Original Article

Identification of Novel Potential miRNA and mRNA Biomarkers in High Myopia Using Systems Biology Approaches

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Abstract

Background: Myopia, also known as shortsightedness, is a common eye disorder characterized by a refractive error of the eye. It is estimated that by 2050, over 1 billion people will be affected by myopia. Recent studies have shown that miRNAs play a significant role in the genetic factors contributing to myopia. However, the current understanding of the number and role of certain miRNAs in myopia is limited. This study aims to identify unknown miRNAs and their involvement in high myopia.

Materials and Methods: The raw dataset (GSE142359), consisting of miRNAs, was obtained from the GEO database. The dataset includes 10 samples, 5 from patients with high myopia and 5 from controls. Differentially expressed genes were identified using the EdgeR package in R software. The most significant miRNAs were then entered as input into the miRWalk web tool and target genes were identified. Subsequently, the PPI network was extracted using STRING and 10 clusters were identified. After that, we selected the most related cluster with myopia. Finally, we used DGIdb to identify candidate drugs for treatment of high myopia.

Results: A total of 136 miRNAs were identified as significant differentially expressed miRNAs (DEmiRNAs). Then, we constructed miRNA-mRNA bipartite network with 564 interactions including 490 genes and 50 miRNAs. Afterwards, 5 miRNAs including: hsa-miR-195-5p, hsa-miR-24-3p, hsa-miR-16-5p, hsa-miR-181b-5p, and hsa-miR-130a-3p and 8 proteins including ESR1, BCL2, VEGFA, FGF2, MAPK14, H2AX, APP, and MAP3K10 were identified. Finally, two new miRNAs including hsa-miR-195-5p and hsa-miR-24-3p, and three novel proteins including: ESR1, BCL2, and MAPK14 were proposed as potential biomarker in high myopia.

Conclusion: This study revealed new miRNAs previously unknown in highly myopic eyes through bioinformatics analysis. The identified candidate miRNAs and their related drug targets and genes can serve as potential biomarkers, improving the understanding of new therapeutic targets for highly myopic eyes.

Keywords: Myopia; NGS Data; MicroRNA; Differential Gene Expression; Drug- Gene Network.

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Introduction

Myopia, also known as nearsightedness, is one of the leading causes of visual impairment, affecting nearly 163 million people worldwide [1]. Its prevalence is projected to increase to 9.8 % of the world population, or approximately 1 billion people by 2050 [1-4]. Furthermore, myopia, the most prevalent form of refractive error, is becoming a major cause of blindness globally. Myopia can be classified into common myopia (with a refractive error less than 6 diopters) and high myopia (with a refractive error equal to or more than 6 diopters). High myopia is considered a syndromic form of myopia, as it is associated with other eye diseases such as cataracts, glaucoma, and neovascular membranes [5]. Both genetic factors and environmental factors play a vital role in causing myopia [6] Yi </ author >< author > Li, Weiran < /author >< author > Zhu, Dongqing </ author >< author > Zhou, Jibo </ author ></ authors ></ contributors >< titles >< title > microRNA profiling in the aqueous humor of highly myopic eyes using next generation sequencing < /title >< secondary-title > Experimental eye research </ secondary-title >< /titles >< periodical >< full-title > Experimental eye research </full-title >< /periodical >< pages > 108034 < / pages >< volume > 195 < /volume >< dates >< year > 2020 < /year >< /dates >< isbn > 0014-4835 < /isbn >< urls ></ urls ></ record ></ Cite ></ EndNote >.

miRNAs are small, endogenous, noncoding, single-stranded RNA molecules consisting of about 22 nucleotides [4, 7]. They play critical roles in the regulation of gene expression at the post-transcriptional level, impacting various biological processes such as proliferation, differentiation, apoptosis, developmental timing, and metabolism [4, 8]. miRNAs bind to complementary sequences in the 3' untranslated region (UTR) of target mRNAs, affecting the pathogenesis of various eye diseases through suppression or degradation of these molecules [9].

The role of microRNAs (miRNAs) in high myopia has been investigated using next-generation sequencing (NGS) [6] Yi </ author >< author > Li, Weiran </ author >< author > Zhu, Dongqing </ author >< author > Zhou, Jibo </ author ></ authors ></ contributors >< titles >< title > microRNA profiling in the aqueous humor of highly myopic eyes using next generation sequencing </ title >< secondary-title > Experimental eye research </ secondary-title ></ titles >< periodical >< full-title > Experimental eye research </ full-title ></ periodical >< pages > 108034 </ pages >< volume > 195 </ volume >< dates >< year > 2020 </ year ></ dates >< isbn > 0014-4835</ isbn >< urls ></ urls ></ record ></ Cite ></ EndNote >. In previous studies, for example, in [10], the high expression level of miR-328 in the peripheral circulation and the presence of miR-29a were related to high myopia. In [7], the role of PAX6 and its association with microRNA-328 in myopia was investigated. This study reported that the single nucleotide polymorphism (SNP) rs662702 in PAX6 is located in the microRNA-328 binding site, which causes susceptibility to high myopia. Also, in [11] miR-29a expression is significantly increased in the aqueous humor of highly myopic patients. This miRNA inhibits collagen type I synthesis in human scleral fibroblast cells and increases cell migration, however, it has no significant effect on cell proliferation.

MiRNAs in aqueous humor, an intraocular fluid that provides nutrients, are thought to play important roles in ocular diseases, particularly in highly myopic eyes [1, 6, 12]. The goal of this study was to uncover novel miRNAs involved in high myopia using miRNA-mRNA-drug

tripartite network, so that the candidate miRNAs can serve as new key biomarkers and improve our understanding of therapeutic targets for this disease.

Material and Methods

Dataset and pre-processing

The next generation sequencing (NGS) dataset of miRNAs (GSE142359), was obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo) and consisted of 10 samples, including 5 patients with high myopia and 5 cataract eyes used as controls at the onset of surgery. The miRNAs were obtained from aqueous humor, a fluid secreted by the epithelial cells of the body. At first, the raw miRNA data was normalized using the edgeR package in R software [13, 14]. The hierarchical clustering and heatmap analyses were performed for quality control and outlier removing [13, 14].

DEmiRNA analysis

A Differential Expression analysis was performed on miRNAs using the edgeR package (version 3.32.1). Differentially expressed miRNAs between myopic patients and control samples were selected using the following criteria:

1- Filtering genes with a False Discovery Rate (FDR) below 0.05.

2- Filtering genes with a Log Fold Change (LogFC) greater than 1 or less than -1.

The miRNAs that met these criteria were considered as significant DEmiRNAs.

miRNA-mRNA bipartite network

miRWalk (http://mirwalk.umm.uni-heidelberg.de/) [15] is publicly database that generates predicted and validated miRNA binding sites of some known organisms such as human, mouse, rat, dog, and cow genes. To identify related genes with our selected miRNAs, we used the miRWalk web tool. This tool helped us identify mRNAs that interact with our selected miRNAs. We just selected mRNAs that were reported experimentally.

Protein-Protein interaction network

We reconstructed the protein-protein interaction (PPI) network using the genes obtained in the previous section. We used STRING (https://string-db.org/) [16] to extract interaction between proteins. In STRING, the minimum required interaction score parameter was considered to be 0.9, and we selected only experimental interactions and excluded the rest of the interactions. Proteins in the generated network are represented as nodes and the interactions between these nodes are represented as edges. Then, we clustered the proteins using K-means clustering.

Functional enrichment analysis

ToppGene (https://toppgene.cchmc.org/) [17, 18], is a free web tool for (1) gene list functional enrichment (gene ontology, pathway, disease enrichment, and etc.), (2) candidate gene prioritization and (3) identification and prioritization of novel disease candidate. We used ToppGene to identify the enriched genes in all clusters. We selected a cluster as a candidate relative to other clusters whose genes were more significant in terms of adj-p value in the biological process, pathways and diseases associated with high myopia.

MicroRNA-mRNA-Drug Targets identification

The Drug-Gene Interaction Database (DGIdb) (https://www.dgidb.org/) [19, 20] is a public online database that combines a variety of data sources describing drug-gene interactions and gene-drug susceptibility. It collects and integrates interactions from articles, databases and web

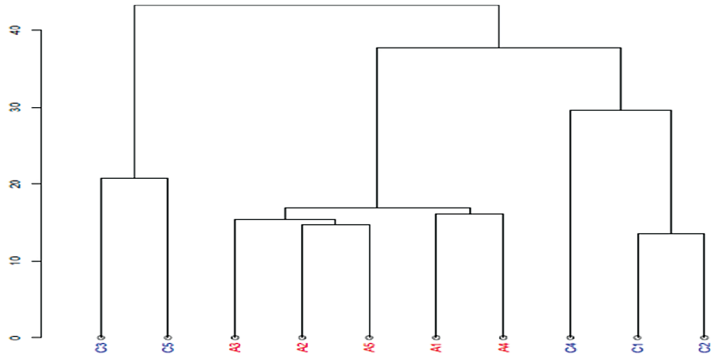

Figure 1: Hierarchical Clustering of samples. Red samples are indicated controls and blue samples pointed to high myopia sample

resources, including DrugBank, PharmGKB, ChEMBL, Drug Target Commons, TTD, text mining, etc. We used DGIdb to extract drugs that interact with identified gene targets in selected clusters. Finally, we visualized the relationship between miRNAs and drugs using the Cytoscape software.

Results

Clinical features of high myopia and cataract as controls

The demographic characteristics of the patients and the controls group are shown in Table 1. Patients with high myopia were diagnosed, with an average age of 29.93 ± 13.85 years, and their aqueous humor was used as the tissue sample. And also, the control group were samples with a mean age of 70.27 ± 9.55 years.

Normalization and differentially expressed miRNAs analyses

We obtained the expression data of GSE142359 from the GEO database. Then we normalized the raw data using quantitative normalization and also displayed the hierarchical clustering and heat map of the samples in Figures 1 and 2, respectively.

Figure 1 clearly has differentiated between high myopia and control samples. Control samples (A1–A5) have fallen into one cluster, while high myopia samples (C1–C5) have fallen into separate clusters.

We generated a heatmap plot of the samples to show the relative expression levels of each samples. This plot helps identify patterns between high myopia and control samples. By clustering the samples based on their expression profiles, we can also identify groups that share similar expression patterns and may be related to each other biologically. Finally, this analysis also confirmed that the normalization has performed successfully.

After normalization, we performed DEmiRNA analysis. Then, we filtered miRNAs with LogFC greater than 1 and less than -1 and

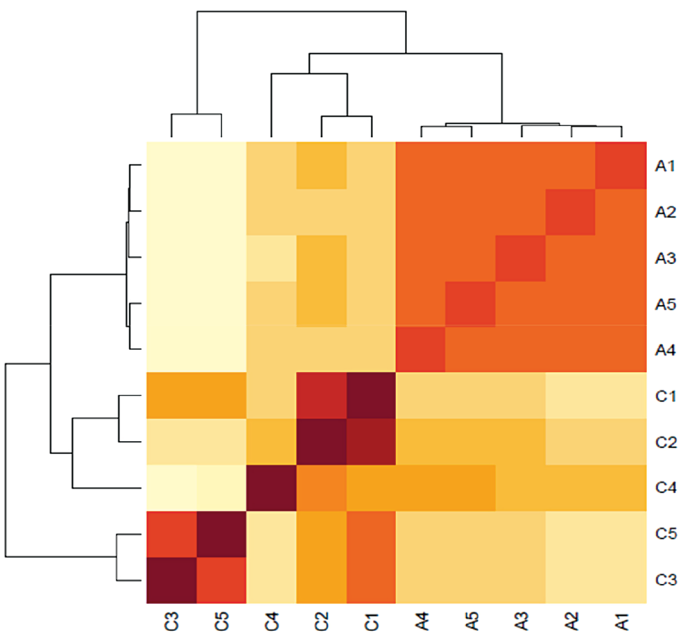

Figure 2: Heatmap plot. A1-5 samples are indicated controls and C1-5 samples pointed to high myopia samples

Table 1: Clinical dataset in high myopia and control samples

Samples	Number of Samples	mean age (years)	Source name /tissue	Extracted molecule
High myopia	5	29.93 ± 13.85	aqueous humor	total RNA
Control	5	70.27 ± 9.55	aqueous humor	total RNA

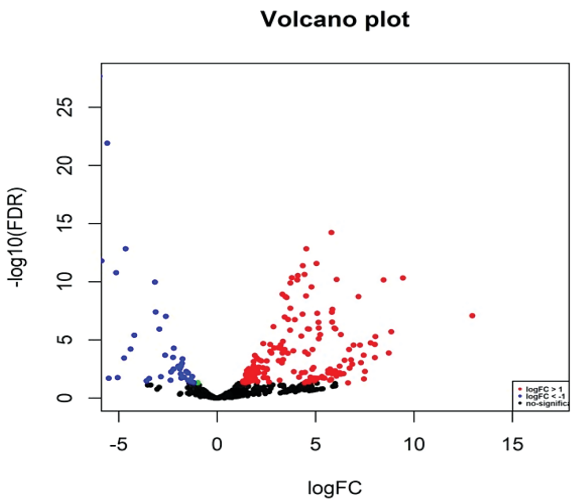

Figure 3: Volcano plot of DEmiRNA. Up-regulated genes are shown in red, down-regulated ones are shown in blue and also no-significant genes are visualized in black

an FDR of less than 0.05. A total of 136 miRNAs were identified as DEmiRNAs, with both up/ down-regulated miRNAs. The result of DEmiRNA analysis is reported in Supplementary 1 and also, the volcano plot of this analysis is shown in Figure 3.

miRNA-mRNA bipartite network

The identified DEmiRNAs (136 miRNAs) entered as input into miRWalk database to extract target genes. Finally, we identified 564 miRNA-mRNA bipartite interactions including 490 genes and 50 miRNAs. The result of this analysis is reported in supplementary 2. We ranked miRNAs based on degree (A degree in biological networks refers to the number of connections that a node (miRNA) has within a network) and just selected 10 miRNAs with high degree including: hsa-miR-16-5p, hsa-miR-130a-3p, hsa-miR-34a-5p, hsa-miR-30c-5p, hsa-miR-181b-5p, hsa-miR-181a-5p, hsa-miR-195-5p, hsa-miR-24-3p, hsa-miR-92b-3p, and hsa-let-7d-5p , and finally showed 10 miRNAs and their linked mRNAs (No. 316 mRNAs) in figure 4.

Protein-Protein interaction network

We entered 316 genes as STRING input. We extracted the PPI network with 83 proteins and 102 interactions. The result of this analysis is reported in supplementary 3. Then we performed k-means clustering. Finally, we identified 10 distinct clusters. The result of clustering is reported in table 2 and full detail of this are in supplementary 4.

Functional enrichment analysis

We used the ToppGene database to identify the enriched genes in all clusters. Therefore, the genes belonging to each cluster were enriched in terms of biological processes, pathways, and diseases related to the studied disease. We identified the enriched genes in purple module in terms of biological processes (including: negative regulation of cell cycle, regulation of response to stress, response to wounding, regulation of retinal cell programmed cell death, and etc.), pathways (including: VEGF signaling pathway, EGFR tyrosine kinase inhibitor resistance, Kit receptor signaling pathway, TGF-beta signaling pathway, Innate immune system, and etc.) and diseases (including: severe myopia,

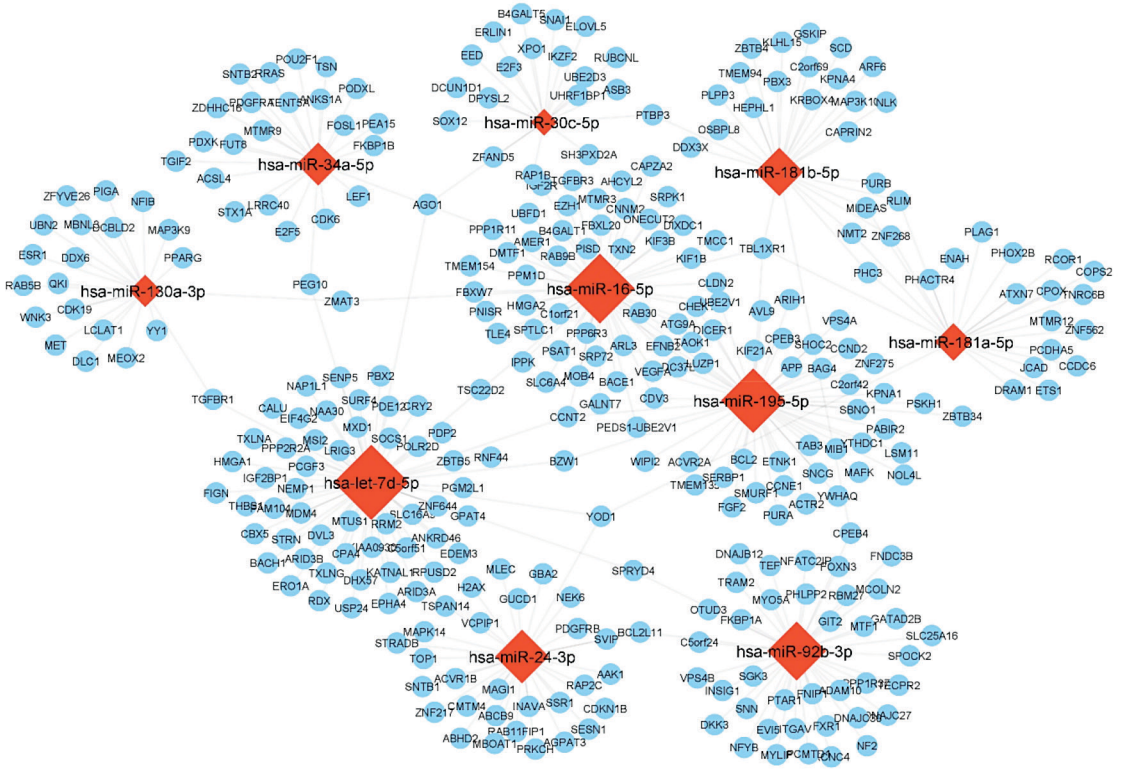

Figure 4: miRNA-mRNA bipartite network. Circles indicate mRNAs and diamonds represent miRNAs. The size of diamonds represents the degree of the nodes

myopic choroidal neovascularization, corneal neovascularization, retinopathy of prematurity, blindness of one eye, retinoblastoma, and etc.). Finally, the purple cluster genes were selected for further analysis. The details resulting from enriched the selected cluster are reported in supplementary 5.

MicroRNA-mRNA-Drug Targets identification

We entered the purple cluster genes as entries to the DGIdb database to extract drug-target interactions. The result of this analysis showed 167 interactions including 8 genes and 151 drugs. Details of this analysis are reported in supplementary 6. Additionally, as shown in Figure 5, the relationship between miRNAs, mRNAs, and drugs was depicted using the Cytoscape software.

Two miRNAs (including: hsa-miR-195-5p and hsa-miR-24-3p) interacted the most with 4 and 2, respectively. Also, five proteins (including: ESR1, BCL2, VEGFA, FGF2, and MAPK14) had the highest interaction with 84, 28, 24, 15, and 13, respectively. These miRNAs and proteins are known as hub. Furthermore, 9 drugs (including: Doxorubicin, Vincristine, Lenalidomide, Cisplatin, Sorafenib, Carboplatin, Docetaxel, and Oxaliplatin) are shared at least between two hub proteins.

Discussion

Myopia is a prevalent refractive error that can cause gradual blindness and is associated with other ocular diseases, such as cataract. High myopia is increasing globally, and genetic and environmental play a role in causing this eye disease. Among genetic factors, miRNAs have been reported to play a critical role in myopia development [12, 21]. The aim of this study was to identify new potential biomarkers that play a significant role in high myopia.

One of the potential hub genes selected in

Table 2: List of protein names related to 10 clusters

Cluster name	Protein name
Blue	BACH1,BCL2L11,CALU,CDK19,CPEB3,CPEB4,CPOX,CRY2,DCBLD2,DPYSL2,ELMSAN1,ELOVL5,ERLIN1,FAM46A,GUCD1,HMGA2,IKZF2,KIAA0195,KIF1B,LRRC40,MOB4,MYLIP,MYO5A,NAP1L1,NEK6,NFATC2IP,NFYB,NOL4L,PBX3,PCDHA5,PDE12,PDP2,PNISR,RPUSD2,SGK3,SH3PXD2A,SLC6A4,SOCS1,SOX12,SPOCK2,SPTLC1,SRP72,SRPK1,TMCC1,TMEM135,TOP1,TSC22D2,TSPAN14,TXLNA,ZBTB34,ZNF562
Brown	ABHD2,ANKS1A,C1orf106,C2orf69,C5orf24,C5orf51,CAPZA2,CDV3,CHEK1,DLC1,DNAJC30,EFNB2,EPHA4,EVI5,FKBP1B,FNIP1,GSKIP,KIAA1462,KIF3B,KPNA4,LRIG3,LUZP1,MTUS1,NFIB,ONECUT2,PDXK,PEG10,PRKCH,PSAT1,PTAR1,QKI,RNF44,RRAS,SCD,SESN1,SNTB2,TLE4,TMEM154,TMEM189-UBE2V1,TMEM194A,WNK3,YOD1,ZBTB4,ZNF275
Dark Golden Rod	ABCB9,B4GALT5,CAPRIN2,CLDN2,DMTF1,DNAJB12,FIGN,FXR1,KATNAL1,KRBOX4,PHACTR4,PPP2R2A,PPP6R3,PSKH1,PTBP3,RDX,RLIM,SBNO1,SERBP1,SHOC2,SVIP,TECPR2,UBE2D3,UBN2,ZDHHC16,ZNF217,ZNF268
Green	ACSL4,AGPAT3,AGPAT6,AHCYL2,AVL9,CCDC6,DVL3,ENAH,ERO1L,FAM104A,FNDC3B,FOSL1,GATAD2B,LCLAT1,LSM11,MAP3K9,MBOAT1,MXD1,NAA30,NF2,PBX2,PISD,PPAP2B,PURA,PURB,RAP2C,SENP5,SLC16A9,SNCG,SNTB1,SURF4,UBFD1,VPS4B,YTHDC1
Green2	AAK1,AMER1,ARF6,DRAM1,E2F3,EED,EZH1,KIAA0930,MEOX2,MET,MSI2,PCGF3,PHC3,POLR2D,RAP1B,STRADB,TBL1XR1,TXLNG,TXN2,YY1,ZFAND5
Green Yellow	ANKRD46,BZW1,CDC37L1,DIXDC1,DKK3,EDEM3,EIF4G2,GALNT7,GIT2,INSIG1,MBNL3,MCOLN2,MDM4,MIB1,MTF1,MTMR12,NMT2,PGM2L1,PHLPP2,POU2F1,PPARG,PPP1R11,RAB30,SLC25A16,SNN,UBE2V1,USP24,VCPIP1
Light Sky Blue	ACTR2,ACVR1B,ACVR2A,ARID3B,ARIH1,ATG9A,ATXN7,BACE1,BAG4,CMTM4,DDX3X,ETNK1,FKBP1A,IGF2R,KCNC4,KIF21A,KLHL15,MLEC,OSBPL8,PDGFRB,PIGA,PPM1D,RAB11FIP1,RBM27,SMURF1,SSR1,TAB3,TAOK1,TEF,TGFBR1,TGFBR3,ZBTB5,ZMAT3
Medium Blue	C1orf21,C2orf42,CCND2,CCNE1,CCNT2,CDK6,CDKN1B,CNNM2,COPS2,DCUN1D1,E2F5,FBXW7,FOXN3,ITGAV,KPNA1,MTMR3,PDGFRA,PLAG1,RAB5B,STX1A,TGIF2,XPO1,YWHAQ,ZFYVE26
Purple	ADAM10,APP,ARL3,B4GALT1,BCL2,CBX5,CPA4,ESR1,ETS1,FGF2,FUT8,H2AFX,MAP3K10,MAPK14,MTMR9,PHOX2B,PPP1R37,RAB9B,RCOR1,SNAI1,STRN,TRAM2,UHRF1BP1,VEGFA,VPS4A
Red	AGO1,ARID3A,ASB3,DDX6,DHX57,DICER1,DNAJC27,FBXL20,GBA2,HEPHL1,HMGA1,IGF2BP1,IPPK,KIAA0226L,LEF1,MAFK,MAGI1,NLK,OTUD3,PCMTD1,PEA15,PODXL,RRM2,SPRYD4,THBS1,TNRC6B,TSN,WIPI2,ZNF644

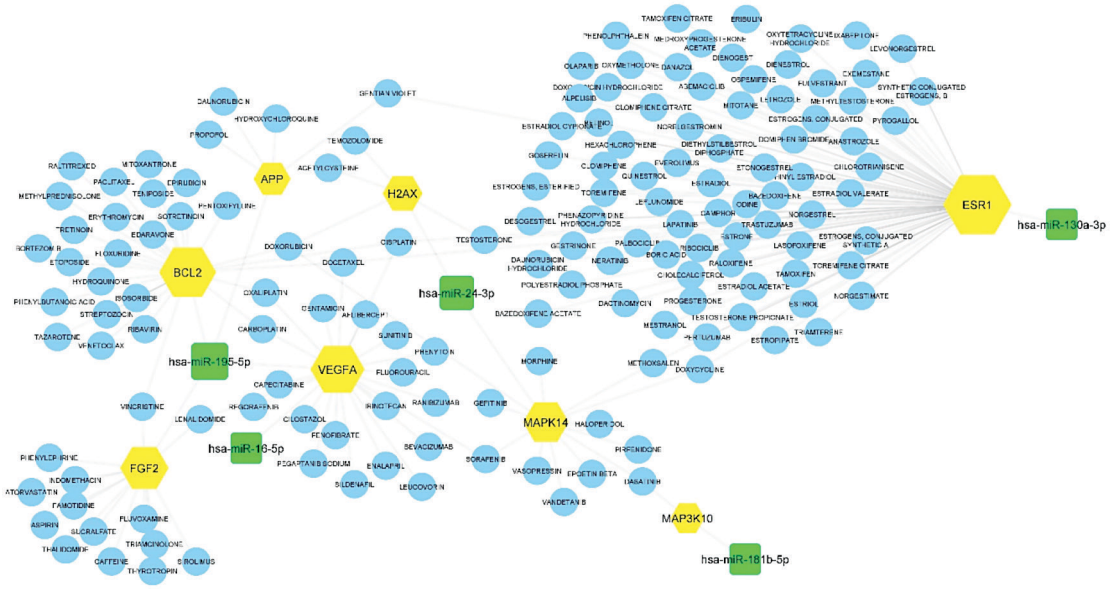

Figure 5: miRNA-mRNA-Drug network. The squares, hexagons, and circles represent miRNAs, mRNAs, and drugs, respectively. The size of squares and hexagons indicate the degree of the nodes

this study (FGF2) played a vital role in the development of myopia. According to the examination of FGF2 in the sclera and retinal pigment epithelium, it has been reported in previous studies that fibroblast growth factor 2 (FGF2) plays an effective role in the development of myopia [22, 23].

VEGFA is a glycosylated mitogen that especially affects endothelial cells and has severla effects, such as: 1- increasing vascular permeability, 2- inducing angiogenesis, 3- angiogenesis and growth of endothelial cells, 4- promotion of migration. Cellular and 5- restraint apoptosis. VEGFA has been shown to play a key role in myopic choroidal neovascularization, which can lead to irreversible vision loss [24].

Another hub gene identified in this study is ESR1. The ESR1 gene is located on chromosome 6q25 and has two well-known SNPs including rs2234693 and rs9340799 [25]. ESR1 is present in almost all layers of the retina, cornea, lens, conjunctiva, lacrimal glands, and meibomian. So that the synthesis and signaling of ESR1 is essential for the eye. It has been reported that this protein has a neuroprotective effect in the retina and optic nerve. In addition, it appears to be involved in several eye pathologies such as glaucoma, myopia, age-related maculopathy and cataracts [26].

The BCL2 gene is the principle member of the Bcl-2 family of regulatory proteins that regulate cell death by inhibiting or inducing apoptosis. It was the first apoptosis regulator identified in any living organism [27]. Apoptosis plays an important role in the regulation of variousdiseases including cancers, autoimmune diseases, retinal diseases and etc. For example, apoptosis has been observed in retinal ischemia. Decreased expression of anti-apoptotic genes such as BCL2 and BCLXL causes retinal ischemia and these changes increase apoptosis in this disease [28]. This gene has been selected as a hub in this study. Hence, expression changes in this protein may be effective in high myopia. Therefore, this protein is suggested as a potential biomarker of high myopia.

MAPK14 is another hub gene identified in this study. MAPK14 is the ortholog of MAPK14A. MAPK14A activates MAP kinase activities. It is involved in cellular response to UV, embryonic cleavage and p38MAPK cascade. This protein is predicted to be active in the cytoplasm and nucleus and is expressed in several structures, for example, 1-digestive system, 2-immature eye, 3-nervous system, 4-otic vesicle and 5-pronephric duct. Besides, MAP kinases are implicated in cell survival/apoptosis, proliferation, differentiation, migration, mRNA stability, and inflammatory response in different cell types through variety of different target molecules. Hence, this protein can be effective for treatment of high myopia.

In the miRNA-mRNA-drug network, two miRNAs were also proposed as biomarkers. Hsa-miR-195-5p is one of them that interacts with three proteins including: FGF2, VEGFA and BCL2. Considering that the two proteins FGF2 [22, 23] and VEGFA [24] were identified in previous studies for myopia, so this protein and Hsa-miR-195-5p can be seriously considered as biomarkers in high myopia. In addition, this miRNA had more interaction with the proteins in the network and is known as the highest rank among miRNAs in terms of degree.

Another miRNA identified as a biomarker in this study was hsa-miR-24-3p. This miRNA interacts with two proteins including MAPK14 and H2AX. MAPK14 is one of the identified potential biomarkers, which is known as a hub in the network. Therefore, this miRNA may also cause high myopia. Hence; hsa-miR-24-3p is proposed as a potential biomarker in high myopia.

Lenalidomide is one of the proposed drugs that shows promise as a treatment for ocular angiogenesis due to its anti-proliferative and anti-inflammatory properties. This drug has been shown to inhibit retinal endothelial cell viability in both normal and pathological conditions, as well as VEGF-induced endothelial cell migration and tube formation in vitro. Additionally, lenalidomide has been found to reduce the expression of angiogenesis- and inflammation-related proteins, effectively inhibiting ocular angiogenesis in vivo [29].

Conclusion

In conclusion, our research proposed two new miRNAs including hsa-miR-195-5p and hsa-miR-24-3p, and three novel proteins including ESR1, BCL2, and MAPK14 as potential biomarkers in high myopia. These potential biomarkers will help expand our understanding of the genetics of high myopia for future research.

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Footnotes and Financial Disclosures

Conflict of interest:

The authors have no conflict of interest with the subject matter of the present manuscript.