Observational Study

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Integrated bioinformatic analysis of the molecular mechanisms between type 2 diabetes mellitus and osteoarthritis

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Abstract

Type 2 diabetes mellitus (T2DM) is a metabolic syndrome that has been identified as an independent risk factor for osteoarthritis (OA) and may even trigger and exacerbate the progression of OA. However, the relationship between T2DM and OA is complex and has not yet been fully clarified by current research. In this study, we analyzed the potential mechanism of action between T2DM and OA by bioinformatics. Transcriptome sequencing data of T2DM (GSE25724) and OA (GSE55235) were downloaded from the gene expression omnibus. Differential expression analysis was performed for different subgroups to obtain differentially expressed genes. The protein–protein interaction network was constructed using overlapping genes and screened for hub targets. Then the enrichment analysis was performed separately for overlapping and hub targets. The GeneMANIA is used to predict functionally similar genes of hub genes. Differential expression analyses revealed that 184 genes are involved in both diseases together. The Kyoto Encyclopedia of Genes and Genomes pathway enrichment results showed that the overlapping genes were mainly involved in the advanced glycation end products-receptor of advanced glycation end products signaling pathway, the NF-kappa B signaling pathway, the mitogen-activated protein kinases signaling pathway, and the interleukin-17 signaling pathway in diabetic complications. The functions of genes similar to the hub genes are focused on cell chemotaxis, positive regulation of cell migration, positive regulation of RNA polymerase II transcription, regulation of leukocyte migration, epithelial cell proliferation, and integrated stress response signaling. The transcription factor Jun and C-X-C motif chemokine 8 may play an important role in the inflammatory response caused by advanced glycation end products. This study improves our understanding of T2DM complicating OA and helps to stimulate more effective treatments.

Abbreviations: AGE = advanced glycation end products, CXCL8 = C-X-C motif chemokine 8, GEO = gene expression omnibus, GO = gene ontology, IL-17 = interleukin-17, KEGG = Kyoto Encyclopedia of Genes and Genomes, OA = osteoarthritis, PPI = protein–protein interaction, RAGE = receptor of advanced glycation end products, T2DM = type 2 diabetes mellitus.

Keywords: advanced glycation end products, bioinformatics, inflammation, osteoarthritis, type 2 diabetes mellitus

1. Introduction

Osteoarthritis (OA) is a common clinical chronic disease of the joint tissues that often causes pain, loss of function, and reduced quality of life. Currently, there are approximately 344 million patients with OA worldwide, and the average annual incidence is significantly increasing.[1] Although OA has become a global public health problem, there is no effective treatment for OA cartilage damage. Risk factors for OA can be categorized into systemic factors (age, gender, obesity, genetics, and diet) and localized joint factors (injury, poor joint alignment, and abnormal loading).[2,3] Multiple risk factors interact in a complex manner, ultimately leading to structural damage and a range of clinical manifestations.

The study found that knee cartilage in patients with type 2 diabetes mellitus (T2DM) showed accelerated degeneration compared to healthy people without T2DM.[4] Hyperglycemia and insulin resistance are important factors in the development of OA in patients with T2DM.[5] On the one hand, hyperglycemia is able to promote the development of OA by affecting tissue structures such as articular cartilage, synovium, and subchondral bone.[6–8] On the other hand, insulin resistance weakens the protective effect of insulin on the joints, thus promoting the development of OA.[9] However, the relationship between T2DM and OA is complex and has not yet been fully clarified by current research.

High-throughput microarray platforms have emerged as a promising and useful tool for the discovery of biomarkers for

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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a wide range of diseases. By combining differentially expressed genes from multiple microarray datasets analyzed, exploration of disease mechanisms will become more reliable. In this study, we analyzed the potential mechanism of action between T2DM and OA by bioinformatics. It is hoped that this will provide a new direction for effective prevention and treatment of T2DM and OA.

2. Materials and methods

2.1. GEO datasets

Transcriptome sequencing data of T2DM and OA were downloaded from the gene expression omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). The datasets were searched using "osteoarthritis" and "type 2 diabetes mellitus" as keywords. GSE55235 and GSE25724 were used as follow-up analyses (Table 1).

2.2. Differential expression analysis

The differential expression analysis between the 2 groups was performed by using the "limma" package of R software. The differentially expressed genes in the dataset were screened out by setting $|\log2(FC)| > 1$ and P value $< .05$. The results of differential expression analysis were visualized as volcano plots.

2.3. Protein–protein interaction (PPI) networks

The resulting OA genes and T2DM genes were mapped to obtain overlapping genes and plotted in a Venn diagram. The overlapping genes were analyzed by the STRING online database (https://www.string-db.org/), and the PPI network was constructed by setting the required minimum interaction score (0.400). The above data were imported into Cytoscape 3.8.2 software to analyze this PPI network and screen for hub genes using cytoHubba.

2.4. Enrichment analysis

DAVID platform (https://david.ncifcrf.gov/) was used to perform gene ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis on the overlapping genes and hub genes, respectively. $P < .05$ was set to obtain the relevant biological functions and signaling pathways.

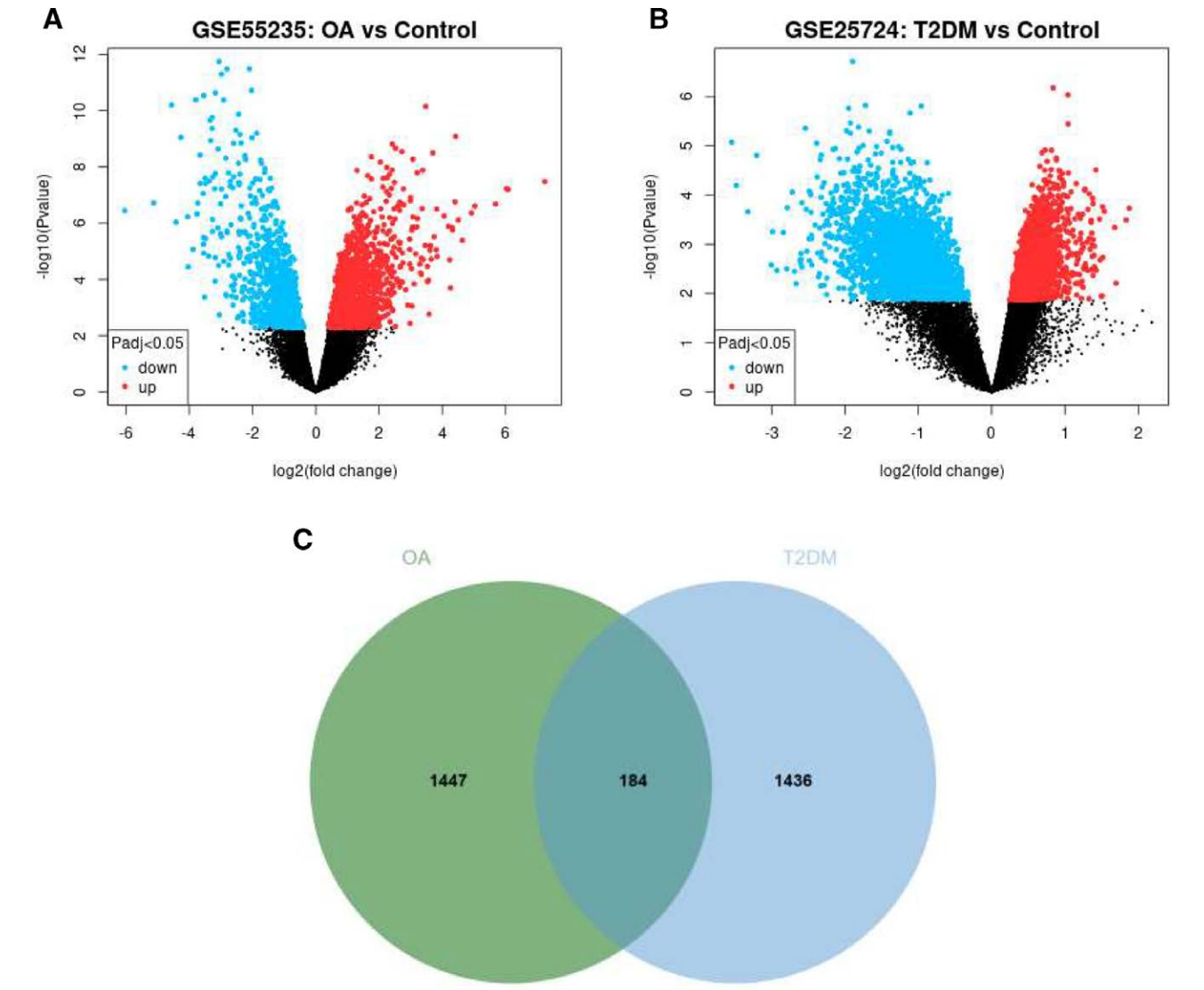

Figure 1. Differentially expressed genes. (A) Differentially expressed genes of GSE55235. (B) Differentially expressed genes of GSE55235. (C) Intersecting genes GSE55235 and GSE25724. OA = osteoarthritis, T2DM = type 2 diabetes mellitus.

2.5. GeneMANIA

The GeneMANIA website (http://genemania.org) is used to predict functionally similar genes of hub genes and construct the PPI network among them. It can also predict the relationships among functionally similar genes and hub genes, including protein–protein, protein–DNA interactions, pathways, physiological and biochemical reactions, co-expression, and co-localization.[10] In this study, we explored functionally similar genes of hub genes and performed functional enrichment analysis.

3. Results

3.1. "OA-T2DM" targets

Differential analysis of GSE55235 resulted in 1659 genes significantly associated with the development of OA, including 1075 up-regulated genes and 584 down-regulated genes (Fig. 1A). In addition, differential analysis of GSE25724 yielded 1623 T2DM-related genes, including 117 up-regulated genes and 1506 down-regulated genes (Fig. 1B). Finally, 184 overlapping genes of OA and T2DM were mapped by Venn diagram (Fig. 1C).

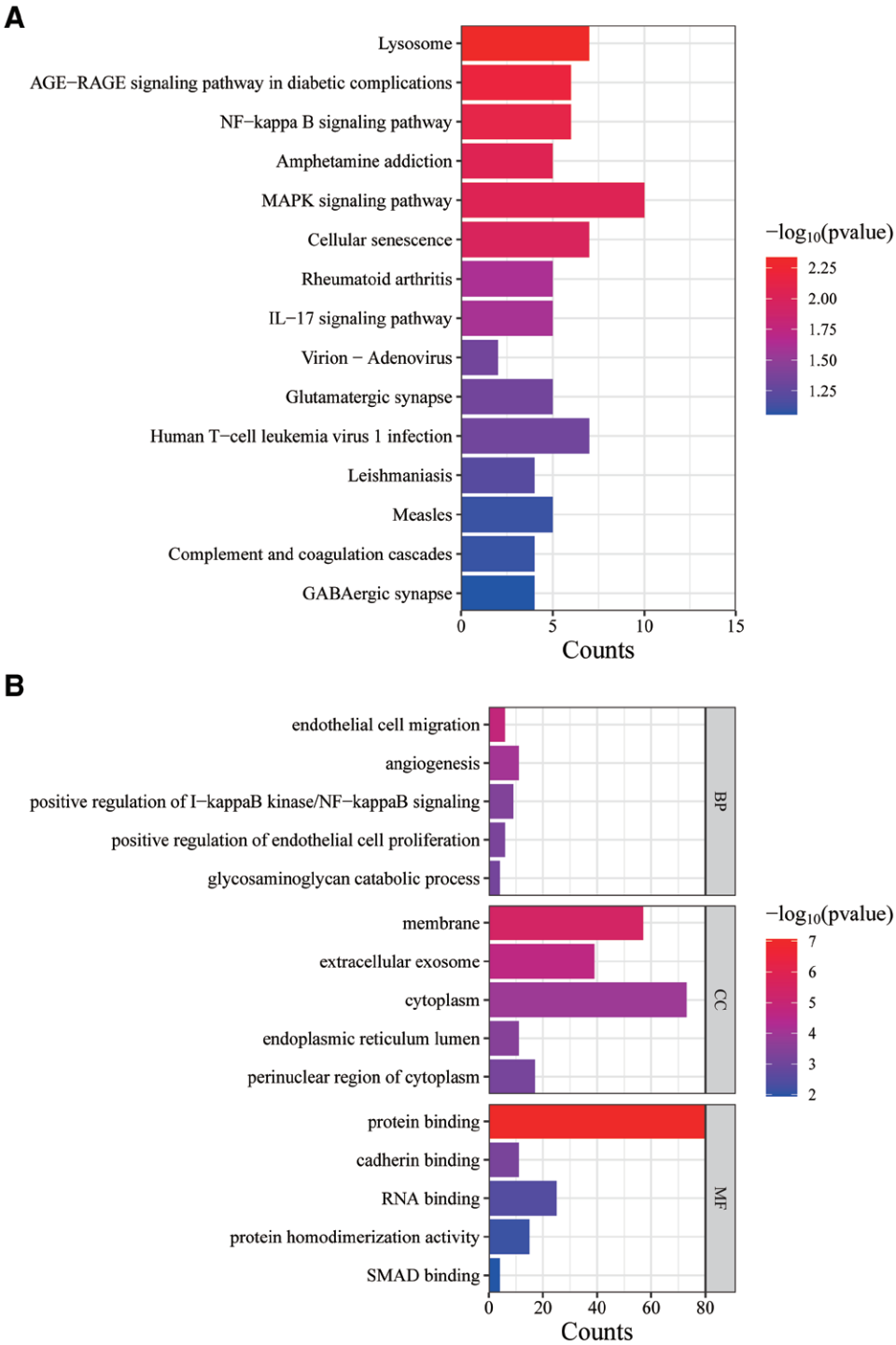

Figure 2. Enrichment analysis of overlapping genes. (A) KEGG pathway enrichment analysis. (B) GO functional enrichment analysis. GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes.

3.2. Enrichment analysis of "OA-T2DM" genes

To further systematically elucidate the common mechanisms between OA and T2DM, 184 overlapping genes were analyzed by GO functional enrichment and KEGG pathway enrichment. The results showed that the overlapping genes were mainly involved in the advanced glycation end products (AGE)—receptor of AGEs (RAGE) signaling pathway in diabetic complications, NF-kappa B signaling pathway, mitogen-activated protein kinases signaling pathway, and interleukin-17 (IL-17) signaling pathway (Fig. 2A and Table 2). In addition, these overlapping genes are involved in endothelial cell migration, angiogenesis, positive regulation of I-kappaB kinase/NF-kappaB signaling, positive regulation of endothelial cell proliferation, and glycosaminoglycan catabolic processes (Fig. 2B). Cellular components mainly include membrane, extracellular exosome, cytoplasm, endoplasmic reticulum lumen, and perinuclear region of cytoplasm (Fig. 2B). Molecular functions mainly include protein binding, collagen binding, RNA binding, protein homodimerization activity, and SMAD binding (Fig. 2B).

3.3. Construction of PPI network and screening of hub genes

The STRING online database was used to construct the PPI network, which contains 144 nodes and 293 edges (Fig. 3A). Then, the top 10 genes with the highest degree value were screened and considered as hub genes. The color of the nodes in the network varies according to the degree value. Hub-genes contain transcription factor SOX-9, C-X-C motif chemokine 8 (CXCL8), signal transducer and activator of transcription 1, prostaglandin G/H synthase 2, early growth response protein 1, transcription factor Jun (JUN), HLA class II histocompatibility antigen gamma chain, fibroblast growth factor receptor 1, bromodomain-containing protein 4, and SERPINE1 mRNA-binding protein 1.

3.4. Enrichment analysis of hub genes

We further analyzed the hub genes for GO function enrichment and KEGG pathway enrichment. Hub genes are involved in positive regulation of transcription, DNA-templated, angiogenesis, positive regulation of gene expression, and positive regulation of transcription from RNA polymerase II promoter (Fig. 4A). Consistent with previous results, JUN and CXCL8 were equally involved in the AGE-RAGE signaling pathway in diabetic complications and IL17 signaling pathways. This indicates that AGEs in T2DM may contribute to inflammation in OA through the JUN/CXCL8 axis (Fig. 4B).

3.5. PPI of hub genes at the GeneMANIA

The GeneMANIA is used to predict functionally similar genes of hub genes. We obtained 20 similar genes of hub genes (Fig. 5). The hub genes were located in the inner circle, while the

Table 1

Information of gene expression omnibus datasets.

GSE number	Platform	Samples	Tissue	Disease
GSE55235	GPL96	10 patients and 10 controls	Synovial	OA
GSE25724	GPL96	6 patients and 7 controls	Islets	T2DM

OA = osteoarthritis, T2DM = type 2 diabetes mellitus.

Table 2

KEGG pathway enrichment analysis of overlapping genes.

Pathway	Count	*P* value	Genes
AGE-RAGE signaling pathway in diabetic complications	6	0.006442854	EGR1, COL3A1, JUN, CXCL8, STAT1, SERPINE1
NF-kappa B signaling pathway	6	0.007585016	CXCL8, GADD45A, CD14, LTB, CFLAR, PTGS2
MAPK signaling pathway	10	0.008980691	PPM1B, JUN, PPP3CC, GADD45A, PDGFC, MKNK2, CACNA1D, CD14, PGF, FGFR1
IL-17 signaling pathway	5	0.024826938	JUN, CXCL8, CCL20, FOSB, PTGS2

MAPK = mitogen-activated protein kinases.

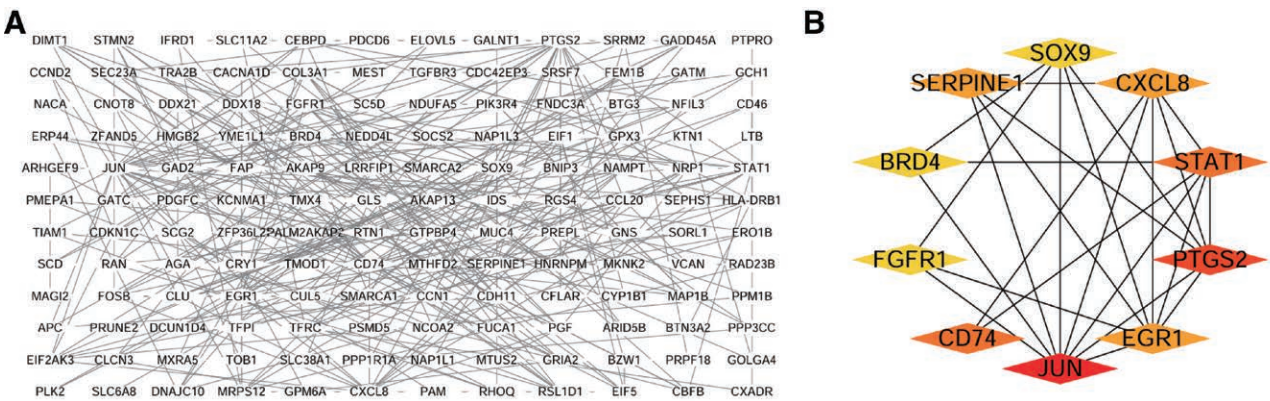

Figure 3. PPI network analysis. (A) The PPI network was constructed based on the STRING database. (B) The top 10 genes with the highest degree value. PPI = protein–protein interaction.

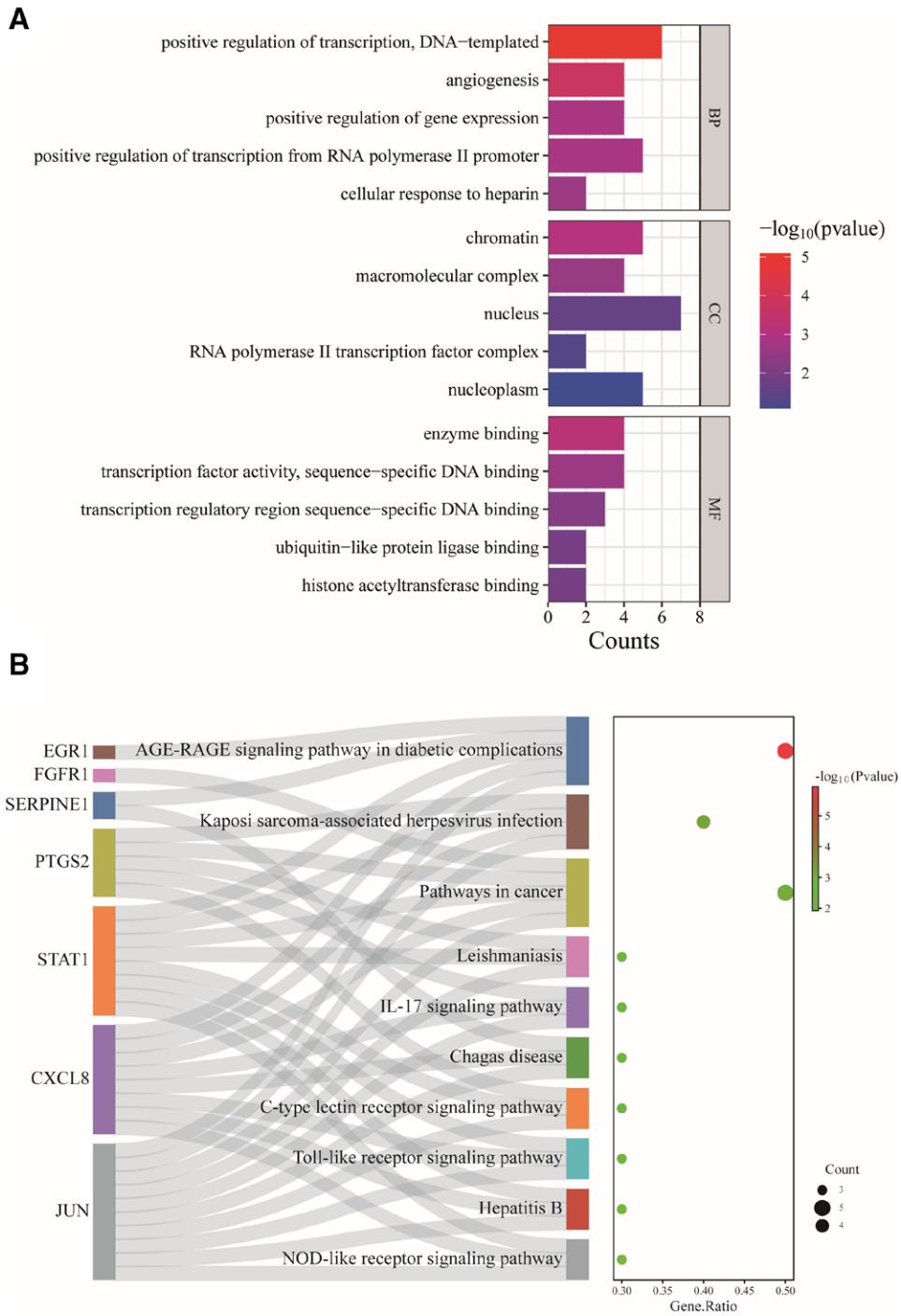

Figure 4. Enrichment analysis of hub genes. (A) GO functional enrichment analysis. (B) KEGG pathway enrichment analysis. GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes.

predicted genes were in the outer circle. Their functions focused on cell chemotaxis, positive regulation of cell migration, positive regulation of transcription by RNA polymerase II, regulation of leukocyte migration, epithelial cell proliferation, and integrated stress response signaling.

4. Discussion

T2DM is a metabolic syndrome that has been identified as an independent risk factor for OA and may even trigger and exacerbate the progression of OA.[11] Animal experiments also demonstrated an insulin-like growth factor 1-resistant state in the cartilage of rats with T2DM induced by streptozotocin.[12] However, whether and how hyperglycemia aggravates or even triggers the pathogenesis of OA remains unclear.

In this study, we used the bioinformatics approach to explore the potential mechanisms by which T2DM leads to OA. Differential expression analysis of the GEO dataset revealed many overlapping differentially expressed genes between T2DM and OA, which suggests a strong correlation between the diseases. KEGG enrichment analysis of overlapping genes and hub genes together revealed that AGE-RAGE signaling pathway in diabetic complications and IL-17 signaling pathway play a crucial role in disease development.

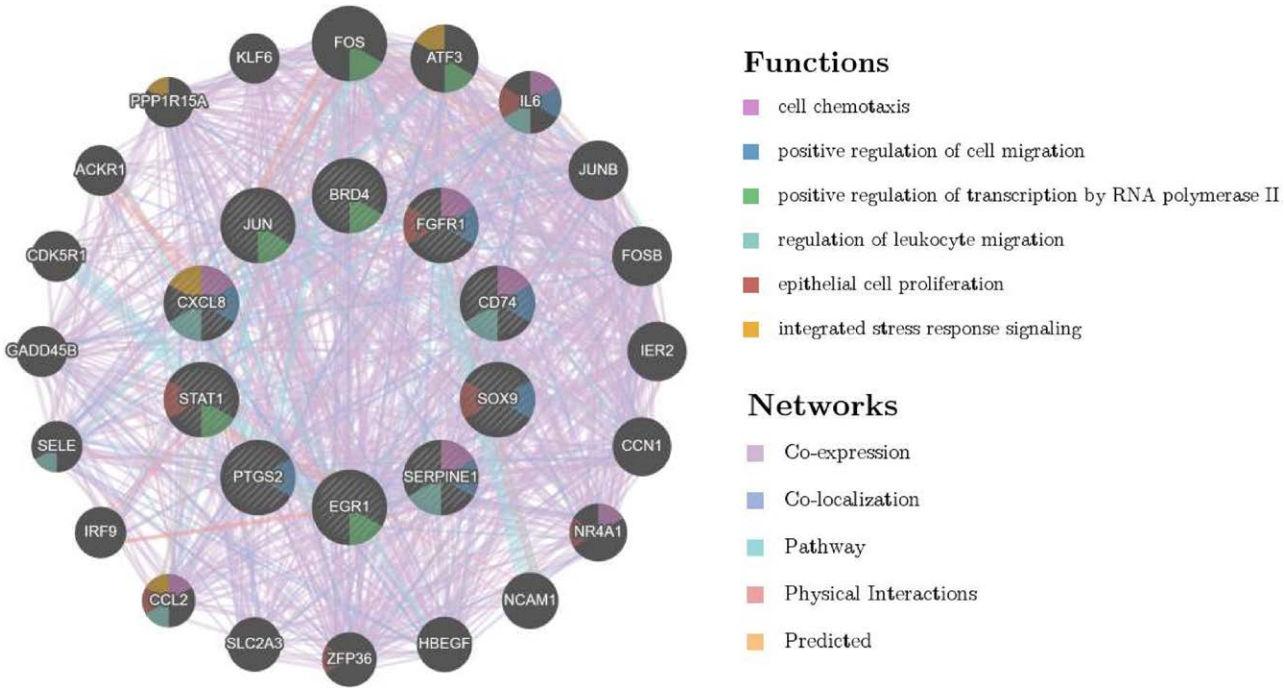

Figure 5. Prediction of similar function genes.

The RAGE is the receptor for AGEs, which triggers endoplasmic reticulum stress and downstream inflammatory signaling upon stimulation by AGEs.[13] T2DM is directly associated with the accumulation of AGE. At the same time, AGE reacts irreversibly with proteins to form cross-linked proteins, leading to stiffness of joint tissues and hardening of cartilage.[14] It has been shown that AGE may be involved in the activation of the mitogen-activated protein kinases pathway in chondrocytes.[15] This leads to down-regulation of peroxisome proliferator-activated receptor γ in chondrocytes and up-regulation of MMP and pro-inflammatory cytokines, which ultimately accelerates the destruction of articular cartilage tissue.[16] On the other hand, hyperglycemia also induces the accumulation of AGEs in fibroblast-like synoviocytes, which increases the release of inflammatory factors and subsequently induces chondrocyte degradation and promotes OA progression.[17]

IL-17 is a key pro-inflammatory cytokine produced primarily by Th17 cells.[18] Although, IL-17 plays a substantial role in the defense against microbial infections through enhanced induction of proinflammatory cytokines and chemokines, it is engaged in many inflammatory disorders such as autoimmune and metabolic diseases as well as cancer.[19] Recent investigations indicated that IL-17 has a crucial role in the creation of inflammation in adipose tissues of obese individuals. Circulating levels of IL-17 are significantly higher in obese and diabetic patients.[20] Elevated circulating IL-17 levels further activate the production of pro-inflammatory mediators leading to inflammatory arthritis.[21] In the serum of patients with knee OA, there was a significant correlation between IL-17 expression and cartilage defects and bone marrow lesions, as well as a positive correlation between IL-17 expression and the severity of knee OA.[22] On the one hand, IL-17 leads to impaired chondrocyte autophagy, premature senescence and disruption of cartilage matrix metabolic homeostasis.[23] On the other hand, IL-17 amplifies the synovial inflammatory effects in OA by promoting the expression of a range of pro-inflammatory factors and chemokines.[24]

However, both AGEs and IL-17 by themselves have limited pro-inflammatory effects, and they exert stronger inflammatory effects by augmenting and synergising with other cytokines and inflammatory mediators. In this study, we found that both JUN and CXCL8 are involved in the AGE-RAGE signaling pathway in diabetic complications and IL-17 signaling pathway. The results indicate that AGEs in T2DM may contribute to inflammation in OA through the JUN/CXCL8 axis. In the future, we will further investigate the specific mechanisms of these signaling pathways in the inflammatory microenvironment of osteoarthritis.

This study has some limitations. Firstly, all work has been performed based on bioinformatics, and subsequent validation of the expression of these hub genes in tissue samples from patients is needed. In addition, more in vivo and in vitro studies are needed to validate the specific mechanisms by which T2DM leads to OA.

5. Conclusion

In conclusion, our study explored the possible signaling pathways of T2DM leading to OA through bioinformatics. The AGE-RAGE signaling pathway in diabetic complications and IL-17 signaling pathway may play important roles in pathogenesis. AGEs in T2DM may contribute to inflammation in OA via the JUN/CXCL8 axis. This study improves our understanding of T2DM complicating OA and helps to stimulate more effective treatments. However, the association and mechanisms between T2DM and OA require further study.

Author contributions

Conceptualization: Bao Zhang, Deding Liu.
Data curation: Bao Zhang, Deding Liu.
Methodology: Bao Zhang, Deding Liu.
Visualization: Bao Zhang, Deding Liu.
Writing – original draft: Bao Zhang.
Writing – review & editing: Deding Liu.

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