side effects, they should be appropriately recommended.

Abbreviations

NSARDs: nonsteroidal anti-inflammatory drugs;

KOA: knee osteoarthritis;

GEO: Gene Expression Omnibus;

RMA: robust multi-array;

DEGs: differential expression genes;

GO: gene ontology;

KEGG: Kyoto Encyclopedia of Genes and Genomes;

DAVID: Database for Annotation, Visualization and Integrated Discovery;

PPI: protein-protein interaction;

FC: fold change;

PGs: prostaglandin synthetase;

COX: cox-oxidase;

BP: biological processes;

CC: cell components;

MF: molecular functions;

Declarations

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Conflict of Interest:

The authors declare that they have no competing interests.

Authors Contributions:

Fei Wang contributed to the conception of the study; Chao Zhao contributed significantly to the analysis and wrote the manuscript; Han Wang collected the data; Conglei Dong and Huijun Kang helped perform the analysis with constructive discussions. The authors read and approved the final manuscript.

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Availability of data and materials:

All of the data and materials are available online.

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Figures

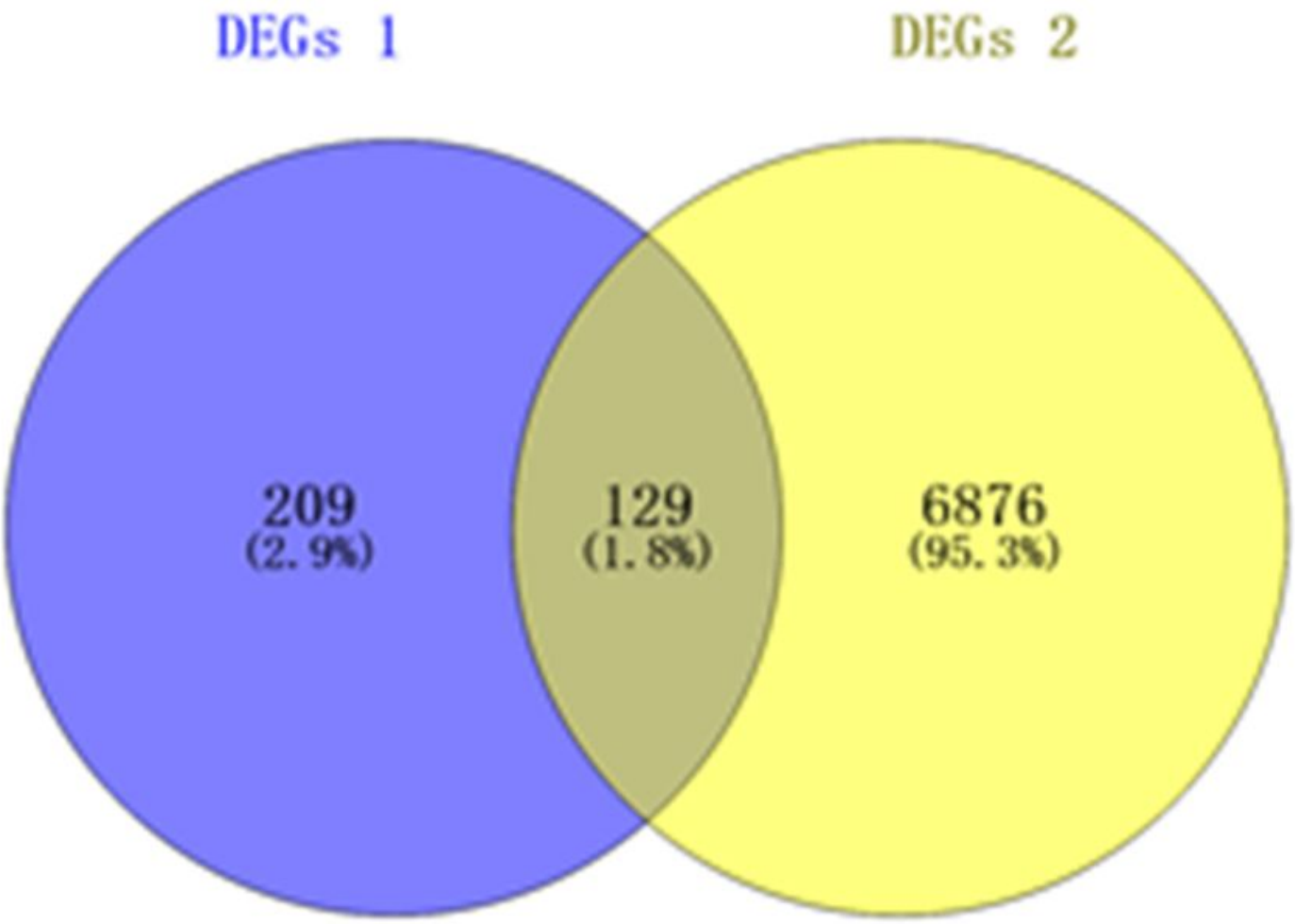

Figure 1

Screening for common DEGs from main analysis and cooperative analysis DEGs 1 for main analysis; DEGs 2 for cooperative analysis.

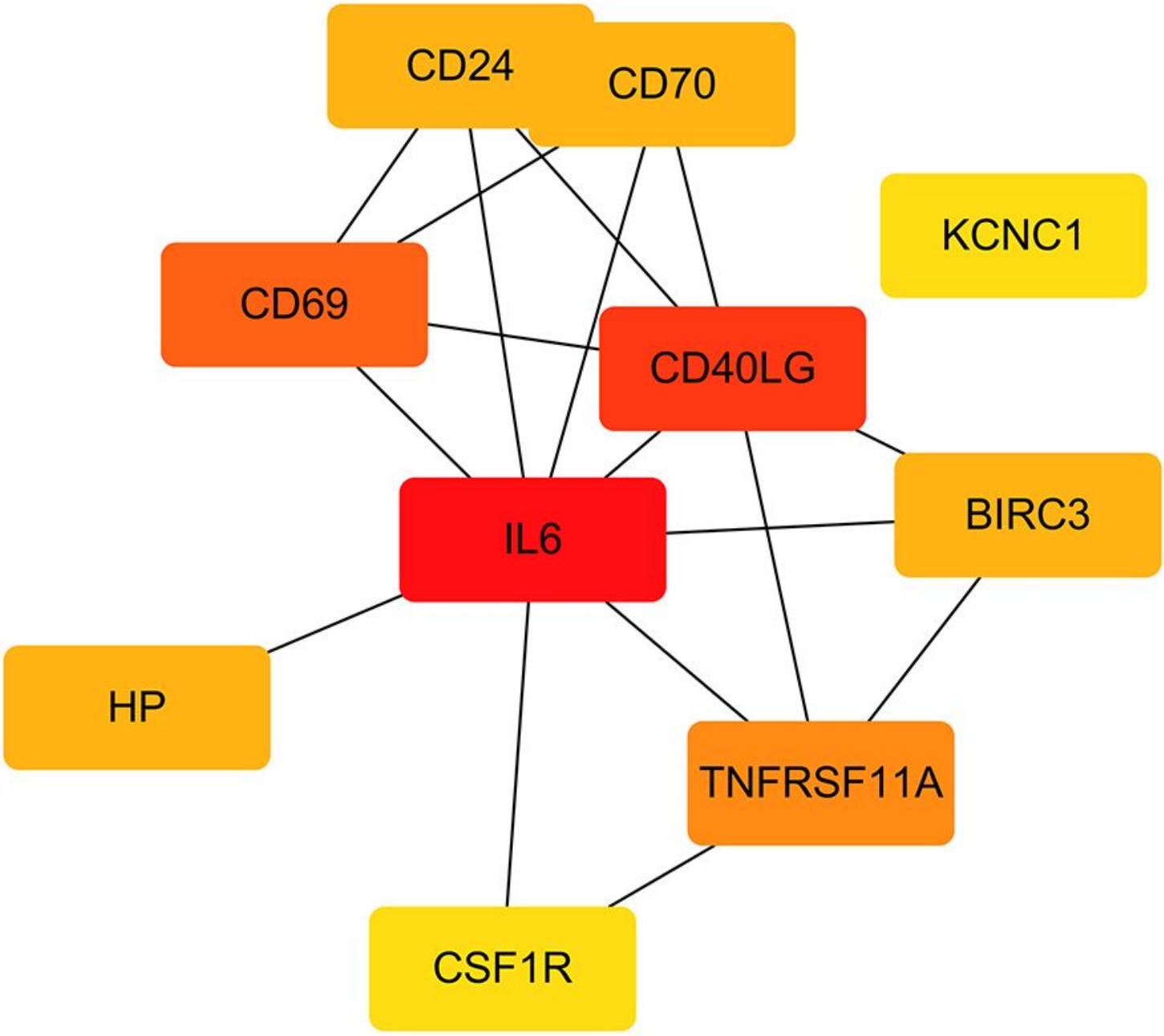

Figure 2

The top 10 hub nodes from common DEGs CD24: cluster of differentiation 24; CD70: cluster of differentiation 70; KCNC1: potassium voltage-gated channel subfamily C member 1; BIRC3: baculoviral IAP repeat containing 3; TNFRSF11A: tumor necrosis factor receptor superfamily member 11a; CSF1R: olony stimulating factor 1 receptor; HP: haptoglobin; CD69: cluster of differentiation 69; CD40LG: cluster of differentiation 40 ligand; IL6: interleukin 6.