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Original Article

Identification and analysis of immune aging related biomarkers in cartilage and meniscus tissues of osteoarthritis

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Abstract: This study aimed to investigate the relationship between immunosenescence and osteoarthritis (OA) and analyze its potential clinical implications. Thus, we conducted transcriptome sequencing by collecting clinical meniscus (Aging_meniscus:Control_meniscus = 3:7) and cartilage tissues (Aging_cartilage:Control_cartilage = 2:6). Meanwhile, immune-related genes (IRGs) and aging-related genes (ARGs) were included in this research. The differentially expressed genes (DEGs) between Aging_meniscus and Control_meniscus as well as Aging_cartilage and Control_cartilage were analyzed by differential analysis, respectively. Then, differentially expressed IRGs (DEIRGs) were generated by crossing DEG with IRGs. Similarly, differentially expressed ARGs (DEARGs) were achieved by intersecting DEG and ARGs. To obtain genes simultaneously associated with immune and aging in both meniscus and cartilage samples, biomarkers were screened out by crossing share.IRGs and share.ARGs overlapped by DEIRGs1 and DEIRGs2 as well as DEARGs1 and DEARGs2, respectively. In addition, the biomarkers' functions were analyzed by gene set enrichment analysis (GSEA). To detect the regulatory mechanism, a miRNA-mRNA-transcription factors (TFs) regulatory network and a X2K network were constructed. Moreover, disease association analysis and potential small molecule drugs for biomarkers were also performed to further reveal the possible role of biomarkers for OA. Then, 3 biomarkers, namely Insulin-like Growth Factor 1 Receptor (IGF1R), Interleukin 7 receptor (IL7R) and Leptin receptor (LEPR), were selected out through the intersection of 14 share.IRGs and 4 share.ARGs. And they were all enriched in 'ribosome' from both meniscus and cartilage samples, and had complex regulatory networks. In all, the expression of IGF1R was markedly up-regulated in OA ($P < 0.05$). Eventually, mecasermin could stably bind to IGF1R and simvastatin could stably bind to LEPR. It suggested that mecasermin and simvastatin may exhibit significant clinical potential in treating immunosenescence-related OA.

Keywords: Osteoarthritis, meniscus, cartilage, aging, immune

Introduction

Osteoarthritis (OA) is a degenerative joint disease characterized by cartilage degeneration, osteophyte formation, subchondral bone sclerosis abnormalities, as well as synovial fibrosis and proliferation [1-3]. According to statistics, approximately 240 million people worldwide suffer from OA and are deeply troubled, resulting in huge medical costs and mortality rates [4]. The current methods for treating OA include: nonsteroidal anti-inflammatory drugs (NSAIDs) [5], intra-articular injection of corticosteroids [6], intra-articular injection of hyaluronic acid (HA) [7], central nervous system inhibitors [8], Biological inhibitors such as IL-1 or TNF-α [9] and joint replacement surgery [10] are not widely accepted due to poor long-term efficacy of drugs and biologics, and joint replacement faces various postoperative complications and other disadvantages. At present, various risk factors for OA have been recognized from the etiology, such as age, obesity, joint trauma, biomechanical changes, and

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developmental diseases. However, the exact pathogenesis of OA is still unknown, so finding biomarkers for OA is crucial.

In recent years, researchers have been trying to uncover the biological information related to human aging, thereby inhibiting the progression of age-related diseases [11], including neurological diseases, cardiovascular diseases, and osteoarticular diseases [12, 13]. In OA, aging cells increase with age, while the proliferation of chondrocytes and meniscus cells decreases, leading to tissue regeneration and impaired function, thereby exacerbating the progression of the disease [14]. In addition to aging cells, immune cells (neutrophils, macrophages, monocytes, dendritic cells, and natural killer cells) play a crucial role in the pathogenesis of OA [15]. They not only induce the expression of inflammatory factors, but also activate key degrading enzymes to degrade chondrocytes and extracellular matrix [16]. In OA, there is not yet sufficient understanding of the combined analysis of biomarkers for aging and immune cells. Currently, biomarkers can provide clinical diagnostic guidance and prognosis for specific causes of OA, such as hemophilia [17], alkaptonuria/ochronosis and Kashin Beck Disease [18], and rheumatoid arthritis [19]. Regarding patients with OA without specific causes, studying aging and immune cells may be the best approach. Therefore, it is urgent to research and develop biomarkers for aging and immune cells.

Although various tissue cells are involved in the pathological process of OA, chondrocytes are considered a key factor in the occurrence and development of OA [20]. At the same time, the meniscus is an important stable and mechanical buffer structure between the femur and tibia, which is crucial for maintaining knee joint function [21]. Therefore, this study is based on transcriptome data of cartilage, meniscus, and normal samples from patients with OA. A series of bioinformatics methods are used to screen for immune and age-related biomarkers in OA. Through their functions, regulatory mechanisms, and drug prediction, further understanding of the driving mechanism of OA is achieved, and theoretical reference is provided for the clinical treatment of OA.

Materials and methods

Data source

Transcriptome sequencing data were collected from samples of meniscus (Aging_meniscus:Control_meniscus = 3:7) and cartilage (Aging_cartilage:Control_cartilage = 2:6). Aging_meniscus and Aging_cartilage were collected from OA over 60 years of age. Control_meniscus and Control_cartilage were obtained from 18-50 year olds with lower extremity injuries or more than a disarticulated knee. The external validation set GSE114007, containing 20 OA and 18 Control from cartilage tissues, was mined from Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). And 1,793 immune-related genes (IRGs) [22] were retrieved from the Immunology Database and Analysis Portal database (ImmPort, https://www.immport.org), along with 307 aging-related genes (ARGs) from the Human Aging Genomic Resources (HAGR, https://www.genomics.senescence.info/).

Acquisition of differentially expressed genes (DEGs) from the samples of meniscus and cartilage

To obtain the DEGs1, differential expression analysis was performed using *DESeq2* package (v 1.34.0, [23]) for the 2 meniscus groups (Aging_meniscus *vs* Control_meniscus) with $P < 0.05$, $|\log_2 FC| > 0.5$. Then, differentially expressed IRGs1 (DEIRGs1) were generated by crossing DEG1 with IRGs. Similarly, differentially expressed ARGs1 (DEARGs1) were achieved by overlapping DEG1 with ARGs. To explore the biological functions and processes in which DEIRGs1 and DEARGs1 might be involved, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed on the up-regulated and down-regulated genes of these two sets of genes, respectively, using *ClusterProfiler* package (v 4.7.1, [24]). Similarly, DEGs2 from Aging_cartilage and Control_cartilage groups were also obtained by *DESeq2* with same parameters. In addition, differentially expressed IRGs2 (DEIRGs2) and differentially expressed ARGs2 (DEARGs2) were acquired by taking the intersection of DEG2 with IRGs as well as DEG2 with ARGs, respectively. The functions of DEIRGs2

and DEARGs2 were also detected by GO and KEGG.

Identification and enrichment analysis of biomarkers

To obtain genes simultaneously associated with immune in both meniscus and cartilage samples, DEIRGs1 and DEIRGs2 were overlapped, resulting in share.IRGs. In the same way, share.ARGs were obtained by overlapping DEARGs1 and DEARGs2. Additionally, share.IRGs and share.ARGs were taken to intersection, so as to get biomarkers that were simultaneously related to immune and aging in both meniscus and cartilage samples. Functional enrichment of the biomarkers was then performed using GO and KEGG. Moreover, a co-expression for biomarkers was constructed for biomarkers using GeneMANIA (http://genemania.org/) to further explore protein interactions of biomarkers.

Subcellular localization and gene set enrichment analysis (GSEA) further explore biomarker functions

Based on the mRNA fasta files of the biomarkers obtained from National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/), subcellular localization analysis of the biomarkers was performed to provide further insight into gene expression and cellular function using the mRNALocater database (http://bio-bigdata.cn/mRNALocater/) [25]. The GSEA for biomarkers were performed on cartilage samples and meniscus samples, respectively. These analyses were conducted based on the ranking of correlation coefficients of the biomarkers with all genes utilizing *ClusterProfiler* package. The c2.cp.kegg.v7.5.1.symbols.gmt from the Molecular Signatures Database (MSigDB, https://www.gsea-msigdb.org/gsea/msigdb) was used as background gene set.

Construction of regulatory networks

To explore the molecular regulation of biomarkers, the Encyclopedia of RNA Interactomes (ENCORI, http://starbase.sysu.edu.cn/index.php) and miRWalk (http://mirwalk.umm.uni-heidelberg.de/) were used to predict the upstream miRNAs of biomarkers, respectively. We intersected the pairs of mRNA-miRNA relationships from both databases and retained miRNAs that target two or more mRNAs simultaneously as core miRNAs. The transcription factors (TFs) were then obtained by identifying the intersection of predicted mRNA-TF relationship pairs from JASPAR (https://jaspar.genereg.net) and ChIP-X Enrichment Analysis (ChEA, https://amp.pharm.mssm.edu/ChEA3) databases. Finally, the core mRNA-miRNA network and mRNA-TF network were integrated and visualized using *CytoScape* (v 3.9.1, [26]) to obtain the miRNA-mRNA-TF regulatory network. Furthermore, TFs, kinases, and related proteins associated with biomarkers were analyzed by eXpression2Kinases (X2K, https://amp.pharm.mssm.edu/X2K/) to explore potential regulatory mechanisms of biomarkers.

Disease association analysis and drug prediction

In order to analyze the role of biomarkers in other orthopedic diseases, the relationship between biomarkers and orthopedic diseases was analyzed using the Comparative Toxicogenomics Database (CTD, https://ctdbase.org/), and the Top 5 diseases of each biomarker were selected for presentation according to inference score. In addition, to screen for small molecule drugs associated with biomarkers, the Drug-Gene Interaction database (DGIdb, https://dgidb.org/) was used to predict potential drugs for biomarkers. To further validate the potential role of biomarkers in drug therapy, the highest scoring drugs with available molecular structures were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) according to inference score. Meanwhile, the 3D protein structures of the corresponding biomarkers were downloaded from the Protein Data Bank (PDB, https://www.rcsb.org/) database. Finally, molecular docking was performed for the selected drugs and biomarkers.

Expression of biomarkers

Biomarkers expression was shown separately in meniscus and cartilage samples based on transcriptome sequencing data. To further investigate the role of biomarkers in OA, the expression of biomarkers was validated by reverse transcription quantitative polymerase chain reaction (RT-qPCR). In this experiment, a total of 6 clinical cartilage samples were

obtained from control and OA patients in people's liberation army joint logistic support force 920th hospital, including 3 control and 3 OA samples. Additionally, 6 clinical meniscus samples were collected, of which 3 samples were from OA patients and 3 samples from control individuals. This study was approved by the Ethics Committee of Hospital 920 of the Joint Logistics Support Force (No. 2021-067 (Department) -01). Total RNA was prepared using TRIZol reagent. Reverse transcription was performed using the SureScript First-Strand cDNA Synthesis Kit to obtain cDNAs. RT-qPCR was performed as follows: a total of 40 cycles, 95°C for 1 min, 95°C for 20 s, 55°C for 20 s, and 72°C for 30 s. GAPDH was used as the internal reference genes. The RT-qPCR primers were listed in Table S1. The relative expression levels of biomarkers were calculated by $2^{-\Delta\Delta ct}$ method.

Statistical analysis

R software (v 4.2.3) was engaged to implement statistical analysis. Unless otherwise specified, $P < 0.05$ was guessed as statistically significant.

Results

There were 4,199 DEGs1 between Aging_meniscus and Control_meniscus groups

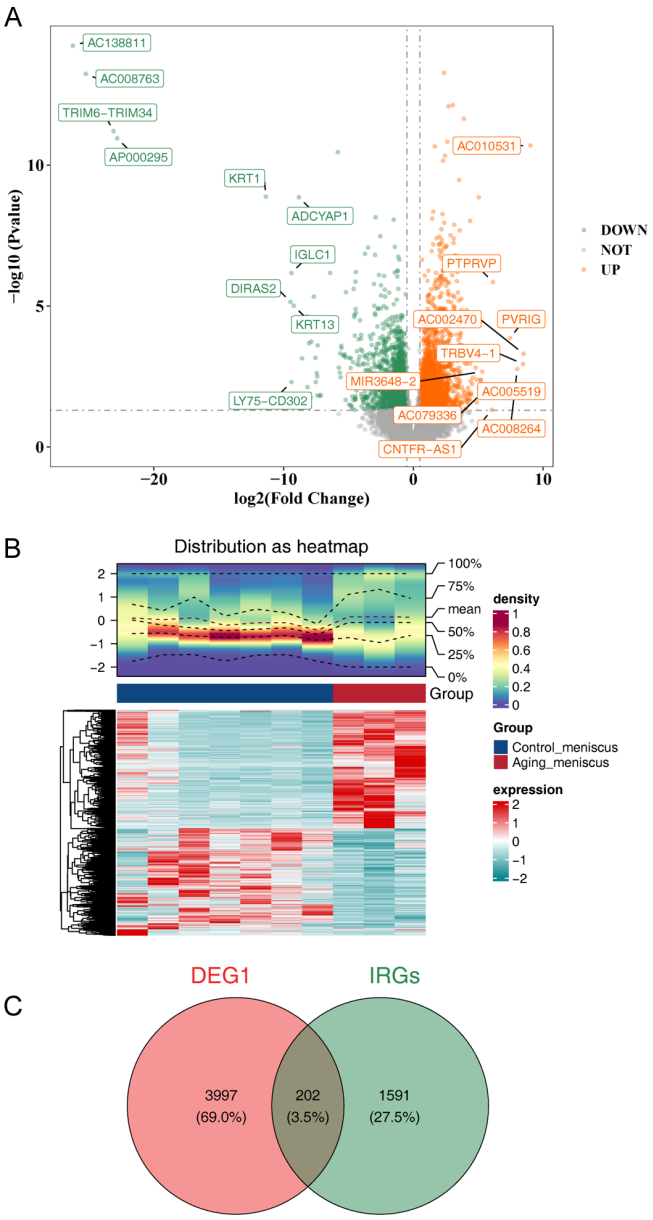
In total, 4,199 DEGs1 (2,247 up-regulated and 1,952 down-regulated) between Aging_meniscus and Control_meniscus groups were gained (**Figure 1A**, **1B**). Furthermore, 202 DEIRGs1 (75 up-regulated and 127 down-regulated) and 72 DEARGs1 (23 up-regulated and 49 down-regulated) were obtained by intersecting DEGs1 with 1,793 IRGs and 307 ARGs, respectively (**Figure 1C**, **1D**). In GO, 75 up-DEIRGs1 (like 'positive regulation of cytokine production') and 127 down-DEIRGs1 ('cytokine-mediated signaling pathway', 'cytokine activity' and so on) were enriched for a number of cytokine-related pathways, which were closely linked to immune and inflammatory responses (**Figure 1E**). In KEGG, both up- and down-DEIRGs1 were enriched in 'cytokine-cytokine receptor interaction' (**Figure 1F**). In other hand, 23 up-DEARGs1 enriched in 'cellular response to abiotic stimulus', 'response to UV', 'p53 binding' and so forth. And 49 down-DEARGs1 involved in 'response to oxidative stress', 'D repair complex', 'phosphatidylinositol 3-kinase complex', etc. (**Figure 1G**). In KEGG, both up- and down-DEARGs1 were related to 'C-type lectin receptor signaling pathway', 'FoxO signaling pathway' and other and other cellular senescence-related pathways (**Figure 1H**).

There were 934 DEGs2 between Aging_cartilage and Control_cartilage groups

Altogether 934 DEGs2 (216 up-regulated and 718 down-regulated) were got between Aging_cartilage and Control_cartilage groups (**Figure 2A**, **2B**). Moreover, 88 DEIRGs2 (15 up-regulated and 73 down-regulated) and 8 DEARGs2 (3 up-regulated and 5 down-regulated) were obtained by intersecting DEGs2 with 1,793 IRGs and 307 ARGs, respectively (**Figure 2C**, **2D**). In addition, 15 up-DEIRGs2 enriched in 'response to ketone', 'response to alcohol', 'response to prostaglandin', etc. and 73 down-DEIRGs2 enriched to multiple immune system-related pathways by GO, e.g., 'B cell receptor signaling pathway', 'immunoglobulin complex', and 'antigen binding' (**Figure 2E**). Both up- and down-DEIRGs2 were also enriched in 'cytokine-cytokine receptor interaction' in KEGG (**Figure 2F**). Meanwhile, 'neuronal cell body', 'peptide hormone binding', 'hormone binding' etc. in GO might be connected to 3 up-DEARGs2, and 'ERK1 and ERK2 cascade', 'positive regulation of MAP kinase', 'cytokine receptor binding' and so on in GO were associated with 5 down-DEARGs2 (**Figure 2G**). For KEGG pathways, up-DEARGs2 involved in 'longevity regulating pathway', 'HIF-1 signaling pathway', 'FoxO signaling pathway' and so on, and down-DEARGs2 involved in 'C-type lectin receptor signaling pathway', 'FoxO signaling pathway', 'Cellular senescence' etc. (**Figure 2H**).

IGF1-R, IL-7R and LEPR were recognized as biomarkers and were mainly localized in cytoplasm, endoplasmic reticulum, and extracellular region, respectively

A total of 14 share.IRGs and 4 share.ARGs were identified by overlapping DEIRGs1 and DEIRGs2 as well as DEARGs1 and DEARGs2, respectively (**Figure 3A**, **3B**). Then, 3 biomarkers, namely insulin-like growth factor 1 receptor (*IGF1-R*), interleukin 7 receptor (*IL-7R*) and leptin receptor (*LEPR*), were selected out through the intersection of 14 share.IRGs and 4 share.ARGs (**Figure 3C**). The 3 biomarkers


A
AC138811
AC008763
TRIM6-TRIM34
AP000295
KRT1
ADCYAP1
IGLC1
DIRAS2
KRT13
LY75-CD302
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PTPRVP
AC002470
PVRIG
TRBV4-1
MIR3648-2
AC005519
AC079336
AC008264
CNTFR-AS1
DOWN
NOT
UP
−log10 (Pvalue)
log2(Fold Change)
B
Distribution as heatmap
100%
75%
mean
50%
25%
0%
Group
density
Group
Control_meniscus
Aging_meniscus
expression
C
DEG1
IRGs
3997
(69.0%)
202
(3.5%)
1591
(27.5%)
E
GO Terms Enrich
Counts
type
Biological Process
Cellular Component
Molecular Function
F
Th17 cell differentiation
T cell receptor signaling pathway
RIG-I-like receptor signaling pathway
PD-L1 expression and PD-1 checkpoint pathway in cancer
Non-small cell lung cancer
Lipid and atherosclerosis
Insulin resistance
Hepatitis B
Hepatitis C
ErbB signaling pathway
EGFR tyrosine kinase inhibitor resistance
Cytokine-cytokine receptor interaction
Axon guidance
AGE-RAGE signaling pathway in diabetic complications
Adipocytokine signaling pathway
Description
−log10(p.adjust)
Toll-like receptor signaling pathway
Ras signaling pathway
Prolactin signaling pathway
Phospholipase D signaling pathway
Neurotrophin signaling pathway
Human cytomegalovirus infection
Gastric cancer
EGFR tyrosine kinase inhibitor resistance
Colorectal cancer
Chronic myeloid leukemia
C-type lectin receptor signaling pathway
G
GO Terms Enrich

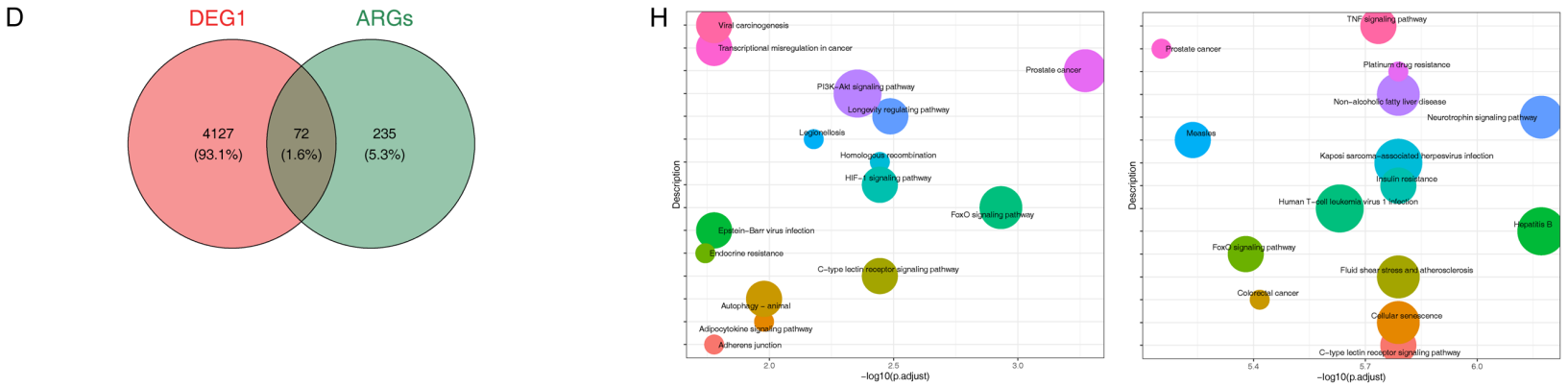
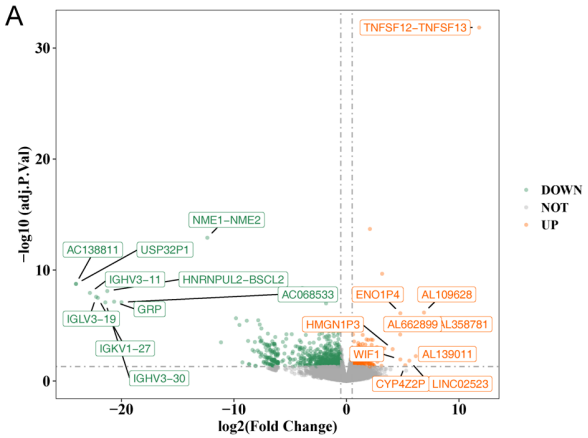
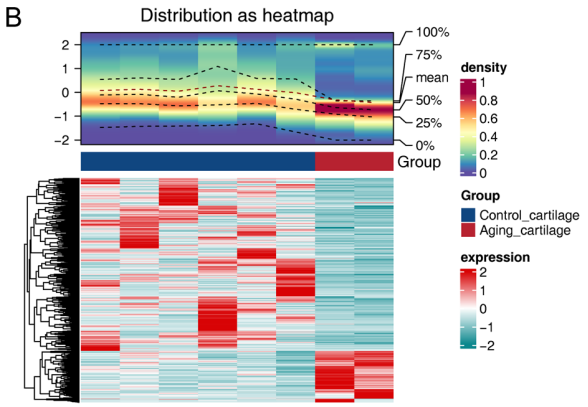
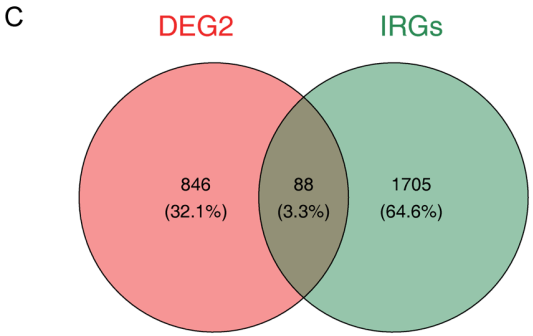
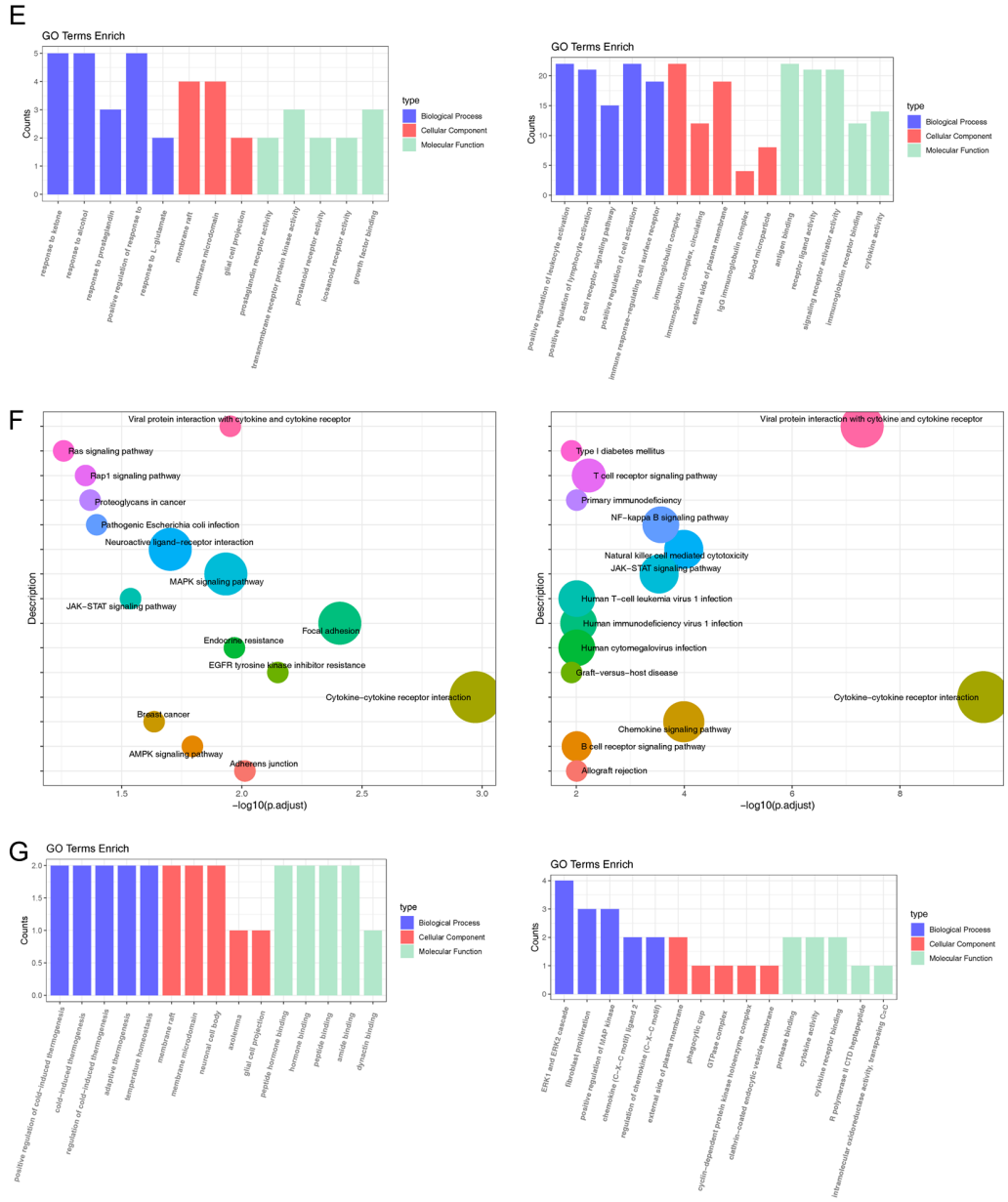


Figure 1. A. Volcano map of DEGs1 between Aging_meniscus and Control_meniscus. Green represents a significant up-regulation of DEGs1, represents downward adjustments, orange refers to a down-regulation of DEGs1 and gray represents no significant difference in gene expression. B. Heat map of DEGs1. Red indicates high expression and green indicates low expression in Aging_meniscus. C. Venn map for 202 DEIRGs1 by intersecting DEGs1 and IRGs. D. Venn map of 72 DEARGs1 by intersecting DEGs1 and ARGs. E. GO term enrichment of up- (left) and down-DEIRGs1 (right). F. KEGG pathway analysis of up- (left) and down-DEIRGs1 (right). G. GO term enrichment of up- (left) and down-DEARGs1 (right). H. KEGG pathway analysis of (left) and down-DEARGs1 (right).


A
TNFSF12−TNFSF13
NME1−NME2
AC138811
USP32P1
IGHV3−11
HNRNPUL2−BSCL2
AC068533
ENO1P4
AL109628
GRP
IGLV3−19
HMGN1P3
AL662899
AL358781
IGKV1−27
WIF1
AL139011
IGHV3−30
CYP4Z2P
LINC02523
DOWN
NOT
UP
−log10 (adj.P.Val)
log2(Fold Change)


B
Distribution as heatmap
100%
75%
mean
50%
25%
0%
Group
density
Group
Control_cartilage
Aging_cartilage
expression


C
DEG2
IRGs
846
(32.1%)
88
(3.3%)
1705
(64.6%)


E
GO Terms Enrich
Counts
type
Biological Process
Cellular Component
Molecular Function
F
Viral protein interaction with cytokine and cytokine receptor
Ras signaling pathway
Rap1 signaling pathway
Proteoglycans in cancer
Pathogenic Escherichia coli infection
Neuroactive ligand−receptor interaction
MAPK signaling pathway
JAK−STAT signaling pathway
Focal adhesion
Endocrine resistance
EGFR tyrosine kinase inhibitor resistance
Cytokine−cytokine receptor interaction
Breast cancer
AMPK signaling pathway
Adherens junction
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T cell receptor signaling pathway
Primary immunodeficiency
NF−kappa B signaling pathway
Natural killer cell mediated cytotoxicity
JAK−STAT signaling pathway
Human T−cell leukemia virus 1 infection
Human immunodeficiency virus 1 infection
Human cytomegalovirus infection
Graft−versus−host disease
Chemokine signaling pathway
B cell receptor signaling pathway
Allograft rejection
G
GO Terms Enrich
Counts
type
Biological Process
Cellular Component
Molecular Function

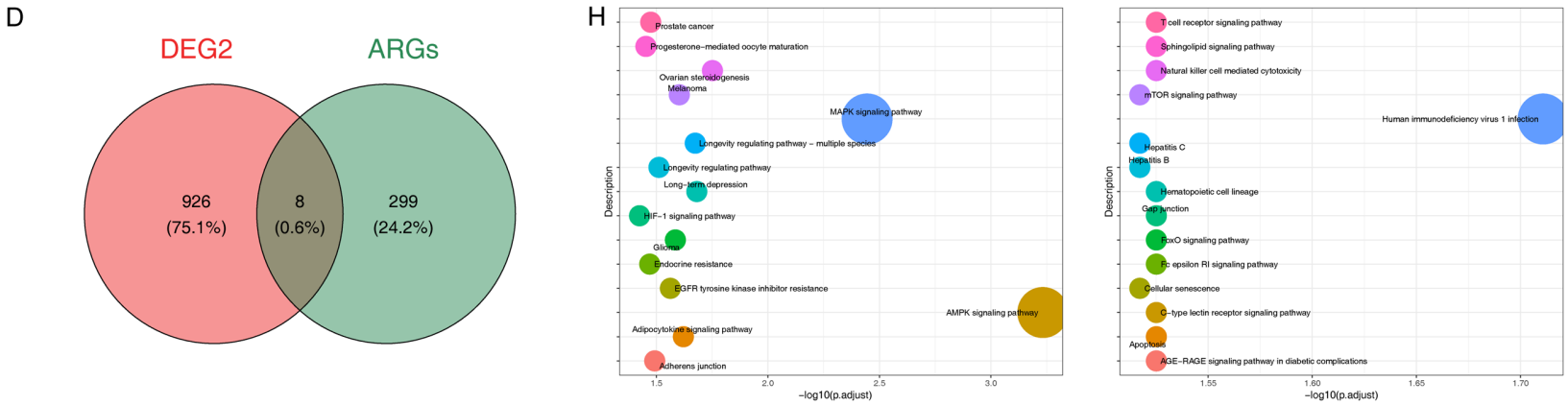

Figure 2. A. Volcano map of DEGs2 between Aging_cartilage and Control_cartilage groups. Green represents a significant up-regulation of DEGs2, represents downward adjustments, orange refers to a down-regulation of DEGs2 and gray represents no significant difference in gene expression. B. Heat map of DEGs2. Red indicates high expression and green indicates low expression in Aging_cartilage. C. Venn map for 88 DEIRGs2 by intersecting DEGs2 and IRGs. D. Venn map of 8 DEARGs2 by intersecting DEGs2 and ARGs. E. GO term enrichment of up- (left) and down-DEIRGs2 (right). F. KEGG pathway analysis of up- (left) and down-DEIRGs2 (right). G. GO term enrichment of up- (left) and down-DEARGs2 (right). H. KEGG pathway analysis of up- (left) and down-DEARGs2 (right).

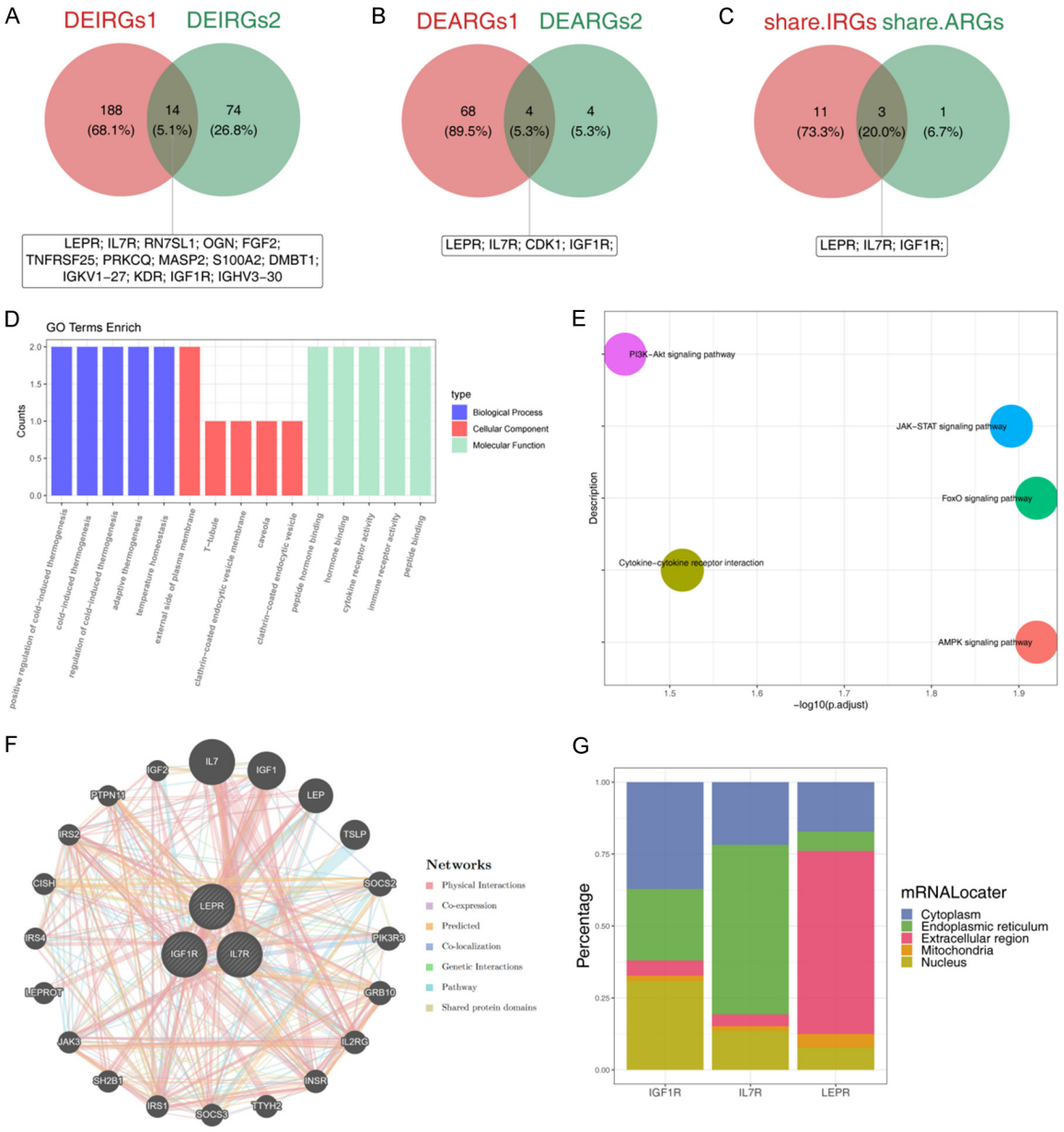

Figure 3. A. Venn map for 14 share.IRGs by overlapping DEIRGs1 and DEIRGs2. B. Venn map for 4 share.ARGs by overlapping DEARGs1 and DEARGs2. C. Three biomarkers (IGF1-R, IL-7R and LEPR) were obtained by 14 share.IRGs and 4 share.ARGs. D. GO term enrichment of 3 biomarkers. E. KEGG pathway analysis of 3 biomarkers. F. Co-expression network for 3 biomarkers. The inner circle is 3 biomarkers, and the outer circle is other proteins that have interaction or co-expression relationships with the 3 biomarkers, with different colored lines representing different relationships. G. Subcellular localization for 3 biomarkers. Different colors represent various organelles.

enriched in 'hormone binding', 'cytokine receptor activity', 'immune receptor activity', etc. in GO (**Figure 3D** and Table S2) and 'FoxO signaling pathway', 'cytokine-cytokine receptor interaction', 'longevity regulating pathway', etc. in KEGG (**Figure 3E** and Table S3). Additionally, the co-expression indicated 3 biomarkers with diverse forms of interactions or co-expression with 20 proteins (e.g. IL-7, IGF1, LEP) (**Figure 3F**). Subcellular localization could enhance comprehension of gene expression and cellular function. In this study, *IGF1-R* was predominantly located in the cytoplasm and might play an important role in regulating intracellular signaling processes. On the other hand, *IL-7R* was mainly sited in the endoplasmic reticulum and

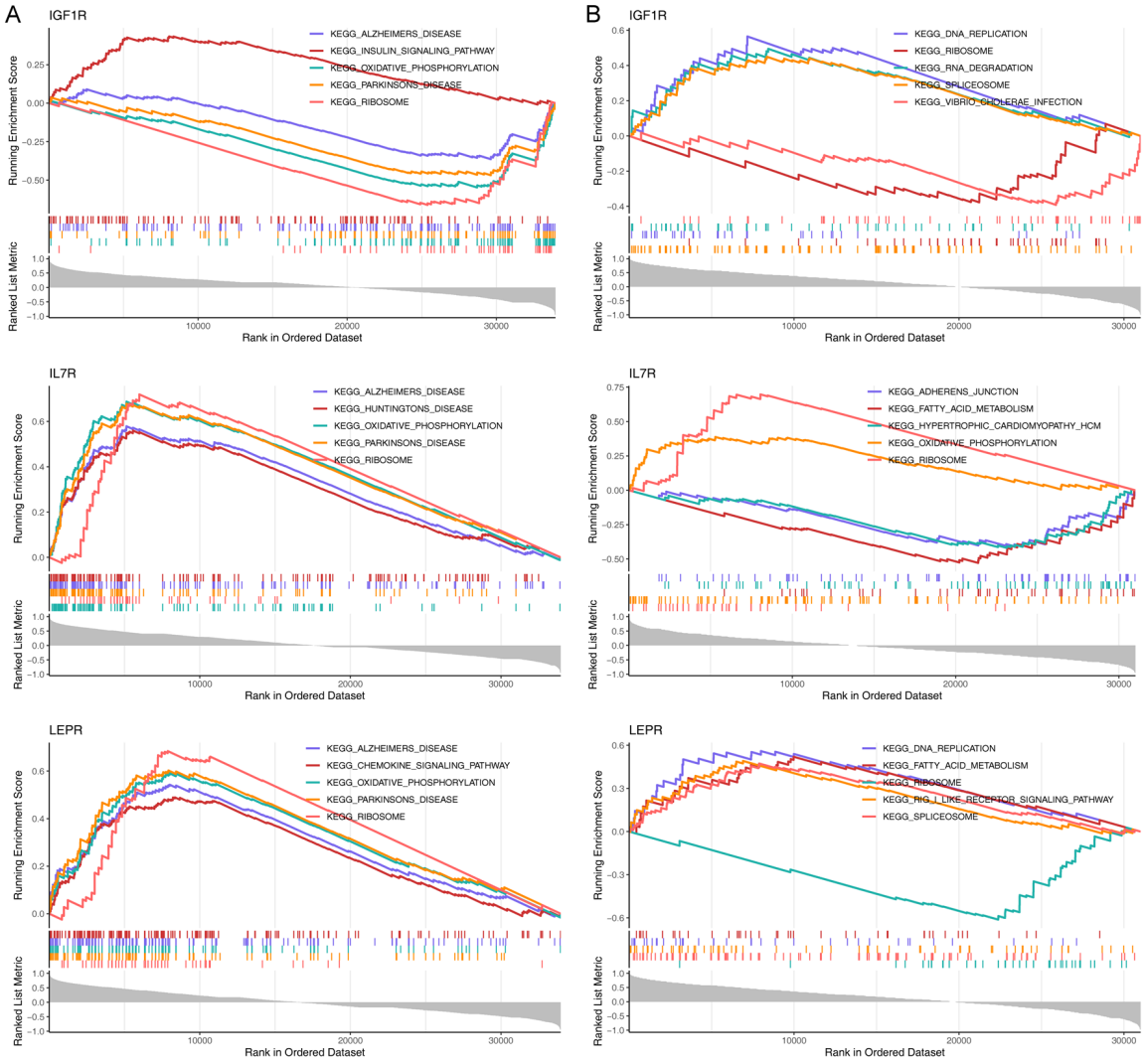

Figure 4. A. GSEA enrichment analysis for KEGG geneset in IGF1-R, IL-7R, and LEPR in meniscus. B. GSEA enrichment analysis for KEGG geneset in IGF1-R, IL-7R, and LEPR in cartilages.

probably participated in protein synthesis and folding. *LEPR* was mainly situated in the extracellular region, presumed to exist on the cell membrane and connected to extracellular signaling and intercellular communication (**Figure 3G**).

IGF1-R, IL-7R and LEPR were all enriched in 'ribosome' from both meniscus and cartilage samples

In meniscus, *IGF1-R*, *IL-7R*, and *LEPR* were all enriched in 'oxidative phosphorylation' and 'ribosome' pathways by GSEA (**Figure 4A**). In cartilage, *IGF1-R*, *IL-7R*, and *LEPR* were all also enriched in 'ribosome' pathway, while *IGF1-R* and *LEPR* were both enriched in 'DNA replication' and 'spliceosome' (**Figure 4B**).

Three biomarkers had complex regulatory networks

On the basis of upstream miRNAs of biomarkers predicted by ENCORI and miRWalk, a total of 275 relationship pairs were obtained by 265 miRNAs and 3 mRNAs (*IGF1-R*, *IL-7R*, and *LEPR*). Further, we identified 19 relationship pairs involved 3 mRNAs and 9 core miRNAs (hsa-miR-124-3p, hsa-miR-183-5p, hsa-miR-186-5p etc.) by retaining miRNAs that target two or more mRNAs simultaneously (Table S4). Meanwhile, 4 relationship pairs (GATA2-*LEPR*, STAT3-*LEPR*, STAT3-*IL-7R*, E2F1-*IGF1-R*) from the 3 mRNAs and 3 TFs (GATA2, STAT3 and E2F1) were acquired by JASPAR and ChEA databases. Finally, we integrated the core mRNA-miRNA network and mRNA-TF network to ob-

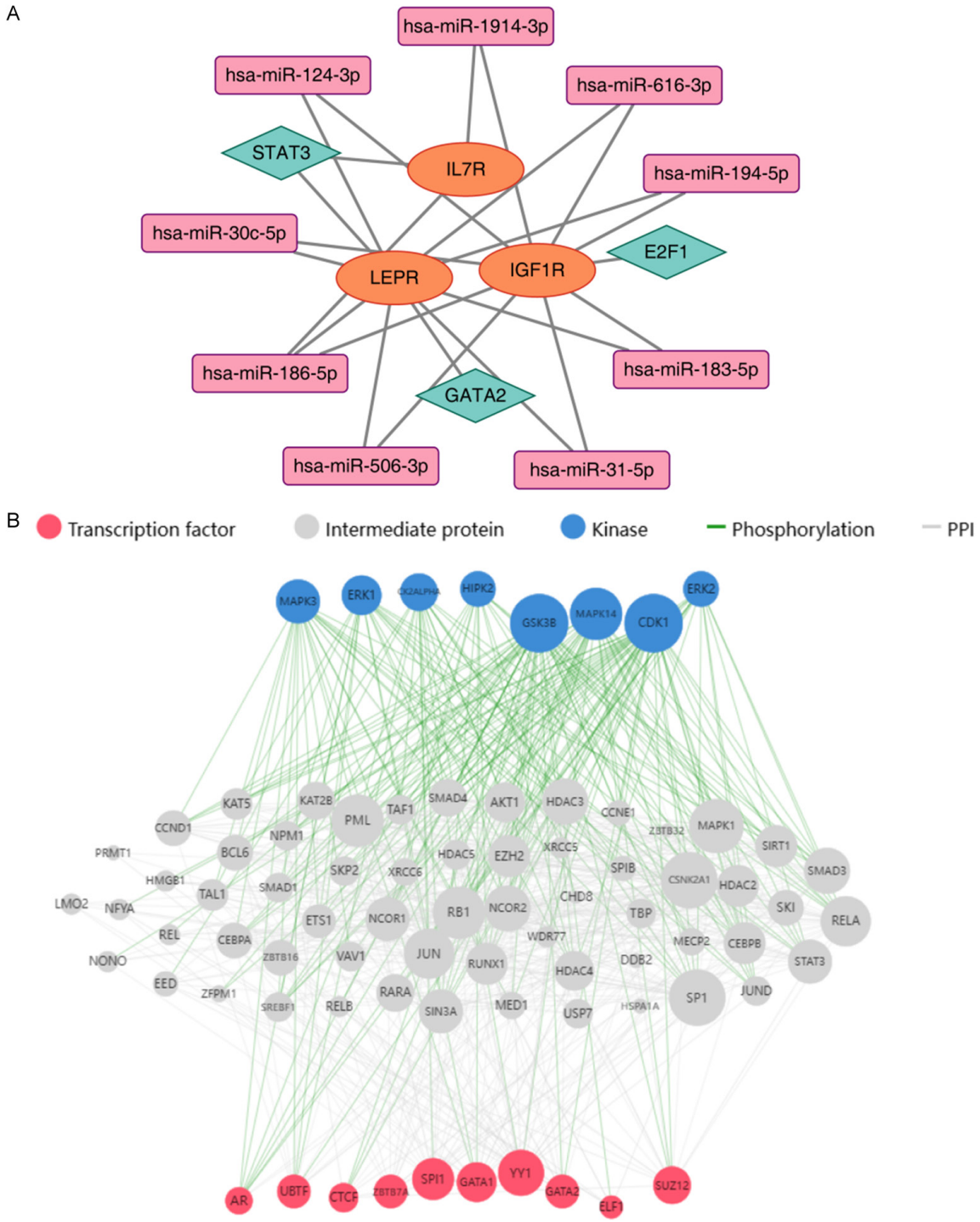

Figure 5. A. The miRNA-mRNA-TF regulatory network. Orange labels are biomarkers, green labels are TFs, and pink labels are miRNAs. B. X2K regulatory network. Red labels are TFs, gray labels are intermediate proteins, blue labels are kinases, green lines represent phosphorylation, and gray lines represent protein-protein interactions.

tain the miRNA-mRNA-TF regulatory network using CytoScape (**Figure 5A**). In addition, a X2K network was constructed, which included 8 kinases (e.g. CDK1, GSK3B and MAPK14), 10 TFs (e.g. YY1, GATA1 and SPI1) and lots of intermediate proteins (**Figure 5B**).

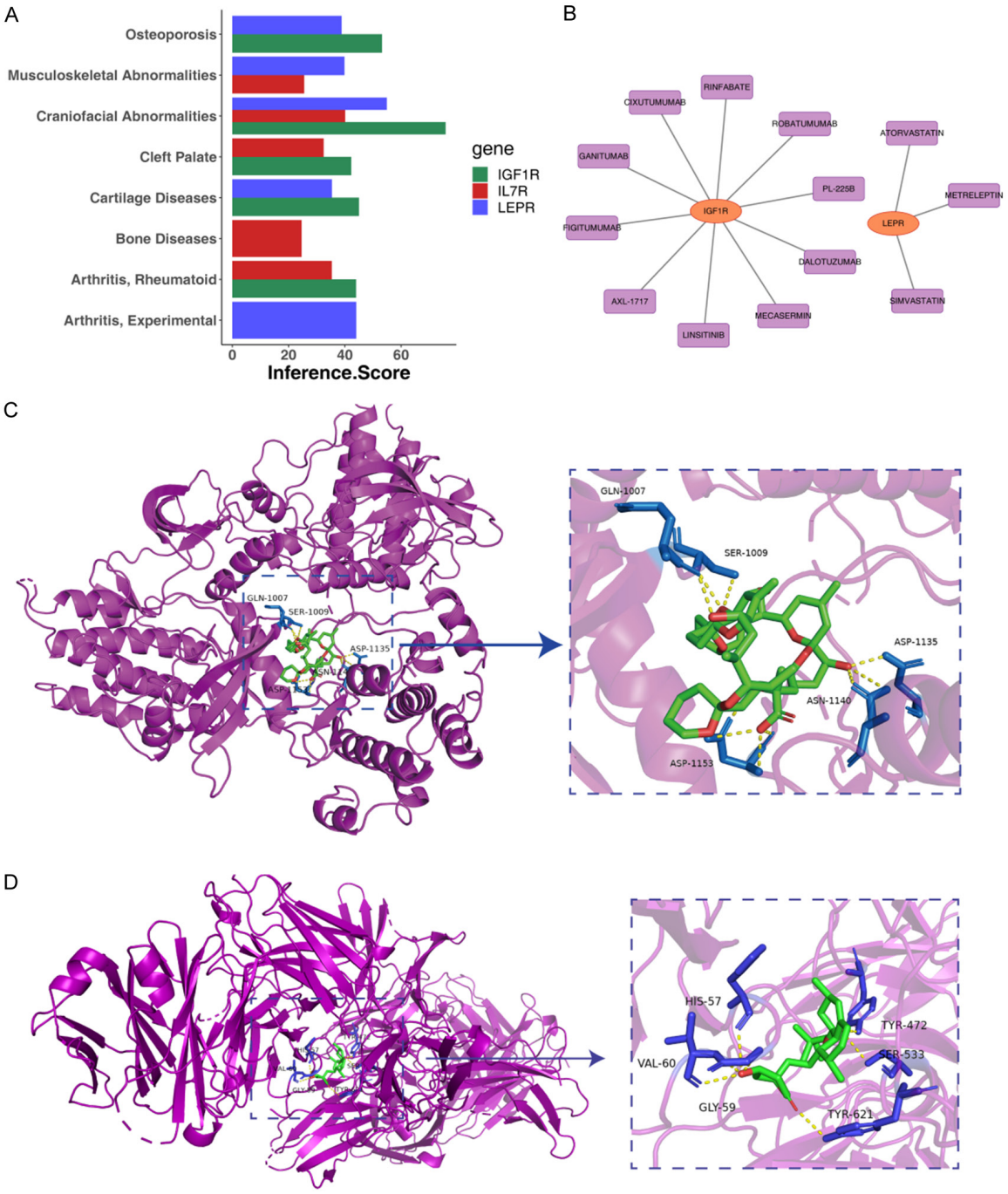

Figure 6. A. Association analysis of 3 biomarkers with other orthopedic diseases. Green, red, and blue indicate IGF1-R, IL-7R, and LEPR, respectively. B. Drug prediction for biomarkers. Orange labels refer to biomarkers, purple labels refer to predicted drugs. C. The molecular docking of Mecasermin with IGF1-R (ΔG = -9.5 KJ/mol). D. The molecular docking of Simvastatin with LEPR (ΔG = -8.4 KJ/mol).

Mecasermin could stably bind to IGF1R and LEPR

The results of disease association analysis showed that craniofacial abnormalities were predicted by all 3 biomarkers, and cleft palate and rheumatoid arthritis were predicted by both IL-7R, and LEPR. Thus, there was a strong association between the biomarkers obtained and orthopedic diseases (**Figure 6A**). Based on

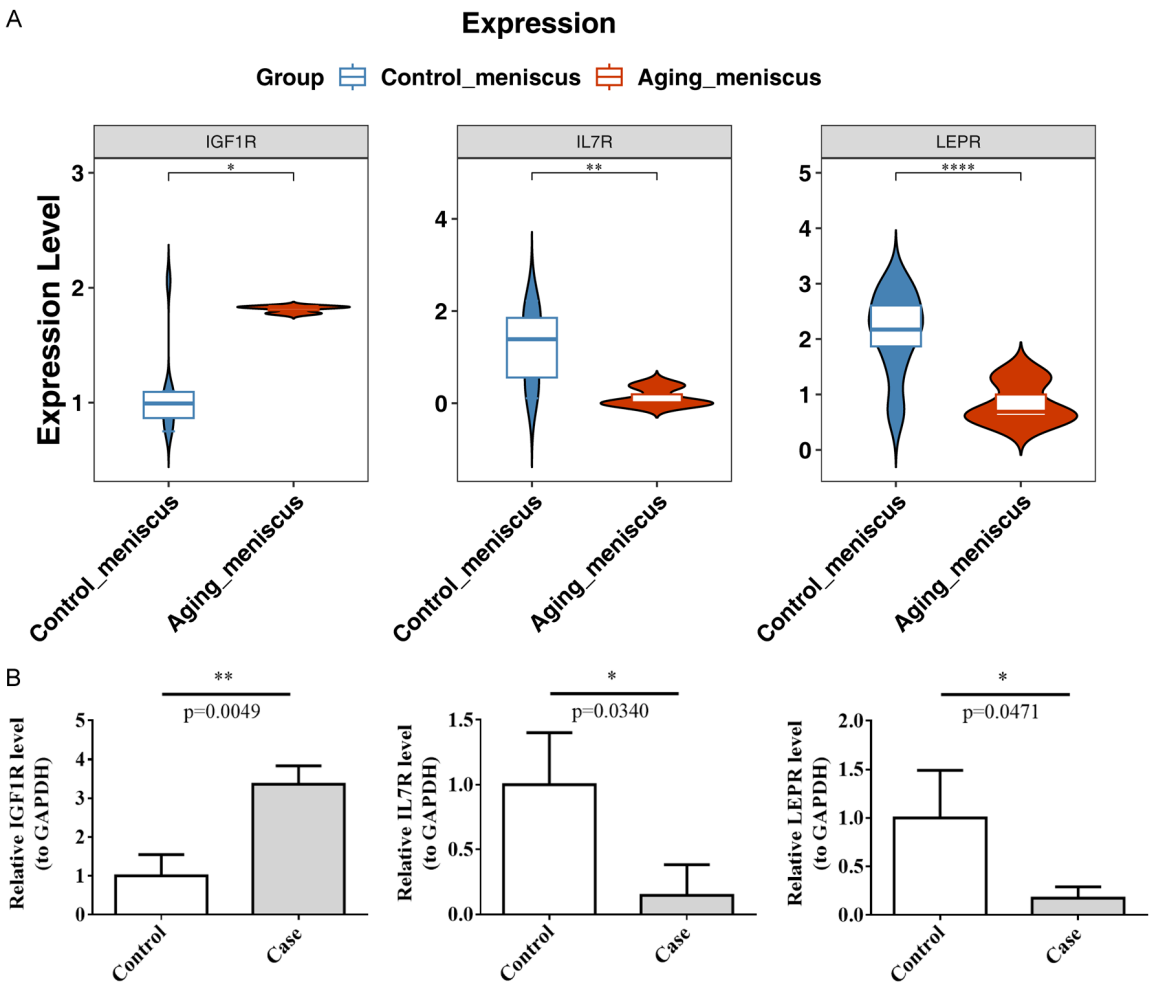

Figure 7. A. Expression levels of biomarkers (IGF1-R, IL-7R, and LEPR) in meniscus with transcriptome sequencing data. B. RT-qPCR for validation of biomarkers expression in meniscus. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.001$, ns: non-significance.

the DGIdb database, we predicted potential small molecule drugs for three biomarkers, and only 2 biomarkers had outcomes, with 10 drugs (Rinfabate, Cixutumumab, Mecasermin, etc.) for IGF1-R and 3 drugs (Atorvastatin, Metreleptin and Simvastatin) for LEPR (**Figure 6B**). Ultimately, Mecasermin was selected to interface with IGF1-R (ΔG = -9.5 KJ/mol, **Figure 6C**) and Simvastatin was selected to interface with LEPR (ΔG = -8.4 KJ/mol, **Figure 6D**).

Expression of IGF1-R, IL-7R, and LEPR in the meniscus of OA

In transcriptome sequencing data, *IGF1R* expression was up-regulated, while *IL7R* and *LEPR* expressions were down-regulated in Aging_meniscus (**Figure 7A**). The expression of all 3 biomarkers was verified in RT-qPCR (**Figure 7B**).

Expression of IGF1-R, IL-7R, and LEPR in the cartilage of OA

In Aging_cartilage, *IGF1-R* and *LEPR* expression was up-regulated and *IL-7R* expression was down-regulated (**Figure 8A**). In the validation set, IGF1-R was significantly highly expressed in OA, and IL7R was also lowly expressed in OA but the difference was not significant (**Figure 8B**). The expression of *IGF1-R* and *LEPR* exhibited a marked up-regulation in OA cartilage via RT-qPCR, aligning with the sequencing findings (**Figure 8C**). Although not statistically significant, *IL-7R* expression was also down-regulated in OA cartilage by RT-qPCR.

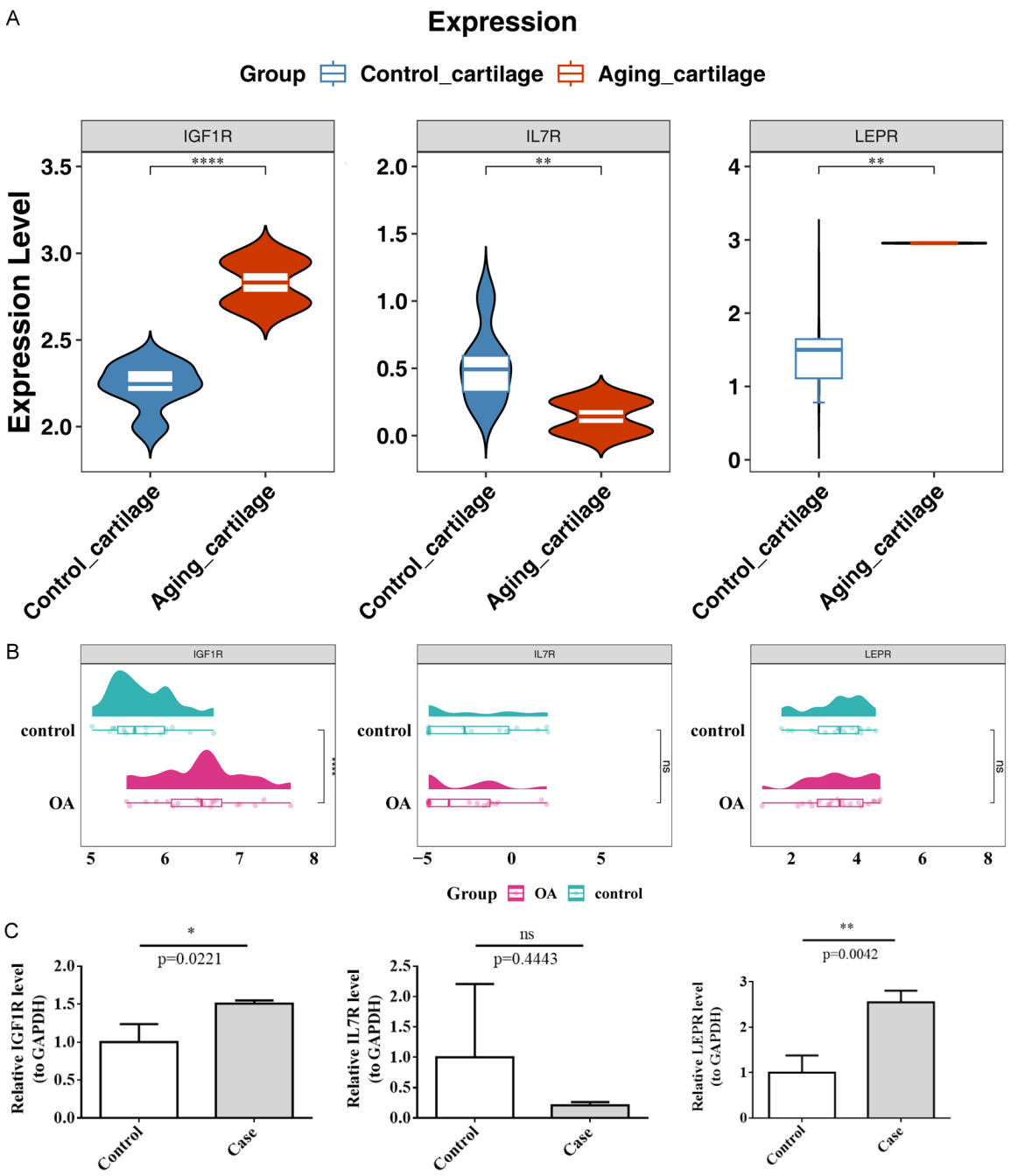

Figure 8. A. Expression levels of biomarkers (IGF1-R, IL-7R, and LEPR) in cartilages with transcriptome sequencing data. B. Expression levels of biomarkers in GSE114007. C. RT-qPCR for validation of biomarkers expression in cartilages. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.001$, ns: non-significance.

Discussion

OA is a chronic disease characterized by pain, cartilage loss, and joint inflammation, in which immune cells and aging play important roles in its progression. So far, there has been extensive research on individual immune cells or aging cells, such as immune cells (T and B cells, macrophages) involved in osteoclastogenesis, bone erosion, degradation and destruction of chondrocytes in OA [27, 28]. TLR7, CSF1R, APOE, C1QA, and CCL5 are involved in immune response to promote the progression of OA [29]. Oxidative stress leads to chondrocyte

aging by increasing the expression of p53 and p21 and activating the p38 MAPK and PI3K/Akt signaling pathways [30]. Mechanical stress can lead to chondrocyte aging and accelerate cartilage breakdown metabolism by downregulating FBXW7 [31]. However, there are no studies on genes that function in both immune cells and aging cells. Therefore, this study aims to identify potential aging and immune related biomarkers in OA, and explore the roles and mechanisms of aging and immunity in OA cartilage and meniscus tissue, thereby providing new directions and ideas for the treatment of OA.

In this study, three biomarkers related to aging and immunity were screened based on self sequencing data combined with bioinformatics, namely IGF1R, IL7R, and LEPR. Through GO and KEGG enrichment analysis, it was found that biomarkers mainly affect cell aging through energy metabolism. Hormones and their receptors play a role in immune, metabolic, and cellular signaling. The AMPK signaling pathway, FoxO signaling pathway, JAK-STAT signaling pathway, and PI3K-Akt signaling pathway affect the progression of OA, which is consistent with previous studies [32-35]. Some have suggested that aging and immune cells may be involved in inflammation regulation, cell metabolism, cell cycle regulation, transcriptional regulation, and other mechanisms that promote OA progression [35, 36]. GSEA analysis in meniscus and cartilage shows that the aging and immune function of cartilage and meniscus cells are affected through chemokine signaling pathways, oxidative phosphorylation, RIG-1 like receptor signaling pathways, and fatty acid metabolism, thereby affecting their physiological function and health status.

By analyzing the disease correlation through the CTD database, it was found that all genes were screened to be associated with OA. IGF1-R is a member of the insulin-like growth factor 1 (IGF) signaling pathway and is crucial for cell growth and tissue differentiation [37]. This receptor mainly mediates the effects of IGF1 and IGF2, and IGF1-R is also believed to participate in the regulation of articular cartilage metabolism through IGF-1 binding [38]. Previous studies had shown that the expression of IGF1-R increased in OA, and the increased expression was positively correlated with the degree of cartilage lesions in OA [39, 40]. In the cartilage and meniscus tissues of patients with OA, negative regulation is used to promote the expression of IGF1-R and inhibit the progression of osteoarthritis. In our experiment, we found through screening biomarkers and qPCR studies that IGF1-R is highly expressed in both cartilage and meniscus tissues of the bone and joint, making it a biomarker for OA. The IL-7R is heterodimeric, consistent of IL-7R α Chain (IL-7R α, CD127) and the common γ Chain [41]. In the progress of OA, there is significant controversy surrounding IL-7R [42, 43]. The signal mediated by IL-7R promotes lymphatic development and immune balance in the body. Currently, extensive research has been conducted on cancer and autoimmune diseases [44, 45]. We have innovatively discovered low expression in cartilage and meniscus tissues of OA. LEPR determines the role of leptin signaling [46]. In the presence of leptin, LEPR is overexpressed in human chondrocytes, increasing chondrocyte aging by activating mTOR [47]. Leptin can also induce the release of pro-inflammatory agents in cartilage, thereby accelerating the progression of OA [48], however, research has not yet been conducted in meniscus tissue, and our experiment has made up for this drawback. In the experiment, it was found that LEPR was upregulated in cartilage, which is consistent with previous studies. However, in meniscus tissue, LEPR expression was downregulated, which may be related to the differential expression of the same disease in different tissues.

As the human body ages and the innate and adaptive immune system activates, it plays a crucial role in various aspects of the pathogenesis of OA [49]. To investigate the molecular regulation of OA pathogenesis by biomarkers, we used ENCORI and miRWalk to predict upstream miRNAs of biomarkers, and obtained a total of 19 relationship pairs between biomarkers and miRNAs. At the same time, we used the JASPAR and ChEA databases of miRNet online tools to predict biomarkers and transcription factors, and obtained four pairs of relationships between biomarkers and transcription factors. We found that LEPR/STAT3 plays an important role in inflammation regulation [50], hormone regulation, and cell regeneration [51]. Finally, in this study, we identified two potential small molecule compounds,

Mecasermin, which is a recombinant human insulin-like growth factor-1 (rh-IGF-1). Studies have shown that IGF-1 is considered the main synthetic metabolic factor in articular cartilage, capable of stimulating the synthesis of proteoglycans and collagen [52]. When IGF-1 expression is inhibited, it can promote the progression of OA [53]. Our study shows that Mecasermin can bind to IGF-1R, suggesting that the drug could work to treat OA by supplementing IGF-1 in combination with IGF-1R. Simvastatin is an effective hydroxymethylglutaryl CoA (HMG CoA) inhibitor commonly used as a cholesterol lowering drug for hypercholesterolemia and cardiovascular disease. It plays an important role in inhibiting chondrocyte aging [54], death, and catabolism [55], as well as the expression of interleukin and matrix metalloproteinase [56, 57]. In the future, Mecasermin and Simvastatin may become important drugs for the treatment of OA.

Based on transcriptome sequencing data and using bioinformatics methods, this study obtained immune and aging biomarkers (IGF1-R, IL-7R, LEPR). These genes have good diagnostic and therapeutic effects on OA, laying a certain direction for the next basic research. The biological functions of these biomarkers were also explored, and disease-related drugs were predicted. Meanwhile, our research also has the following limitations: limited sample size has a potential impact on the accuracy of the results, and larger sample size and prospective study design are needed to validate and strengthen the model's findings. The specific regulatory mechanisms and functions of biomarkers require further clinical research, but we will continue to pay attention to the mechanisms of action of genes related to OA diagnosis/treatment.

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All patients signed an informed consent form to acquire and use discarded tissue.

Disclosure of conflict of interest

None.

Abbreviations

OA, osteoarthritis; IRGs, immune-related genes; ARGs, aging-related genes; DEGs, differentially expressed genes; DEIRGs, differentially expressed IRGs; DEARGs, differentially expressed ARGs; GSEA, gene set enrichment analysis; TF, transcription factor; NSAIDs, nonsteroidal anti-inflammatory drugs; HA, hyaluronic acid; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; NCBI, National Center for Biotechnology Information; RT-qPCR, reverse transcription quantitative polymerase chain reaction; IGF1-R, insulin-like growth factor 1 receptor; IL-7R, interleukin 7 receptor; LEPR, leptin receptor.

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Table S1. RT-qPCR primers

primers	sequences
LEPR F	TGTCGTCTATCGGGAAGGAG
LEPR R	CGGTAAGCTACATCGTGCATTA
IL7R F	AGTGGGGCTATTGGACTGAG
IL7R R	TCCAGCAGGCAAAAGGAAGT
IGF1R F	GTCTTGGGTGGAGTCATGGTT
IGF1R R	TGAGCACTCCAGACCAACTG
GAPDH F①	CGAAGGTGGAGTCAACGGATTT
GAPDH R①	ATGGGTGGAATCATATTGGAAC
GAPDH F②	CGAAGGTGGAGTCAACGGATTT
GAPDH R②	ATGGGTGGAATCATATTGGAAC
GAPDH F③	CGAAGGTGGAGTCAACGGATTT
GAPDH R③	ATGGGTGGAATCATATTGGAAC

Table S2. GO enrichment analysis for IGF1-R, IL-7R and ILEPR

ONTOLOGY	ID	Description	BgRatio	pvalue	p.adjust	qvalue	geneID	Count
BP	GO:0120162	positive regulation of cold-induced thermogenesis	98/18903	7.95E-05	0.016456821	0.002601213	LEPR/IGF1R	2
BP	GO:0106106	cold-induced thermogenesis	147/18903	0.000179278	0.016456821	0.002601213	LEPR/IGF1R	2
BP	GO:0120161	regulation of cold-induced thermogenesis	147/18903	0.000179278	0.016456821	0.002601213	LEPR/IGF1R	2
BP	GO:1990845	adaptive thermogenesis	160/18903	0.000212409	0.016456821	0.002601213	LEPR/IGF1R	2
BP	GO:0001659	temperature homeostasis	178/18903	0.000262889	0.016456821	0.002601213	LEPR/IGF1R	2
BP	GO:0042593	glucose homeostasis	248/18903	0.000509855	0.022981581	0.003632535	LEPR/IGF1R	2
BP	GO:0033500	carbohydrate homeostasis	249/18903	0.000513965	0.022981581	0.003632535	LEPR/IGF1R	2
BP	GO:0030217	T cell differentiation	296/18903	0.000725553	0.026729499	0.004224942	LEPR/IL7R	2
BP	GO:0008202	steroid metabolic process	323/18903	0.000863368	0.026729499	0.004224942	LEPR/IGF1R	2
BP	GO:0030098	lymphocyte differentiation	419/18903	0.001448898	0.026729499	0.004224942	LEPR/IL7R	2
BP	GO:0001915	negative regulation of T cell mediated cytotoxicity	10/18903	0.001586294	0.026729499	0.004224942	IL7R	1
BP	GO:0046886	positive regulation of hormone biosynthetic process	10/18903	0.001586294	0.026729499	0.004224942	IGF1R	1
BP	GO:0033089	positive regulation of T cell differentiation in thymus	11/18903	0.001744831	0.026729499	0.004224942	IL7R	1
BP	GO:0033210	leptin-mediated signaling pathway	11/18903	0.001744831	0.026729499	0.004224942	LEPR	1
BP	GO:0071394	cellular response to testosterone stimulus	11/18903	0.001744831	0.026729499	0.004224942	IGF1R	1
BP	GO:1903131	mononuclear cell differentiation	473/18903	0.001843361	0.026729499	0.004224942	LEPR/IL7R	2
BP	GO:1902065	response to L-glutamate	12/18903	0.001903352	0.026729499	0.004224942	IGF1R	1
BP	GO:0019221	cytokine-mediated signaling pathway	496/18903	0.002025516	0.026729499	0.004224942	LEPR/IL7R	2
BP	GO:0033690	positive regulation of osteoblast proliferation	13/18903	0.002061855	0.026729499	0.004224942	IGF1R	1
BP	GO:0048680	positive regulation of axon regeneration	13/18903	0.002061855	0.026729499	0.004224942	IGF1R	1
BP	GO:0071389	cellular response to mineralocorticoid stimulus	13/18903	0.002061855	0.026729499	0.004224942	IGF1R	1
BP	GO:0044849	estrous cycle	14/18903	0.002220342	0.026729499	0.004224942	IGF1R	1
BP	GO:0070572	positive regulation of neuron projection regeneration	14/18903	0.002220342	0.026729499	0.004224942	IGF1R	1
BP	GO:0098760	response to interleukin-7	14/18903	0.002220342	0.026729499	0.004224942	IL7R	1
BP	GO:0098761	cellular response to interleukin-7	14/18903	0.002220342	0.026729499	0.004224942	IL7R	1
BP	GO:1904044	response to aldosterone	14/18903	0.002220342	0.026729499	0.004224942	IGF1R	1
BP	GO:0032352	positive regulation of hormone metabolic process	15/18903	0.002378812	0.027576595	0.004358836	IGF1R	1
BP	GO:0090030	regulation of steroid hormone biosynthetic process	16/18903	0.002537265	0.02791687	0.004412621	IGF1R	1
BP	GO:0110096	cellular response to aldehyde	17/18903	0.002695701	0.02791687	0.004412621	IGF1R	1
BP	GO:0044320	cellular response to leptin stimulus	18/18903	0.002854121	0.02791687	0.004412621	LEPR	1
BP	GO:0045721	negative regulation of gluconeogenesis	18/18903	0.002854121	0.02791687	0.004412621	LEPR	1
BP	GO:0048535	lymph node development	18/18903	0.002854121	0.02791687	0.004412621	IL7R	1
BP	GO:0097284	hepatocyte apoptotic process	19/18903	0.003012524	0.028573331	0.004516383	IGF1R	1
BP	GO:0038083	peptidyl-tyrosine autophosphorylation	21/18903	0.003329279	0.028946232	0.004575325	IGF1R	1
BP	GO:0045056	transcytosis	21/18903	0.003329279	0.028946232	0.004575325	IGF1R	1
BP	GO:0097062	dendritic spine maintenance	21/18903	0.003329279	0.028946232	0.004575325	IGF1R	1
BP	GO:0044321	response to leptin	22/18903	0.003487632	0.029503478	0.004663405	LEPR	1
BP	GO:0010893	positive regulation of steroid biosynthetic process	23/18903	0.003645967	0.029551159	0.004670942	IGF1R	1
BP	GO:0070233	negative regulation of T cell apoptotic process	24/18903	0.003804286	0.029551159	0.004670942	IL7R	1

BP	GO:0001911	negative regulation of leukocyte mediated cytotoxicity	25/18903	0.003962589	0.029551159	0.004670942	IL7R	1
BP	GO:0046885	regulation of hormone biosynthetic process	25/18903	0.003962589	0.029551159	0.004670942	IGF1R	1
BP	GO:0002710	negative regulation of T cell mediated immunity	27/18903	0.004279143	0.029551159	0.004670942	IL7R	1
BP	GO:0033081	regulation of T cell differentiation in thymus	27/18903	0.004279143	0.029551159	0.004670942	IL7R	1
BP	GO:0060259	regulation of feeding behavior	27/18903	0.004279143	0.029551159	0.004670942	LEPR	1
BP	GO:0071549	cellular response to dexamethasone stimulus	27/18903	0.004279143	0.029551159	0.004670942	IGF1R	1
BP	GO:0031342	negative regulation of cell killing	28/18903	0.004437394	0.029551159	0.004670942	IL7R	1
BP	GO:1904385	cellular response to angiotensin	28/18903	0.004437394	0.029551159	0.004670942	IGF1R	1
BP	GO:0045940	positive regulation of steroid metabolic process	30/18903	0.004753848	0.0301465	0.004765043	IGF1R	1
BP	GO:0033688	regulation of osteoblast proliferation	31/18903	0.00491205	0.0301465	0.004765043	IGF1R	1
BP	GO:0048679	regulation of axon regeneration	31/18903	0.00491205	0.0301465	0.004765043	IGF1R	1
BP	GO:0098868	bone growth	31/18903	0.00491205	0.0301465	0.004765043	LEPR	1
BP	GO:1990776	response to angiotensin	32/18903	0.005070234	0.030446677	0.00481249	IGF1R	1
BP	GO:0001782	B cell homeostasis	33/18903	0.005228402	0.030446677	0.00481249	IL7R	1
BP	GO:0070570	regulation of neuron projection regeneration	34/18903	0.005386554	0.030446677	0.00481249	IGF1R	1
BP	GO:0033687	osteoblast proliferation	36/18903	0.005702806	0.030446677	0.00481249	IGF1R	1
BP	GO:0043243	positive regulation of protein-containing complex disassembly	36/18903	0.005702806	0.030446677	0.00481249	IGF1R	1
BP	GO:0003230	cardiac atrium development	37/18903	0.005860907	0.030446677	0.00481249	IGF1R	1
BP	GO:0051385	response to mineralocorticoid	37/18903	0.005860907	0.030446677	0.00481249	IGF1R	1
BP	GO:0070229	negative regulation of lymphocyte apoptotic process	37/18903	0.005860907	0.030446677	0.00481249	IL7R	1
BP	GO:0048009	insulin-like growth factor receptor signaling pathway	38/18903	0.006018991	0.030446677	0.00481249	IGF1R	1
BP	GO:0032350	regulation of hormone metabolic process	39/18903	0.006177059	0.030446677	0.00481249	IGF1R	1
BP	GO:0032570	response to progesterone	39/18903	0.006177059	0.030446677	0.00481249	IGF1R	1
BP	GO:0097242	amyloid-beta clearance	39/18903	0.006177059	0.030446677	0.00481249	IGF1R	1
BP	GO:0071392	cellular response to estradiol stimulus	40/18903	0.006335109	0.030446677	0.00481249	IGF1R	1
BP	GO:0001914	regulation of T cell mediated cytotoxicity	41/18903	0.006493143	0.030446677	0.00481249	IL7R	1
BP	GO:0070232	regulation of T cell apoptotic process	41/18903	0.006493143	0.030446677	0.00481249	IL7R	1
BP	GO:0043029	T cell homeostasis	42/18903	0.006651161	0.030446677	0.00481249	IL7R	1
BP	GO:0033574	response to testosterone	43/18903	0.006809161	0.030446677	0.00481249	IGF1R	1
BP	GO:0071548	response to dexamethasone	43/18903	0.006809161	0.030446677	0.00481249	IGF1R	1
BP	GO:0097009	energy homeostasis	43/18903	0.006809161	0.030446677	0.00481249	LEPR	1
BP	GO:0046427	positive regulation of receptor signaling pathway via JAK-STAT	45/18903	0.007125112	0.030805383	0.004869188	IL7R	1
BP	GO:1904646	cellular response to amyloid-beta	45/18903	0.007125112	0.030805383	0.004869188	IGF1R	1
BP	GO:0010677	negative regulation of cellular carbohydrate metabolic process	46/18903	0.007283062	0.030805383	0.004869188	LEPR	1
BP	GO:0032467	positive regulation of cytokinesis	46/18903	0.007283062	0.030805383	0.004869188	IGF1R	1
BP	GO:0046850	regulation of bone remodeling	48/18903	0.007598912	0.031530937	0.004983871	LEPR	1
BP	GO:0035094	response to nicotine	49/18903	0.007756812	0.031530937	0.004983871	IGF1R	1
BP	GO:1904894	positive regulation of receptor signaling pathway via STAT	49/18903	0.007756812	0.031530937	0.004983871	IL7R	1
BP	GO:0001913	T cell mediated cytotoxicity	51/18903	0.008072562	0.031678001	0.005007116	IL7R	1
BP	GO:0045912	negative regulation of carbohydrate metabolic process	52/18903	0.008230411	0.031678001	0.005007116	LEPR	1
BP	GO:0006111	regulation of gluconeogenesis	53/18903	0.008388244	0.031678001	0.005007116	LEPR	1

BP	GO:0010656	negative regulation of muscle cell apoptotic process	53/18903	0.008388244	0.031678001	0.005007116	IGF1R	1
BP	GO:0014009	glial cell proliferation	54/18903	0.00854606	0.031678001	0.005007116	LEPR	1
BP	GO:0031103	axon regeneration	55/18903	0.00870386	0.031678001	0.005007116	IGF1R	1
BP	GO:0071385	cellular response to glucocorticoid stimulus	55/18903	0.00870386	0.031678001	0.005007116	IGF1R	1
BP	GO:1904645	response to amyloid-beta	55/18903	0.00870386	0.031678001	0.005007116	IGF1R	1
BP	GO:2000107	negative regulation of leukocyte apoptotic process	55/18903	0.00870386	0.031678001	0.005007116	IL7R	1
BP	GO:0002707	negative regulation of lymphocyte mediated immunity	56/18903	0.008861643	0.031719942	0.005013746	IL7R	1
BP	GO:0002823	negative regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	57/18903	0.009019408	0.031719942	0.005013746	IL7R	1
BP	GO:0070231	T cell apoptotic process	57/18903	0.009019408	0.031719942	0.005013746	IL7R	1
BP	GO:0002820	negative regulation of adaptive immune response	62/18903	0.009807987	0.033009677	0.005217605	IL7R	1
BP	GO:0031102	neuron projection regeneration	62/18903	0.009807987	0.033009677	0.005217605	IGF1R	1
BP	GO:0070228	regulation of lymphocyte apoptotic process	62/18903	0.009807987	0.033009677	0.005217605	IL7R	1
BP	GO:1904036	negative regulation of epithelial cell apoptotic process	62/18903	0.009807987	0.033009677	0.005217605	IGF1R	1
BP	GO:0071384	cellular response to corticosteroid stimulus	64/18903	0.010123301	0.03370844	0.005328053	IGF1R	1
BP	GO:0043954	cellular component maintenance	67/18903	0.010596147	0.034700178	0.00548481	IGF1R	1
BP	GO:0002704	negative regulation of leukocyte mediated immunity	68/18903	0.010753729	0.034700178	0.00548481	IL7R	1
BP	GO:0042446	hormone biosynthetic process	68/18903	0.010753729	0.034700178	0.00548481	IGF1R	1
BP	GO:0002260	lymphocyte homeostasis	69/18903	0.010911294	0.034849338	0.005508387	IL7R	1
BP	GO:0042698	ovulation cycle	72/18903	0.011383889	0.035203946	0.005564437	IGF1R	1
BP	GO:0050795	regulation of behavior	73/18903	0.011541387	0.035203946	0.005564437	LEPR	1
BP	GO:0071260	cellular response to mechanical stimulus	73/18903	0.011541387	0.035203946	0.005564437	IGF1R	1
BP	GO:0005977	glycogen metabolic process	75/18903	0.011856334	0.035203946	0.005564437	LEPR	1
BP	GO:0050810	regulation of steroid biosynthetic process	75/18903	0.011856334	0.035203946	0.005564437	IGF1R	1
BP	GO:0006073	cellular glucan metabolic process	76/18903	0.012013782	0.035203946	0.005564437	LEPR	1
BP	GO:0044042	glucan metabolic process	76/18903	0.012013782	0.035203946	0.005564437	LEPR	1
BP	GO:1903036	positive regulation of response to wounding	77/18903	0.012171213	0.035203946	0.005564437	IGF1R	1
BP	GO:0014068	positive regulation of phosphatidylinositol 3-kinase signaling	80/18903	0.012643406	0.035203946	0.005564437	IGF1R	1
BP	GO:0070227	lymphocyte apoptotic process	80/18903	0.012643406	0.035203946	0.005564437	IL7R	1
BP	GO:0097061	dendritic spine organization	81/18903	0.012800771	0.035203946	0.005564437	IGF1R	1
BP	GO:0021766	hippocampus development	82/18903	0.012958118	0.035203946	0.005564437	IGF1R	1
BP	GO:0006094	gluconeogenesis	83/18903	0.013115449	0.035203946	0.005564437	LEPR	1
BP	GO:0034103	regulation of tissue remodeling	85/18903	0.013430061	0.035203946	0.005564437	LEPR	1
BP	GO:0019319	hexose biosynthetic process	86/18903	0.013587342	0.035203946	0.005564437	LEPR	1
BP	GO:0033077	T cell differentiation in thymus	86/18903	0.013587342	0.035203946	0.005564437	IL7R	1
BP	GO:0033273	response to vitamin	86/18903	0.013587342	0.035203946	0.005564437	IGF1R	1
BP	GO:0001910	regulation of leukocyte mediated cytotoxicity	87/18903	0.013744606	0.035203946	0.005564437	IL7R	1
BP	GO:0010232	vascular transport	87/18903	0.013744606	0.035203946	0.005564437	LEPR	1
BP	GO:0010507	negative regulation of autophagy	87/18903	0.013744606	0.035203946	0.005564437	LEPR	1
BP	GO:0010660	regulation of muscle cell apoptotic process	87/18903	0.013744606	0.035203946	0.005564437	IGF1R	1
BP	GO:0150104	transport across blood-brain barrier	87/18903	0.013744606	0.035203946	0.005564437	LEPR	1

BP	GO:0006112	energy reserve metabolic process	88/18903	0.013901853	0.035203946	0.005564437	LEPR	1
BP	GO:0046889	positive regulation of lipid biosynthetic process	88/18903	0.013901853	0.035203946	0.005564437	IGF1R	1
BP	GO:0002709	regulation of T cell mediated immunity	89/18903	0.014059084	0.035203946	0.005564437	IL7R	1
BP	GO:0046849	bone remodeling	89/18903	0.014059084	0.035203946	0.005564437	LEPR	1
BP	GO:0048678	response to axon injury	89/18903	0.014059084	0.035203946	0.005564437	IGF1R	1
BP	GO:0046364	monosaccharide biosynthetic process	90/18903	0.014216298	0.035315089	0.005582005	LEPR	1
BP	GO:0106027	neuron projection organization	91/18903	0.014373495	0.035363798	0.005589704	IGF1R	1
BP	GO:0010657	muscle cell apoptotic process	92/18903	0.014530676	0.035363798	0.005589704	IGF1R	1
BP	GO:0051781	positive regulation of cell division	93/18903	0.014687839	0.035363798	0.005589704	IGF1R	1
BP	GO:2000106	regulation of leukocyte apoptotic process	93/18903	0.014687839	0.035363798	0.005589704	IL7R	1
BP	GO:0032465	regulation of cytokinesis	95/18903	0.015002117	0.035844752	0.005665725	IGF1R	1
BP	GO:0048661	positive regulation of smooth muscle cell proliferation	98/18903	0.015473408	0.036303711	0.005738269	IGF1R	1
BP	GO:0044264	cellular polysaccharide metabolic process	99/18903	0.015630471	0.036303711	0.005738269	LEPR	1
BP	GO:0001776	leukocyte homeostasis	100/18903	0.015787518	0.036303711	0.005738269	IL7R	1
BP	GO:0097306	cellular response to alcohol	100/18903	0.015787518	0.036303711	0.005738269	IGF1R	1
BP	GO:0021549	cerebellum development	101/18903	0.015944548	0.036303711	0.005738269	IGF1R	1
BP	GO:0019218	regulation of steroid metabolic process	102/18903	0.016101562	0.036303711	0.005738269	IGF1R	1
BP	GO:0031341	regulation of cell killing	102/18903	0.016101562	0.036303711	0.005738269	IL7R	1
BP	GO:0043255	regulation of carbohydrate biosynthetic process	103/18903	0.016258558	0.036303711	0.005738269	LEPR	1
BP	GO:0042100	B cell proliferation	104/18903	0.016415538	0.036303711	0.005738269	IL7R	1
BP	GO:0046425	regulation of receptor signaling pathway via JAK-STAT	104/18903	0.016415538	0.036303711	0.005738269	IL7R	1
BP	GO:1901655	cellular response to ketone	105/18903	0.016572502	0.036303711	0.005738269	IGF1R	1
BP	GO:0010906	regulation of glucose metabolic process	107/18903	0.016886378	0.036303711	0.005738269	LEPR	1
BP	GO:0007631	feeding behavior	108/18903	0.017043291	0.036303711	0.005738269	LEPR	1
BP	GO:0062014	negative regulation of small molecule metabolic process	108/18903	0.017043291	0.036303711	0.005738269	LEPR	1
BP	GO:0090398	cellular senescence	108/18903	0.017043291	0.036303711	0.005738269	IGF1R	1
BP	GO:0005976	polysaccharide metabolic process	110/18903	0.017357068	0.036303711	0.005738269	LEPR	1
BP	GO:1904035	regulation of epithelial cell apoptotic process	110/18903	0.017357068	0.036303711	0.005738269	IGF1R	1
BP	GO:0014066	regulation of phosphatidylinositol 3-kinase signaling	111/18903	0.017513931	0.036303711	0.005738269	IGF1R	1
BP	GO:0021761	limbic system development	111/18903	0.017513931	0.036303711	0.005738269	IGF1R	1
BP	GO:0022037	metencephalon development	111/18903	0.017513931	0.036303711	0.005738269	IGF1R	1
BP	GO:0002456	T cell mediated immunity	113/18903	0.017827607	0.036710796	0.005802614	IL7R	1
BP	GO:1904892	regulation of receptor signaling pathway via STAT	115/18903	0.018141216	0.037104466	0.005864839	IL7R	1
BP	GO:0045582	positive regulation of T cell differentiation	117/18903	0.018454759	0.037104466	0.005864839	IL7R	1
BP	GO:0071887	leukocyte apoptotic process	117/18903	0.018454759	0.037104466	0.005864839	IL7R	1
BP	GO:0043200	response to amino acid	118/18903	0.018611505	0.037104466	0.005864839	IGF1R	1
BP	GO:0050830	defense response to Gram-positive bacterium	118/18903	0.018611505	0.037104466	0.005864839	IL7R	1
BP	GO:0051897	positive regulation of protein kinase B signaling	120/18903	0.018924948	0.037401213	0.005911744	IGF1R	1
BP	GO:0032355	response to estradiol	121/18903	0.019081644	0.037401213	0.005911744	IGF1R	1
BP	GO:0002698	negative regulation of immune effector process	122/18903	0.019238324	0.037401213	0.005911744	IL7R	1
BP	GO:0008286	insulin receptor signaling pathway	122/18903	0.019238324	0.037401213	0.005911744	IGF1R	1

BP	GO:0043244	regulation of protein-containing complex disassembly	126/18903	0.019864875	0.0383809	0.006066596	IGF1R	1
BP	GO:0045471	response to ethanol	128/18903	0.020178051	0.038746809	0.006124433	IGF1R	1
BP	GO:0045621	positive regulation of lymphocyte differentiation	130/18903	0.020491159	0.039108127	0.006181544	IL7R	1
BP	GO:0000018	regulation of DNA recombination	133/18903	0.020960698	0.039522279	0.006247006	IL7R	1
BP	GO:0001101	response to acid chemical	133/18903	0.020960698	0.039522279	0.006247006	IGF1R	1
BP	GO:0001909	leukocyte mediated cytotoxicity	134/18903	0.021117177	0.0395789	0.006255955	IL7R	1
BP	GO:0071333	cellular response to glucose stimulus	136/18903	0.021430086	0.039926291	0.006310865	IGF1R	1
BP	GO:1904019	epithelial cell apoptotic process	137/18903	0.021586515	0.039979759	0.006319316	IGF1R	1
BP	GO:0071331	cellular response to hexose stimulus	138/18903	0.021742928	0.040032567	0.006327663	IGF1R	1
BP	GO:0008203	cholesterol metabolic process	139/18903	0.021899324	0.040084727	0.006335908	LEPR	1
BP	GO:0071326	cellular response to monosaccharide stimulus	140/18903	0.022055703	0.04013625	0.006344052	IGF1R	1
BP	GO:0046328	regulation of JNK cascade	141/18903	0.022212066	0.040187148	0.006352097	IGF1R	1
BP	GO:0051384	response to glucocorticoid	142/18903	0.022368412	0.04023743	0.006360044	IGF1R	1
BP	GO:0014065	phosphatidylinositol 3-kinase signaling	145/18903	0.02283735	0.040846231	0.006456273	IGF1R	1
BP	GO:1902652	secondary alcohol metabolic process	149/18903	0.023462367	0.041693086	0.00659013	LEPR	1
BP	GO:0030010	establishment of cell polarity	150/18903	0.02361858	0.041693086	0.00659013	IGF1R	1
BP	GO:0071322	cellular response to carbohydrate stimulus	152/18903	0.023930955	0.041693086	0.00659013	IGF1R	1
BP	GO:0030902	hindbrain development	153/18903	0.024087118	0.041693086	0.00659013	IGF1R	1
BP	GO:0007584	response to nutrient	154/18903	0.024243264	0.041693086	0.00659013	IGF1R	1
BP	GO:0016125	sterol metabolic process	154/18903	0.024243264	0.041693086	0.00659013	LEPR	1
BP	GO:0045834	positive regulation of lipid metabolic process	154/18903	0.024243264	0.041693086	0.00659013	IGF1R	1
BP	GO:0010675	regulation of cellular carbohydrate metabolic process	155/18903	0.024399393	0.041732296	0.006596327	LEPR	1
BP	GO:0010976	positive regulation of neuron projection development	157/18903	0.024711602	0.042036584	0.006644424	IGF1R	1
BP	GO:0001678	cellular glucose homeostasis	158/18903	0.024867682	0.042073429	0.006650248	IGF1R	1
BP	GO:0031960	response to corticosteroid	165/18903	0.025959772	0.043060486	0.006806265	IGF1R	1
BP	GO:0099173	postsynapse organization	166/18903	0.026115718	0.043060486	0.006806265	IGF1R	1
BP	GO:0003205	cardiac chamber development	167/18903	0.026271647	0.043060486	0.006806265	IGF1R	1
BP	GO:0007568	aging	169/18903	0.026583457	0.043060486	0.006806265	IGF1R	1
BP	GO:0007254	JNK cascade	172/18903	0.027051046	0.043060486	0.006806265	IGF1R	1
BP	GO:0007259	receptor signaling pathway via JAK-STAT	172/18903	0.027051046	0.043060486	0.006806265	IL7R	1
BP	GO:1903034	regulation of response to wounding	172/18903	0.027051046	0.043060486	0.006806265	IGF1R	1
BP	GO:0021543	pallium development	174/18903	0.027362689	0.043060486	0.006806265	IGF1R	1
BP	GO:0009267	cellular response to starvation	175/18903	0.027518485	0.043060486	0.006806265	IGF1R	1
BP	GO:0048660	regulation of smooth muscle cell proliferation	175/18903	0.027518485	0.043060486	0.006806265	IGF1R	1
BP	GO:0006694	steroid biosynthetic process	178/18903	0.027985775	0.043060486	0.006806265	IGF1R	1
BP	GO:0048015	phosphatidylinositol-mediated signaling	178/18903	0.027985775	0.043060486	0.006806265	IGF1R	1
BP	GO:0002706	regulation of lymphocyte mediated immunity	179/18903	0.028141505	0.043060486	0.006806265	IL7R	1
BP	GO:0045580	regulation of T cell differentiation	179/18903	0.028141505	0.043060486	0.006806265	IL7R	1
BP	GO:0048659	smooth muscle cell proliferation	179/18903	0.028141505	0.043060486	0.006806265	IGF1R	1
BP	GO:0048771	tissue remodeling	179/18903	0.028141505	0.043060486	0.006806265	LEPR	1
BP	GO:0035265	organ growth	180/18903	0.028297218	0.043060486	0.006806265	LEPR	1

BP	GO:0043409	negative regulation of MAPK cascade	181/18903	0.028452914	0.043060486	0.006806265	IGF1R	1
BP	GO:0002822	regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	182/18903	0.028608594	0.043060486	0.006806265	IL7R	1
BP	GO:0046890	regulation of lipid biosynthetic process	182/18903	0.028608594	0.043060486	0.006806265	IGF1R	1
BP	GO:0048017	inositol lipid-mediated signaling	182/18903	0.028608594	0.043060486	0.006806265	IGF1R	1
BP	GO:0008361	regulation of cell size	183/18903	0.028764258	0.043060486	0.006806265	IL7R	1
BP	GO:0097696	receptor signaling pathway via STAT	183/18903	0.028764258	0.043060486	0.006806265	IL7R	1
BP	GO:1902107	positive regulation of leukocyte differentiation	184/18903	0.028919905	0.043060486	0.006806265	IL7R	1
BP	GO:1903708	positive regulation of hemopoiesis	184/18903	0.028919905	0.043060486	0.006806265	IL7R	1
BP	GO:0006109	regulation of carbohydrate metabolic process	186/18903	0.029231148	0.043060486	0.006806265	LEPR	1
BP	GO:0000910	cytokinesis	187/18903	0.029386745	0.043060486	0.006806265	IGF1R	1
BP	GO:0006006	glucose metabolic process	188/18903	0.029542325	0.043060486	0.006806265	LEPR	1
BP	GO:0001906	cell killing	189/18903	0.029697889	0.043060486	0.006806265	IL7R	1
BP	GO:0051896	regulation of protein kinase B signaling	189/18903	0.029697889	0.043060486	0.006806265	IGF1R	1
BP	GO:0050777	negative regulation of immune response	190/18903	0.029853436	0.043060486	0.006806265	IL7R	1
BP	GO:0051302	regulation of cell division	190/18903	0.029853436	0.043060486	0.006806265	IGF1R	1
BP	GO:0031099	regeneration	192/18903	0.03016448	0.043309552	0.006845633	IGF1R	1
BP	GO:0009749	response to glucose	194/18903	0.030475458	0.043556248	0.006884626	IGF1R	1
BP	GO:0032872	regulation of stress-activated MAPK cascade	195/18903	0.030630922	0.043579447	0.006888293	IGF1R	1
BP	GO:0002819	regulation of adaptive immune response	198/18903	0.031097213	0.043844269	0.006930152	IL7R	1
BP	GO:0070302	regulation of stress-activated protein kinase signaling cascade	198/18903	0.031097213	0.043844269	0.006930152	IGF1R	1
BP	GO:0009746	response to hexose	199/18903	0.031252611	0.043865772	0.006933551	IGF1R	1
BP	GO:1901654	response to ketone	205/18903	0.032184646	0.044972296	0.007108451	IGF1R	1
BP	GO:0034284	response to monosaccharide	207/18903	0.032495192	0.045066671	0.007123368	IGF1R	1
BP	GO:0032869	cellular response to insulin stimulus	208/18903	0.03265044	0.045066671	0.007123368	IGF1R	1
BP	GO:0071383	cellular response to steroid hormone stimulus	209/18903	0.032805671	0.045066671	0.007123368	IGF1R	1
BP	GO:0045619	regulation of lymphocyte differentiation	210/18903	0.032960886	0.045066671	0.007123368	IL7R	1
BP	GO:0009612	response to mechanical stimulus	211/18903	0.033116084	0.045066671	0.007123368	IGF1R	1
BP	GO:0042594	response to starvation	211/18903	0.033116084	0.045066671	0.007123368	IGF1R	1
BP	GO:0016051	carbohydrate biosynthetic process	212/18903	0.033271265	0.045081844	0.007125767	LEPR	1
BP	GO:0043491	protein kinase B signaling	218/18903	0.034202005	0.046143223	0.007293531	IGF1R	1
BP	GO:0046777	protein autophosphorylation	226/18903	0.035442063	0.047611011	0.007525534	IGF1R	1
BP	GO:0019318	hexose metabolic process	229/18903	0.03590681	0.048029195	0.007591633	LEPR	1
BP	GO:0031669	cellular response to nutrient levels	231/18903	0.036216559	0.048237374	0.007624539	IGF1R	1
BP	GO:0060348	bone development	233/18903	0.036526241	0.048240215	0.007624988	LEPR	1
BP	GO:0007163	establishment or maintenance of cell polarity	234/18903	0.036681058	0.048240215	0.007624988	IGF1R	1
BP	GO:0009743	response to carbohydrate	234/18903	0.036681058	0.048240215	0.007624988	IGF1R	1
BP	GO:0051403	stress-activated MAPK cascade	239/18903	0.037454891	0.049043985	0.007752034	IGF1R	1
BP	GO:0002703	regulation of leukocyte mediated immunity	241/18903	0.037764308	0.049043985	0.007752034	IL7R	1
BP	GO:0042445	hormone metabolic process	241/18903	0.037764308	0.049043985	0.007752034	IGF1R	1
BP	GO:0032984	protein-containing complex disassembly	242/18903	0.037918992	0.049043985	0.007752034	IGF1R	1

BP	GO:0031098	stress-activated protein kinase signaling cascade	246/18903	0.038537561	0.049426957	0.007812568	IGF1R	1
BP	GO:0031330	negative regulation of cellular catabolic process	247/18903	0.038692161	0.049426957	0.007812568	LEPR	1
BP	GO:0033002	muscle cell proliferation	247/18903	0.038692161	0.049426957	0.007812568	IGF1R	1
BP	GO:0005996	monosaccharide metabolic process	248/18903	0.038846746	0.049426957	0.007812568	LEPR	1
BP	GO:0050870	positive regulation of T cell activation	251/18903	0.039310399	0.049814392	0.007873807	IL7R	1
CC	GO:0009897	external side of plasma membrane	462/19869	0.001593595	0.03505909	0.003354937	LEPR/IL7R	2
CC	GO:0030315	T-tubule	52/19869	0.00783129	0.049855143	0.004770827	IGF1R	1
CC	GO:0030669	clathrin-coated endocytic vesicle membrane	72/19869	0.010832403	0.049855143	0.004770827	IL7R	1
CC	GO:0005901	caveola	82/19869	0.012330687	0.049855143	0.004770827	IGF1R	1
CC	GO:0045334	clathrin-coated endocytic vesicle	90/19869	0.013528225	0.049855143	0.004770827	IL7R	1
CC	GO:0044853	plasma membrane raft	113/19869	0.016965753	0.049855143	0.004770827	IGF1R	1
CC	GO:0030665	clathrin-coated vesicle membrane	132/19869	0.019799419	0.049855143	0.004770827	IL7R	1
CC	GO:0042383	sarcolemma	136/19869	0.020395286	0.049855143	0.004770827	IGF1R	1
CC	GO:1902911	protein kinase complex	136/19869	0.020395286	0.049855143	0.004770827	IGF1R	1
MF	GO:0017046	peptide hormone binding	52/18432	2.34E-05	0.000467538	NA	LEPR/IGF1R	2
MF	GO:0042562	hormone binding	87/18432	6.59E-05	0.000546332	NA	LEPR/IGF1R	2
MF	GO:0004896	cytokine receptor activity	97/18432	8.19E-05	0.000546332	NA	LEPR/IL7R	2
MF	GO:0140375	immune receptor activity	148/18432	0.000191108	0.000955541	NA	LEPR/IL7R	2
MF	GO:0042277	peptide binding	330/18432	0.000947384	0.003789537	NA	LEPR/IGF1R	2
MF	GO:0033218	amide binding	408/18432	0.001444871	0.004816236	NA	LEPR/IGF1R	2
MF	GO:0031994	insulin-like growth factor I binding	13/18432	0.002114508	0.00528627	NA	IGF1R	1
MF	GO:0043560	insulin receptor substrate binding	13/18432	0.002114508	0.00528627	NA	IGF1R	1
MF	GO:0005520	insulin-like growth factor binding	19/18432	0.003089429	0.006503001	NA	IGF1R	1
MF	GO:0016500	protein-hormone receptor activity	20/18432	0.003251854	0.006503001	NA	IGF1R	1
MF	GO:0005158	insulin receptor binding	22/18432	0.003576651	0.006503001	NA	IGF1R	1
MF	GO:0001965	G-protein alpha-subunit binding	26/18432	0.004226033	0.007043389	NA	IGF1R	1
MF	GO:0043548	phosphatidylinositol 3-kinase binding	30/18432	0.004875134	0.007427968	NA	IGF1R	1
MF	GO:0140318	protein transporter activity	32/18432	0.005199578	0.007427968	NA	IGF1R	1
MF	GO:0004714	transmembrane receptor protein tyrosine kinase activity	60/18432	0.009734397	0.012979196	NA	IGF1R	1
MF	GO:0019199	transmembrane receptor protein kinase activity	79/18432	0.012803733	0.016004667	NA	IGF1R	1
MF	GO:0019838	growth factor binding	132/18432	0.021332032	0.02448013	NA	IGF1R	1
MF	GO:0004713	protein tyrosine kinase activity	138/18432	0.022294393	0.02448013	NA	IGF1R	1
MF	GO:0019955	cytokine binding	144/18432	0.023256123	0.02448013	NA	LEPR	1
MF	GO:0003823	antigen binding	171/18432	0.027576105	0.027576105	NA	IL7R	1

Table S3. KEGG enrichment analysis for IGF1-R, IL-7R and ILEPR

ID	Description	BgRatio	pvalue	p.adjust	qvalue	geneID	Count
hsa04152	AMPK signaling pathway	121/8586	0.000585496	0.012007975	0.004694847	LEPR/IGF1R	2
hsa04068	FoxO signaling pathway	131/8586	0.00068617	0.012007975	0.004694847	IL7R/IGF1R	2
hsa04630	JAK-STAT signaling pathway	166/8586	0.001100563	0.012839905	0.005020113	LEPR/IL7R	2
hsa04060	Cytokine-cytokine receptor interaction	297/8586	0.003496008	0.030590071	0.011960028	LEPR/IL7R	2
hsa04151	PI3K-Akt signaling pathway	359/8586	0.005085764	0.035600349	0.013918934	IL7R/IGF1R	2
hsa05340	Primary immunodeficiency	38/8586	0.013220285	0.064882178	0.025367468	IL7R	1
hsa04913	Ovarian steroidogenesis	51/8586	0.01771612	0.064882178	0.025367468	IGF1R	1
hsa04730	Long-term depression	60/8586	0.020820609	0.064882178	0.025367468	IGF1R	1
hsa04213	Longevity regulating pathway - multiple species	61/8586	0.021165147	0.064882178	0.025367468	IGF1R	1
hsa04920	Adipocytokine signaling pathway	69/8586	0.023918549	0.064882178	0.025367468	LEPR	1
hsa05218	Melanoma	72/8586	0.024949742	0.064882178	0.025367468	IGF1R	1
hsa05214	Glioma	75/8586	0.025980208	0.064882178	0.025367468	IGF1R	1
hsa01521	EGFR tyrosine kinase inhibitor resistance	79/8586	0.027353034	0.064882178	0.025367468	IGF1R	1
hsa04211	Longevity regulating pathway	89/8586	0.030779453	0.064882178	0.025367468	IGF1R	1
hsa04520	Adherens junction	93/8586	0.032147764	0.064882178	0.025367468	IGF1R	1
hsa05215	Prostate cancer	97/8586	0.033514786	0.064882178	0.025367468	IGF1R	1
hsa01522	Endocrine resistance	98/8586	0.033856341	0.064882178	0.025367468	IGF1R	1
hsa04640	Hematopoietic cell lineage	99/8586	0.034197815	0.064882178	0.025367468	IL7R	1
hsa04914	Progesterone-mediated oocyte maturation	102/8586	0.035221754	0.064882178	0.025367468	IGF1R	1
hsa04066	HIF-1 signaling pathway	109/8586	0.03760813	0.065814228	0.025731879	IGF1R	1
hsa04114	Oocyte meiosis	131/8586	0.045082545	0.074622207	0.0291756	IGF1R	1
hsa04550	Signaling pathways regulating pluripotency of stem cells	143/8586	0.049143138	0.074622207	0.0291756	IGF1R	1
hsa05224	Breast cancer	147/8586	0.050494107	0.074622207	0.0291756	IGF1R	1
hsa04932	Non-alcoholic fatty liver disease	155/8586	0.053192204	0.074622207	0.0291756	LEPR	1
hsa04150	mTOR signaling pathway	156/8586	0.053529106	0.074622207	0.0291756	IGF1R	1
hsa04140	Autophagy - animal	165/8586	0.056557632	0.074622207	0.0291756	IGF1R	1
hsa05225	Hepatocellular carcinoma	168/8586	0.057565702	0.074622207	0.0291756	IGF1R	1
hsa05202	Transcriptional misregulation in cancer	193/8586	0.065938382	0.080842531	0.031607606	IGF1R	1
hsa04510	Focal adhesion	203/8586	0.069273519	0.080842531	0.031607606	IGF1R	1
hsa05205	Proteoglycans in cancer	205/8586	0.069939592	0.080842531	0.031607606	IGF1R	1
hsa04015	Rap1 signaling pathway	210/8586	0.071603385	0.080842531	0.031607606	IGF1R	1
hsa04014	Ras signaling pathway	236/8586	0.080223129	0.087744048	0.034305943	IGF1R	1
hsa04144	Endocytosis	250/8586	0.084842352	0.089984312	0.035181836	IGF1R	1
hsa04010	MAPK signaling pathway	301/8586	0.101538707	0.10452514	0.040866972	IGF1R	1
hsa04080	Neuroactive ligand-receptor interaction	367/8586	0.12284264	0.12284264	0.048028701	LEPR	1

Table S4. miRNA-mRNA relationship pairs

miRNAname	geneName	link
hsa-miR-31-5p	LEPR	hsa-miR-31-5p-LEPR
hsa-miR-30c-5p	LEPR	hsa-miR-30c-5p-LEPR
hsa-miR-183-5p	LEPR	hsa-miR-183-5p-LEPR
hsa-miR-124-3p	LEPR	hsa-miR-124-3p-LEPR
hsa-miR-186-5p	LEPR	hsa-miR-186-5p-LEPR
hsa-miR-194-5p	LEPR	hsa-miR-194-5p-LEPR
hsa-miR-506-3p	LEPR	hsa-miR-506-3p-LEPR
hsa-miR-616-3p	LEPR	hsa-miR-616-3p-LEPR
hsa-miR-186-5p	IL7R	hsa-miR-186-5p-IL7R
hsa-miR-1914-3p	IL7R	hsa-miR-1914-3p-IL7R
hsa-miR-31-5p	IGF1R	hsa-miR-31-5p-IGF1R
hsa-miR-30c-5p	IGF1R	hsa-miR-30c-5p-IGF1R
hsa-miR-183-5p	IGF1R	hsa-miR-183-5p-IGF1R
hsa-miR-124-3p	IGF1R	hsa-miR-124-3p-IGF1R
hsa-miR-186-5p	IGF1R	hsa-miR-186-5p-IGF1R
hsa-miR-194-5p	IGF1R	hsa-miR-194-5p-IGF1R
hsa-miR-506-3p	IGF1R	hsa-miR-506-3p-IGF1R
hsa-miR-616-3p	IGF1R	hsa-miR-616-3p-IGF1R
hsa-miR-1914-3p	IGF1R	hsa-miR-1914-3p-IGF1R