Mechanism of Myopic Defocus or Atropine for Myopia Control: Different or Similar Ways?

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Key words
Myopia · RNA sequencing profiling · Atropine · Myopic defocus

Abstract
Introduction: Myopia is usually caused by excessive elongation of the eye during development. This condition is common worldwide. In clinical practice, the progression of myopia is commonly controlled through optical or drug measures, but the specific mechanisms underlying these two treatments remain unclear. To verify whether the effects of these two treatments on posterior-pole tissues are similar or different, we studied a set of common transcriptional changes in chicken models. Methods: Chicks were divided into four groups, and they were given the intervention measures of plus-lens induction, minus-lens induction, minus-lens induction with atropine injection, and minus-lens induction with saline injection. Then, the genetic changes in each tissue at the posterior pole were detected, and the results of different genes were compared. A semiquantitative real-time polymerase chain reaction method was used to further study the visually induced changes in the transcription of potential candidate genes. Results: Based on RNA sequencing (RNA-seq) analysis of the transcriptome, we identified variations between the differentially expressed transcripts in three tissues from the two treatment groups. Through Kyoto Encyclopedia of Genes and Genomes enrichment analyses, eukaryotic protein translation elongation factor 1α2 (EEF1A2) was enriched in the “leishmaniasis” pathway in the choroid and showed increased expression in both the plus-lens induction and injection atropine groups. The expression levels of selected genes verified by quantitative real-time PCR were concordant with the RNA-seq data. Conclusions: Overlapping differentially expressed mRNAs of only one-tenth could suggest a different mechanism of myopic defocus and intravitreal injection of atropine controlling myopia. EEF1A2 might play an important role in the choroid during the treatment of myopia.

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Introduction

In 2010, the global prevalence of myopia was close to 2 billion people (28.3% of the global population), of whom 277 million were highly myopic (4.0% of the global population). It is estimated that, by 2050, the prevalence of myopia will increase to 4.76 billion people (49.8% of the global population), while the prevalence of high myopia will increase to nearly 1 billion people (9.8% of the global population) [1]. Myopia will present further vision challenges since high myopia increases the risk of pathological ocular changes, such as cataracts, glaucoma, retinal detachment, and myopic macular degeneration, all of which can lead to irreversible vision loss [2]. As the severity of myopia increases, treatments to prevent the occurrence and development of this condition are urgently needed.

Visually induced changes in eyeball growth are the results of the locally driven retina to choroid-sclera molecular signal cascade. This signal cascade is initiated by visual stimuli, followed by biochemical and structural changes in the retina and choroid [3]. In addition, the choroid is believed to synthesize and/or release scleral growth regulators to control the elongation of the eye in response to visual stimuli since it is close to the sclera [4, 5]. Therefore, the three posterior tissues all play important roles in the occurrence, development, and control of myopia.

Useful clinical measures to reduce or slow the progression of myopia include orthokeratology (ortho-k) and the application of atropine eye drops at concentrations ranging between 0.01% and 1% [6–11]. Interestingly, combining ortho-k and atropine is likely to enhance the efficacy of clinical myopia control [12]. Although the mechanism behind the ortho-k’s control effect is still unclear, it is well-documented that the lenses convert relative peripheral defocus of the eye from being hyperopic pre-treatment to being myopic post-treatment, regardless of the wearing time [13]. This myopic defocus is believed to be the mechanism that slows myopia progression during ortho-k wear [14]. Besides, the exact mechanism of topical atropine is still not known [15]. Interestingly, some patients who use ortho-k lenses [16] or atropine [17] will have the phenomenon of choroidal thickening in clinical practice, which arouses our thinking and wants to further explore the common pathways involved in the myopic defocus and atropine in animal experiments.

In animal models, the defocus phenomenon was demonstrated in chicks as early as 1990 [18]: the application of plus lenses resulted in the formation of an image in front of the retina. This imposed myopic defocus will slow the elongation speed of the eye and the thickening of the choroid, thereby effectively pushing the retina to the image plane and minimizing the imposed refractive error [19]. Many cell or animal experiments have focused on hyperopia defocusing [20–23] and atropine [24–26] to control myopia, but no unified conclusions have yet been reached. Many studies have focused on the early stages of therapeutic intervention or selected one tissue or a mixture of multiple tissues. To date, no experiments have separately sequenced the three posterior tissues and compared the sequencing results after these two treatment methods. To verify whether the effects of these two treatments on posterior pole tissues are similar or different, we chose myopic defocus (plus-lens induction) and intravitreal injection of atropine to delay the progression of myopia and used RNA sequencing (RNA-seq) to analyze the transcriptomes of retinal, choroidal, and scleral tissues in these eyes.

Materials and Methods

Animals and Housing

Four-day-old white Leghorn chickens (Gallus gallus) were purchased from Beijing Boehringer Ingelheim Vital Biotechnology Co., Ltd., (Beijing, China) (each group n = 10). The chickens had access to unlimited amounts of food and water and were given 3 days to become accustomed to their environment before the initiation of experiments on post-hatching day 7. The chickens were housed in temperature-controlled rooms and kept under normal laboratory lighting (∼500 lux) on a 12:12 h light:dark cycle, with lights on at 8:00 a.m. and off at 8:00 p.m. This study adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. All of the experimental procedures were approved by the Institutional Animal Care and Use Committee of the Peking University People’s Hospital (permit number: 2018PHC059).

Lens Induction

PMMA plastic lenses (with a back optic radius of 7 mm) were used. The lenses (diameter = 12 mm) were glued between rigid plastic rings and Velcro rings attached to mating Velcro rings glued to the feathers around the chicks’ eyes. Lenses were cleaned at least twice per day. In both experiments, chicks had monocular lens wear, leaving the other eye uncovered.

Experimental Design

To study the changes in the transcriptome in response to different forms of inhibition, chicks were split into one of the following four experimental groups: plus-minus lens induction group, (1) hyperopia induction: chick was fitted with a +10D lens (Fig. 1a);...
myopia induction: chick was fitted with a −10D lens (Fig. 1b); (3) atropine-saline injection group; (4) saline injection: chick was fitted with a −10D lens and intravitreal injection of saline (Fig. 1d); for each of the four experimental groups, posterior samples were collected at the end of post-hatching day 12 (Fig. 1).

Drug Preparation and Administration

Injections were performed under light isoflurane anaesthesia (5% in 1 L of medical-grade oxygen per minute, RWD Science Life, Shenzhen, China) using a vaporizer gas system (Anesthetic Gas Machine [ZS-M], Beijing, China). For the drug administration, a 12.5 µL intravitreal injection was administered using a 33-gauge needle fitted to a Hamilton syringe, at the following concentrations:

1. 25 μg atropine sulphate monohydrate (98%) (Macklin, Beijing, China) was dissolved in 12.5 µL physiological saline (LDBIO, Beijing, China), a concentration equivalent to 0.2% atropine.
2. The contralateral control eyes received 12.5 µL saline.

Drug concentrations were chosen based on previously reported data on their effectiveness against the development of experimental myopia in chickens [27].

Fig. 1. Chicks were given 6 days post-hatching (P6) to become accustomed to their surrounding (white bars) before the commencement of experiments. a, b For the development of lens-induced hyperopia (LIH)/lens-induced myopia (LIM), chicks were fitted with +10 D/-10 D lenses at the commencement of the light phase on P7, with tissues collected after 6 days following lens attachment (arrows). c, d For the intravitreal administration of atropine/saline, measurements and first injections were administered immediately before the fitment of translucent diffusers on P7, and each subsequent treatment was injected at the same time in the next 6 days.

Measurements of Ocular Parameters

Ocular dimensions and refractive errors were measured before and after 6 days of lens wear. Axial length was defined as the distance from the front of the cornea to the front of the retina [28]. Ocular parameters were examined by a high-frequency A-scan ultrasound system (UTSystem) with a 25-MHz transducer (TECLAB, Beijing, China) sampled at a rate of 160 MHz, while refractive errors were measured using a streak retinoscope (YZ24; Suzhou Liu-liu Vision Technology Co., Ltd., Suzhou, China) as previously described. Refractive and axial length measurements are presented as means ± standard deviation of the means, with the data representing the difference between the diopter after treatment minus the difference before treatment.

RNA Isolation and Library Preparation for Next-Generation Sequencing

For a collection of retinal tissue following 6 days of treatment, chicks were heavily anesthetized using isoflurane and sacrificed by decapitation. Each eye was rapidly removed and hemisected equatorially, with the vitreous body removed and the anterior portion of the eye discarded. The posterior eye cup was floated in chilled phosphate-buffered saline, allowing for removal, and three cryopreservation tubes were used to collect the retina/retinal pigment.
epithelium, choroid, and sclera. Total RNA was extracted from the tissues using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. Total RNA was qualified and quantified using a NanoDrop and Agilent 2100 bioanalyzer (Thermo Fisher Scientific, Waltham, MA, USA). The qualified library was amplified on cBot to generate the cluster on the flowcell. The amplified flowcell was sequenced at the single end on the Illumina Novaseq 6000 platform.

Bioinformatics Analysis
Differential expression analysis was performed using DESeq2 (version 1.4.5) [29] with \( p < 0.05 \) and \( \log_2 \text{(fold change)} > 0.58 \) in the DEseq2 analysis of two RNA-seq biological replicates were taken to identify differentially expressed genes (DEGs) (each group \( n = 3 \)). To take an insight into the change of phenotype, GO (http://www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.kegg.jp/) enrichment analysis of annotated different expressed genes was performed by Phyper (https://en.wikipedia.org/wiki/Hypergeometric_distribution) based on hypergeometric test.

Protein-Protein Interaction Network Creation and Identification of Hub Genes
The protein-protein interaction (PPI) network analysis was conducted using STRING (https://string-db.org/), which is an online database of known and predicted PPIs. These interactions include physical and functional associations, and the data are mainly derived from computational predictions, high-throughput experiments, automated text mining, and co-expression networks. We mapped the DEGs onto the PPI network and set an interaction score of \( >0.4 \) as the threshold value. In addition, Cytoscape v3.8.2 software was used to visualize and construct the PPI network. Nodes with the greatest numbers of interactions with neighbouring nodes were considered hub nodes. To identify the key PPI network modules, the app ClusterONE from the Cytoscape software suite was used to perform the gene network clustering analysis. A \( p < 0.05 \) was set as the significance threshold for identifying key modules.

Validation of RNA-Seq Data by Quantitative Real-Time PCR
To validate the reliability of the RNA-seq results, we selected twenty-six mRNAs in each group for real-time PCR. Total RNA (1.5 \( \mu \text{g} \)) extracted from the samples used for the transcriptome analysis was reverse-transcribed to first-strand cDNA in a 20 \( \mu \text{L} \) reaction using the Maxima First Strand cDNA Synthesis Kit for sqRT-PCR (TOYOBO [Shanghai] Biotech Co., Ltd., Shanghai, China). One-and-a-half microliters of the first-strand cDNA template were used in a 15 \( \mu \text{L} \) sqRT-PCR reaction. sqRT-PCR was undertaken using a Fast SYBR green kit following the manufacturer’s instructions (TOYOBO [Shanghai] Biotech Co., Ltd.). All reactions were run on a real-time thermal cycler (pikoreal 96; Thermo Fisher Scientific, Vantaa, Finland). The primer sequences are shown in the online supplementary material (available at www.karger.com/doi/10.1159/000525744). The experiments were repeated using at least 3 biological and 3 technical replicates. Relative mRNA expression levels were analysed by the \( 2^{-\Delta\Delta CT} \) method using GAPDH as an endogenous control.

Data Analysis
SPSS 26 (IBM, Armonk, NY, USA) software was used for data analysis. Data for treated and control eyes, as well as derived differences at different time points are reported as the mean ± SEM. For multiple groups of data with repeated measurements, if the homogeneity test of variance was satisfied, analysis of variance with Bonferroni’s post hoc test was applied to longitudinal data. If the data did not meet the homogeneity test of variance, the Kruskal-Wallis test was used. Independent Student \( t \) tests were performed to reveal the differences between groups (plus lens vs. minus lens; atropine group vs. saline group).
Results

Ocular Growth Is Suppressed by the Two Treatments

Prior to the transcriptomic analysis, we confirmed that lenses-induced experimental ametropia, while the two preventive treatments inhibited this myopic growth (shown in Fig. 2). As expected, the −10D lens induced a significant myopic shift in refraction ($p < 0.001$) and excessive axial elongation ($p < 0.001$) compared to those of the untreated contralateral controls. Conversely, the +10D lens induced the development of hyperopia. In the atropine-saline group, the development of lens-induced myopia was suppressed by intravitreal injection of atropine. Except that there was no statistical difference between the −10D induction group and the saline injection group, the differences between the other groups were statistically significant ($p < 0.05$).

Gene Expression

Based on RNA-seq analysis of the transcriptome, the differentially expressed transcripts (DETs) of three tissues from the two treatment groups were identified. There were 3,323 DETs in various tissues in the posterior part of the chicks (Fig. 3). In the plus-minus group, there were 107 genes with upregulated expression in the retina and 86 genes with downregulated expression; there were 251 DETs with upregulated expression and 140 DETs with downregulated expression in the choroid; and in the sclera, 619 DETs had upregulated and 731 had downregulated expression. When the atropine group was com-

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Fig. 3. Analyses of RNA-seq data from the plus-minus group and the atropine-saline group of differentially expressed genes (DEGs) of three tissues. a1–a3 Volcano plot representation of DEGs in the plus-minus group between the three different tissues: retina (a1); choroid (a2); sclera (a3). b1–b3 Volcano plot representation of DEGs in the atropine-saline group between the three different tissues: retina (b1); choroid (b2); sclera (b3). The x-axes show log2 (fold change), and the negative log10 ($p$ value) is plotted on the y-axes. Each point represents a single gene. Red points represent upregulated DEGs with $p < 0.05$ and log2 (fold change) $>0.58$, and green points are DEGs that downregulated expressed. c1–c3 Venn results of DEGs between the two groups: retina (c1); choroid (c2); sclera (c3). *The number of the overlapping DEGs which showed changes in opposite directions.
Fig. 4. The top 10 GO terms in biological process (BP), cellular component (CC), and molecular function (MF) for the plus-minus group (retina, a; choroid, b; sclera, c) and the atropine-saline group (retina, d; choroid, e; sclera, f).

(Figure continued on next page.)
Fig. 5. Statistically significant KEGG terms in the plus-minus group (a) and the atropine-saline group (b).
pared with the saline group, there were 186 genes with upregulated expression in the retina and 64 with downregulated expression were found; there were 143 DETs with upregulated expression and 96 DETs with downregulated expression in the choroid; and 331 with upregulated expression and 569 with downregulated expression in the sclera.

Both treatment methods resulted in the greatest differences at the scleral level, and the overlapping differentially expressed mRNAs in the two groups accounted for approximately 10% of the whole number in the plus-minus lens or atropine-saline group (Fig. 3a1–a3). In addition, in the retina and choroid tissues, most of the differentially expressed mRNAs that overlapped in the two treatment groups had the same direction of change (upregulation or downregulation). In the sclera, most of the overlapping differentially expressed mRNAs showed changes in the opposite directions (Fig. 3, asterisk).

**Gene Ontology Analysis**
Gene Ontology (GO) enrichment analysis was conducted to analyse the target gene functions in three categories, including cell components, biologic process, and molecular function. Each top 20 GO terms in every category of three posterior tissues were analysed (in attachment) and approximately half of the GO pathways enriched by the two treatments in different tissues had been overlapped (overlap pathway number: retina 30, choroid 28, sclera 40). In each treatment group, there were 15 and 12 overlapping pathways in the three tissues; 6 pathways were enriched in every group: cell proliferation, extracellular space, positive regulation of cell proliferation, protein dimerization activity, purine ribonucleoside binding, and transcription factor binding. The top 30 GO terms are shown in Figure 4.

**KEGG Pathway Analysis**
Similar to the GO analysis, the KEGG pathway analysis (shown in Fig. 5) after the two treatments showed that the common pathways at the retinal level were ECM-receptor interaction and focal adhesion, but the two pathways were enriched by different genes. Common pathways at the choroidal level were neuroactive ligand-receptor interaction and leishmaniaisis. The leishmaniasis pathway was enriched by upregulated expression of *eukaryotic protein translation elongation factor 1a2* (*EEF1A2*). Oxidative phosphorylation and cardiac muscle contraction pathways were found in the sclera between the two treatment groups. Interestingly, the expression trends of genes enriched in these two pathways at the scleral level showed the opposite pattern. Thus, the effects of the two treatments at the late stage in the sclera are quite different.

**PPI Network**
The PPI network depicts the relationships of biomolecules that play important roles in biological processes. Identifying the functional modules in a complex interactome could therefore help us to understand the pathogenesis of myopia. The STRING database was used to assess functional associations, and a PPI network was constructed to include many nodes and edges and was visualized using NetworkAnalyst (Table 1). To identify the hub genes among these DEGs, we constructed a PPI network using STRING11.0. The genes were ranked by a degree in the network. In this network, the top 5 hub genes are displayed in Table 2.

**Validation of RNA-Seq Data by Quantitative Real-Time PCR**
We further verified the expression levels of mRNAs using quantitative real-time PCR analysis. As shown in Figure 6, the expression trend of mRNAs was consistent with the results of gene sequencing. Moreover, the correlation analysis of the log$_2$(fold change) between RNA-seq and quantitative real-time PCR data of these genes indicated a high correlation ($R^2 = 0.8491$, Fig. 6c).

### Table 1. The results of the PPI network analysis

|          | Gene  | Node | Edge |
|----------|-------|------|------|
| Retina   |       |      |      |
| Plus, versus minus lens | 193   | 51   | 44   |
| Atropine versus saline   | 250   | 85   | 73   |
| Choroid  |       |      |      |
| Plus, versus minus lens | 391   | 189  | 338  |
| Atropine versus saline   | 239   | 81   | 69   |
| Sclera   |       |      |      |
| Plus, versus minus lens | 1350  | 967  | 6045 |
| Atropine versus saline   | 900   | 683  | 2653 |

### Discussion
In our study, treatment with atropine and the positive lens had a significant effect on the diopter changes and the growth of the eye axis of the chicks. Compared with simple negative lens induction or normal saline treatment, these two treatment methods effectively inhibited the progression of myopia. Next-generation sequencing
was used to detect the changes in gene expression. We aimed to further identify genes whose expression differences remained in the late stage of controlling myopia.

During the development of myopia, various changes in the retina (including the retina/retinal pigment epithelium), choroid, and sclera suggest the existence of a cascade of cell signals arising from the retina that modulates scleral biochemistry and regulates eye growth [3]. The results of this experiment demonstrated that the number of DEGs between the various tissues caused by different treatments also increased from the retina to the sclera (the number of choroidal gene differences in the atropine-saline group was smaller than that in the retina, but the difference was small, 239 vs. 250). These results support the cascade hypothesis and indicate that different treatment methods also cause increasing differences as the signal is transmitted from tissue to tissue.

By observing the difference in DETs, we found that the proportion of overlapping DETs between the plus-minus lens or the atropine-saline group in each tissue was almost maintained at approximately 10%. However, if we focus on the number of overlapping genes with the same expression trend between the positive and negative lens or atropine-saline groups, it decreases from the retina to the sclera. The number of genes with the same expression trend was 24 in the retina, 22 in the choroid, and 5 in the sclera. Meanwhile, the opposite result was observed in the number of overlapping genes with the opposite trend of expression. Therefore, as the signal propagates from tissue to tissue, the differences in the effects of the two treatments on each tissue increase.

When we analysed the GO terms between the plus-minus lens treatment group and the atropine-saline group, 6 pathways were enriched in every group, which mainly involved the cellular components, such as extracellular space. Moreover, the target genes in biological processes such as cell proliferation and positive regulation of cell proliferation. The target genes were also involved in molecular functions, including protein dimerization activity, purine ribonucleoside binding, and transcription factor binding, and this one drew our attention.

**Table 2.** Top 5 hub genes identified by the cytoHubba plug-in of Cytoscape

| Tissue   | Hub gene/overlapping | Hub gene name top 5                          |
|----------|----------------------|----------------------------------------------|
| Retina   |                      |                                              |
| Plus, versus minus lens | 51                   | 6 (KCNQ1, NR2E3, GNRH1, LRAT, ALDH1A3, SAMD7) ITGA8, IGF1, FGF22, KCNQ1, GLI2 |
| Atropine versus saline  | 89                   | CYBA, ATF3, CEPA, BEST1, COL1A2               |
| Choroid  |                      |                                              |
| Plus, versus minus lens | 189                  | 11 (LOC422308, VWA2, RGS8, EEF1A2, IGJ, GJA5, KCNQ2, PCDHA2, SAMD11, CHAT, NOG2) MPEG1, ITGB2, RSFR, IL2RB, ANG |
| Atropine versus saline  | 82                   | RHO, CXCL12, LOC422308, PPL, VILL            |
| Sclera   |                      |                                              |
| Plus, versus minus lens | >500; top 500        | 57 (ATP5H, UQCRH, ATP5L, NDUFS5, NDUFA1, NDUFA2, NDUF84, COX6A1, USMG5, POLR2L, PTPRC, CD80, FGL2, MRPL54, MRPL15, MRPL18, CCL15, TIMM13, LSMEM1, PIN4, MYEOV2, ROMO1, HYPK, KRAS, RPL38, SHFM1, RPL37A, HSPB7, CNND1, IL18, PPARG, RHOQ, SOCS6, GDP1L2, ARHGAP5, BRAF, MTG1, OGN, HDAC4, PTPRJ, MINOS1, ZEB2, RBP7, PDE5A, HSPB1, MYCBP, BST, ADAM19, MBOAT2, ID1, RAP2B, TET2, GALK2, KCNNQ, PFDN1, RAB24, FOXP2) |
| Atropine versus saline  | >500; top 500        | UQCRFS1, ATP5H, NDUFA5, NDUFBS, COX5A        |

[Fig. 6.](#) Quantitative real-time PCR verification and correlation analysis between the RNA-seq and qPCR data of selected genes. a Quantitative real-time PCR verification for thirteen genes in each group. Statistical analysis of the difference in the bar plots between the plus and minus group is performed with Student’s t test. *p < 0.05, **p < 0.01, ***p < 0.001. Biological replicates for each test ≥3. b The data between the atropine and saline group. c Correlation analysis between RNA-seq and qPCR data of these genes; Log₂ (fold change) was calculated using qPCR data. Linear regression model (grey line): y = 0.5915x + 0.1492, R² = 0.8491. qPCR, quantitative real-time PCR.

(For figure see next page.)
Transcription factors are key cellular components that recognize specific DNA sequences to control chromatin and transcription [30], control gene expression, and their activities determine how cells function and respond to the environment [31]. Recent research based on insights into gene networks controlling myopia prevention has indicated that many transcription factors show universal changes in expression across growth-inhibitory processes [32]. These results also remind us that we could analyse myopia control from transcription factors in the future.

KEGG pathway analysis after the two treatment methods showed that the common pathways at the retinal level were ECM-receptor interaction and focal adhesion, but these two pathways were enriched by different genes. One study [33] combined the results of several GWAS and animal experiments, and through pathway analysis, it identified these two biological processes in the development of refractive errors. In the study by Karouta et al. [32], enrichment of the focal adhesion pathway appeared after 24 h of treatment. When combined with the results of this study, these findings indicate that this pathway could play a sustained role in the retina during myopic control. Common pathways at the choroidal level were neuroactive ligand-receptor interaction and leishmaniasis. Previous studies have reported similar results, confirming that neuroactive ligand-receptor interactions could contribute to the development of myopia by modifying dopamine (DA) signalling, and its potential target may be the DA D2-like receptor [34]. In the sclera, we found that oxidative phosphorylation and cardiac muscle contraction were common pathways in the plus-minus lens or atropine-saline group. A recent analysis of single-cell sequencing results [35] of scleral fibroblasts in myopic mice confirmed that the significantly enriched signalling pathways of different genes were mainly oxidative phosphorylation pathways related to hypoxia. Another study [26] showed a significant decrease in oxidative phosphorylation in scleral fibroblasts after atropine treatment. However, the results of our study confirmed that most of the differentially expressed overlapping genes of the plus-minus and atropine-saline groups in the sclera showed the opposite trend. We believe that the reason for this result is that optical intervention and drug intervention act through channels that are not very similar. Therefore, when the signal reaches the ultimate target, the sclera, the difference between the two mechanisms shows an increase.

By analysing the pathway results of the above KEGG, we screened the pathways of the retina, choroid, and sclera and found that the two ways of treating myopia only have the same pathway that the genes with the same expression trend are enriched in the choroid tissue, that is, EEF1A2 gene enrichment to the leishmaniasis pathway, which successfully drew our attention.

Although the exact mechanisms involved in myopia progression are unclear, increasing evidence has suggested that the choroid response to therapy might be related to the rate of myopia progression [36]. Studies have shown that the choroid can adjust the position of the retina by changing the thickness of the choroid, as well as releasing growth factors involved in the regulation of blood vessel formation, scleral remodelling, and eye growth [37, 38]. In conclusion, the choroid plays an important role in the process of emmetropia, the regulation of eye growth, and the development of refractive errors. The expression of EEF1A2 was increased at the choroidal level in both treatments.

By reviewing the literature, we found that the pathways that EEF1A2 effects in other diseases are highly overlapping with myopia, giving us reason to believe that it might be an important gene in the treatment of myopia. (1) TGF-β signalling: EEF1A2 interacts with HSP90AB1 to promote lung adenocarcinoma metastasis by enhancing TGF-β/SMAD signalling [39]. In myopia, the TGF-β superfamily is mainly involved in regulating scleral remodelling, and it is an important regulator in this process [40]. When building guinea pig [41] and tree shrew [42] myopia model, researchers found that the protein expression level of TGF-β was reduced. (2) ERK pathway: EEF1A2 is also positively associated with ER receptors in breast cancer and is involved in its transcriptional regulation. It induced a robust metastatic program in breast cancer cells and induced dramatic changes in their invasive and migratory properties by activating the ERK pathway [43]. In myopia, the ERK pathway is a downstream component of the DA signalling pathway closely related to the control of myopia. DA activates ERK signalling through D2 receptors, and this signalling could trigger multiple transduction cascades [44, 45]. (3) Acetylcholine signalling: researchers [46] discovered an approximately 52-kDa protein from rat brain that specifically binds to M4mAChR, and sequencing identified the protein as EEF1A2. By co-immunoprecipitation, EEF1A2 was identified as a novel specific binding partner of the M4 receptor subtype [47]. In myopia, it is well-known that acetylcholine signalling regulates the development of myopia through its different receptors, and it is called a “mediator of myopia-related pathways” [40].

It is worth noting that ERK and TGF-β [32], as well as mAChR [48], are all considered to be related to the transcription factor early growth response 1 (EGR1), which is a short-lived nuclear protein with a zinc finger-binding domain. Its expression is usually induced quickly and...
briefly by extracellular stimuli. Altering EGR1 is one of the most consistent findings observed during experimentally induced changes in the eye growth rate [32]. However, the gene was not differentially expressed in this experiment. Based on this finding, we speculate that EEF1A2 and EGR1 may also regulate the pathways at different stages to affect the transcriptional process and control myopia. Moreover, the innovation of this article is the discovery that the EEF1A2 gene is first expressed in all tissues of the posterior pole, and the expression of this protein in the choroid after intervention with different myopic control methods is significantly different, indicating that it may play an important role in the control of myopia. Then, we used the cytoHubba plug-in of Cytoscape to screen out the top five hub genes of the PPI network with the highest connectivity degrees, and these genes may play a key role in the prevention and control of myopia that can be used as the research focus in follow-up experiments.

This report studied three types of posterior pole tissues. Compared with previous studies, this report has certain advantages, but there are still limitations. One limitation is that we cannot distinguish which cell populations showed the observed transcriptional changes in all three tissues. In addition, we could not evaluate molecules that were not encoded by the genome. In this comparison, EEF1A2 did not show opposite expression patterns in the positive and negative mirror groups. Both the positive and negative mirror groups showed a decrease in EEF1A2 compared to the blank group. Thus, this protein is not a defocus recognition gene, and there may be a compensatory reaction during its expression.

**Conclusion**

The results showed that the proportion of overlapping DETs between optical treatment and pharmaceutical treatment was basically maintained at approximately only one-tenth, and this maybe reveals a different mechanism of controlling myopia by them. Meanwhile, we speculate that EEF1A2 may play an important role in the choroid during the treatment of myopia. However, the precise mechanism requires further research.

**Statement of Ethics**

This study complied with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Procedures were approved by the Institutional Animal Care and Use Committee of the Peking University People’s Hospital (permit number: 2018PHC059).

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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**Author Contributions**

Liyuan Sun wrote this manuscript. Liyuan Sun, Li Zhu, Sitong Chen, Jiarui Li, and Xuewei Li performed the experiment process. Liyuan Sun conducted data processing and bioinformatic analysis. Kai Wang and Mingwei Zhao provided the idea of this study and revised the manuscript. Correspondence should be addressed to Kai Wang and Mingwei Zhao.

**Data Availability Statement**

All data generated or analysed during this study are included in this article and its online supplementary material. Further enquiries can be directed to the corresponding author.

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