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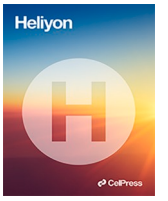

Research article

Machine learning and single-cell analysis identify the mitophagy-associated gene TOMM22 as a potential diagnostic biomarker for intervertebral disc degeneration

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ABSTRACT

Background: Mitophagy selectively eliminates potentially cytotoxic and damaged mitochondria and effectively prevents excessive cytotoxicity from damaged mitochondria, thereby attenuating inflammatory and oxidative responses. However, the potential role of mitophagy in intervertebral disc degeneration remains to be elucidated.

Methods: The GSVA method, two machine learning methods (SVM-RFE algorithm and random forest), the CIBERSORT and MCPcounter methods, as well as the consensus clustering method and the WGCNA algorithm were used to analyze the involvement of mitophagy in intervertebral disc degeneration, the diagnostic value of mitophagy-associated genes in intervertebral disc degeneration, and the infiltration of immune cells, and identify the gene modules that were closely related to mitophagy. Single-cell analysis was used to detect mitophagy scores and TOMM22 expression, and pseudo-temporal analysis was used to explore the function of TOMM22 in nucleus pulposus cells. In addition, TOMM22 expression was compared between human normal and degenerated intervertebral disc tissue samples by immunohistochemistry and PCR.

Results: This study identified that the mitophagy pathway score was elevated in intervertebral disc degeneration compared with the normal condition. A strong link was present between mitophagy genes and immune cells, which may be used to typify intervertebral disc degeneration. The single-cell level showed that mitophagy-associated gene TOMM22 was highly expressed in medullary cells of the disease group. Further investigations indicated the upregulation of TOMM22 expression in late-stage nucleus pulposus cells and its role in cellular communication. In addition, human intervertebral disc tissue samples established that TOMM22 levels were higher in disc degeneration samples than in normal samples.

Conclusions: Our findings revealed that mitophagy may be used in the diagnosis of intervertebral disc degeneration and its typing, and TOMM22 is a molecule in this regard and may act as a potential diagnostic marker in intervertebral disc degeneration.

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1. Introduction

Despite advances in surgery and medicine, low back pain (LBP) remains a major economic burden and a key reason for health-related spending [1]. Intervertebral disc degeneration (IDD) is considered to be the main pathologic cause of LBP [2]. The pathogenesis of IDD is complex and varied, and multiple factors, such as genetics, age, and environment, are involved [3]. The nucleus pulposus (NP), which is the interior of the intervertebral disc, maintains disc height and matrix homeostasis as well as distributes the pressure across the endplates. Therefore, NP plays a vital role in maintaining disc functionality both biologically and mechanically [4,5]. Excessive apoptosis of NP cells (NPCs) is widely recognized as the main cause of IDD [6]. Certain studies have reported that inhibition of NPCs apoptosis is a viable treatment option for IDD [7,8]. However, the apoptotic pathway of NPCs remains poorly understood.

Autophagy exerts anti-aging and quality control effects by eliminating senescent or damaged organelles and proteins [9,10]. The dysregulation of this process is commonly associated with a wide range of diseases, including cancer, liver disease, cardiovascular disease, and neurodegeneration [11–13]. Mitophagy is a key selective autophagic process that protects cells from mitochondrial apoptosis and dysfunction [14]. However, excessive mitophagy can eliminate several mitochondria and lead to apoptosis [15]. As the pathogenesis of IDD includes oxidative stress, mitochondrial dysfunction, and apoptosis [16], mitophagy may play a potential role in it. However, limited information is available in the literature in this regard, and the specific mechanism is yet to be clarified.

More and more diseases can be found for their biomarkers by using machine learning and single-cell analysis [17–19], thereby improving diagnostic accuracy, and as a gene related to mitophagy, TOMM22 has been reported and studied in many diseases [20,21], and some studies have reported that TOMM22 plays an important role in maintaining mitochondrial dynamic homeostasis [22,23].

Therefore, this study, aimed to explore the role of mitophagy and its mitophagy-associated molecule TOMM22 in IDD and verify the differences in the expression of TOMM22 using human tissue samples.

2. Materials and methods

2.1. Data collection and processing

Two gene expression profiles of intervertebral disc (GSE23130, GSE70362) were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) [24,25]. The two datasets were integrated using the sva package. GSE165722, which contains single-cell RNA sequencing (scRNA-seq) data of IDD, was also downloaded from the GEO database [26]. Seurat pipeline was performed on the scRNA-seq data and included cell filtering, normalization, scaling, and dimensional reduction.

2.2. Differential expression analysis, feature selection, and machine learning

Differential expression analysis of the microarray data was performed using the limma package. SVM-RFE algorithm and random forest were applied to select the key diagnostic markers [27]. In both methods, the top 10 genes with the largest importance were chose and underwent intersection to screen out the most crucial and robust diagnostic factors. Subsequently, a logistic model was created with the selected features using the glm function. Its predictive efficacy was evaluated using the receiver operating characteristic curve. Furthermore, 1000-time bootstrap validation was performed using the boot package [28]. A nomogram was plotted using the regplot package. The rms package was used to visualize the calibration curve, and ggDCA was applied to perform decision curve analysis.

2.3. Gene set scoring and immune cell evaluation

Gene set scoring was performed using the GSVA package for the microarray data and using the AddModuleScore function for the scRNA-seq data. The mitophagy gene set was downloaded from the Reactome database. CIBERSORT and MCPcounter were used to determine immune cell infiltration in the samples [29]. The correlation between immune cells and genes was calculated using the psych package and visualized with a heatmap.

2.4. Clustering and weighted correlation network analysis

Consensus cluster analysis was performed using “ConsensusClusterPlus” to obtain information on the patients’ heterogeneity. The km method and 100-time repetitions were used [30]. Clinical traits and immune cell proportions were compared between clusters [31]. Furthermore, the weighted gene correlation network analysis (WGCNA) package was used to identify crucial gene modules between clusters [32]. The scale-free topology criterion was used to select the soft threshold parameter of the power adjacency function, and the optimal value was accepted based on the SFT criterion recommendation. The significant gene module was subsequently extracted for gene function enrichment, which was performed using the clusterProfiler package [33].

2.5. Single-cell analysis

Cell types were annotated by combining the SingleR package and previous studies. To elucidate the function of TOMM22 at the

single-cell level, cell developmental trajectory analysis was done using Monocle2 [34]. The relationship between the expression and pseudotime was plotted. The cells were then separated into TOMM22+ and TOMM22− based on TOMM22 expression. Cell–cell communications were analyzed using the Cellchat package [35].

2.6. Tissue sample collection

5 human Non-IDD samples (simple lumbar isthmus fracture without degeneration, age 35–76 years, Non-IDD) and 12 human IDD samples (37–75 years, Grade III) and 10 human IDD samples (age 41–76 years, Grade V) were collected from patients who had undergone diskectomy at the Sixth People's Hospital of Shanghai Jiao Tong University School of Medicine. The extent of IDD was graded according to the Pfirrmann Grading System [36].This research was approved by the Accreditation Committee of the Shanghai Sixth People's Hospital, and all patients agreed to participate in the study and signed an informed consent form. Tissue samples were frozen and stored in liquid nitrogen immediately after separation until use.

2.7. RNA extraction and reverse transcription-polymerase chain reaction validation

RNA was extracted from intervertebral disc tissues using the Total RNA Extraction Kit (Shabio, Shanghai, China) according to the manufacturer's protocol. cDNA was reverse transcribed from RNA using the RevertAid™ H Minus First Strand

cDNA Synthesis Kit. Reverse transcription-polymerase chain reaction (RT-PCR) was performed using the SYBR® Green MasterMix in the ABI PRISM® 7500 Sequence Detection System (Applied Biosystems Inc: Foster City, CA). Three replicate wells were set up for each sample. Glyceraldehyde 3-phosphate dehydrogenase served as the internal standardization control, and TOMM22 primer sequences were synthesized by Sangon Biotech (Shanghai, China). The relative fold expression of RNA was determined using the $2^{-\Delta\Delta ct}$

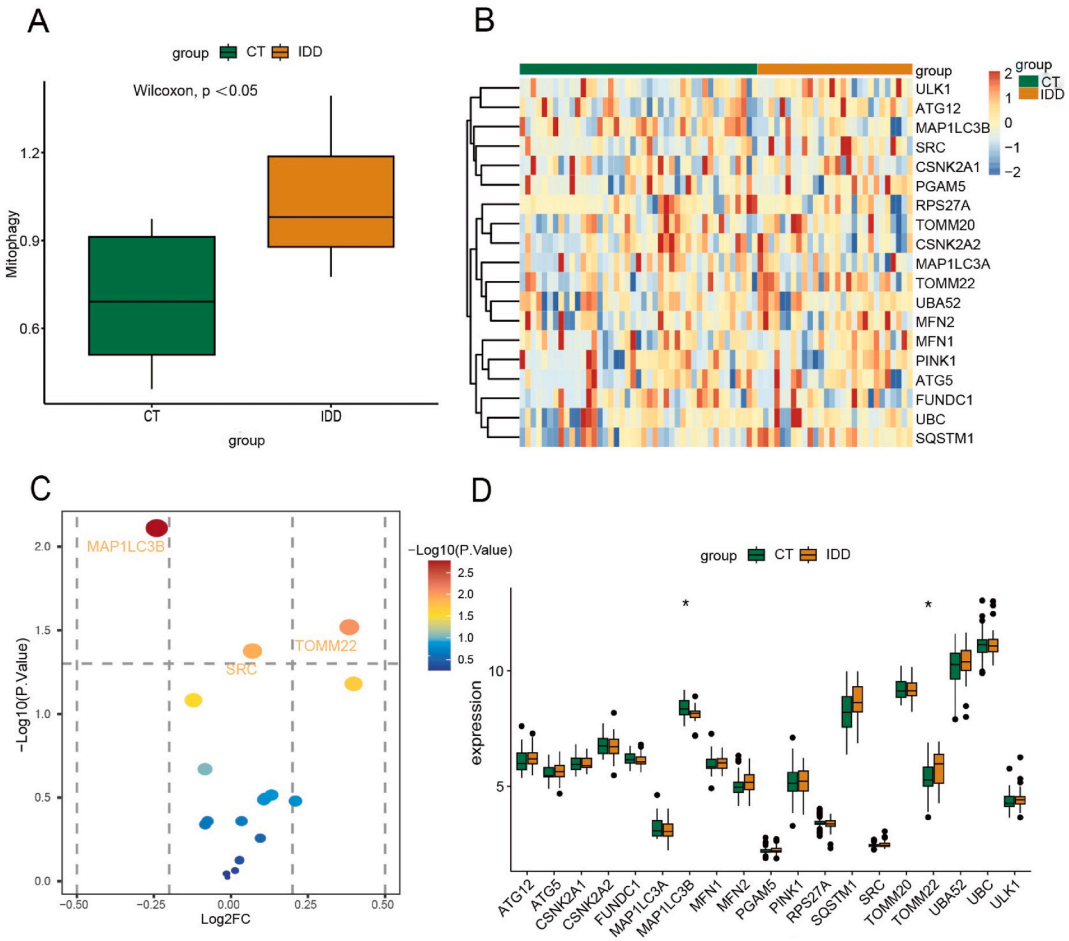

Fig. 1. (A) A higher GSVA score was detected in IDD than in normal control. (B) The heat map displays the expression of mitophagy genes. (C) The volcano plot displays the expression differences of mitophagy genes. (D) The box plot described the expression differences of mitophagy genes in IDD. *$p < 0.05$.

method.

2.8. Western blotting

Proteins were extracted and their content was determined using the bicinchoninic acid assay method (Thermo Scientific, USA). Equal amounts of protein lysate were separated using 10 % sodium dodecyl sulphate-polyacrylamide gel electrophoresis and then transferred to a poly (vinylidene fluoride) membrane. After being closed with fresh 5 % skimmed milk in Tris-buffered saline with 0.1 % Tween 20 (TBST) for 2 h at room temperature, the membranes were incubated with the primary antibody against TOMM22 at 4 °C overnight. Subsequently, after washing with TBST, the membranes were incubated with the corresponding enzyme-labeled secondary antibody for 2 h at room temperature. Proteins on the membrane were assayed using the ECL Western blotting (WB) detection system (Thermo Scientific, USA) and quantified using the ImageJ software (Rawak Software, Germany).

2.9. Histological evaluation

Paraffin sections were prepared from isolated human intervertebral disc samples and stained with hematoxylin–eosin (HE) and safranin O-fast green (SO) according to the manufacturer's instructions. The HE and SO-stained images were observed under a microscope (Olympus Corp, Japan).

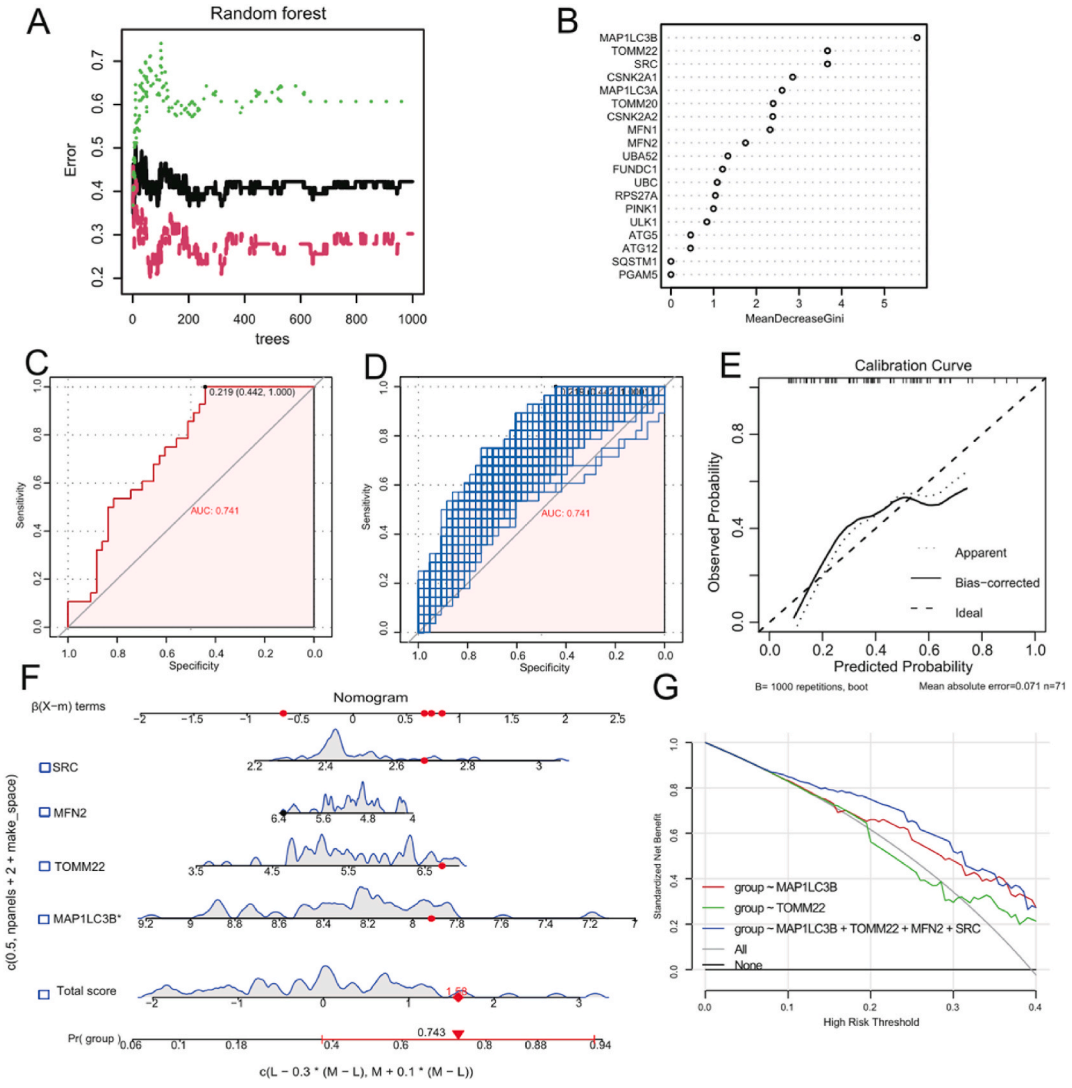

Fig. 2. (A) The error-rate confidence intervals for the random-forest model. (B) The relative importance of genes in the random-forest model. (C) Logistic regression model constructed with 4 genes. (D) Bootstrap validation based on 4 gene constructs. (E) Calibration curve to assess the predictive power of the logistic model. (F) Nomogram to predict the occurrence of IDD. (G) Decision curve analysis of the logistic model.

2.10. Immunohistochemistry

The isolated human intervertebral disc samples were embedded in paraffin, followed by sectioning, dewaxing, hydration, antigen repair, sealing, antibody incubation, and color development. The prepared samples were then observed microscopically and photographed (Olympus Corp, Japan).

2.11. Statistical analysis

SPSS software (version 22.0) was utilized for statistical analysis. All raw data were processed on the R software (version 4.2.1). Independent samples *t*-test was employed to compare the statistical differences between the two groups, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Identification of mitophagy-associated differentially expressed genes in IDD

To investigate the involvement of the mitophagy pathway in IDD, the GSVA method was initially employed. The results demonstrated that the mitophagy pathway score was higher in the IDD group than in the control (CT) group with normal tissues (Fig. 1A). The 19 mitophagy-associated genes were visualized using a heatmap (Fig. 1B) and a volcano plot (Fig. 1C). These studies further delineated

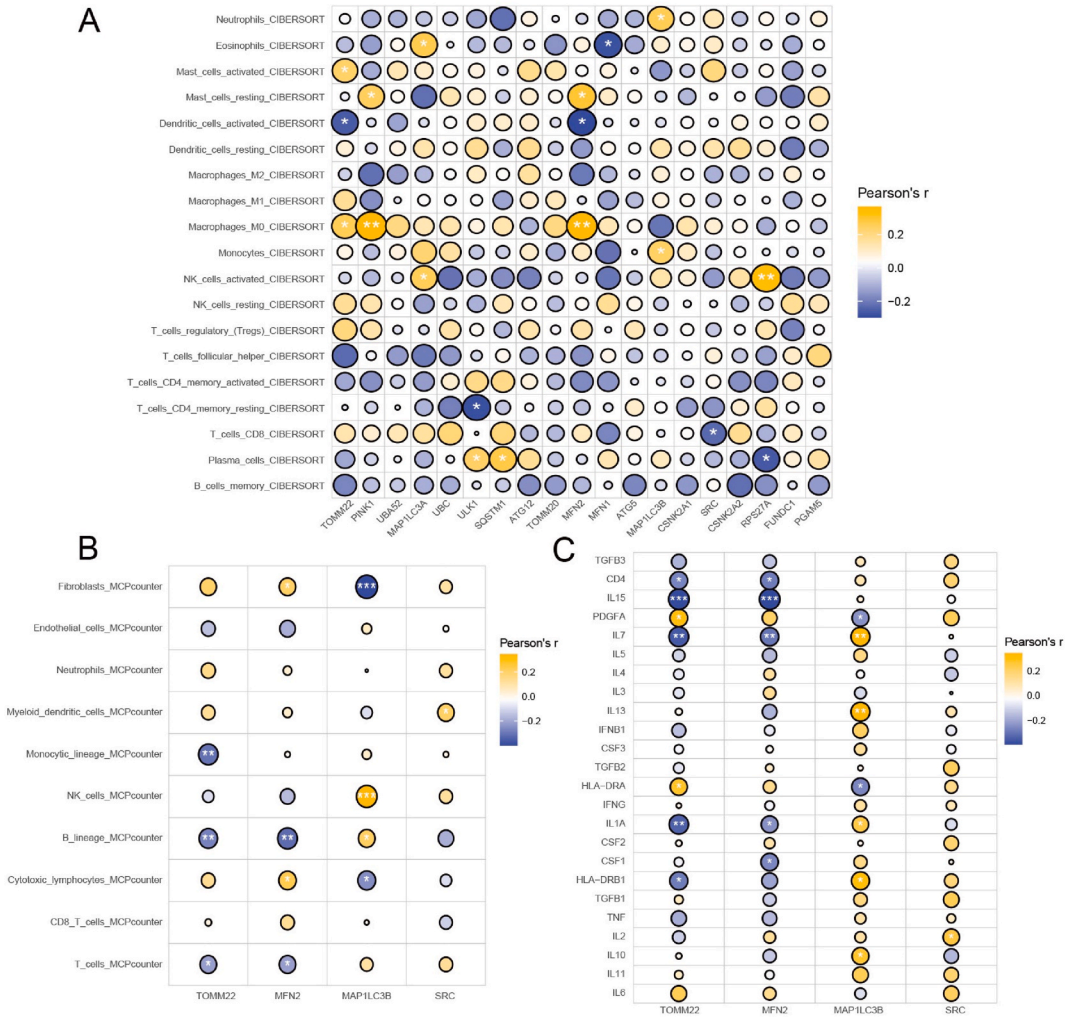

Fig. 3. (A) Correlation map of genes and immune cells based on CIBERSORT algorithm. (B) Correlation map of four diagnostic genes and immune cells based on the MCP counter algorithm. (C) Correlation diagram of four diagnostic genes and inflammatory factors. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

the differences between the IDD and CT groups and revealed that the expressions of MAP1LC3B and TOMM22 were elevated in the CT and IDD groups, respectively. With regard to differences in the expressions of mitophagy genes, TOMM22, MAP1LC3B, and SRC showed significance (Fig. 1C and D).

3.2. Mitophagy-associated diagnostic model for predicting IDD

To discern the diagnostic value of mitophagy-associated genes in IDD, two machine learning algorithms were applied to identify the diagnostic genes. The top 10 genes were identified using SVM-RFE and random forest algorithms (Fig. 2A and B). Intersecting the results of the two algorithms revealed four genes, namely, MAP1LC3B, TOMM22, MFN2, and SRC. In the subsequent analysis, a multifactor logistic regression model that incorporated the four identified genes was applied. The model showed an area under the curve (AUC) value of 0.741 (Fig. 2C). The performance of the model was further validated using 1000 bootstrap samples (Fig. 2D). The calibration curve, used to assess the predictive accuracy of the classifier, indicated negligible differences between the predicted and observed IDD risks (Fig. 2E), which underscored the robustness of the model. A nomogram derived from the logistic regression model was then generated (Fig. 2F), and the decision curve suggested the potential clinical value of the model (Fig. 2G).

3.3. Immune cell infiltration and mitophagy-associated genes

Immune characteristics were assessed using the CIBERSORT method. Of the 19 genes, MFN2 exhibited a positive correlation with M0 macrophages and resting mast cells (Fig. 3A). Conversely, MFN2 showed a negative correlation with activated dendritic cells.

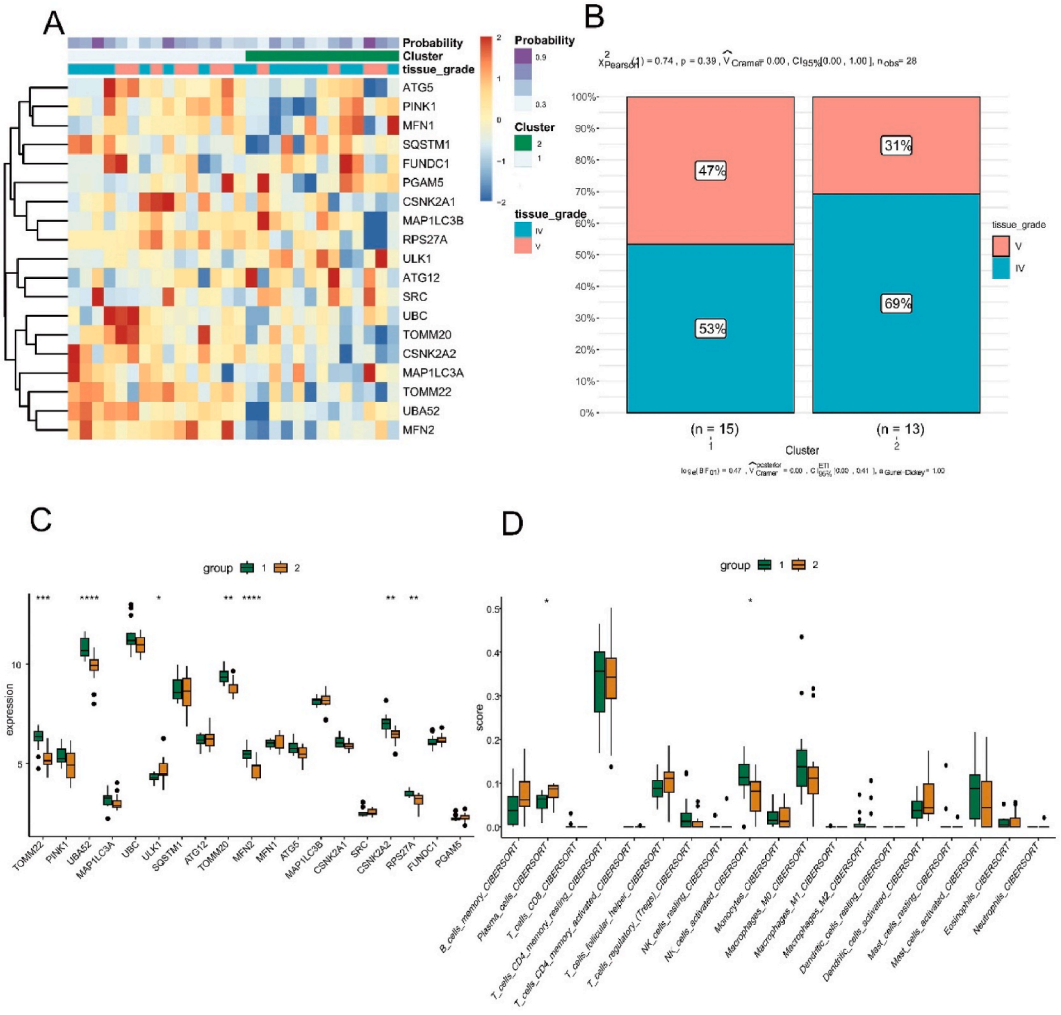

Fig. 4. (A) Heat map of the analysis of differences among the 2 subtypes and correlation analysis between subtypes and clinicopathological features. (B) The proportion of tissue grade in the 2 clusters. (C) Box line plots describing the mRNA expressions of mitophagy-associated DEGs between clusters. (D) Box line plots describing the expressions of immune cells between clusters. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

TOMM22 was also positively associated with M0 macrophages and resting mast cells but negatively correlated with activated dendritic cells (Fig. 3A). Furthermore, according to the MCP-counter method, the diagnostic genes were strongly linked to NK cells, fibroblasts, B lineage, and monocytic lineage (Fig. 3B). Correlation curves were subsequently generated for the four diagnostic genes and inflammatory factors, which highlighted that the four selected genes were strongly associated with IL7, IL15, IL1A, and IL13 (Fig. 3C).

3.4. Differences in mitophagy regulators and immune infiltration characteristics between mitophagy clusters

To comprehend the heterogeneity in patients with regard to mitophagy, 28 IDD samples were categorized based on the expression profiles of 19 metabolism-related genes (MRGs) using a consensus clustering method. The findings indicated that the stability of the cluster numbers peaked when the k-value was set to two (k = 2). To delineate the molecular differences between clusters, variations in the expressions of 19 MRGs between Cluster 1 and Cluster 2 were analyzed. Clear differences in MRG expression were noted between the two mitophagy patterns (Fig. 4A and C). Specifically, Cluster 1 displayed elevated expressions of TOMM22, UBA52, TOMM20, MFN2, CSNK2A2, and RPS27A. Furthermore, Cluster 1 showed a higher disease degree than Cluster 2 (Fig. 4B). In contrast, Cluster 2 predominantly showed increased expression of ULK1. Plasma_cells were expressed to a greater extent in Cluster 1 than in Cluster 2, whereas NK_cells_activated were expressed to a lesser extent in Cluster 1 than in Cluster 2 (Fig. 4D).

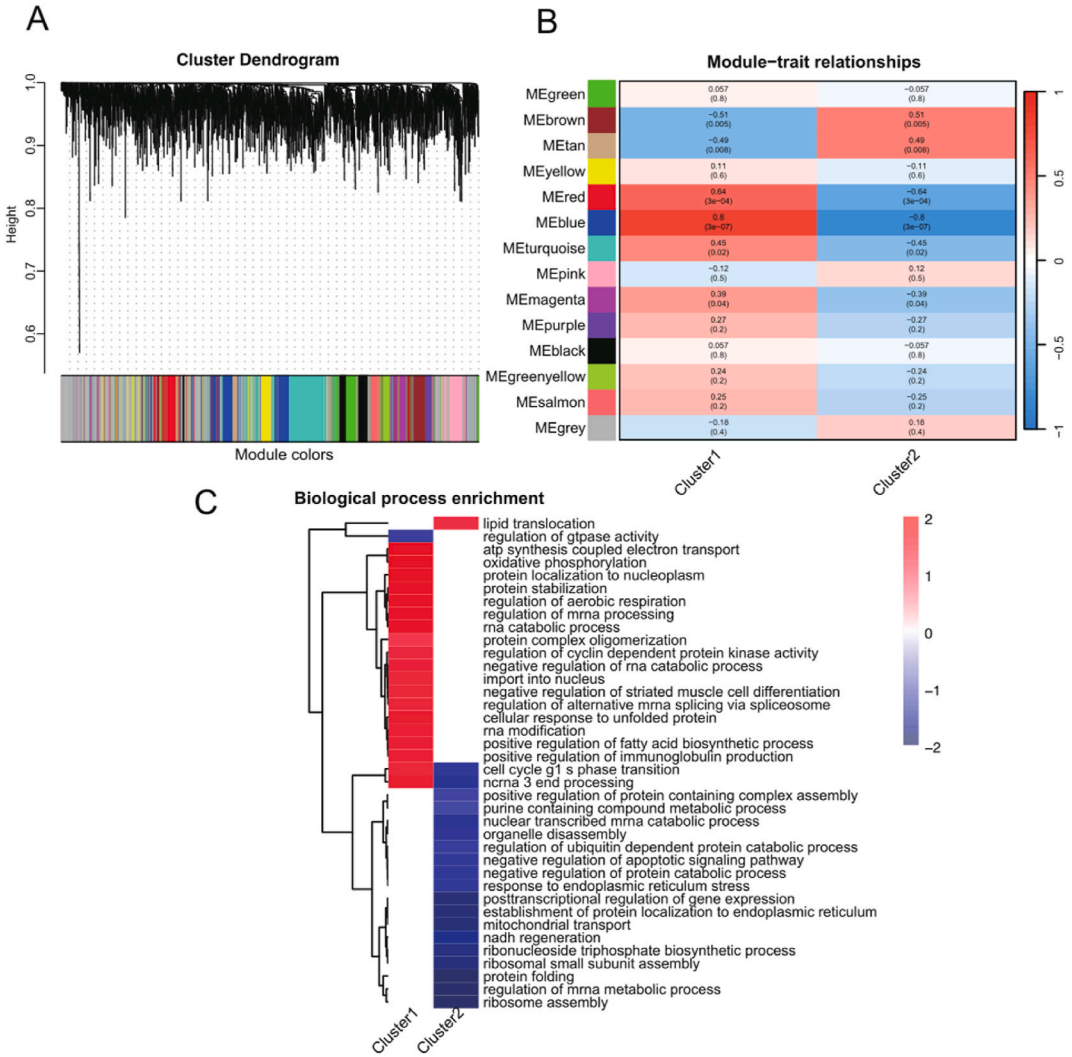

Fig. 5. (A) Cluster tree dendrogram of the co-expression modules. (B) Correlation analysis between module eigengenes and clinical status. Each row represents a module; each column represents a clinical status. (C) Differences in the biological functions between Cluster 1 and Cluster 2 samples ranked by the t-value of the GSVA method.

3.5. Screening of gene modules and construction of coexpression network

To identify gene modules intricately linked to mitophagy clusters, the WGCNA algorithm was employed. 14 significant modules encompassing 4422 genes were recognized (Fig. 5A), and the heatmap illustrated the TOM of all module-associated genes. When the relationship between module and clinical features (Cluster 1 and Cluster 2) was analyzed, it signified a strong correlation between the blue module, which contained 1115 genes, and IDD clusters (Fig. 5B). Furthermore, functional enrichment analysis suggested the prominent association of Cluster 1 with protein synthesis and oxidative stress (Fig. 5C). Hence, Cluster 1 might play a pivotal role in these processes.

3.6. Examination of mitophagy scores and TOMM22 expression using single-cell analysis

The GSE165722 dataset was evaluated to determine the mitophagy scores at the single-cell level. Initially, cell populations were categorized into 21 clusters. Based on the expressions of marker genes, these clusters were subsequently consolidated into 9 distinct cell populations: myelocytes, neutrophils, promyelocytes, pre-B cells, NPCs, erythroblasts, T cells, monocytes, and macrophages (Fig. 6A and B). In-depth analysis revealed that cells with high mitophagy scores predominantly resided within NPCs (Fig. 6C). Notably, TOMM22 showed significant distributional differences in NPCs, which largely agreed with the distribution of mitophagy (Fig. 6C and D), and the NPCs_IDD had slightly higher TOMM22 levels than the NPCs_CT (Fig. 6 E).

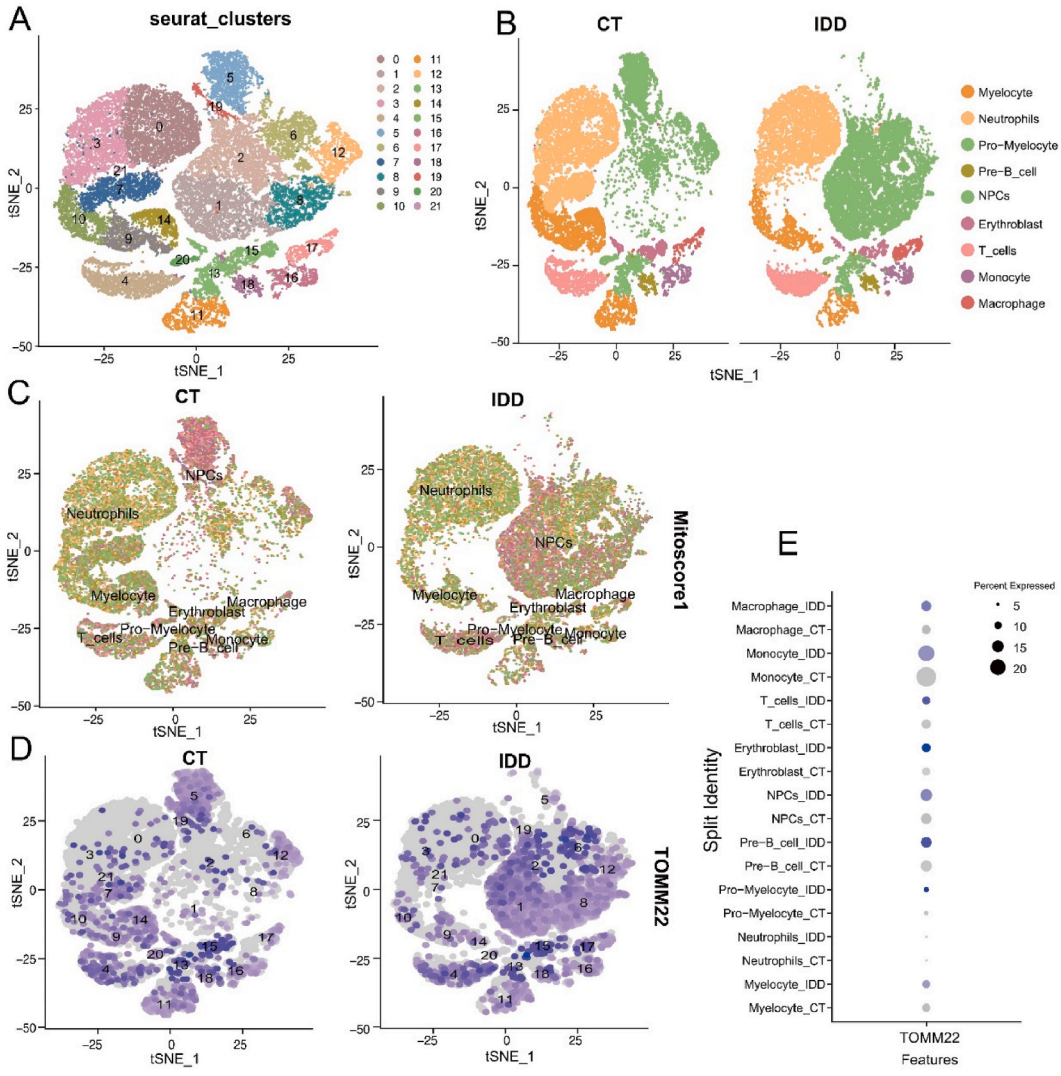

Fig. 6. (A) Cell clusters for GSE165722 of 8 IDD samples. (B) Cell markers for clusters' annotation. (C) The mitophagy scores between IDD and CT. (D–E) The TOMM22 expression between IDD and CT.

3.7. The crosstalk of TOMM22+ cells with monocytes was the strongest

To further explore the function of TOMM22 in NPCs, pseudotime analysis was performed using Monocle2, which indicated that TOMM22 was expressed in late developmental NPCs (Fig. 7A). Two redefined subclusters of NPCs, namely, TOMM22+ and TOMM22−, were discerned. The network approach was employed to determine the weight and strength of their interactions, which revealed that TOMM22+ NPCs exhibited enhanced communication with monocytes (Fig. 7B–D). Delving deeper into the strength of secretory signals across diverse cell types using heatmaps signified the pronounced activation of pivotal pathways in the extracellular matrix (Fig. 7E).

3.8. Validation of TOMM22 expression in human intervertebral disc tissue samples

To evaluate the expression of TOMM22 in human intervertebral disc tissue samples, a total of 27 patients who underwent discectomy were included, of which 22 patients had lumbar disc herniation with degeneration (12 cases of Grade III intervertebral disc degeneration and 10 cases of Grade V intervertebral disc degeneration) and the remaining 5 patients had simple lumbar isthmus fracture without intervertebral disc degeneration (Non-IDD). Intervertebral discs with varying levels of degeneration show differences in signal intensities on T2-weighted magnetic resonance imaging (Fig. 8A). HE and SO staining were performed on Non-IDD, Grade III,

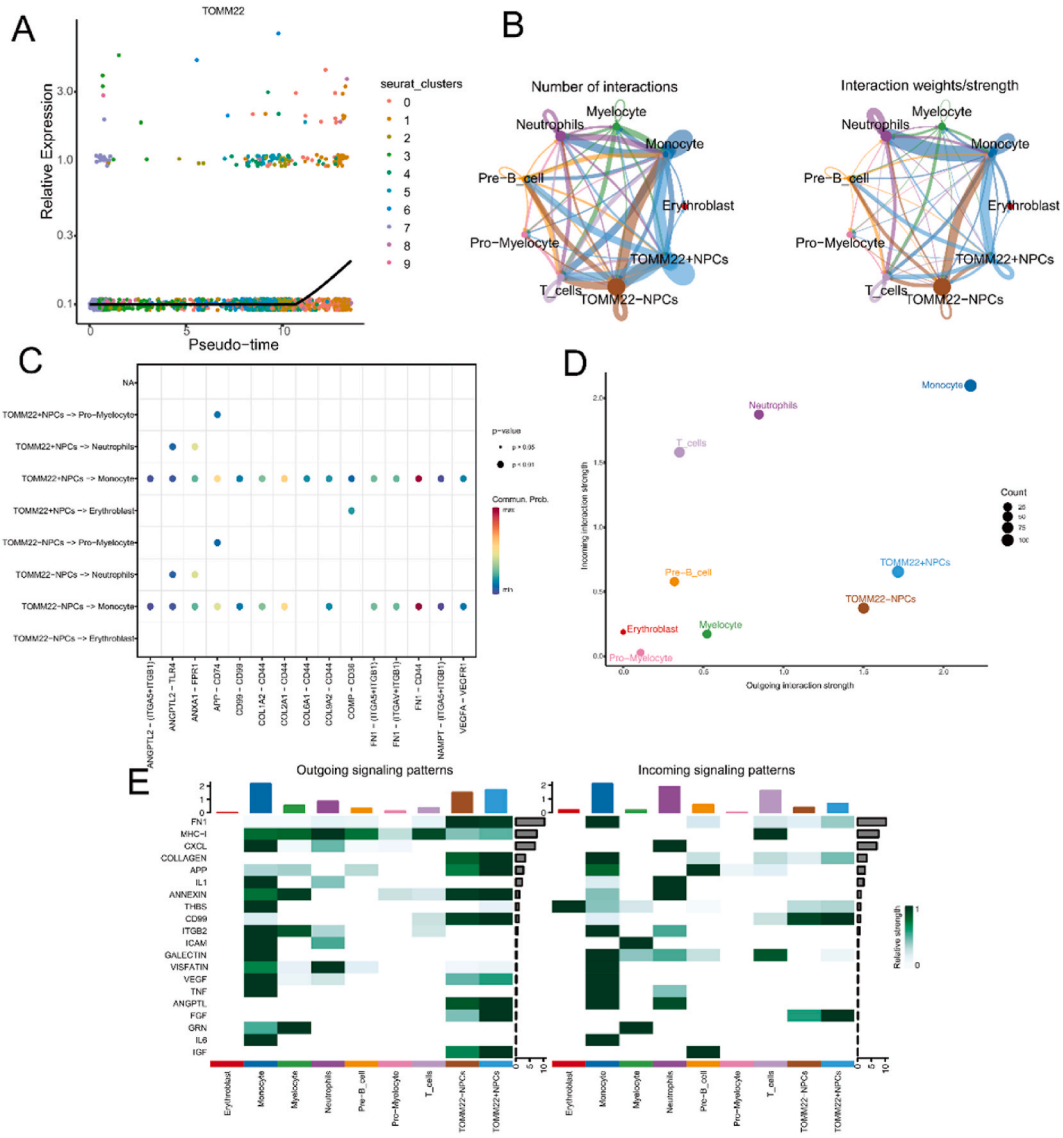

Fig. 7. (A) Pseudotime trajectory inferred by monocle and colored according to the cell subpopulation. (B–D) Cellular communication of TOMM22-positive and other cells. (E) Heatmap demonstrating the significant activation of key efferent signals.

and Grade V intervertebral disc tissues. The results implied that the degenerated intervertebral disc tissues (Grade III and Grade V) exhibited reduced elasticity and volume shrinkage compared with normal tissues (Non-IDD). The greater the degree of degeneration, the more pronounced the reduction in elasticity and volume (Fig. 8B). In addition, immunohistochemical analysis was performed, which showed that Grade III and Grade V intervertebral disc tissues had higher TOMM22 staining intensity and TOMM22+ cell rate compared with Non-IDD (Fig. 8C and E). Moreover, the findings of PCR showed that the expression of TOMM22 in Grade III and Grade V intervertebral disc tissues were significantly higher than that in Non-IDD (Fig. 8D), which agreed with our previous analysis. All these results indicated that the IDD group had a higher TOMM22 expression.

4. Discussion

As a leading cause of LBP, IDD is a major contributor to the burden of health expenditures worldwide owing to a limited understanding of its pathogenesis and the lack of effective prevention and treatment methods [37]. IDD therapy can be classified into conservative and surgical treatments [38]. Anti-inflammatory drugs, analgesics, and physiotherapy are symptomatic treatments that can only alleviate symptoms and retard the process but cannot truly treat or reverse IDD. Surgical interventions are the last resort as these are often associated with intraoperative and postoperative complications [39]. Therefore, new tools for the early diagnosis and pharmacologic treatment of IDD that address the pathologic mechanisms of the condition are urgently needed.

Mitophagy is a selective autophagy that promotes the degradation of damaged mitochondria and prevents excessive inflammation [40]. This process plays a pivotal role in regulating mitochondrial integrity and maintaining mitochondrial dynamic homeostasis [41]. Various risk factors have been reported to promote IDD events by inducing oxidative stress [42]. The role of mitophagy in IDD has been investigated, which revealed that NPCs apoptosis due to disc degeneration is associated with mitochondrial dysfunction and mitophagy inhibition [43]. PINK1 prevents mitochondrial dysfunction and apoptosis by inducing mitophagy. PINK1 deficiency leads to mitochondrial dysfunction, increased oxidative stress, and aggravates cellular senescence in NPCs, alluding that mitophagy plays a key role in preventing NPCs senescence [44]. In addition, LRRK2 is involved in the pathogenesis of IDD, and its knockdown promotes mitophagy to inhibit oxidative stress-induced NPCs apoptosis [45]. Based on these findings, mitophagy was hypothesized to be associated with the presence of IDD.

To investigate the role of mitophagy in IDD, both normal and degenerative disc tissues were included in this study, and the mitophagy pathway score was found to be higher in IDD. Further investigations revealed that of the 19 mitophagy-associated genes, only TOMM22, MAP1LC3B, and SRC were associated with IDD. Of these, TOMM22 was positively correlated with IDD, and MAP1LC3B was negatively correlated. Random forest model, logistic regression model bootstrap validation, calibration curve, and nomogram asserted the diagnostic value of these mitophagy-associated genes in predicting IDD. The 4 mitophagy-associated genes cumulatively

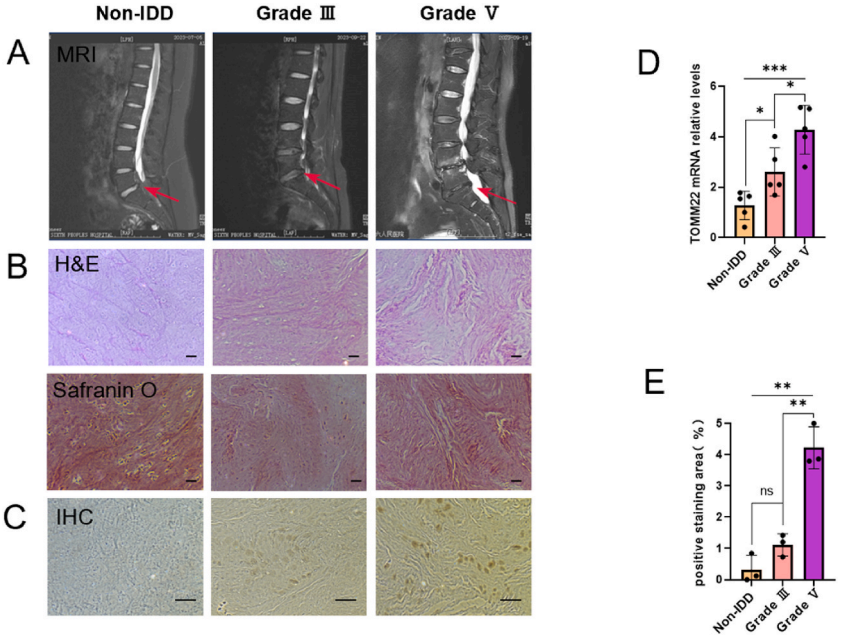

Fig. 8. (A) Magnetic resonance T2-weighted images of the Non-IDD, Grade III, and Grade V human intervertebral disc tissues. The red arrows indicate degenerated intervertebral disc tissues. Scale bar = 5 cm. (B) H&E and Safranin O staining of the NP from human intervertebral disc tissues can be seen. Scale bar = 200 μm. (C) IHC staining for TOMM22 in the NP from human intervertebral disc tissues. Positively stained cells appear brown in color. Scale bar = 200 μm. (D) The mRNA expression of TOMM22 in the NP from human intervertebral disc tissues, $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$, $n = 5$. (E) The expression level of TOMM22 was shown as the percentage of positively stained area, $^{**}p < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

had a better diagnostic effect, which further suggests that mitophagy pathway scores may be used for the diagnosis of IDD.

Mitophagy plays a crucial role in the regulation of inflammation by eliminating damaged mitochondria, inhibiting the inflammatory response, and reducing further damage [46–48]. Immunocyte analysis confirmed the close association of the mitophagy-associated genes TOMM22, MFN2, MAP1LC3B, and SRC with NK cells, fibroblasts, B lineage, and monocytic lineage as well as the immune factors IL7, IL15, IL1A, and IL13. This finding implies that mitophagy-associated genes may be involved in immune regulation during the pathogenesis of IDD. However, further experimental studies are needed to confirm this hypothesis.

The 28 IDD samples were classified into 2 categories using the consensus clustering method, with Cluster 1 having a higher degree of disease than Cluster 2. Cluster 1 showed elevated expressions of TOMM22, UBA52, TOMM20, MFN2, CSNK2A2, RPS27A, and plasma cells, whereas Cluster 2 showed increased expression of ULK1 and activated NK cells. Subsequently, the WGCNA algorithm was applied, which suggests the association of Cluster 1 with protein synthesis and oxidative stress. IDD has previously been reported to be associated with the onset of oxidative stress and proteoglycan loss [49], which is in line with our findings.

Single-cell analysis indicated that NPCs were predominantly the cells with high mitophagy scores, and the IDD group had high TOMM22 expression. This expression increased with age, and TOMM22+ NPCs exhibited improved communication with monocytes and significantly activated key pathways in the extracellular matrix.

To identify the important regulators associated with mitophagy, the 19 key genes linked to mitophagy obtained by examining the disc samples were analyzed, which revealed that TOMM22 is one of the core genes in the entire network.

TOMM22 is the central component of the TOMM (mitochondrial outer membrane translocase) receptor complex, which is involved in the recognition and translocation of synthetic mitochondrial precursor proteins and plays a cruial role in mitophagy [50,51]. Phosphorylated TOMM22 is thought to be a vital switch in mitophagy [52]. However, reports on the role of TOMM22 in mitophagy are limited. Some scientists have suggested that mutations in the TOMM22 gene are associated with mitochondrial dysfunction [53]. A study showed that TOMM22 gene overexpression plays an important role in the pathophysiology of pancreatic cancer by altering mitochondrial input and function, and that the degree of TOMM22 expression is positively correlated with the degree of malignancy of pancreatic cancer, which can be used as a potential diagnosis and predictor of pancreatic cancer [54], which corresponds to our study. In our study, the distribution of TOMM22 and mitophagy was roughly consistent, and both were mainly concentrated in NPCs, which were linearly correlated. The IDD group exhibited higher TOMM22 and mitophagy, especially a significant increase in TOMM22, which suggests that both play negative roles in the regulated progression of IDD, and apoptosis of NPCs is an important cause of IDD progression [55,56], so we hypothesized that the mechanism of TOMM22 and mitophagy regulating IDD might be related to NPCs. Furthermore, TOMM22 increased significantly in the late stage of development, and IDD is a degenerative disease [57]. This finding implies that the trend of TOMM22 and IDD is basically consistent, and hence, we have reason to believe that TOMM22 has the potential as a diagnostic indicator for IDD.

The role of mitophagy in IDD remains controversial [58,59], and although most of studies have reported that mitophagy is involved in the development of IDD [60,61], the effects of mitophagy on IDD are not entirely positive. Excessive mitophagy leads to the apoptosis of NPCs, whereas the upregulation of NDUFA4L2 expression can ameliorate apoptosis of NPCs by inhibiting excessive mitophagy [62]. While appropriate mitophagy eliminates damaged and dysfunctional mitochondria, averts their undesirable stimuli, and promotes cell survival, excessive mitophagy causes excessive mitochondrial clearance and induces inflammation [49], which in turn induces apoptosis [63,64]. Therefore, the rational regulation of mitophagy is crucial for the prevention and treatment of IDD [65]. Our findings suggested that mitophagy seem to be more detrimental than beneficial. We hypothesize that as IDD progresses, excessive mitochondrial clearance triggers inflammation and induces apoptosis of NPCs, which worsens IDD progression. The inhibition of mitophagy may slow down this progression, but further studies are required to confirm these hypotheses.

Based on the current findings, TOMM22 appears to be a molecule in mitophagy and has a linear relationship with IDD, but our study still has shortcomings. Firstly, the role of mitophagy in IDD and its mechanism have not been fully confirmed, and the association between TOMM22 and mitophagy still needs further research. Secondly, the sample sizes of machine learning and single-cell analysis were from GEO database, which were relatively weakly persuasive. In addition, previous studies on TOMM22 were fewer and lacked the support of relevant literature, which required further in vivo and in vitro experiments to confirm the relationship between TOMM22 and IDD. More importantly, although the linear relationship between TOMM22 and IDD was verified by immunohistochemistry and PCR, this was not sufficient, which required larger samples and more clinical data to confirm the relationship.

5. Conclusion

This study identified a link between mitophagy and IDD by combining machine learning algorithms, single-cell analysis and validation with human tissue samples. Mitophagy pathway scores and the expression of TOMM22, a molecule involved in mitophagy, were shown to be elevated in patients with IDD. This study provided new insights into the relationship between mitophagy and IDD, and the findings confirmed that TOMM22 may be used as a potential predictive and diagnostic indicator of IDD.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Ethical approval and consent to participate

This work was conducted in accordance with the Declaration of Helsinki and approved by the Accreditation Committee of the Shanghai Sixth People's Hospital.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Yinghao Wu: Conceptualization, Writing – original draft, Writing – review & editing. **Shengting Wu:** Conceptualization, Data curation. **Zhiheng Chen:** Data curation, Formal analysis. **Erzhu Yang:** Conceptualization, Project administration, Resources. **Haiyue Yu:** Software. **Guowang Zhang:** Conceptualization, Data curation, Formal analysis. **XiaoFeng Lian:** Conceptualization, Data curation. **JianGuang Xu:** Funding acquisition, Validation.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37378.

References

[1] R. He, Z. Wang, M. Cui, S. Liu, W. Wu, M. Chen, Y. Wu, Y. Qu, H. Lin, S. Chen, B. Wang, Z. Shao, HIF1A Alleviates compression-induced apoptosis of nucleus pulposus derived stem cells via upregulating autophagy, Autophagy 17 (11) (2021) 3338–3360.
[2] D. Sakai, J. Schol, Cell therapy for intervertebral disc repair: clinical perspective, J Orthop Translat 9 (2017) 8–18.
[3] L. Yu, Y. Liu, J. Wu, S. Wang, J. Yu, W. Wang, X. Ye, Genipin cross-linked decellularized nucleus pulposus hydrogel-like cell delivery system induces differentiation of ADSCs and retards intervertebral disc degeneration, Front. Bioeng. Biotechnol. 9 (2021) 807883.
[4] S. Roberts, H. Evans, J. Trivedi, J. Menage, Histology and pathology of the human intervertebral disc, J Bone Joint Surg Am 88 (Suppl 2) (2006) 10–14.
[5] P.-P.A. Vergroesen, A.J. van der Veen, B.J. van Royen, I. Kingma, T.H. Smit, Intradiscal pressure depends on recent loading and correlates with disc height and compressive stiffness, Eur. Spine J. 23 (11) (2014) 2359–2368.
[6] Y. Li, S. Liu, D. Pan, B. Xu, X. Xing, H. Zhou, B. Zhang, S. Zhou, G. Ning, S. Feng, The potential role and trend of HIF-1α in intervertebral disc degeneration: friend or foe? Mol. Med. Rep. 23 (4) (2021) (Review).
[7] D. Zhou, C. Song, Y. Mei, K. Cheng, F. Liu, W. Cai, S. Gao, Z. Wang, Z. Liu, A review of Duhuo Jisheng decoction mechanisms in intervertebral disc degeneration in vitro and animal studies, J. Orthop. Surg. Res. 18 (1) (2023) 436.
[8] H. Sudo, A. Minami, Caspase 3 as a therapeutic target for regulation of intervertebral disc degeneration in rabbits, Arthritis Rheum. 63 (6) (2011) 1648–1657.
[9] Y. Feng, D. He, Z. Yao, D.J. Klionsky, The machinery of macroautophagy, Cell Res. 24 (1) (2014) 24–41.
[10] Y. Ohsumi, Historical landmarks of autophagy research, Cell Res. 24 (1) (2014) 9–23.
[11] D.P. Panigrahi, P.P. Praharaj, C.S. Bhol, K.K. Mahapatra, S. Patra, B.P. Behera, S.R. Mishra, S.K. Bhutia, The emerging, multifaceted role of mitophagy in cancer and cancer therapeutics, Semin. Cancer Biol. 66 (2020) 45–58.
[12] X. Ma, T. McKeen, J. Zhang, W.-X. Ding, Role and mechanisms of mitophagy in liver diseases, Cells (2020).
[13] J.M. Bravo-San Pedro, G. Kroemer, L. Galluzzi, Autophagy and mitophagy in cardiovascular disease, Circ. Res. 120 (11) (2017) 1812–1824.
[14] M. Saberi, X. Zhang, A. Mobasheri, Targeting mitochondrial dysfunction with small molecules in intervertebral disc aging and degeneration, Geroscience 43 (2) (2021) 517–537.
[15] K. Sun, X. Jing, J. Guo, X. Yao, F. Guo, Mitophagy in degenerative joint diseases, Autophagy 17 (9) (2021) 2082–2092.
[16] Y. Chen, Y. Wu, H. Shi, J. Wang, Z. Zheng, J. Chen, X. Chen, Z. Zhang, D. Xu, X. Wang, J. Xiao, Melatonin ameliorates intervertebral disc degeneration via the potential mechanisms of mitophagy induction and apoptosis inhibition, J. Cell Mol. Med. 23 (3) (2019) 2136–2148.
[17] S. He, L. Liu, X. Long, M. Ge, M. Cai, J. Zhang, Single-cell analysis and machine learning identify psoriasis-associated CD8(+) T cells serve as biomarker for psoriasis, Front. Genet. 15 (2024) 1387875.
[18] Z. Yang, Y. Yang, X. Han, J. Hou, Novel AT2 cell subpopulations and diagnostic biomarkers in IPF: integrating machine learning with single-cell analysis, Int. J. Mol. Sci. 25 (14) (2024).
[19] P. Zhang, J. Feng, M. Rui, J. Xie, L. Zhang, Z. Zhang, Integrating machine learning and single-cell analysis to uncover lung adenocarcinoma progression and prognostic biomarkers, J. Cell Mol. Med. 28 (13) (2024) e18516.
[20] Q. Ke, Y. Wang, Y. Deng, J. Wang, M. Yuan, A. Liang, J. Wang, Q. Gong, Identification and validation of mitophagy-related signatures as a novel prognostic model for colorectal cancer, Transl. Cancer Res. 13 (2) (2024) 782–797.
[21] W. Xu, D. Zhao, X. Huang, M. Zhang, M. Yin, L. Liu, H. Wu, Z. Weng, C. Xu, The prognostic value and clinical significance of mitophagy-related genes in hepatocellular carcinoma, Front. Genet. 13 (2022) 917584.

[22] N.V. Yang, S. Rogers, R. Guerra, D.J. Pagliarini, E. Theusch, R.M. Krauss, TOMM40 and TOMM22 of the Translocase Outer Mitochondrial Membrane Complex rescue statin-impaired mitochondrial dynamics, morphology, and mitophagy in skeletal myotubes, bioRxiv (2023).
[23] Y. Dou, Y. Tan, Presequence protease reverses mitochondria-specific amyloid-β-induced mitophagy to protect mitochondria, Faseb j 37 (5) (2023) e22890.
[24] H.E. Gruber, G.L. Hoelscher, J.A. Ingram, E.N. Hanley Jr., Genome-wide analysis of pain-, nerve- and neurotrophin -related gene expression in the degenerating human annulus, Mol. Pain 8 (2012) 63.
[25] Z. Kazezian, R. Gawri, L. Haglund, J. Ouellet, F. Mwale, F. Tarrant, P. O'Gaora, A. Pandit, M. Alini, S. Grad, Gene expression profiling identifies interferon signalling molecules and IGFBP3 in human degenerative annulus fibrosus, Sci. Rep. 5 (2015) 15662.
[26] J. Tu, W. Li, S. Yang, P. Yang, Q. Yan, S. Wang, K. Lai, X. Bai, C. Wu, W. Ding, J. Cooper-White, A. Diwan, C. Yang, H. Yang, J. Zou, Single-cell transcriptome profiling reveals multicellular ecosystem of nucleus pulposus during degeneration progression, Adv. Sci. 9 (3) (2022) e2103631.
[27] D. Chen, J. Liu, L. Zang, T. Xiao, X. Zhang, Z. Li, H. Zhu, W. Gao, X. Yu, Integrated machine learning and bioinformatic analyses constructed a novel stemness-related classifier to predict prognosis and immunotherapy responses for hepatocellular carcinoma patients, Int. J. Biol. Sci. 18 (1) (2022) 360–373.
[28] N. Li, S. Zhan, C. Liu, T. Li, T. Tu, B. Chen, Y. He, M. Chen, Z. Zeng, X. Zhuang, Development and validation of a nomogram to predict indolent course in patients with ulcerative colitis: a single-center retrospective study, Gastroenterol Rep (Oxf) 10 (2022) goac029.
[29] X.G. Wu, J.J. Chen, H.L. Zhou, Y. Wu, F. Lin, J. Shi, H.Z. Wu, H.Q. Xiao, W. Wang, Identification and validation of the signatures of infiltrating immune cells in the eutopic endometrium endometria of women with endometriosis, Front. Immunol. 12 (2021) 671201.
[30] M.D. Wilkerson, D.N. Hayes, ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking, Bioinformatics 26 (12) (2010) 1572–1573.
[31] S. Mo, B. Jin, Y. Tseng, L. Lin, L. Lin, X. Shen, H. Song, M. Kong, Z. Luo, Y. Chu, C. Jiang, Z. Cao, J. Liu, F. Luo, A precise molecular subtyping of ulcerative colitis reveals the immune heterogeneity and predicts clinical drug responses, J. Transl. Med. 21 (1) (2023) 466.
[32] P. Langfelder, S. Horvath, WGCNA: an R package for weighted correlation network analysis, BMC Bioinf. 9 (2008) 559.
[33] G. Yu, L.G. Wang, Y. Han, Q.Y. He, clusterProfiler: an R package for comparing biological themes among gene clusters, OMICS 16 (5) (2012) 284–287.
[34] A.A. Ionkina, G. Balderrama-Gutierrez, K.J. Ibanez, S.H.D. Phan, A.N. Cortez, A. Mortazavi, J.A. Prescher, Transcriptome analysis of heterogeneity in mouse model of metastatic breast cancer, Breast Cancer Res. 23 (1) (2021) 93.
[35] S. Jin, C.F. Guerrero-Juarez, L. Zhang, I. Chang, R. Ramos, C.H. Kuan, P. Myung, M.V. Plikus, Q. Nie, Inference and analysis of cell-cell communication using CellChat, Nat. Commun. 12 (1) (2021) 1088.
[36] C.W. Pfirrmann, A. Metzdorf, M. Zanetti, J. Hodler, N. Boos, Magnetic resonance classification of lumbar intervertebral disc degeneration, Spine 26 (17) (2001) 1873–1878.
[37] J. Jia, S.Z. Wang, L.Y. Ma, J.B. Yu, Y.D. Guo, C. Wang, The differential effects of leukocyte-containing and pure platelet-rich plasma on nucleus pulposus-derived mesenchymal stem cells: implications for the clinical treatment of intervertebral disc degeneration, Stem Cells Int 2018 (2018) 7162084.
[38] Z. Wang, H. Yang, X. Xu, H. Hu, Y. Bai, J. Hai, L. Cheng, R. Zhu, Ion elemental-optimized layered double hydroxide nanoparticles promote chondrogenic differentiation and intervertebral disc regeneration of mesenchymal stem cells through focal adhesion signaling pathway, Bioact. Mater. 22 (2023) 75–90.
[39] B. Costăchescu, A.G. Niculescu, R.I. Teleanu, B.F. Iliescu, M. Rădulescu, A.M. Grumezescu, M.G. Dabija, Recent advances in managing spinal intervertebral discs degeneration, Int. J. Mol. Sci. 23 (12) (2022).
[40] S. Chen, J. Ma, P. Yin, F. Liang, The landscape of mitophagy in sepsis reveals PHB1 as an NLRP3 inflammasome inhibitor, Front. Immunol. 14 (2023) 1188482.
[41] F. Zhang, S. Wang, M. Gao, B. Li, Z. He, T. Tang, Z. Zhu, S. Liu, Z. Zhou, Hyaluronic acid ameliorates intervertebral disc degeneration via promoting mitophagy activation, Front. Bioeng. Biotechnol. 10 (2022) 1057429.
[42] Y. Song, S. Lu, W. Geng, X. Feng, R. Luo, G. Li, C. Yang, Mitochondrial quality control in intervertebral disc degeneration, Exp. Mol. Med. 53 (7) (2021) 1124–1133.
[43] S. Shi, X.J. Kang, Z. Zhou, Z.M. He, S. Zheng, S.S. He, Excessive mechanical stress-induced intervertebral disc degeneration is related to Piezo1 overexpression triggering the imbalance of autophagy/apoptosis in human nucleus pulpous, Arthritis Res. Ther. 24 (1) (2022) 119.
[44] Y. Wang, J. Shen, Y. Chen, H. Liu, H. Zhou, Z. Bai, Z. Hu, X. Guo, PINK1 protects against oxidative stress induced senescence of human nucleus pulposus cells via regulating mitophagy, Biochem. Biophys. Res. Commun. 504 (2) (2018) 406–414.
[45] J. Lin, X. Zheng, Z. Zhang, J. Zhuge, Z. Shao, C. Huang, J. Jin, X. Chen, Y. Chen, Y. Wu, N. Tian, L. Sun, W. Gao, Y. Zhou, X. Wang, X. Zhang, Inhibition of LRRK2 restores parkin-mediated mitophagy and attenuates intervertebral disc degeneration, Osteoarthritis Cartilage 29 (4) (2021) 579–591.
[46] Q. Yuan, N. Sun, J. Zheng, Y. Wang, X. Yan, W. Mai, Y. Liao, X. Chen, Prognostic and immunological role of FUN14 domain containing 1 in pan-cancer: friend or foe? Front. Oncol. 9 (2019) 1502.
[47] Z. Ma, J. Wu, Y. Wu, X. Sun, Z. Rao, N. Sun, Y. Fu, Z. Zhang, J. Li, M. Xiao, Q. Zeng, Y. Wu, C. Han, D. Ding, H. Zhang, H. Yuan, J. Zhang, S. Yang, Y. Chen, Parkin increases the risk of colitis by downregulation of VDR via autophagy-lysosome degradation, Int. J. Biol. Sci. 19 (5) (2023) 1633–1644.
[48] J. Wang, X. Qiu, J. Huang, Z. Zhuo, H. Chen, R. Zeng, H. Wu, K. Guo, Q. Yang, H. Ye, W. Huang, Y. Luo, Development and validation of a novel mitophagy-related gene prognostic signature for glioblastoma multiforme, BMC Cancer 22 (1) (2022) 644.
[49] Y. Li, L. Chen, Y. Gao, X. Zou, F. Wei, Oxidative stress and intervertebral disc degeneration: pathophysiology, signaling pathway, and therapy, Oxid. Med. Cell. Longev. 2022 (2022) 1984742.
[50] M. Merholz, Y. Jian, J. Wimberg, L. Gessler, S. Hashemolhosseini, In skeletal muscle fibers, protein kinase subunit CSNK2A1/CK2α is required for proper muscle homeostasis and structure and function of neuromuscular junctions, Cells 11 (24) (2022).
[51] N. Wiedemann, N. Pfanner, Mitochondrial machineries for protein import and assembly, Annu. Rev. Biochem. 86 (2017) 685–714.
[52] B. Kravic, A.B. Harbauer, V. Romanello, L. Simeone, F.N. Vögtle, T. Kaiser, M. Straubinger, D. Huraskin, M. Böttcher, C. Cerqua, E.D. Martin, D. Poveda-Huertes, A. Buttgereit, A.J. Rabalski, D. Heuss, R. Rudolf, O. Friedrich, D. Litchfield, M. Marber, L. Salviati, D. Mougiakakos, W. Neuhuber, M. Sandri, C. Meisinger, S. Hashemolhosseini, In mammalian skeletal muscle, phosphorylation of TOMM22 by protein kinase CSNK2/CK2 controls mitophagy, Autophagy 14 (2) (2018) 311–335.
[53] S. Curado, E.A. Ober, S. Walsh, P. Cortes-Hernandez, H. Verkade, C.M. Koehler, D.Y. Stainier, The mitochondrial import gene tomm22 is specifically required for hepatocyte survival and provides a liver regeneration model, Dis Model Mech 3 (7–8) (2010) 486–495.
[54] M.O. Haastrup, K.S. Vikramdeo, S. Anand, M.A. Khan, J.E. Carter, S. Singh, A.P. Singh, S. Dasgupta, Mitochondrial translocase TOMM22 is overexpressed in pancreatic cancer and promotes aggressive growth by modulating mitochondrial protein import and function, Mol. Cancer Res. 22 (2) (2024) 197–208.
[55] T. Kamatani, H. Hagizawa, S. Yarimitsu, M. Morioka, S. Koyamatsu, M. Sugimoto, J. Kodama, J. Yamane, H. Ishiguro, S. Shichino, K. Abe, W. Fujibuchi, H. Fujie, T. Kaito, N. Tsumaki, Human iPS cell-derived cartilaginous tissue spatially and functionally replaces nucleus pulposus, Biomaterials 284 (2022) 121491.
[56] J. Antoniou, T. Steffen, F. Nelson, N. Winterbottom, A.P. Hollander, R.A. Poole, M. Aebi, M. Alini, The human lumbar intervertebral disc: evidence for changes in the biosynthesis and denaturation of the extracellular matrix with growth, maturation, ageing, and degeneration, J. Clin. Invest. 98 (4) (1996) 996–1003.
[57] W. Guo, K. Mu, B. Zhang, C. Sun, L. Zhao, Z.Y. Dong, Q. Cui, The circular RNA FAM169A functions as a competitive endogenous RNA and regulates intervertebral disc degeneration by targeting miR-583 and BTRC, Cell Death Dis. 11 (5) (2020) 315.
[58] M.Y. Liu, L. Zhang, W.D. Zang, K.G. Zhang, H.J. Li, Y.Z. Gao, Pharmacological effects of resveratrol in intervertebral disc degeneration: a literature review, Orthop. Surg. 14 (12) (2022) 3141–3149.
[59] N.A. Shnayder, A.V. Ashhotov, V.V. Trefilova, Z.A. Nurgaliev, M.A. Novitsky, E.E. Vaiman, M.M. Petrova, R.F. Nasyrova, Cytokine imbalance as a biomarker of intervertebral disk degeneration, Int. J. Mol. Sci. 24 (3) (2023).
[60] Z. Zhang, T. Xu, J. Chen, Z. Shao, K. Wang, Y. Yan, C. Wu, J. Lin, H. Wang, W. Gao, X. Zhang, X. Wang, Parkin-mediated mitophagy as a potential therapeutic target for intervertebral disc degeneration, Cell Death Dis. 9 (10) (2018) 980.
[61] T. Lan, Y.C. Zheng, N.D. Li, X.S. Chen, Z. Shen, B. Yan, CRISPR/dCas9-Mediated parkin inhibition impairs mitophagy and aggravates apoptosis of rat nucleus pulposus cells under oxidative stress, Front. Mol. Biosci. 8 (2021) 674632.
[62] W.N. Xu, H.L. Zheng, R.Z. Yang, T. Liu, W. Yu, X.F. Zheng, B. Li, S.D. Jiang, L.S. Jiang, Mitochondrial NDUFA4L2 attenuates the apoptosis of nucleus pulposus cells induced by oxidative stress via the inhibition of mitophagy, Exp. Mol. Med. 51 (11) (2019) 1–16.

[63] X. Guo, X. Sun, D. Hu, Y.J. Wang, H. Fujioka, R. Vyas, S. Chakrapani, A.U. Joshi, Y. Luo, D. Mochly-Rosen, X. Qi, VCP recruitment to mitochondria causes mitophagy impairment and neurodegeneration in models of Huntington's disease, Nat. Commun. 7 (2016) 12646.
[64] R. Wang, Y. Dong, Y. Lu, W. Zhang, D.W. Brann, Q. Zhang, Photobiomodulation for global cerebral ischemia: targeting mitochondrial dynamics and functions, Mol. Neurobiol. 56 (3) (2019) 1852–1869.
[65] Z. Zeng, X. Zhou, Y. Wang, H. Cao, J. Guo, P. Wang, Y. Yang, Y. Wang, Mitophagy-A new target of bone disease, Biomolecules 12 (10) (2022).