Hsp90-associated DNA replication checkpoint protein and proteasome-subunit components are involved in the age-related macular degeneration

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Abstract

Background: Age-related macular degeneration (AMD) is the leading cause of vision loss worldwide. However, the mechanisms involved in the development and progression of AMD are poorly delineated. We aimed to explore the critical genes involved in the progression of AMD.

Methods: The differentially expressed genes (DEGs) in AMD retinal pigment epithelial (RPE)/choroid tissues were identified using the microarray datasets GSE99248 and GSE125564, which were downloaded from the gene expression omnibus database. The overlapping DEGs from the two datasets were screened to identify DEG-related biological pathways using gene ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses. The hub genes were identified from these DEGs through protein-protein interaction network analyses. The expression levels of hub genes were evaluated by quantitative real-time polymerase chain reaction following the induction of senescence in ARPE-19 with FK866. Following the identification of AMD-related key genes, the potential small molecule compounds targeting the key genes were predicted by PharmacoDB. Finally, a microRNA-gene interaction network was constructed.

Results: Microarray analyses identified 174 DEGs in the AMD RPE compared to the healthy RPE samples. These DEGs were primarily enriched in the pathways involved in the regulation of DNA replication, cell cycle, and proteasome-mediated protein polyubiquitination. Among the top ten hub genes, *HSP90AA1*, *CHEK1*, *PSMA4*, *PSMD4*, and *PSMD8* were upregulated in the senescent ARPE-19 cells. Additionally, the drugs targeting HSP90AA1, CHEK1, and PSMA4 were identified. We hypothesize that Hsa-miR-16-5p might target four out of the five key DEGs in the AMD RPE.

Conclusions: Based on our findings, HSP90AA1 is likely to be a central gene controlling the DNA replication and proteasome-mediated polyubiquitination during the RPE senescence observed in the progression of AMD. Targeting *HSP90AA1*, *CHEK1*, *PSMA4*, *PSMD4*, and/or *PSMD8* genes through specific miRNAs or small molecules might potentially alleviate the progression of AMD through attenuating RPE senescence.

Keywords: Age-related macular degeneration; Retinal pigment epithelium; Cell senescence; HSP90AA1; DNA damage checkpoint; Proteasomal subunit components

Introduction

Age-related macular degeneration (AMD) is a disease that affects the macular region of the retina and causes progressive loss of central vision. It is the third leading cause of severe irreversible vision loss worldwide.[1] AMD is clinically classified as early-stage and late-stage. The early-stage AMD is characterized by the accumulation of medium-sized drusen, yellow deposits which form under the retina, and abnormalities of the retinal pigment epithelium (RPE). The late-stage AMD is defined by the presence of choroidal neovascularization (CNV, neovascular or wet AMD) or geographic atrophy (GA, atrophic or dry AMD) in the Beckman classification.[2] Late AMD results in severe and permanent vision impairment and blindness. The global prevalence of AMD has been steadily increasing over the past years and is expected to reach 288 million by 2040.[3] Although the incidence of vision impairment and blindness caused by

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001773

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Chinese Medical Journal 2021;134(19)
Received: 18-05-2021 **Edited by:** Yan-Jie Yin and Xiu-Yuan Hao

AMD have decreased since the introduction of vascular endothelial growth factor (VEGF)-targeted therapies,[4] no proven therapies for atrophic disease are currently available. Thus, novel regenerative therapies are required to attenuate the progression of neovascular AMD and GA.

There are several risk factors and biomarkers associated with AMD. Age is an important risk factor for AMD, since nearly all the late AMD cases have been reported in people aged >60 years.[1] Additionally, smoking and diet are known risk factors for AMD.[5] Stress or tissue damage, inflammation, immune response, and pathological angiogenesis have been found to cause AMD through driving CNV.[6,7] However, the exact etiology of AMD remains unclear. Elucidation of the molecular mechanisms involved in the development and progression of AMD will provide new strategies for AMD treatment.

The RPE plays an essential role in maintaining normal retinal health through protecting the retina from systemic insults.[8] RPE acts as a physiological barrier between the photoreceptor cells and the choroidal blood supply, facilitating the delivery of nutrients and ions by the choroidal blood and the transport of photoreceptor-derived waste products to the choroid.[9] The onset of AMD is characterized by initial damage to RPE, resulting in subsequent loss of photoreceptor cells over time.[10] The senescence of RPE induced by the depletion of nicotinamide adenine dinucleotide (NAD^+) has been found to play a critical role in the progression of AMD.[8] Phenotypic and functional studies on cultured human RPE cells have found that impaired autophagy contributes to the development of AMD.[11] Consistent with the important role of the RPE in AMD, gene mutations or gene expression alterations in RPE have been shown to contribute to the pathogenesis of AMD. Although the expression profiles of RPE signature genes in mouse and human tissues have been characterized,[12] large-scale functional genomic analysis of the RPE from AMD patients and healthy subjects is still limited.

Given that it is rather difficult to get the RPE biopsies from either AMD patients or healthy people, pre-existing high-throughput gene expression microarray databases, and RNA sequencing from clinical biopsies provide us precious resources to explore the molecular changes in AMD.[13,14] Therefore, we pooled the existing high-throughput gene expression data from RPE/choroid of human AMD and healthy controls to evaluate the differential gene expression and the potential biological pathways involved in the development of AMD.

In the present study, we have downloaded two datasets, GSE99248 and GSE125564, from gene expression omnibus (GEO) database and identified the differentially expressed genes (DEGs) in the RPE from AMD samples and healthy controls. Gene Ontology (GO) enrichment analysis, protein-protein interaction (PPI) network construction, and hub gene analysis were conducted to uncover the key genes involved in the development of AMD. We further examined the expression levels of the top ten hub genes in human RPE cell line, ARPE-19. The key genes altered in the AMD were determined following the induction of senescence in ARPE-19 with FK866, a highly specific inhibitor of nicotinamide phosphoribosyl transferase (NAMPT). Thereafter, we constructed the key gene-miRNA interaction and the key gene-drug interaction networks to explore potential strategies for attenuating the progression of AMD.

Methods

Cell culture and FK866 treatment

ARPE-19 cells were obtained from the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. ARPE-19 cells were grown in DMEM/F12 medium supplemented with 10% fetal bovine serum. For FK866 (Sigma-Aldrich; St Louis, MO, USA) treatment, ARPE-19 cells were serum starved overnight and treated with different doses (0.01–10 μmol/L) of FK866 as described previously.[15] Cells were collected at different time intervals (24, 48, and 72 h) after FK866 treatment and total RNA was extracted for the evaluation of hub gene expression.

Quantitative real-time polymerase chain reaction (RT-qPCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) from FK866-treated cells. Reverse transcription was performed to synthesize cDNA with 2 μg of total RNA using the Superscript First-Strand Synthesis System (Invitrogen). RT-qPCR was performed to evaluate the expression of the hub genes. The primer sequences for PCR are shown in Supplementary Table S1, http://links.lww.com/CM9/A769.

Data collection

The gene expression profiles (GSE99248)[16] of 16 eye samples obtained from the AMD donors and 15 healthy control samples were downloaded from the GEO (http://www.ncbi.nlm.nih.gov/geo/) database. The sequencing was previously performed on the GPL11154 Illumina HiSeq 2000 platform (Illumina, San Diego, USA). The mRNA profile for human AMD samples (GSE125564)[17] is available on the GEO database derived from platform GPL23159 (The Human Affymetrix Clariom S Assay).

Identification of DEGs

The Limma package, a prominent tool for analyzing microarray and RNA-Seq data in R software,[18] was used to detect differentially expressed mRNAs by calculating the adjusted *P* value (adj. *P*) and the absolute log value of fold change (log|FC|) after extracting the gene expression data from the AMD and control groups. Cut-off values of adj. $P < 0.05$ and $\log|FC| > 1$ were set to screen the DEGs as described previously.[19] The DEGs from GSE99248 and GSE125564 were pooled to select the common DEGs.

GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

GO and KEGG pathway enrichment analyses of the common DEGs between GSE99248 and GSE125564 were conducted using the Database for Annotation, Visualization, and Integrated Discovery (DAVID, https://david.

ncifcrf.gov/).[20] The biological entities and pathways exhibiting $P < 0.05$ were regarded as statistically significant in GO analysis, including biological processes (BP), cellular components (CC), and molecular functions (MF) were used for pathway analysis. The GO enrichment analysis and KEGG pathway analysis results were visualized using the GO plot package.[21]

PPI network and hub gene analyses

To analyze PPI network, we first submitted the DEGs to Search Tool for the Retrieval of Interacting Genes (STRING) v11 (https://string-db.org/),[22] an online database which contains comprehensive information on multiple proteins. The confidence score ≥0.4 was used as the cut-off criteria for selecting significant PPI, yielding PPIs of DEGs as shown in Supplementary Table S2, http://links.lww.com/CM9/A769. The PPI networks of DEGs were visualized using Cytoscape software (http://www.cytoscape.org/) (version 3.7.1).[23] To identify the critical DEGs among PPIs, the cytoHubba plug-in was used to calculate the topological properties, including node degree, betweenness, and stress. Finally, the hub genes were selected according to the node degree and the interaction network of DEGs and visualized using Cytoscape software (version 3.7.1).

Construction of target gene-miRNA regulatory network

The regulatory relationships between target genes and miRNAs were identified using online tool miRNet (https://www.mirnet.ca/).[24] The target gene-miRNA regulatory network was visualized using Cytoscape (http://www.cytoscape.org/).

Drug prediction

The data for netrin family-related drugs were downloaded from PharmacoDB (https://pharmacodb.pmgenomics.ca/).[25] The target gene-drug regulatory network was visualized using Cytoscape (http://www.cytoscape.org/).

Results

Identification of DEGs

To uncover novel molecules involved in AMD, we first analyzed the DEGs between the AMD RPE and healthy samples from datasets GSE99248 and GSE125524 using the cut-off values of adj. $P < 0.05$ and $\log|FC| > 1$ as previously described.[19] As shown in the volcano plot of GSE99248, 3671 DEGs, including 2873 upregulated and 798 downregulated genes, were identified in the RPE samples from AMD patients compared with those from the healthy controls [Figure 1A]. From GSE125524, 802 DEGs, including 313 upregulated and 489 downregulated genes, were identified in the AMD RPE samples [Figure 1B]. By merging these two DEG datasets, 174 common genes were obtained [Figure 1C].

GO and KEGG pathway enrichment analyses

Thereafter, the GO and KEGG pathway enrichment analyses were conducted on the selected 174 overlapping genes using the DAVID database. The BP extracted from the GO analysis showed that the DEGs were mainly enriched in the regulation of protein polyubiquitination, mitotic cell cycle regulation, and cellular amino acid metabolism [Figure 2A]. The top three enriched CC were nucleoplasm, mitochondrial membrane, and cytosol [Figure 2B]. The MF analysis showed that the top three enriched functions were protein binding, poly-A RNA binding, and DNA helicase activity [Figure 2C]. We further conducted the KEGG pathway enrichment analysis with the 174 DEGs. It showed that the critical pathways were enriched in the DNA replication, cell cycle regulation, and proteasomal degradation [Figure 2D]. Integration of both GO and KEGG analyses indicated that the AMD-related genes might primarily function in the DNA replication-associated cell cycle regulation and proteasome-mediated protein polyubiquitination.

PPI network and hub gene analysis

To further confirm the key biological pathways involved in the AMD, PPI network was constructed to explore the potential interactions between the 174 DEGs using STRING database and Cytoscape. The PPI networks are shown in the Supplementary Table S2, http://links.lww.com/CM9/A769. The hub genes with top ten node degrees in the PPI network were *HSP90AA1* (degree = 24), pre-mRNA processing factor 19 (*PRPF19*) (degree = 18), *PSMD4* (degree = 17), *PSMD8* (degree = 17), *PSMA4* (degree = 17), *CHEK1* (degree = 16), *SF3B5* (degree = 16), *CCT7* (degree = 17), *PSMB5* (degree = 15), and *PSMB6* (degree = 15) [Figure 3A and Table 1]. The expression levels of these top ten hub genes in the AMD and healthy RPE samples were plotted from GSE99248 and GSE125524. As shown Figure 3B and 3C, most of these genes were upregulated to different extents in the AMD RPEs compared with healthy samples. With the exception that *HSP90AA1* has been found to be involved in AMD,[26] the remaining nine hub genes were not directly related to AMD. It is of interest that five out of these nine hub genes, including *PSMD4*, *PSMD8*, *PSMA4*, *PSMB5*, and *PSMB6*, are functionally related with proteasomal function [Figure 2D], suggesting that proteasome-mediated protein degradation might play an essential role in the development of AMD. Additionally, the hub gene *CHEK1* plays a key role in the DNA replication checkpoint of cell cycle,[27] suggesting that DNA replication checkpoint might be super-activated in the AMD development.

Expression of five hub genes is upregulated in RPE cellular senescence

Since the senescence of RPE cells is critical to the initiation and progression of AMD and ARPE-19 cell line structurally and functionally possesses the properties of RPE cells *in vivo*,[28] we induced senescence in ARPE-19 cell line and evaluated the expression of the top ten hub genes. Senescence was induced with FK866, a selective NAMPT inhibitor, thereby reducing the NAD^+ levels in ARPE-19 cells, as described previously.[8] The mRNA levels of the AMD-related top ten hub genes were evaluated in the FK866-treated ARPE-19 cells. We showed

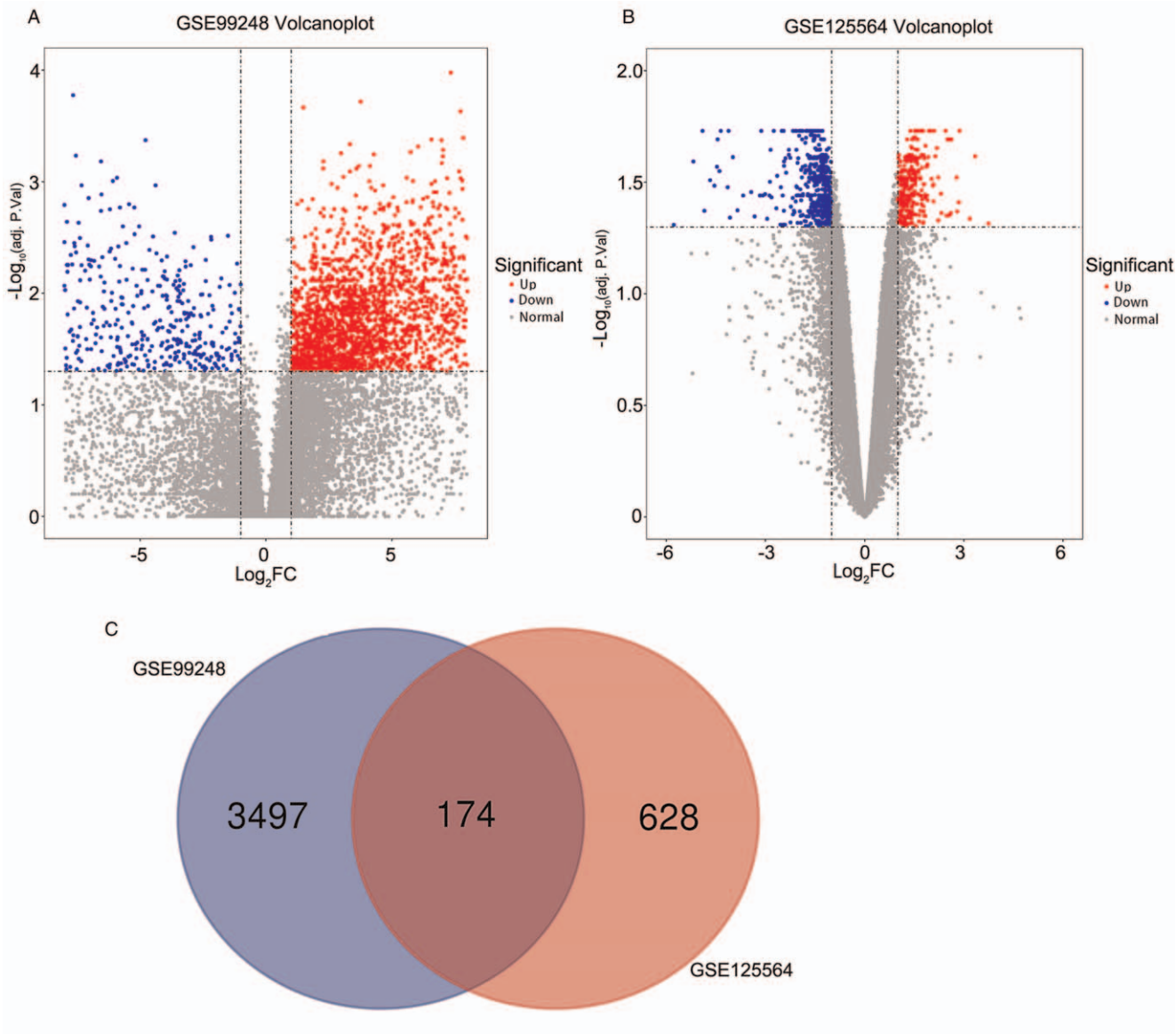

Figure 1: Volcano plots of DEGs identified from datasets GSE99248 and GSE125564. (A) DEGs from GSE99248; (B) DE-mRNAs from GSE125564; (C) The Venn diagrams of overlapping DEGs from GSE99248 and GSE125564. A total of 174 overlapping genes were identified. DEGs: Differentially expressed genes; DE-mRNAs: Differentially expressed mRNAs.

that the expression levels of *HSP90AA1*, *CHEK1*, *PSMA4*, *PSMD4*, and *PSMD8* were upregulated when ARPE-19 cells were treated with 10 μmol/L FK866 [Figure 4A], indicating that these genes may play key roles in the development of AMD. To further confirm the altered expression of these AMD-related key genes, we evaluated the mRNA levels of these genes at different time points upon FK866 treatment. As shown in Figure 4B–F, *HSP90AA1*, *CHEK1*, *PSMD4*, *PSMD8*, and *PSMA4* were upregulated at different time points upon FK866 treatment, confirming that these genes were indeed AMD-related key genes functioning in the RPE senescence.

HSP90AA1 might play a central role in RPE cellular senescence

It has been found that FK866 induces senescence in ARPE-19 cells with a broad range of dose from 0.001 to 10 μmol/L. We wanted to explore whether the AMD-related key genes respond to the low dose of FK866. We showed that only *HSP90AA1* was upregulated at low dose (0.1 μmol/L) of FK866, while *CHEK1*, *PSMD4*, *PSMD8*, and *PSMA4* were upregulated at the dose of 1.0 μmol/L FK866 or above [Figure 5A–E], indicating that *HSP90AA1* might be the most sensitive gene to senescence induction and potentially a central regulator of the RPE senescence. We thereafter analyzed the interaction among the AMD-related key genes. As shown in Figure 5F, *HSP90AA1* was indeed at the center of the interaction network.

Prediction of drugs targeting AMD-related key genes

To predict small molecule drugs targeting the AMD-related key genes, we downloaded the list of cancer drugs related to these genes from the cancer pharmacogenomics research database PharmacoDB49 [Figure 5G]. Certain

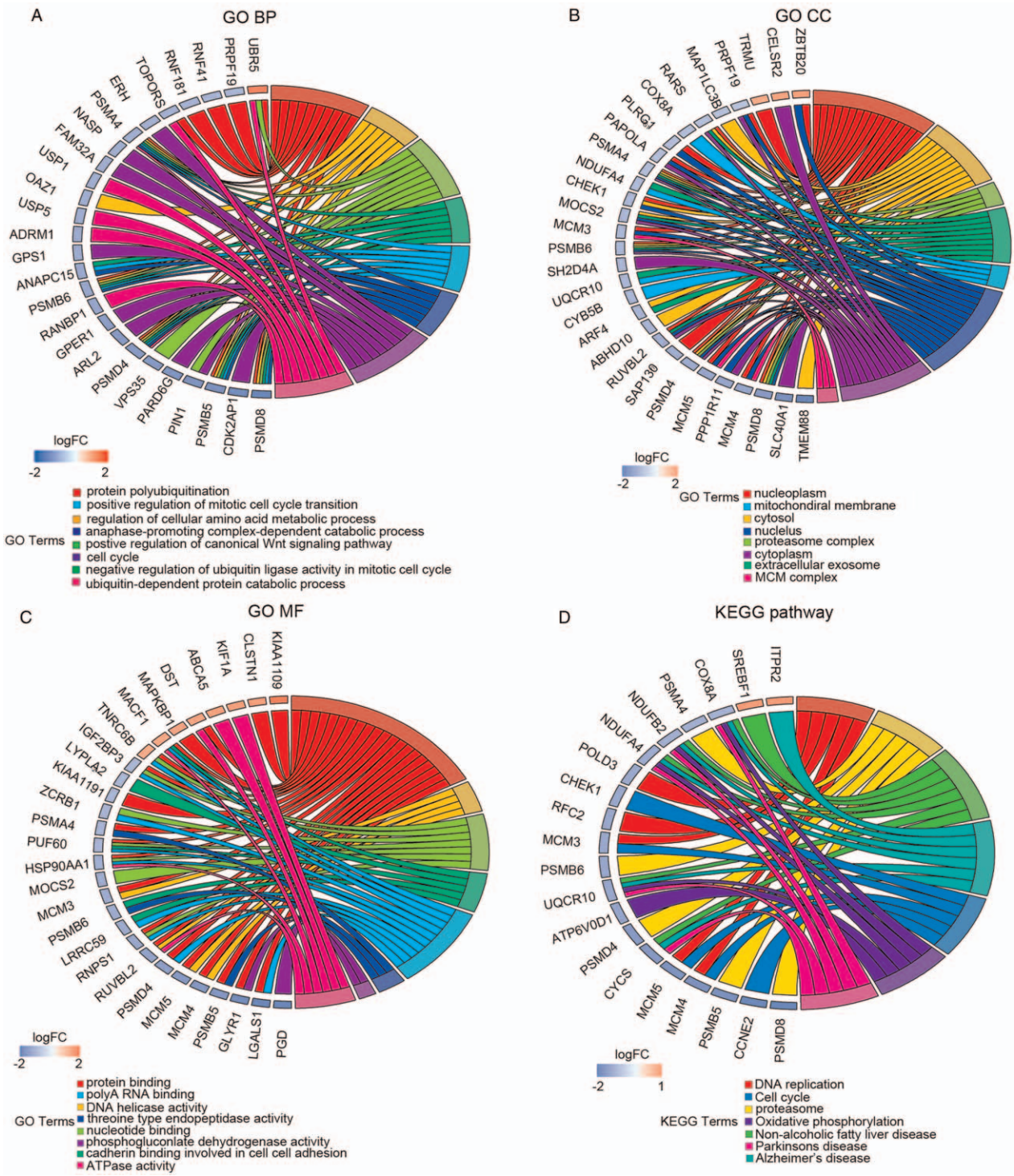

Figure 2: Molecular function and pathway enrichment analyses for the 174 overlapping genes. (A) Biological process (BP); (B) cellular component (CC); (C) molecular function (MF); (D) Kyoto Encyclopedia of Genes and Genomes (KEGG).

compounds were found to target HSP90AA1 (Geldanamycin, CCT018159, AT13387, and SNX-2112) and CHEK1 (BX795, AZD7762, BX-912, and PD98059). Bortezomib was predicted to target PSMA4. No drugs were found to directly target PSMD4 and PSMD8. Among these drugs, Hsp90 inhibitors have been used in the clinical trials for AMD treatment.[26] BX795 is a multiple kinase inhibitor which suppresses the inflammatory response and is safe for eye treatment.[29,30] Bortezomib and a few other drugs have shown potential anti-tumor activities.[31]

Construction of target gene-miRNA regulatory network

Since gene expression is regulated by miRNAs, we next wanted to determine the specific miRNAs that could target all of the AMD-related key genes. We first analyzed the

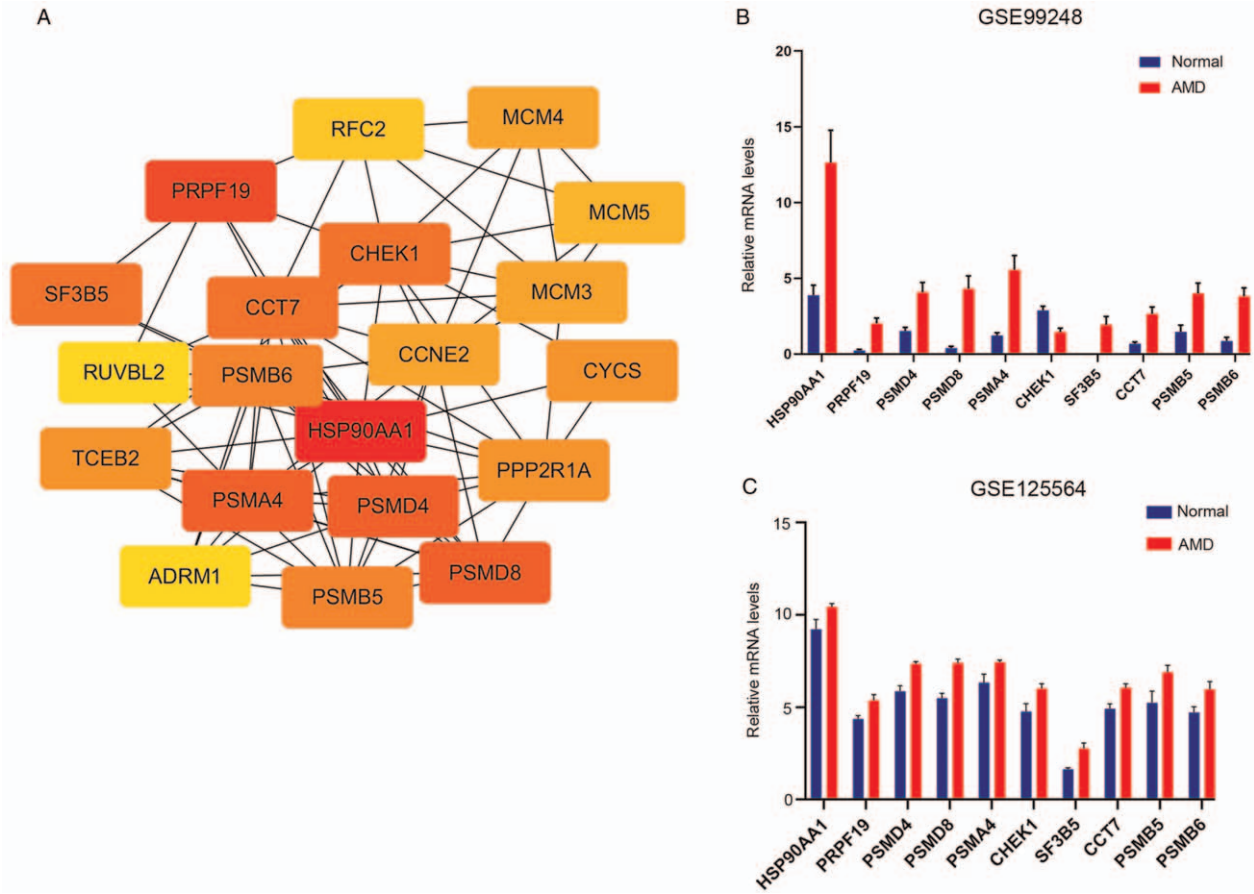

Figure 3: Identification of hub genes in the overlapping DEGs. (A) Visualization of PPI networks for the top 20 hub DEGs. (B) The expression levels of the top ten hub genes in GSE99248 dataset. (C) The expression levels of the top ten hub genes in GSE125564 dataset. DEGs: Differentially expressed genes; PPI: Protein-protein interaction.

Table 1: AMD-related top ten hub genes identified from cytoHubba analysis.

Node name	MCC	DMNC	MNC	Degree
HSP90AA1	2285	0.29055	23	24
PRPF19	795	0.29953	17	18
PSMD4	3612	0.43715	17	17
PSMD8	3612	0.43715	17	17
PSMA4	3629	0.50256	16	17
CHEK1	344	0.34999	16	16
SF3B5	740	0.41901	10	16
CCT7	1642	0.43918	14	16
PSMB5	3602	0.62588	13	15
PSMB6	1327	0.42792	14	15

DMNC: Density of Maximum Neighborhood Component; MCC: Maximal Clique Centrality; MNC: Maximum Neighborhood Component.

gene-miRNA network with single genes. *HSP90AA1*, *CHEK1*, *PSMD4*, *PSMD8*, and *PSMA4* were predicted to interact with several miRNAs [Supplementary Table S3, http://links.lww.com/CM9/A769; Figure 6A]. We further constructed the miRNA-gene network to screen the miRNAs simultaneously targeting two genes. As shown in Figure 6B, some miRNAs were found to target two of the five key genes, but the network still included abundant miRNAs. We thus constructed the miRNA-gene network with the miRNAs targeting more than three genes [Figure 6C]. Specifically, hsa-miR-16-5p was identified to interact with four out of the five key genes, including *HSP90AA1*, *CHEK1*, *PSMD4*, and *PSMD8*, predicting that hsa-miR-16-5p might potentially interfere with the progression of AMD through targeting the RPE senescence-related genes.

Discussion

AMD is a common retinal degenerative disease in older adults. It is reported that the occurrence of AMD is influenced by individual differences, environmental fac-

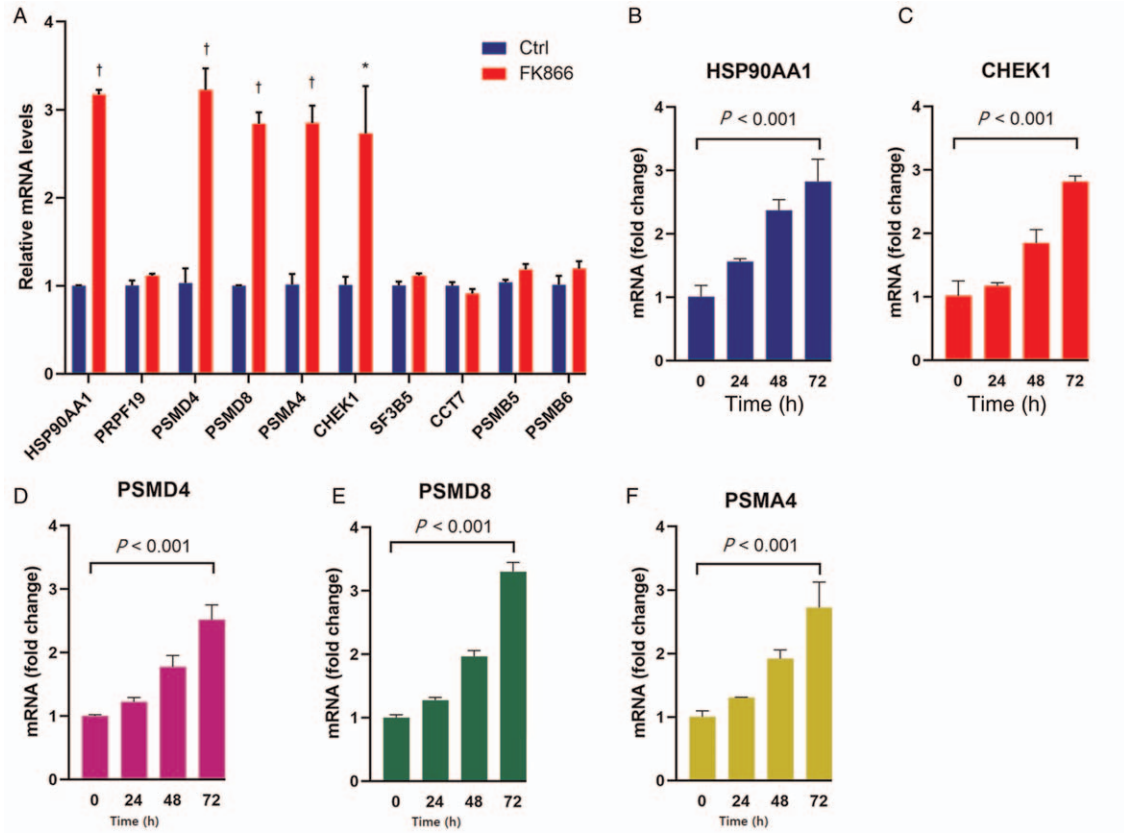

Figure 4: Five out of the top ten hub genes were upregulated in the FK866-treated ARPE-19 cells. (A) ARPE-19 cells were treated with 10 μmol/L FK866 for 72 h. Total RNA was extracted and RT-qPCR was performed to evaluate the expression levels of the top ten hub genes. $^{*}P < 0.05$, $^{\dagger}P < 0.001$. (B–F) ARPE-19 cells were treated with 10 μmol/L FK866 for 0, 24, 48, and 72 h. Total RNA was extracted and the expression levels of *HSP90AA1* (B), *CHEK1* (C), *PSMD4* (D), *PSMD8* (E), and *PSMA4* (F) were evaluated by RT-qPCR. RT-qPCR: Quantitative real-time polymerase chain reaction.

tors, and genetic factors. However, the risk for AMD is significantly elevated with increasing age. Currently, wet AMD is primarily treated with anti-VEGF drugs, whereas antioxidants are utilized for the management of dry AMD to attenuate the progression of symptoms.[32,33] Therefore, it is important to identify novel genes involved in the development of AMD, which can be targeted to provide more strategies for the treatment of AMD.

In the present study, we set out from database analysis to determine the AMD-related critical genes. Recently, RHO, PDE6A, 3′,5′-cyclic-GMP phosphodiesterase, and G protein alpha have been identified as AMD-related genes by canonical pathway analysis using the Ingenuity Pathway Analysis database.[34] We additionally identified *HSP90AA1*, *PRPF19*, *PSMD4*, *PSMD8*, *PSMA4*, *CHEK1*, *SF3B5*, *CCT7*, *PSMB5*, and *PSMB6* as the top ten hub genes associated with AMD. Importantly, in both of the analyzed cohorts, these genes were mostly upregulated in AMD RPE samples compared to healthy control samples. Thus, these genes are likely to promote the progression of AMD. *HSP90AA1*-encoded protein (Hsp90) has been found to be associated with AMD development.[26,35] However, it remains unknown whether the remaining nine hub genes are related to AMD. As cells age, the synthesis of NAD^+ gradually decreases and the resulting depletion of NAD^+ may cause certain ageing-related diseases, with some of them resulting in vision impairment.[8] FK866 is an inhibitor of NAMPT, a crucial enzyme catalyzing the synthesis of NAD^+.[8,15] Therefore, it is widely used to induce senescence in the RPE cells. We, therefore, evaluated the expression of the top ten hub genes in the FK866-treated ARPE-19 cells. We showed that *HSP90AA1*, *CHEK1*, *PSMD4*, *PSMD8*, and *PSMA4* were upregulated in the senescent ARPE-19 cells, indicating that these genes are AMD-related key genes. However, we cannot rule out the functions of *PRPF19*, *SF3B5*, *CCT7*, *PSMB5*, and *PSMB6* genes in the progression of AMD. PRPF19 is a key splicing factor involved in DNA damage response, ubiquitin-proteasome system, cell proliferation, and apoptosis.[36] Additionally, PRPF19 regulates p53-dependent cellular senescence through modulating *MDM4* mRNA splicing.[37] As a spliceosomal protein, SF3B5 might participate in the tumorigenesis and aging.[38,39] Therefore, PRPF19 and SF3B5 might contribute to the pathogenesis of AMD through regulating RPE aging. CCT7/TCP-1 ring complex is involved in the maturation of G protein-coupled receptors (GPCRs).[40] CCT7 might act in the AMD by participating in the GPCR-mediated signaling pathway. However, the precise functional role of PRPF19, SF3B5, and CCT7 in AMD needs to be investigated further. Additionally, the evaluation of hub gene expression in the primary RPE cells obtained from human donor eyes with different types of AMD will provide important functional insights.

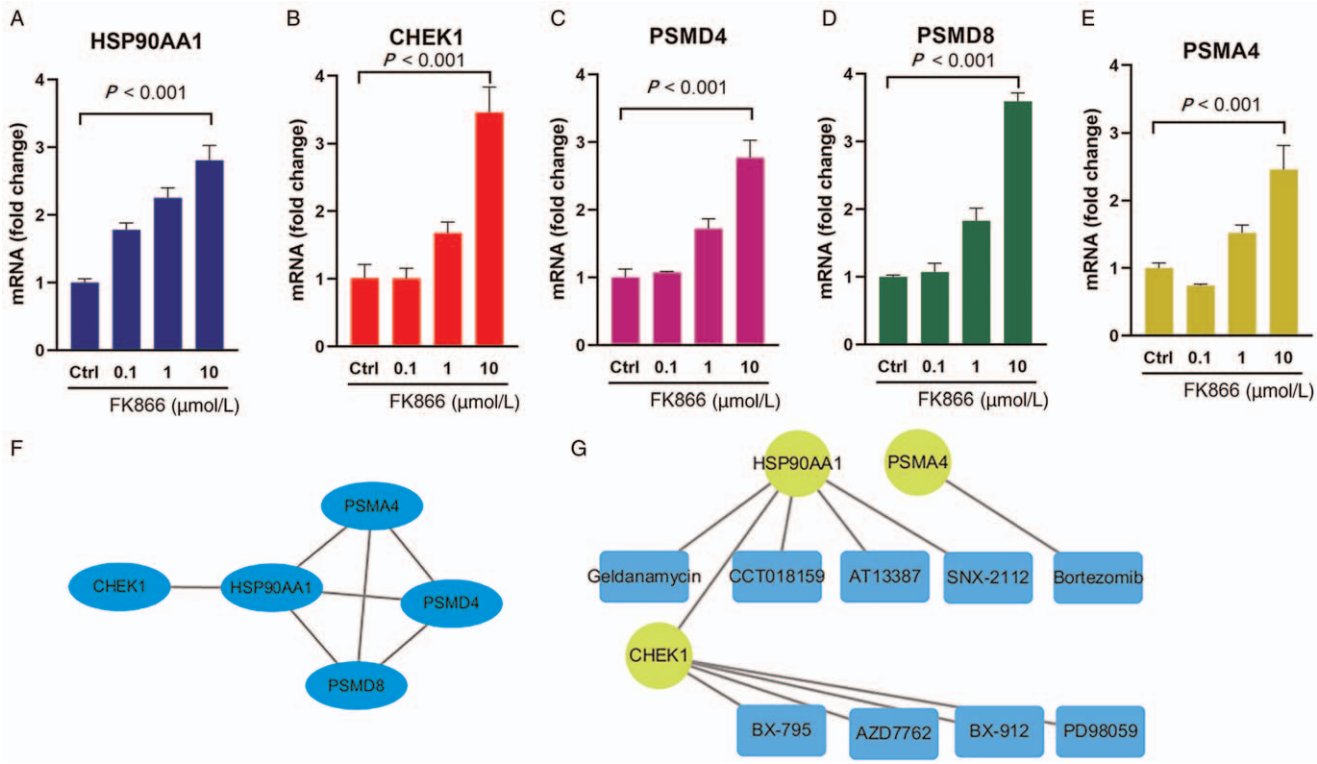

Figure 5: Interaction among the key DEGs and prediction of potential drugs targeting the critical DEGs. (A–E) ARPE-19 cells were treated with different doses of FK866 as indicated. Total RNA was extracted and the expression levels of the key genes were evaluated by RT-qPCR. F. The interactions between the proteins encoded by the indicated genes were analyzed by constructing a PPI network. (G) Potential drugs targeting the AMD-related key genes were predicted using PharmacoDB. HSP90AA1, CHEK1, and PSMA4 were found to be directly targeted by small molecules as indicated. AMD: Age-related macular degeneration; DEGs: Differentially expressed genes; PPI: Protein-protein interaction; RT-qPCR: Quantitative real-time polymerase chain reaction.

Among the proteins encoded by these AMD-related key genes, Hsp90 is a chaperone which is essential for the correct folding and stabilization of various cellular proteins. Importantly, Hsp90 is a potential modulator of cell senescence.[41] Hsp90 plays an essential roles in the retina and Hsp90 inhibitors such as geldanamycin, and its derivatives, such as 17-allylamino-17-demethoxy-geldanamycin and 17-dimethylaminoethylamino-17-demethoxygeldanamycin, can prevent retinal degeneration in the AMD models and have been used in AMD treatment clinical trials.[26] However, prolonged Hsp90 inhibition can also induce the degradation of Hsp90 client proteins, leading to photoreceptor cell death.[42] Thus, combinational therapy might alleviate the side effect by decreasing the dosage or the duration of Hsp90 inhibition. We found that CHEK1 interacts with HSP90AA1 and is upregulated in the senescent RPE cells. *CHEK1* encodes the cell cycle checkpoint kinase Chk1, which modulates aging through regulating the DNA replication.[43] Chk1 is an Hsp90 client and requires Hsp90 to acquire its kinase activity.[44] Thus, targeting Chk1 alone or in combination with Hsp90 inhibitors might attenuate the development of AMD through regulating cell senescence and reducing the dose or duration of Hsp90 inhibition to lower the toxicity on the photoreceptor cells. This hypothesis needs to be validated further.

We further analyzed the potential drugs targeting Hsp90 and Chk1. Among the potential Chk1 inhibitors, BX795 is known to suppress the inflammatory response and is safe for eye treatment.[29,30] Thus, BX795 possesses therapeutic potential in AMD treatment. The combination of Hsp90 inhibitors and potential Chk1 inhibitors could improve the AMD treatment landscape.

In addition to *HSP90AA1* and *CHEK1*, we identified proteasomal subunit components *PSMD4*, *PSMD8*, and *PSMA4* as AMD-related key genes. Since Hsp90 controls the spatial and temporal order of protein interactions and regulates the ubiquitin-mediated proteasomal degradation,[45] Hsp90 might interact with these proteasomal subunit components to regulate the RPE senescence. The *PSMD4*-encoded protein, proteasome 26S subunit non-ATPase 4, recruits phosphorylated and ubiquitinated nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα) to the proteasome, resulting in nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation and induction of cellular senescence.[46] PSMD8 is a 19S proteasomal regulatory complex subunit. We propose that Hsp90 might interact with PSMD4 and PSMD8 to regulate the degradation of misfolded proteins during AMD progression.

MicroRNAs play an important role in regulating the development of AMD.[47] *In vivo* studies found that dysregulated miRNAs might affect AMD progression by targeting genes involved in neurodegeneration and

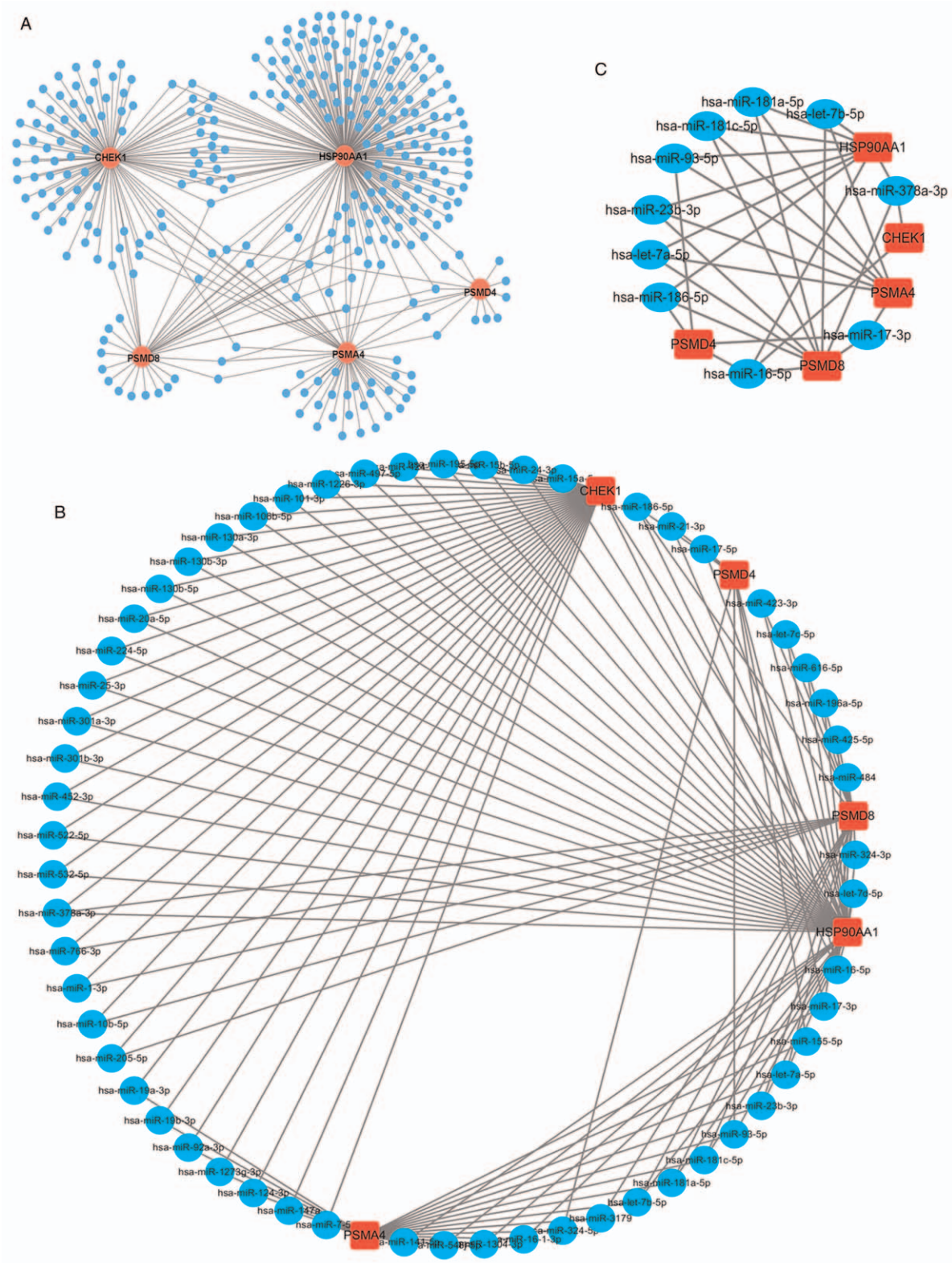

Figure 6: Constructions of the AMD-related gene-miRNA network. (A) Visualization of the indicated gene-miRNA networks. (B) MicroRNAs in (A) targeting two or more genes were selected for network analysis. (C) MicroRNAs in (A) targeting three or more genes were used for network analysis. AMD: Age-related macular degeneration.

inflammation.[48] As senescent cells cause inflammation by senescence-associated secretory phenotype, miRNAs that inhibit inflammation could be effective in the treatment of AMD. For instance, miRNA-191-5p ameliorates amyloid-β1-40-mediated RPE cell injury by suppressing the NLRP3 inflammation pathway.[49] In this study, we found that miR-16-5p targeted four of the AMD-associated key genes, including *HSP90AA1*, *CHEK1*, *PSMD4*, and

PSMD8. Previous studies revealed that miR-16-5p is associated with the regulation of inflammation.[50] Therefore, miR-16-5p might regulate the senescent RPE-induced inflammation and retard the progression of AMD. However, the specific mechanism underlying the role of miR-16-5p in AMD progression requires further investigation.

Acknowledgements

This study was supported by grant from the National Natural Science Foundation of China (No. 81670841).

Conflicts of interest

None.

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How to cite this article: Xing C, Liu XF, Zhang CF, Yang L. Hsp90-associated DNA replication checkpoint protein and proteasome-subunit components are involved in the age-related macular degeneration. Chin Med J 2021;134:2322–2332. doi: 10.1097/CM9.0000000000001773