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Identification of a novel ferroptosis-related gene signature associated with retinal degeneration induced by light damage in mice

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ABSTRACT

Background: Neurodegenerative retinal diseases such as retinitis pigmentosa are serious disorders that may cause irreversible visual impairment. Ferroptosis is a novel type of programmed cell death, and the involvement of ferroptosis in retinal degeneration is still unclear. This study aimed to investigate the related ferroptosis genes in a mice model of retinal degeneration induced by light damage.

Methods: A public dataset of GSE10528 deriving from the Gene Expression Omnibus database was analyzed to identify the differentially expressed genes (DEGs). Gene set enrichment analysis between light damage and control group was conducted. The differentially expressed ferroptosis-related genes (DE-FRGs) were subsequently identified by intersecting the DEGs with a ferroptosis genes dataset retrieved from the FerrDb database. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were further performed using the DE-FRGs. A protein-protein interaction (PPI) network was constructed to identify hub ferroptosis-related genes (HFRGs). The microRNAs (miRNAs)-HFRGs, transcription factors (TFs)-HFRGs networks as well as target drugs potentially interacting with HFRGs were analyzed utilizing bioinformatics algorithms.

Results: A total of 932 DEGs were identified between the light damage and control group. Among these, 25 genes were associated with ferroptosis. GO and KEGG analyses revealed that these DE-FRGs were mainly enriched in apoptotic signaling pathway, response to oxidative stress and autophagy, ferroptosis, necroptosis and cytosolic DNA-sensing pathway. Through PPI network analysis, six hub ferroptosis-related genes (Jun, Stat3, Hmox1, Atf3, Hspa5 and Ripk1) were ultimately identified. All of them were upregulated in light damage retinas, as verified by the GSE146176 dataset. Bioinformatics analyses predicated that 116 miRNAs, 23 TFs and several potential therapeutic compounds might interact with the identified HFRGs.

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Conclusion: Our study may provide novel potential biomarkers, therapeutic targets and new insights into the ferroptosis landscape in retinal neurodegenerative diseases.

1. Introduction

Retinitis pigmentosa (RP) is a retinal degenerative and inherited blinding disease with a prevalence of approximately 1 in 4000 individuals worldwide, which is manifested by initially a loss of night vision and progressively end up with complete blindness [1–3]. To date, mutations in around 100 genes have been reported associated with RP by different inherited manners including autosomal recessive, autosomal dominant, and X-linked inheritance [4–6]. Multiple molecular mechanisms have been shown to be involved in the pathogenesis of RP such as genetic aberrance, autoimmunity and autophagy [7–9]. Genetic defect causing retinal degeneration increases the photoreceptor fragility to light, meanwhile, excessive light exposure evokes the photoreceptor apoptosis, therefore, the retinal light damage mice model has been widely used to study pathogenesis of neurodegenerative retinal diseases including RP [10–12].

Ferroptosis is a novel type of programmed cell death caused by the accumulation of intracellular iron and reactive oxygen species (ROS) generating by lipid peroxidation [13]. Glutathione peroxidase 4 (GPX-4), a critical enzyme that catalyzes glutathione (GSH) to reduced lipid peroxides, is a central repressor of ferroptosis [14]. 4-hydroxy-2-nonenal (4-HNE), a major end-product of lipid peroxidation, is a general used biomarker for oxidative stress [15]. Transferrin, a specific ferroptosis biomarker, is an iron transport protein locating on cellular membrane responsible for transporting iron into the cell via binding to transferrin receptor 1 [16]. When undergoing oxidative stress, the expression level of GPX-4 in the retina is decreased [17]. Intravitreal injection of Fe^{2+} leads to photoreceptor degeneration with an increasing level of GPX-4 and 4-HNE [18]. In the Rd10 mice, a classical and widely used genetic aberrance causing mice model of RP, the decreased GPX and increased HNE are also observed, meanwhile, the total retinal iron as well as transferrin level are improved [19]. Several recent studies suggest the protective effects of iron chelators in multiple RP models [20–22]. These evidences indicate that there are strong association between the ferroptosis and retinal degeneration.

Despite signs of ferroptosis involved in the pathogenesis of retinal degeneration, the underlying mechanisms are not fully elucidated. Hence, we aimed to identify the novel ferroptosis-related genes that are involved in the pathogenesis of retinal degeneration in mice induced by light damage. Datasets were downloaded from the Gene Expression Omnibus (GEO) database. Furthermore, bioinformatics analyses were performed to screen differentially expressed genes (DEGs). After interacting with ferroptosis-related genes and PPI network analysis, six hub genes (Jun, Stat3, Hmox1, Atf3, Hspa5 and Ripk1) were ultimately identified and validated. Our study provides new insights into ferroptosis and retinal neurodegenerative diseases.

2. Methods

2.1. Data acquisition and process in

Microarray data was downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), which contained microarray-based and high-throughput sequencing gene expression datasets. The raw data of GSE10528 for retinal light damage was downloaded from GEO database [23]. It contained the platform of GPL1261 (Affymetrix Mouse Genome 430 2.0 Array), 3 dark adapted control retinas and 3 light damage retinas derived from BALB/C mice. We used the R software (version 3.6.3) to normalize the data. Probes were converted to gene symbols according to the annotation information of normalized data in the platform. The PCA plot was conducted to evaluate the reproducibility by the limma package [24].

2.2. Identification of differentially expressed ferroptosis-related genes

We analyzed differentially expressed genes (DEGs) by the limma package in R software. DEGs were screened with the threshold of p value < 0.05 and $|\text{Fold Change}| \geq 1.5$ between control and light exposure group. The result was visualized in volcano plots using the ggplot2 R package. 259 ferroptosis-related genes that drive, suppress or mark ferroptosis were retrieved from the FerrDb database (http://www.zhounan.org/ferrdb), a database for regulators, markers, and diseases associated with ferroptosis. A venn diagram was shown for the number of differentially expressed ferroptosis-related genes (DE-FRGs) using Venndiagram R package [25]. Thereafter the heatmap of DE-FRGs was generated by the pheatmap R package [26].

2.3. Gene set enrichment analysis

Gene set enrichment analysis (GSEA) was performed to identify the related signaling pathway between the two groups utilizing the clusterProfiler R package [27]. The significant gene sets were confirmed with false discovery rate (FDR) < 0.25 and the nominal (NOM) p-value <0.05.

2.4. Functional enrichment analysis of DE-FRGs

Biological process (BP) of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DE-FRGs were conducted to using the clusterProfiler R package. The top results of enrichment analysis were visualized by the ggplot2 R package.

Construction of protein-protein interaction network and identification of hub ferroptosis-related genes.

The protein-protein interaction (PPI) network of DE-FRGs was constructed and visualized with GENEMANIA platform [28]. Then the CytoHubba plugin of Cytoscape was applied to identify hub genes and key modules in the PPI network. The top 6 genes of PPI network, which were calculated using Maximal Clique Centrality (MCC) algorithm, were considered hub ferroptosis-related genes (HFRGs). The correlation of 6 HFRGs was performed and visualized using the igraph (version 1.3.4) and ggraph (version 2.1.0) R package.

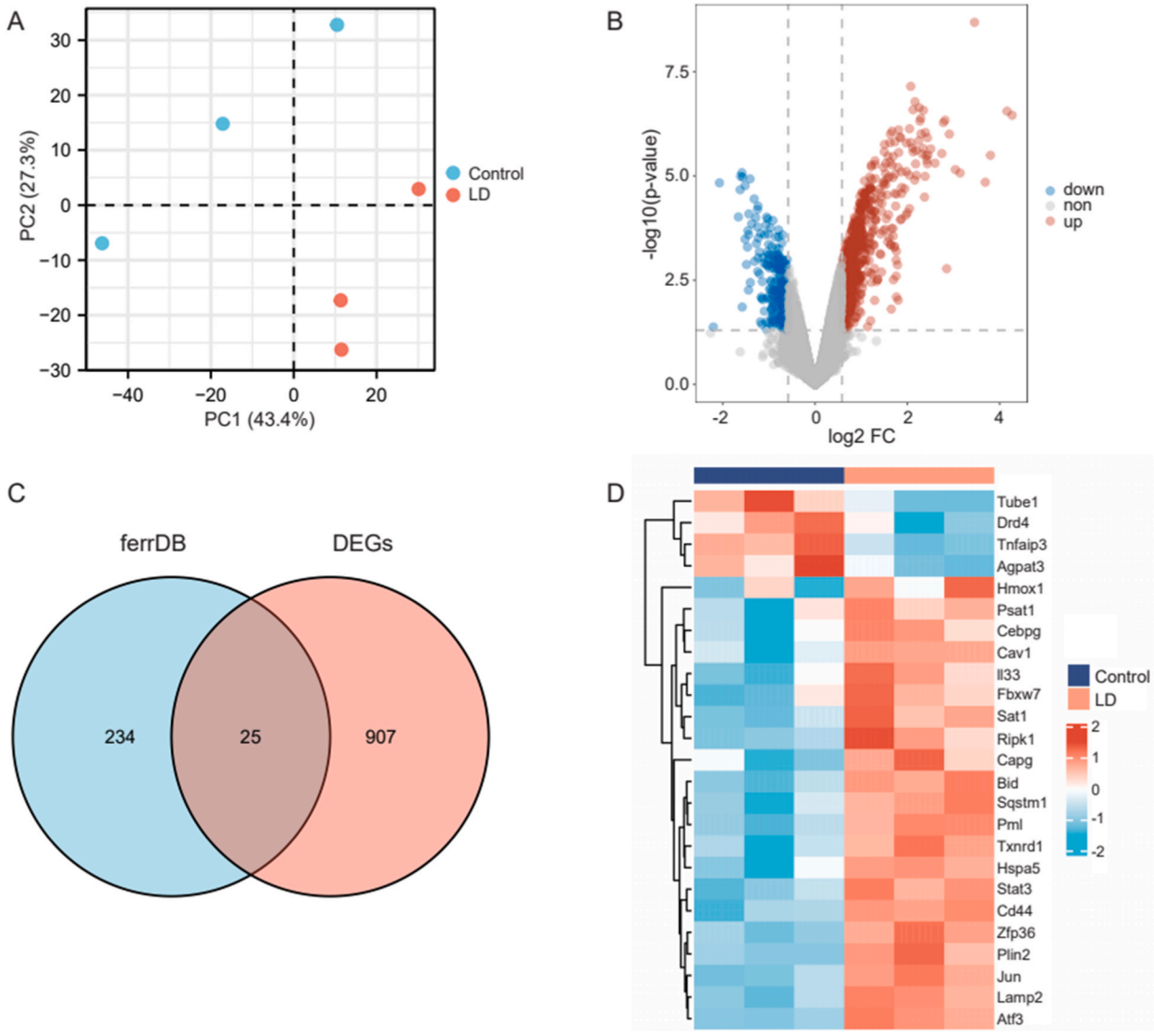

Fig. 1. Identification of differentially expressed ferroptosis-related genes (FRGs). (A) Principal component analysis of the GSE10528 dataset. (B) Volcano plots presenting significantly differentially expressed genes. Red, blue and grey nodes separately indicate up-regulated, down-regulated and none significantly changed genes. (C) Venn diagram displaying the DE-FRGs. Blue circle indicates DEGs in GSE10528, pink circle indicates FRGs, and the intersection shows DE-FRGs. (D) Heatmap presents the expressions of the 25 DE-FRGs in light damage retinas. Red bricks represent genes with high expression and blue bricks represent genes with low expression, pink annotation bar indicates light damage group, blue annotation bar indicates control group. LD, light damage; DEGs, differentially expressed genes; DE-FRGs, differentially expressed ferroptosis-related genes.

2.5. Regulatory network construction of miRNA-HFRGs and transcription factor (TF)-HFRGs

We used miRNet 2.0 database (https://www.mirnet.ca/) that was a miRNA-centric network visual analytics platform to predict the miRNAs targeting and binding to the six HFRGs. miRNA-mRNA regulation network was visualized by miRNet 2.0 platform. Thereafter, we used NetworkAnalyst 3.0 database to investigate the TFs capable of interacting with the HFRGs, and then the regulatory networks of TFs-HFRGs were mapped using Cytoscape software [29,30].

2.6. Prediction of target drugs regulating HFRGs

The DSigDB database is a web resource that provides information on drug signatures for gene set analysis [31]. We used the DSigDB to predict the drugs and molecular compounds that can be potential interacting with the six HFRGs.

2.7. Expression validation of hub genes

The expressions of 6 HFRGs were extracted from GSE146176 between light damage and control group [32]. The results were visualized in box plots using the ggplot2 R package, and the statistical significance was evaluated utilizing Student's t-test. $P < 0.05$

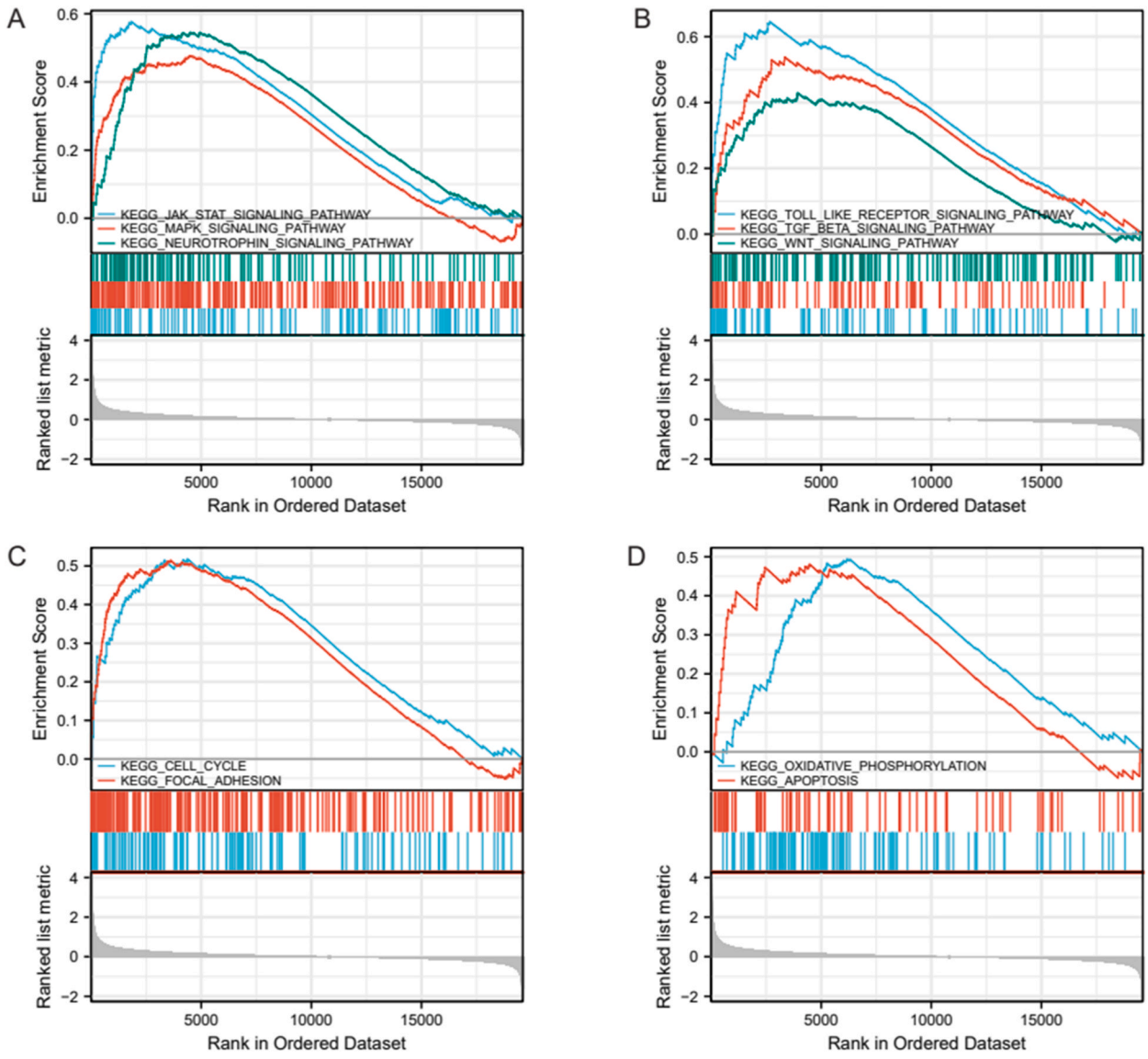

Fig. 2. Gene set enrichment analysis. A-D GSEA reveals the following KEGG pathways are enriched in the retinas undergoing light damage. NOM p < 0.05, FDR <25 %. NOM p, nominal p-value; FDR, false discovery rate.

was considered to be statistically significant.

3. Results

3.1. Identification of differentially expressed ferroptosis-related genes (DE-FRGs) in light damage retinas

To assess the reproducibility of the data in GSE10528, a principal component analysis was conducted (Fig. 1A). Based on a threshold of p-value<0.05 and |fold change| ≥ 1.5, a total of 932 genes were significantly differentially expressed in light damage retinas, of which 707 were upregulated and 225 were downregulated (Fig. 1B). To investigate FRGs differentially expressed in light damage retinas, 259 ferroptosis-related genes were extracted from FerrDb database. After intersection between DEGs and FRGs, a total of 25 DE-FRGs were identified (Fig. 1C), and the expressions of all DE-FRGs were visualized in a heatmap (Fig. 1D).

3.2. Gene set enrichment analysis

For the purpose of comparing the distinct pathways between the two groups, GSEA was subsequently conducted. As a result, the janus kinase (JAK)/signal transducer and activator of transcription (STAT) signal pathway, mitogen-activated protein kinase (MAPK) signaling pathway, neurotrophin signaling pathway, Toll like receptor signaling pathway, transforming growth factor beta (TGF-β) signaling pathway, Wnt signaling pathway, cell cycle, focal adhesion, oxidative phosphorylation, and apoptosis were significantly enriched in light damage retinas (Fig. 2A–D).

3.3. Functional enrichment analysis of DE-FRGs

Then we explored the potential biological functions and pathways of the identified 25 DE-FRGs. The top biological process (BP) results of GO analyses indicated that the DE-FRGs were significantly enriched in related processes such as the regulation of apoptotic signaling pathway, response to oxidative stress and autophagy (Fig. 3A). The KEGG pathway analyses suggested that the DE-FRGs were involved in ferroptosis, apoptosis, necroptosis and cytosolic DNA-sensing pathway (Fig. 3B).

3.4. Protein–protein interaction network construction and visualization

To explore the interactions between each DE-FRGs, the PPI network for the 25 DE-FRGs was established and visualized using GeneMANIA platform (Fig. 4A). In the PPI network, co-expression, physical interactions, co-localization and predicted separately account for 68.46 %, 11.26 %, 4.20 % and 16.07 %. The numbers of interactions for each gene among the 25 DE-FRGs were shown in Fig. 4B. Subsequently, the top 6 intersecting genes using the MCC algorithm of cytoHubba were selected as the hub ferroptosis-related

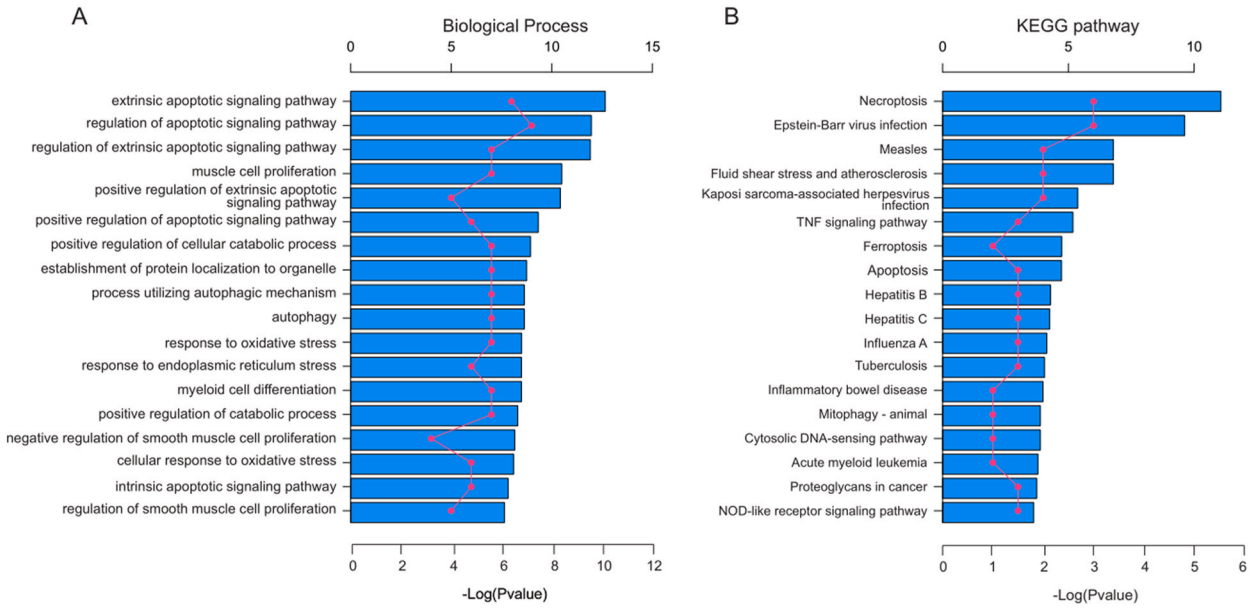

Fig. 3. Functional annotation of DE-FRGs. (A) GO enrichment analysis of biological processes enriched by DE-FRGs. (B) KEGG pathway enrichment analysis enriched by DE-FRGs. Blue bars represent the highest ranked items depending on the DE-FRGs. The red plots represent gene counts in the related item. The upper scale bar represents the gene count number, and the lower scale bar shows the p value. DE-FRGs, differentially expressed ferroptosis-related genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

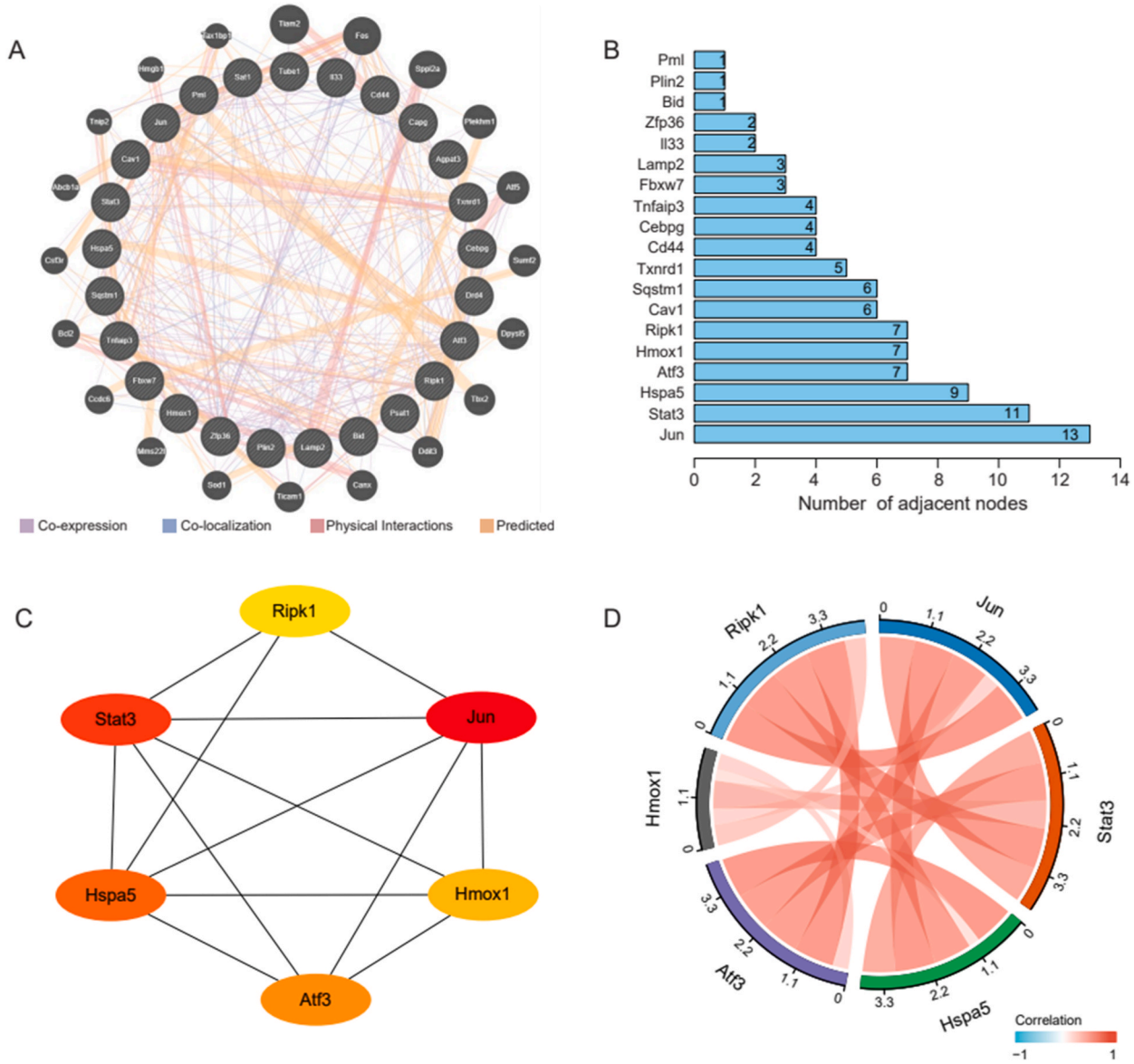

Fig. 4. Protein-protein interaction (PPI) analysis of the DE-FRGs. (A) The PPI network among 25 DE-FRGs constructed by GeneMANIA platform. (B) The interaction number of each DE-FRGs. (C) HFRGs calculated using the maximal clique centrality (MCC) algorithm, the red nodes indicate higher degrees of connectivity, and the yellow nodes indicate lower degrees. (D) Spearman correlation analyses of HFRGs. DE-FRGs, differentially expressed ferroptosis-related genes; HFRGs, hub ferroptosis-related genes.

genes (HFRGs), which included Jun, Stat3, Hspa5, Hmox1, Atf3 and Ripk1 (Fig. 4C). The expression levels of the HFRGs according to GSE10528 dataset was displayed in Table 1. Spearman correlation analysis was used to investigate the correlation of expression in these HFRGs, which indicated that all of the six HFRGs were positively correlated (Fig. 4D). The strong interactions among these

Table 1
Hub ferroptosis-related genes identified by MCC algorithm of cytoHubba.

Gene symbol	Description	Log2FC	p-value	Changes
Stat3	Signal transducer and activator of transcription 3	1.769	2.72E-05	Up
Atf3	Cyclic AMP-dependent transcription factor ATF-3	1.731	1.54E-06	Up
Jun	Transcription factor Jun	1.573	1.15E-05	Up
Hmox1	Heme oxygenase 1	0.876	0.024	Up
Hspa5	Endoplasmic reticulum chaperone BiP	0.679	0.003	Up
Ripk1	Receptor-interacting serine/threonine-protein kinase 1	0.667	0.002	Up

HFRGs suggested that these genes may be involved in the pathogenesis of retinal degeneration induced by light damage.

3.5. Predictions of miRNAs, TFs, and drugs targeting the HFRGs

We used miRNet database to predict target miRNAs of HFRGs. Finally, we obtained 116 target miRNAs of the 6 HFRGs and determined 160 mRNA-miRNA pairs. According to the prediction results, a co-expressed network of mRNAs and miRNAs, which comprised 122 nodes and 160 edges, was constructed (Fig. 5A). There were 48 miRNAs regulating Stat3, 43 miRNAs regulating Hspa5, 31 miRNAs regulating Jun, 22 miRNAs regulating Hmox1, 9 miRNAs regulating Ripk1, and 7 miRNAs regulating Atf3. MiRNAs with high number of gene cross-links ($\geq$3) were displayed in Table 2. To better understand the effect of HFRGs on the pathogenesis of light exposure retinas, we further predicted potential TFs capable of regulating with 6 HFRGs using the ENCODE database and a TFs-hub gene regulatory network was conducted (Fig. 5B).

Subsequently, potential target drugs that may be useful for treating light damage retinas by regulating HFRGs were predicted using DSigDB database. A total of 1189 molecular compounds drugs were finally predicted, and the top 10 predicted drugs according to the combined scores are shown in Table 3.

3.6. Verification of the 6 HFRGs by a dataset from the GEO database

A GSE146176 dataset, which included 5 light-induced damage retinas and 5 control samples, was further selected to verify the expression levels of the six HFRGs. The R software ggplot2 package was used to draw boxplots and the student's t-test statistical analyses were performed. Consistent with our predictions, the mRNA expression levels of the 6 HFRGs in the light damage retinas were significantly increased compared with those in normal controls (Fig. 6A–F).

4. Discussion

Ferroptosis, a novel form of iron-dependent programmed cell death, has been shown to be linked to metabolism, redox biology and a range of diseases [1,33,34]. It is well known that photoreceptor cell death is the major outcome of retinal degeneration, however, the underlying mechanisms are not fully elucidated. Clarifying the correlation between ferroptosis and retinal degeneration may provide new ideas for the pathogenesis and therapy of retinal neurodegenerative disorders.

In this study, we identified the novel ferroptosis-related genes that were involved in the pathogenesis of retinal degeneration in mice induced by light damage. We firstly performed a systematic analysis of an expression profiles from the GSE10528 dataset utilizing bioinformatics analysis. Totally, 932 DEGs were identified in the light damage retinas. By intersection of the DEGs and FRGs derived from the FerrDb database, a total of 25 DE-FRGs were identified. The GO and KEGG analyses revealed that these ferroptosis-related DEGs were mainly enriched in apoptotic signaling pathway, response to oxidative stress and autophagy, ferroptosis, necroptosis

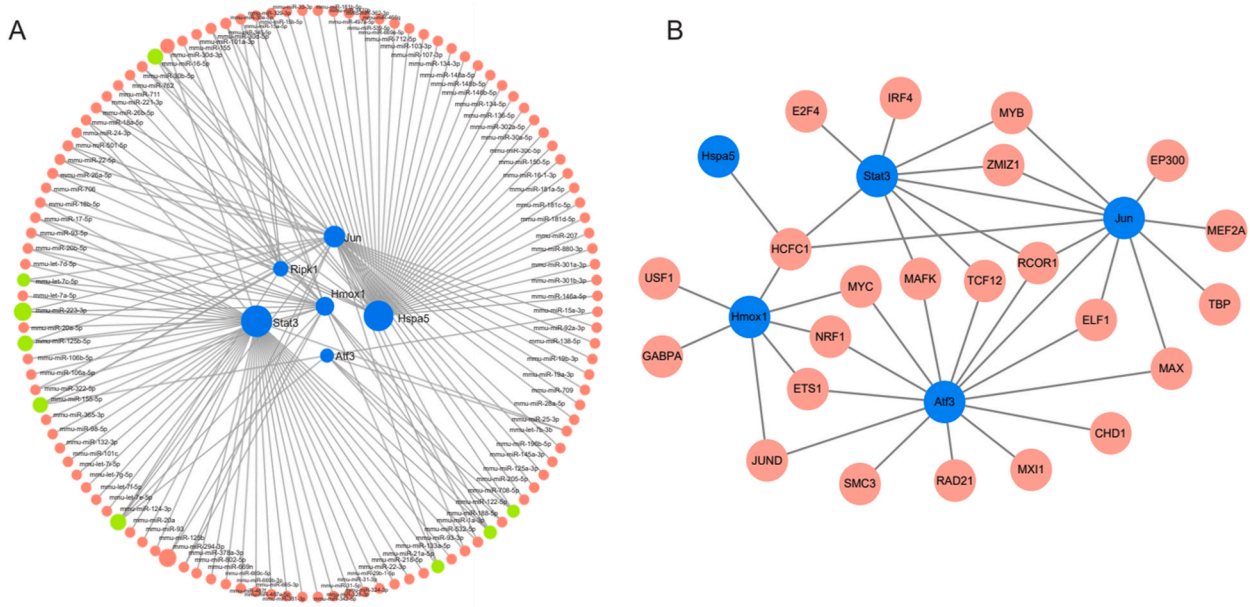

Fig. 5. Interaction network between HFRGs and their targeted miRNAs and TFs. (A) miRNAs-HFRGs network. HFRGs are colored in blue, and node size is adjusted according to number of targeted miRNAs; miRNAs are colored in red; miRNAs simultaneously targeting three or more hub genes are colored in green. (B) TFs-HFRGs network. TFs and HFRGs are separately colored in red and blue. HFRGs, hub ferroptosis-related genes; TFs, transcription factors.

Table 2
Top predicted miRNAs and hub ferroptosis-related genes.

miRNA	Genes targeted by miRNA	Gene counts
mmu-mir-124-3p	Atf3, Jun, Stat3, Ripk1	4
mmu-mir-1a-3p	Atf3, Jun, Stat3, Hmox1	4
mmu-mir-155-5p	Atf3, Jun, Hmox1,Ripk1	4
mmu-mir-21a-5p	Jun, Stat3,Hmox1	3
mmu-mir-122-5p	Atf3, Jun,Hmox1	3
mmu-let-7c-5p	Jun, Stat3,Hmox1	3
mmu-mir-223-3p	Jun, Stat3,Hmox1	3
mmu-mir-125b-5p	Jun, Stat3,Hmox1	3
mmu-mir-16-5p	Jun, Ripk1, Hspa5	3

Table 3
Top 10 candidate drugs associated with hub ferroptosis-related genes.

Index	Name	P value	Odds ratio	Combined score
1	Bortezomib CTD 00003736	7.09E-09	114,726	2152802.909
2	estradiol CTD 00005920	1.04E-04	93,978	862158.551
3	benzo[*a*]pyrene CTD 00005488	1.17E-04	93,450	846045.110
4	Copper sulfate CTD 00007279	7.40E-04	83,898	604789.579
5	VALPROIC ACID CTD 00006977	0.005	70,122	369440.441
6	suloctidil PC3 UP	6.07E-10	831.083	17637.871
7	citrinin CTD 00005677	2.65E-08	998.7	17423.238
8	fluphenazine PC3 UP	4.90E-08	798.76	13444.243
9	prochlorperazine PC3 UP	5.47E-08	768	12842.729
10	bepridil PC3 UP	6.07E-08	739.52	12288.619

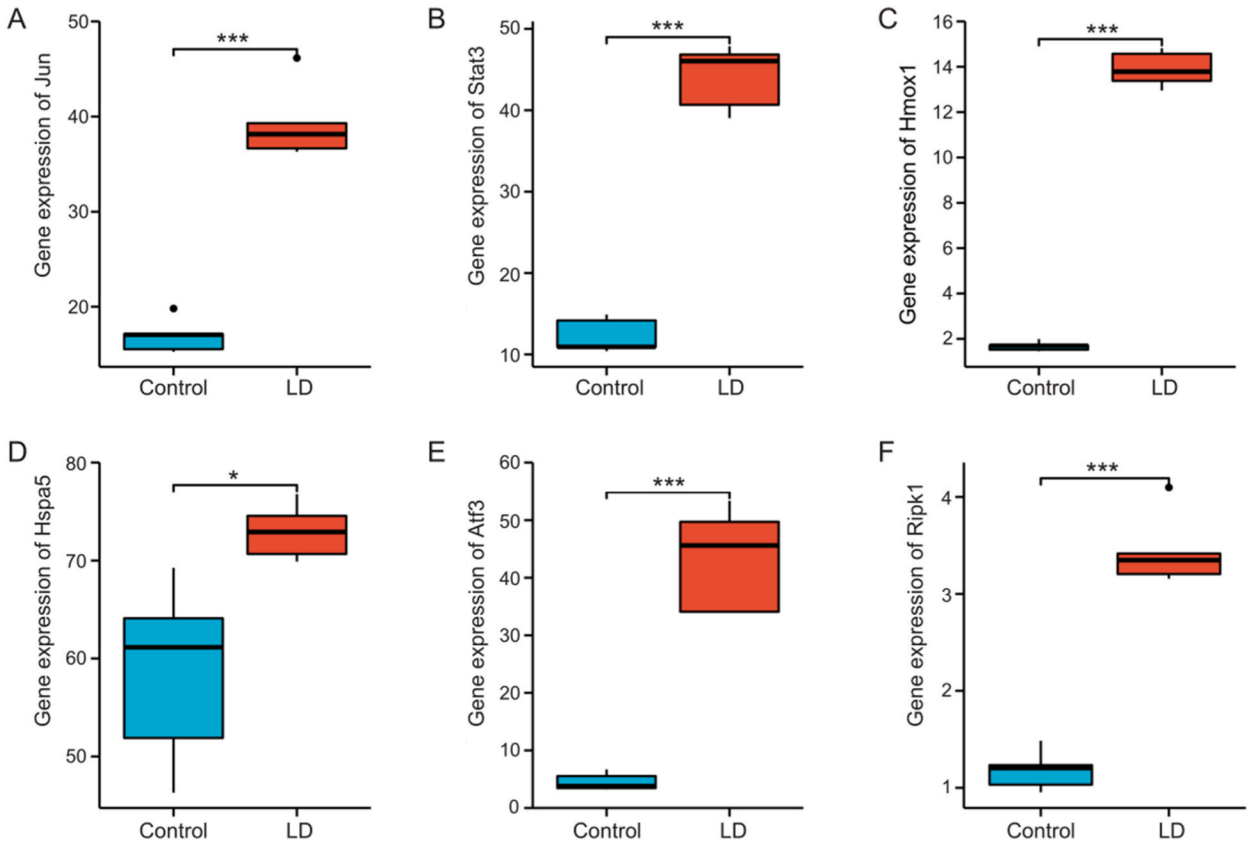

Fig. 6. Verification of the six HFRGs by a dataset of the GEO database. A-F Verification by the GSE146176 dataset. Compared with normal control samples, all of the six HFRGs are significantly unregulated in light damage retinas. HFRGs, hub ferroptosis-related genes; LD, light damage; *P < 0.05, **P < 0.01,***P < 0.001.

and cytosolic DNA-sensing pathway. Furthermore, by constructing a PPI network analysis, we identified six hub ferroptosis-related genes, namely, Jun, Stat3, Hmox1, Atf3, Hspa5 and Ripk1, and subsequently predicted the TFs, miRNAs, and molecular compounds drugs interacting with the six HFRGs. Finally, expression changes of the six HFRGs derived from the GSE146176 dataset proved the accuracy of our analysis.

Signal transducer and activator of transcription 3 (Stat3), a major nuclear transcription factor, belongs to STAT family composed with seven members [35].

It has been shown that the JAK/STAT3 signaling pathway may take part in regulating signaling transduction from Müller cells to photoreceptors [36]. A study of proteomic profiling illustrates that the expression of Stat3 in retinas of Rd10 mice is significantly increased when compared with that in the age matched wild-type individuals [37]. By intravitreal injection of zeaxanthin dipalmitate, a wolfberry-derived carotenoi, the activation of Stat3 and chemokine (C–C motif) ligand 2 (CCL2) can be significantly reduced in Rd10 mice, meanwhile, the improved visual behavior and the delayed photoreceptor degeneration are observed [38]. We also found that the expression of Stat3 was unregulated in the degeneration retinas induced by light damage suggesting its vital role in pathogenesis of retinal degeneration, and using Stat3 inhibitors maybe a latent strategy to delay the progress of retinal degeneration.

HO-1, encoded by the Heme oxygenase-1 (Hmox1) gene, is a key rate-limiting enzyme and plays an important role against oxidative stress and inflammatory activities [39]. Recently, Tang et al. revealed that ferroptosis mediated by HO-1 was involved in the degeneration of retinal pigment epithelium cell (RPE) in the sodium iodate-induced oxidative stress model. The RPE ferroptosis can be significantly blocked by down-regulating HO-1. In addition, using zinc protoporphyrin-9, a HO-1 inhibitor, effectively rescued RPE degeneration and promoted recovery of retinal structure and visual function in vivo [40]. In another study, Li et al. (2021) demonstrated that high dose of Hmox1 was associated with induced expression of DNA damage inducible transcript 3 (Ddit3) and increased endoplasmic reticulum stress associating with rhodopsin mislocalization [41]. In line with these studies, Hmox1 was also found to be a hub ferroptosis related gene in the light damage retinas, hence, we suggested that it may be provided as a novel therapeutic target for retinal degeneration.

Atf3 belongs to the family of basic leucine zipper transcription factors, which plays important biological roles in many different tissues [42,43]. Generally, the basal expression of Atf3 is low in most cell type, however, when responses to various stress conditions, its expression can be rapidly elevated [44]. Kehat et al. (2007) found that high level of acetylcholinesterase (AChE) was involved in the induction of photoreceptor degeneration [45]. The expression of AChE can be directly down-regulated by Atf3 via binding to AChE promoter [46]. Undergoing the photic-stress in the albino mice, the apoptosis almost exclusively displays in the retinal outer nuclear layer, where shows prominent levels of AChE and lack of Atf3, meanwhile, the retinal inter nuclear layer is characterized with high levels of Atf3 and absence of AChE as well as lack of apoptosis, which suggests a protective role of Atf3 in repressing process of retinal degeneration [46].

The heat shock 70 kDa protein 5 (Hspa5) is a member of chaperones located in endoplasmic reticulum (ER) and plays an important role in cell survival by regulating the unfolded protein response [47]. Recent a study showed that the Hspa5 transcript was significantly increased in the retinas of homozygous $Dpagt1^{tvrm76}$ mice model characterized with photoreceptor degeneration [48]. Receptor interacting protein kinase 1 (Ripk1), a key role of mediating cell death and inflammation, is a crucial therapeutic target for neurodegenerative diseases [49]. An imbalanced expression of Ripk1 and Ripk3 were observed in rd10 mouse retinas at postnasal 23 day, meanwhile, both of the two molecules were obviously up-regulated in the retina of P23H rhodopsin mutant rat model [50,51]. Consistent with these studies, Hspa5 and Ripk1 also showed abnormal pattern of expression in the light exposure retinas suggesting that they may attribute to the pathogenesis of retinal degeneration.

MicroRNAs (miRNAs), a kind of small non-coding evolutionarily conserved molecules, have emerged as important regulators in the retinal development and pathogenesis of retinal degeneration [52–54]. In this study, totally116 potential miRNAs were predicted, and 9 of them showed capable of interacting with three or more numbers of HFRGs, including miR-124-3p and miRNA-21a-5p. The miR-124-3p is one of most highly expressed miRNAs in the human retina [55]. Wang et al. found that the expression of Stat3 significantly increased in C57BL/6J mouse retinas when miR-124-3p was knockdown via intravitreal injection of miR-124-3p antagomir, which suggests that Stat3 is directly regulated by miR-124-3p [56]. A study of oxygen-induced retinopathy found that the expression of miR-21a-5p can be highly evoked in mice [57]. Transcription factors are key regulators of gene expression by binding to specific DNA sequence, which are involved in several biological processes and signaling pathways. Some identified TFs in this study such as Smc3, Ets1, Ep300, Stat3 and Atf3 have been shown to attribute to the pathogenesis of retinal degeneration [38,46,58–60]. We also examined multiple previously unrecognized TFs, which may provide new candidates for future mechanistic and therapeutic study of retinal degeneration.

Another finding of our study was that several potential therapeutic compounds interacting to the six HFRGs were identified, for example the Bortezomib and estradiol. Bortezomib is an FDA-approved proteasome inhibitor in the treatment of multiple myeloma via blocking NF-κB activation [61]. Experiments in vitro and vivo suggest that Bortezomib has a potential therapeutic effect in treating retinitis pigmentosa and choroidal neovascularization [62,63]. 17β-estradiol (E2) is an important steroid hormone, which has been recognized as a strong antioxidant in the pathogenesis of several central nervous system diseases [64,65]. A recent study illustrates the protective effect of 17β-E2 against light-induced retinal damage in rat by regulating NRF2/ARE signaling pathway [66].

It is well known that traditional gene therapy is a potential intervention for retinal neurodegenerative diseases including retinitis pigmentosa, however it may has some logistical and financial limitations such as typical genetic heterogeneity and mutations in larger genes untreatable by AAV-mediated gene supplementation. Recently, Xu et al. (2021) demonstrated that treatment with ferrostatin-1, a small molecule inhibiting ferroptosis, led to a substantial decrease in photoreceptor cell death and a significant preservation of both the retinal structure and function in the light exposure rat model of retinal degeneration [67]. As such, anti-ferroptosis shows a great potential in treating retinal neurodegenerative disorders, and the identified HFRGs in our study may provide novel targets for future

therapeutic study.

Inevitably, our study had some limitations. Because of the small samples for the bioinformatics analyses, the accuracy of the result may be affected to some extent, moreover, our study was a retrospective study and lacked of experiment verification. It is desirable to confirm our findings by carrying out experiments in vitro and in vivo with a large sample size to study the functional mechanism in detail in the future.

5. Conclusion

In summary, our study identified six hub ferroptosis-related genes — Jun, Stat3, Hmox1, Atf3, Hspa5 and Ripk1— in retinal degeneration mice induced by light damage through a comprehensive bioinformatics analysis. We also revealed some miRNAs, TFs and several potential target drugs that may interact with the six HFRGs. Our findings may provide potential therapeutic targets and a deeper understanding of the ferroptosis regulatory mechanism underlying retinal neurodegenerative disease.

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Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Xin-Lan Lei: Data curation, Investigation, Software, Writing – original draft, Formal analysis, Methodology. **Qiao-Li Yang:** Data curation, Formal analysis, Software, Methodology, Validation. **Yong-Zhao Wei:** Data curation, Formal analysis, Visualization, Methodology. **Xu Qiu:** Software, Visualization. **Hui-Yi Zeng:** Software, Visualization. **Ai-Min Yan:** Software, Visualization. **Kai Peng:** Software, Visualization. **Ying-Lin Li:** Software, Visualization. **Feng-Qin Rao:** Visualization. **Feng-Hua Chen:** Conceptualization, Writing – review & editing. **Lue Xiang:** Conceptualization, Writing – review & editing. **Kun-Chao Wu:** Conceptualization, Data curation, Funding acquisition, Writing – original draft, Writing – review & editing, Methodology, Project administration, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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