Journal of Translational Medicine

RESEARCH

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# Intraocular pressure is a promising target for myopia control

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## Abstract

**Background** Myopia presents a noteworthy global health concern, urging exploration of innovative treatments. The role of intraocular pressure (IOP) in regulating the progression of myopia has been controversial.

**Methods** To investigate the impact of reducing IOP to varying extents on myopia progression, three groups receiving distinct IOP-lowering medications (Brinzolamide, Latanoprost, and a combination of Brinzolamide and Latanoprost) were designed in a form-deprived myopic guinea pig model. Additionally, proteomics analyses were conducted to identify differentially expressed proteins in the sclera.

**Results** Based on 24-h and 4-week IOP monitoring, the group receiving both Brinzolamide and Latanoprost exhibited the greatest magnitude of IOP reduction and the most significant inhibition of axial length (AL) growth. Moreover, the administration of IOP-lowering medications increased choroidal thickness and induced alterations in the structure of scleral collagen fibrils. Notably, scleral proteomics revealed remodeling processes associated with key mechanisms, including proteolysis, fibrinolysis, and metal ion binding.

**Conclusions** Our findings highlight that pressure-dependent scleral remodeling contributes to the deceleration of AL elongation. These results underscore the efficacy of IOP reduction in mitigating the progression of myopia, providing a promising alternative strategy for myopia management.

**Keywords** Myopia, Intraocular pressure, IOP-lowering medication, Axial elongation, Sclera remodeling, Proteomics

## Background

With the escalating rates of urbanization, myopia has evolved into a significant global health concern over the past three decades, particularly among young adults in East and Southeast Asia, where the estimated prevalence ranges between 80–90% [1–5]. Myopia is characterized by excessive axial length (AL) elongation, defined as a spherical equivalent (SE) ≤ −0.5 diopters (D), resulting in blurred distance vision [6]. Inadequate control of myopia can predispose individuals to the development of high myopia and pathological myopia, leading to irreversible visual impairment or even blindness [7]. Although recent interventions aiming to control myopia have shown promise, they were accompanied by various side effects

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and adverse reactions [8–10]. Furthermore, the current strategies for myopia control predominantly focus on adolescents. For adults with progressive high myopia, apart from posterior scleral surgery, there are limited interventions available [11, 12]. Therefore, there is an urgent need to continually explore innovative approaches for effectively managing the progression of myopia.

Intraocular pressure (IOP) is involved in regulating eyeball development and preserving normal ocular morphology. Elevated IOP stands as a major risk factor of glaucoma, which was closely associated with all degrees of myopia, forming a foundational basis for exploring the relationship between IOP and myopia progression [13–15]. IOP increases with advancing degrees of myopia, while most of the highly myopic patients exhibit normal IOP levels [16]. It is generally agreed that elevated IOP imposes a distending force on the posterior sclera, compelling excessive eye globe elongation. Conversely, others report that higher IOP is associated with slower axial growth in children or in highly myopic patients [17, 18]. A retrospective study suggested an association between no use of IOP-lowering medications and increased AL, while their causation could not be inferred [19]. However, as reported by the latest digest of International Myopia Institute (IMI), the reduction of IOP has not yet become a standard clinical practice for myopia control [20]. To date, limited longitudinal studies have investigated the impact of IOP on myopia progression, leading to ongoing controversy regarding the potential effect of reducing IOP on delaying myopia progression.

The correlation and precise mechanism between IOP and myopia progression remain incompletely elucidated. Changes in IOP have been linked to alterations in the microstructure, composition and biomechanical properties of the sclera, such as increased scleral stiffness and collagen fiber disorganization, leading to scleral remodeling [21]. At this level, reducing IOP has shown potential in slowing down myopia progression by mitigating scleral remodeling, however, studies have drawn inconsistent conclusions regarding this effect [22–27]. Fully understanding the role of IOP and modulating IOP levels hold promise for the development of effective myopia control strategies. Previous studies have identified signaling molecules such as transforming growth factor-beta (TGF-β), matrix metalloproteinases (MMPs), and hypoxia-inducible factor-1α (HIF-1α) as potential mediators influencing myopia progression, while the intricate mechanisms and omics alterations associated with lowering IOP in ocular growth require further exploration [28–30]. Advancing our comprehension of these interactions will facilitate the development of targeted interventions for myopia management, focusing on modulating IOP and preventing or reversing scleral remodeling processes.

In this study, we investigate the impact of different IOP-lowering medications on reducing IOP, decelerating axial elongation, and influencing the pressure-dependent scleral remodeling, which will enhance our understanding of the efficacy and underlying mechanisms associated with lowering IOP in impeding myopia progression. This study actively explores innovative approaches to mitigate myopia severity, providing a potentially viable approach and valuable insights for clinical interventions in myopia control.

## Methods

### Animal model of form-deprivation myopia

A total of 40 male tricolour guinea pigs (four-week-old, weight 160–200 g) (Changsha Taiping Biotechnology Co., Ltd., China) were used for the study. Slit-lamp biomicroscopy examination was performed to exclude guinea pigs with ocular abnormalities prior to the experiment. Every five guinea pigs were housed in an opaque plastic cage (length: 65 cm; width: 45 cm; height: 20 cm). Room temperature was maintained at 24 °C, and the light intensity was ranged from 400 to 600 lx with a 12-h light/dark cycle.

To induce myopia in the right eye of guinea pigs, monocular form-deprivation myopia (FDM) was employed (Supplementary Fig. 1a, b). Specifically, a customized and adjustable headgear was designed for the experiment, with a semi-transparent frosted film sewn onto the opening of the right eye area, while the left eye retained uncovered. The tightness of the headgear was regularly checked and adjusted based on their head circumference to ensure proper and secure fitting for each guinea pig. The animals were randomly divided into four groups (n = 10): FDM group, with the right eye designated as the FDM eye, while the left eye served as the contralateral control group; FDM_A group, the right eye was subjected to FDM and twice-daily instillations of Brinzolamide (Azopt; Alcon, Switzerland); FDM_B group, the right eye underwent FDM and received daily instillations of Latanoprost (Xalatan; Pfizer, New York, USA); FDM_AB group, the right eye was subjected to FDM, and received twice-daily instillations of Brinzolamide and daily instillations of Latanoprost. This study protocol adhered to the ethical requirements for animal welfare and complied with the relevant provisions of the Helsinki Declaration, and it was approved by the Animal Ethics Committee of ZOC, Sun Yat-sen University.

### Biometric measurements in guinea pig

The IOP of guinea pigs was measured using the iCare rebound tonometer (iCare TonoLab; Helsinki, Finland) according to the method described by El-Nimri et al. [31]. To assess diurnal variations in IOP after treatment,

eight measurements were taken over a 24-h period. Additionally, the IOP was measured over 4 weeks after FDM modeling. The results were presented as the mean value ± standard deviation (SD).

A-scan ultrasonography (Aviso, Quantel Medical, France) was employed to measure the AL as previous described [32]. Briefly, 0.5% proparacaine hydrochloride eye drops (Alcon, Fort Worth, USA) were administered for topical anesthesia ten minutes before the procedure. The probe tip was gently placed in contact with the central cornea of the guinea pig, and the distance from the corneal anterior surface to the retinal anterior surface was measured. Ten repeated measurements were taken for each eye, and the mean value was calculated.

Prior to the RE measurement, 0.5% tropicamide eye drops (Shenyang Xingqi Pharmaceutical Co. Ltd, Shenyang, China) were applied to mydriasis. Retinoscopy was performed under darker light conditions. Three repeated measurements were taken for each eye, and the average value was obtained.

#### Measurement of choroidal thickness in guinea pig

The choroidal thickness (ChT) of the guinea pigs at the fourth week post-induction was assessed using the Spectralis HRA + OCT system (Heidelberg Engineering, Heidelberg, Germany) as previous described [33]. The OCT scan center was positioned at the optic disc. Measurements were obtained at two specific locations: 600 and 1050 μm away from the optic disc. ChT was defined as the distance between the outer surface of the retinal pigment epithelium and the inner surface of the sclera. Measurements of ChT were taken at the intersection between the region of interest and the four quadrants of the concentric circles. The final average value of ChT was calculated.

#### Transmission electron microscopy

At the fourth week of the study, guinea pigs were euthanized by intraperitoneal injection of an overdose of pentobarbital sodium. The posterior scleral tissue, located 2 mm from the optic nerve, was carefully clipped. The dissected tissue was fixed in a 2.5% glutaraldehyde solution, followed by fixed in osmium tetroxide for 1 h, and then dehydrated with ethanol and acetone. The scleral tissue was embedded and sliced into ultrathin sections with 80 nm thickness. The sections were further stained with uranyl acetate and lead citrate and captured by transmission electron microscope (HT7800, Hitachi TEM system, Japan). Transverse and longitudinal images of the intermediate layer of scleral collagen fibrils were captured for each group of guinea pigs. The diameter distribution of scleral collagen fibrils was evaluated using ImageJ software (NIH, Bethesda, MD, United States).

#### Scleral proteomics analysis and bioinformatic analysis

After euthanizing the guinea pigs, posterior scleral tissue located 2 mm adjacent to the optic disc was promptly dissected. Retina, choroid, and extracellular connective tissues were carefully removed from the scleral tissue on ice. The dissected tissue was immediately placed into EP tubes and immersed in liquid nitrogen. The steps of tandem mass tag (TMT)-based proteomics analysis, including protein extraction, quantitative analysis, desalting enzymatic digestion, isobaric labeling, and mass spectrometry, were outsourced to Nohe Biotechnology Information Technology Co., Ltd. The filter criteria of differentially expressed proteins were defined as $p \leq 0.05$ and fold change (FC) >1.2 or <0.83, considered significantly upregulated or downregulated, separately. Functional databases were utilized for the analysis of the identified proteins, employing Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) for enrichment analysis. The discrete clusters of features with similar expression changes based on fuzzy c-means algorithm was applied by Mfuzz through R package. Protein–protein interaction (PPI) was analyzed with Cytoscape, for mapping their respective differential proteins linked to targeted KEGG and GO terms.

#### Statistical analysis

Statistical analysis and data visualization were conducted using SPSS 26.0 software (IBM, Armonk, NY, USA) and Prism GraphPad software (Version 9.5.1, GraphPad Software, La Jolla, CA, USA). ImageJ software was employed for the analysis of scleral collagen fiber quantity and diameter. The distribution of collagen fibrils diameters was plotted using Origin 8.0 software (Origin Software, OriginLab, USA). Data are presented as mean ± SD. One-way analysis of variance (ANOVA) was used to compare differences among multiple groups, followed by Tukey's post hoc test for pairwise comparisons. $p$-value < 0.05 was considered statistically significant.

## Results

#### IOP-lowering agents attenuated myopia progression in FDM guinea pigs

The right eye of the guinea pig successfully established the FDM model, which was confirmed by evaluating the SE and AL. The mean IOP in the FDM eye was slightly higher than that in the contralateral control eye, with no significant difference throughout the experimental period ($p > 0.05$) (Supplementary Fig. 1c). From the second week post-modeling, significant differences were observed in SE ($p = 0.0205$) and AL ($p = 0.0083$) between the two groups. By the fourth week, these differences were more pronounced: the FDM group

exhibited a lower SE (−2.65 ± 1.98 D) in comparison to the control group (−0.35 ± 1.08 D) ($p = 0.0047$), alongside a longer AL in the FDM group (8.73 ± 0.07 mm) versus the control group (8.59 ± 0.05 mm) ($p < 0.0001$) (Supplementary Fig. 1d, e). Specifically, after four weeks modeling, increase of AL in FDM group (0.71 ± 0.07 mm) was significantly higher than the control group (0.57 ± 0.04 mm) ($p < 0.0001$) (Supplementary Fig. 1f).

The IOP fluctuation at different time points following treatment with various IOP-lowering agents over a 24-h period were depicted in Fig. 1a. Compared with FDM group, the three IOP-lowering groups exhibited varying degrees of IOP reduction, particularly within the first 2 h after administration of Brinzolamide or Latanoprost eye drops ($p < 0.0001$). The differences in IOP among the four groups were statistically significant within the initial 8 h after treatment (all $p < 0.05$), but not at the 12-h and 24-h time points (Fig. 1a). The mean IOP profiles within 24 h in FDM_A group (−0.70 ± 0.61 mmHg), FDM_B group (−0.60 ± 1.56 mmHg), and FDM_AB group (−1.62 ± 1.60 mmHg) were all significantly lower than the FDM group (0.96 ± 0.50 mmHg) (all $p < 0.0001$) (Fig. 1b). After 4 weeks of treatment, the IOP at the final follow-up in all three IOP-lowering treatment groups was significantly lower than that in the FDM group (all $p < 0.05$) (Fig. 1c).

With respect to axial elongation, both the FDM_A group (0.59 ± 0.06 mm), FDM_B group (0.62 ± 0.07 mm), and FDM_AB group (0.55 ± 0.07 mm) showed significantly shorter AL growth compared to the FDM group (0.71 ± 0.06 mm) (all $p < 0.05$) (Fig. 1d). Moreover, to minimize interindividual differences, interocular differences in AL were also compared, revealing significant interocular disparities of AL between the three IOP-lowering treatment groups (FDM_A, 0.03 ± 0.03 mm, FDM_B, 0.08 ± 0.04 mm, FDM_AB, 0.01 ± 0.06 mm) and the FDM group (0.16 ± 0.04 mm) (all $p < 0.05$) (Fig. 1e). Although, regarding SE, only the FDM_A group (−3.75 ± 1.18 D) and the FDM_AB group (−3.32 ± 1.37 D) significantly differed from the FDM group (−5.83 ± 1.98 D) (Fig. 1f).

### Structural changes of choroid and sclera after various IOP reduction

This study reveals the differential impact of FDM and the utilization of IOP-lowering agents on peripapillary ChT (Fig. 2a). Specifically, the ChT in the FDM group (62.52 ± 4.50 μm) demonstrated a significant reduction compared to the control group (80.23 ± 7.08 μm) ($p < 0.0001$). Subsequent to the administration of

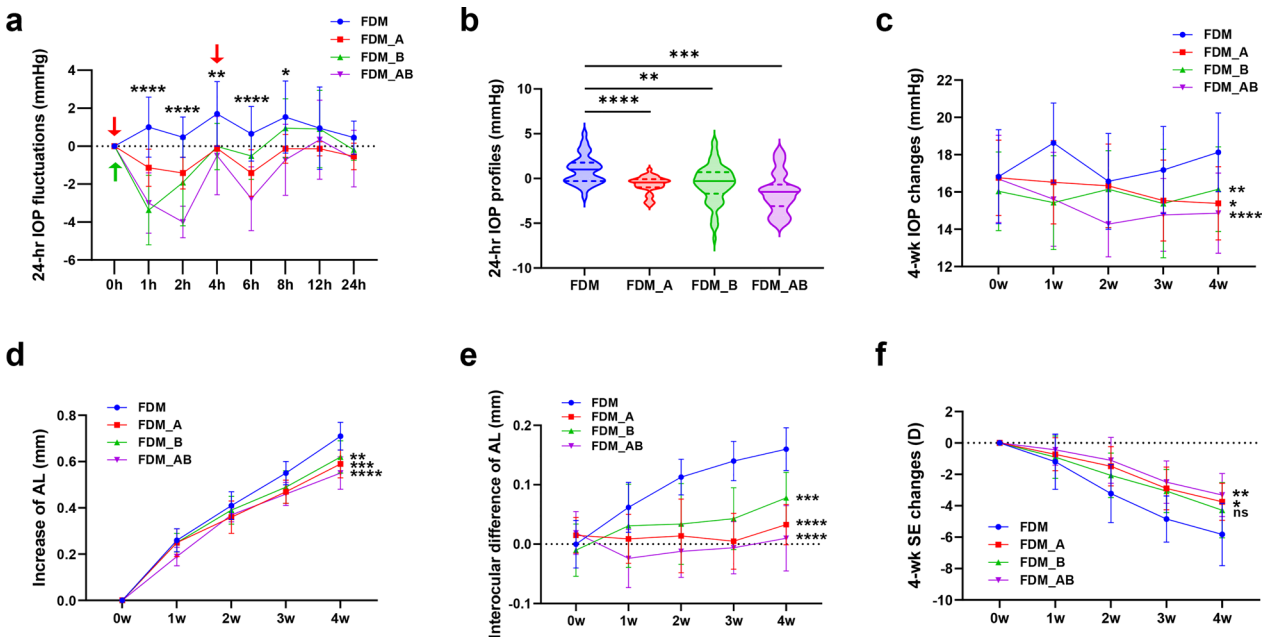


**Fig. 1** Effect of various IOP-lowering agents on myopia progression in guinea pigs. **a** 24-h intraocular pressure (IOP) fluctuations in the form-deprived group and three other groups receiving distinct IOP-lowering treatments. Green and red arrows indicate the time points for application of Latanoprost or Brinzolamide eye drops, respectively. **b** Violin plot depicting the range of 24-h IOP profiles across the four groups. **c** Weekly IOP measurements conducted in the right eyes of guinea pigs during four consecutive weeks. **d** Weekly increases in axial length (AL) compared among the four groups. **e** Analysis of interocular differences in AL changes across the four groups. **f** Changes in spherical equivalent (SE) observed among the four groups during the four-week period. (ns, non-significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, n = 10 per group)

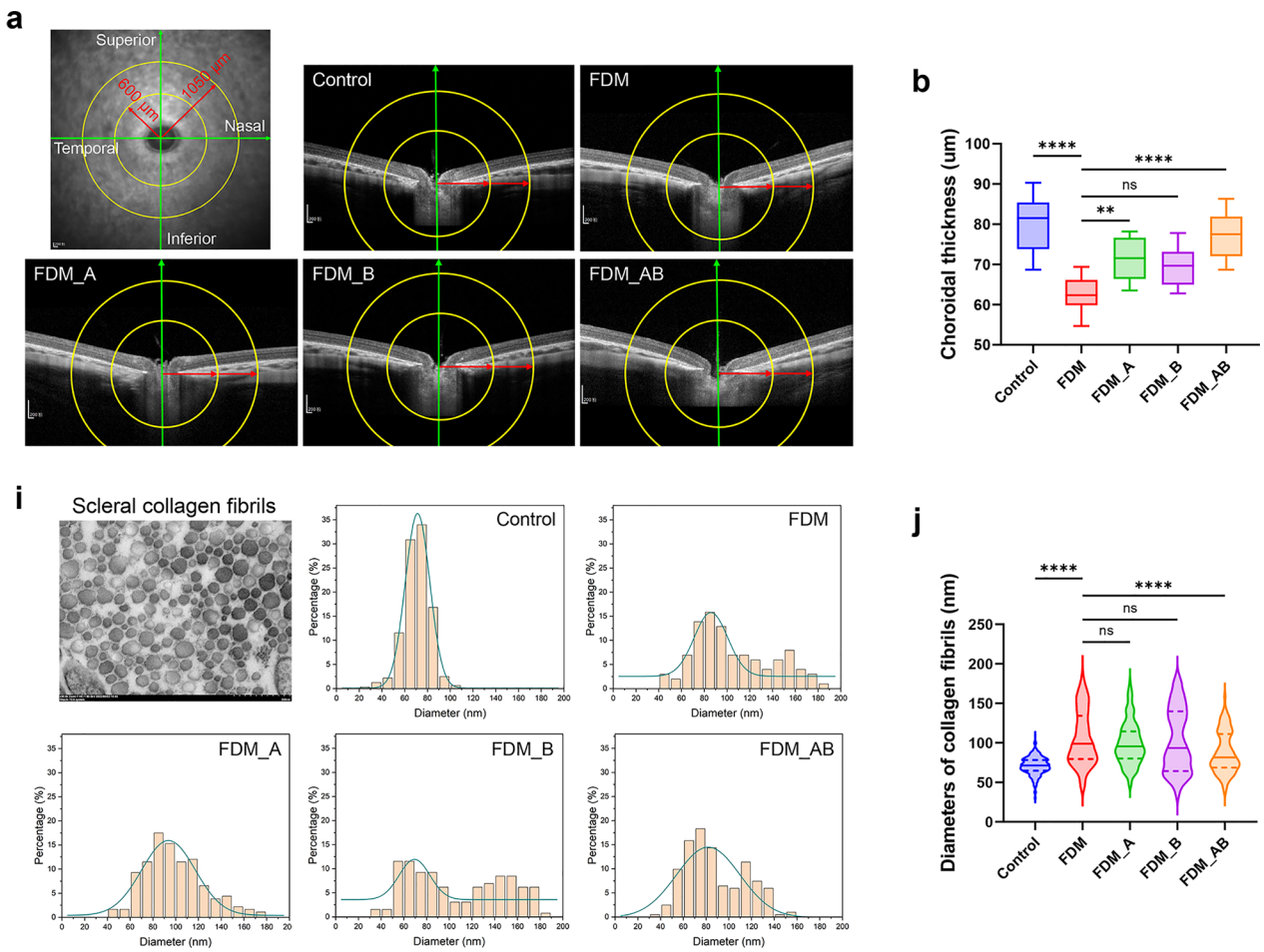


**Fig. 2** Effect of various IOP-lowering agents on choroid and sclera changes. **a** Illustration of the analysis template for optical coherence tomography (OCT) image and representative structural OCT images were displayed. **b** Comparison of mean choroidal thickness among the five groups. **c** Transmission electron microscope images of scleral collagen fibrils and diameter distribution histograms of the middle scleral layer in the five groups. **d** Violin plot displaying diameters of scleral collagen fibrils among the five groups. (ns, non-significant, $^{**}p < 0.01$, $^{****}p < 0.0001$)

IOP-lowering agents, the ChT in both the FDM_A group (71.56 ± 5.20 μm) and the FDM_AB group (77.28 ± 5.78 μm) exhibited an significantly increased thickness relative to the FDM group; however, within the FDM_B group (69.53 ± 4.71 μm), no significant thicken in ChT was observed compared to the FDM group ($p = 0.0508$) (Fig. 2b).

To further elucidate the mechanism by which the reduction of IOP retards axial elongation, we conducted an analysis focusing on alterations in the fibrous structure of the sclera (Fig. 2c). Our findings revealed that in the control group, the transection of posterior scleral collagen fibrils exhibited a dense and uniform arrangement, characterized by a mean diameter of 70.96 ± 11.45 nm. The majority of collagen fibrils displayed diameters primarily distributed within the range of 60–80 nm. Conversely, the FDM group demonstrated an uneven arrangement of scleral collagen fibrils, with a significant increase in diameter (105.3 ± 34.33 nm) compared to the control group ($p < 0.0001$). Additionally, longitudinal sections revealed a more complex and tangled arrangement within the FDM group. In the three IOP-lowering groups, the size and arrangement of collagen fibrils exhibited intermediate characteristics between the FDM group and the control group (Supplementary Fig. 2). The mean diameters of collagen fibrils in the FDM_A group and the FDM_B group were 99.34 ± 26.99 and 102.4 ± 39.59 nm, respectively. Statistical significance was observed between the FDM group and the FDM_AB group (87.75 ± 26.03 nm) ($p < 0.0001$), however, not between the FDM group and FDM_A group ($p = 0.3307$), FDM group and FDM_B group ($p = 0.9241$) (Fig. 2d).

### Proteomic profile of sclera in guinea pigs

To investigate molecular alterations associated with IOP-dependent scleral remodeling, a TMT-based proteomics

analysis was conducted. The heterogeneity of the proteomic profiles among different sample groups was compared using volcano plots and depicted in the heatmap (Fig. 3a, b). Our findings revealed 28 upregulated and 21 downregulated proteins in the comparison between the FDM group and the control group. Moreover, in the FDM_A group versus the FDM group, 52 proteins exhibited upregulation while 44 showed downregulation. In the case of the FDM_B group versus the FDM group, 114 proteins displayed upregulation, while 36 were downregulated. Regarding the FDM_AB group versus the FDM group, 80 proteins were upregulated, whereas 37 were downregulated. To explore pathway enrichment, distinct clusters of differentially expressed proteins were subjected to fuzzy c-means analysis. The proteomic enrichment outcomes (Fig. 3c) unveiled that clusters 1, 3, and 4

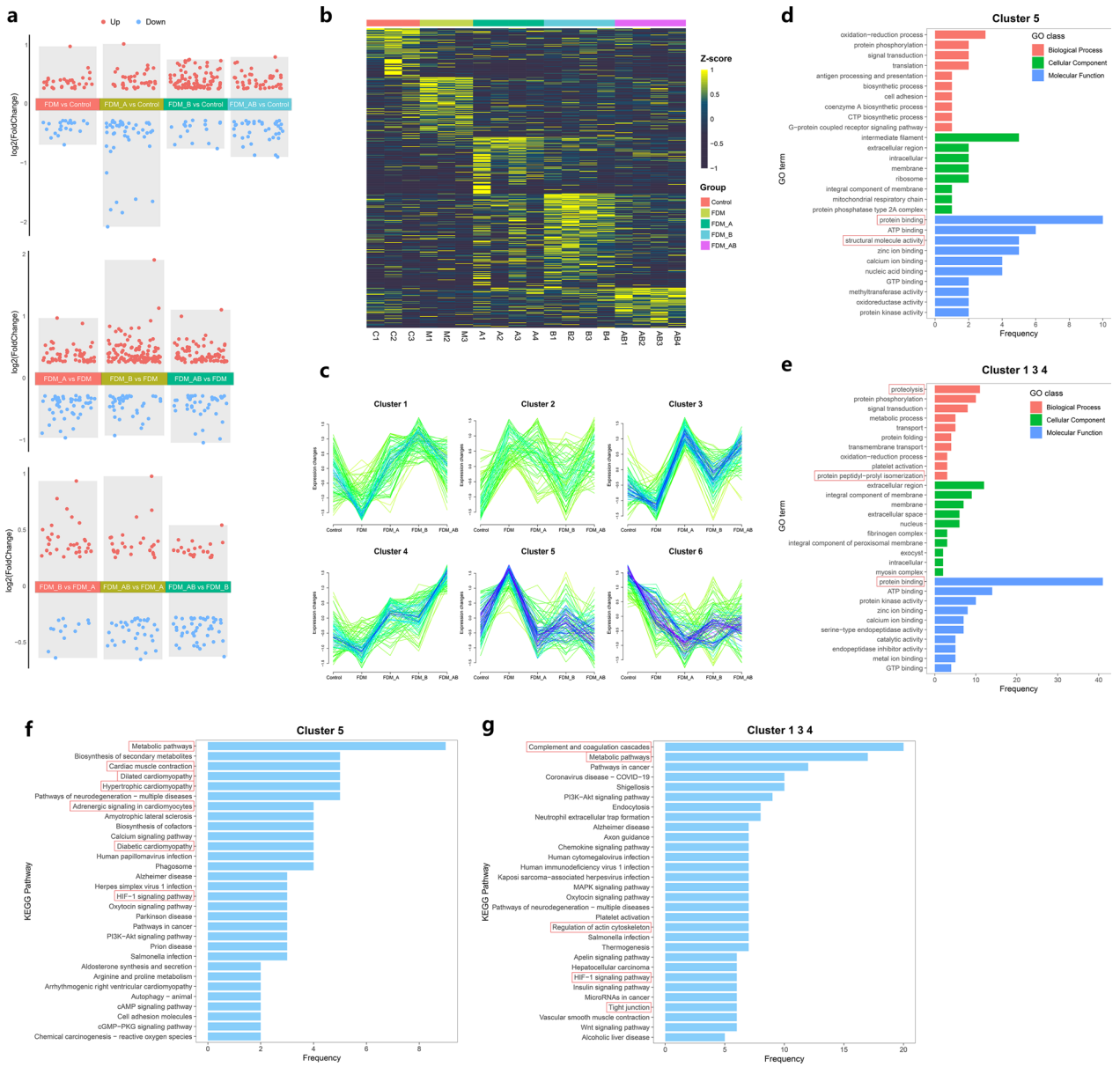


**Fig. 3** GO enrichment and KEGG pathway analysis of protein clusters based on different proteins and clustering. **a** Volcano plots illustrating differential proteins among various groups. **b** Heatmap displaying differential proteins across different groups. **c** Fuzzy c-means clustering of differential proteins. **d** GO enrichment analysis of differential proteins within Cluster 5. **e** GO enrichment analysis of differential proteins across Cluster 1, 3, and 4. **f** KEGG pathway analysis of differential proteins in Cluster 5. **g** KEGG pathway analysis highlighting differential proteins in Clusters 1, 3, and 4. (Proteins corresponding to GO and KEGG terms in the red-framed boxes were chosen for subgroup analysis)

manifested similar fluctuation trends and were therefore consolidated for subsequent analyses. Cluster 5 exhibited a conspicuous difference between the control and FDM groups, with the FDM group expressing higher levels compared to the three IOP-lowering groups, warranting separate analysis.

In cluster 5, GO analysis revealed upregulation of various processes during FDM modeling, including oxidation–reduction processes, protein phosphorylation, intermediate filament formation, protein binding, and structural molecule activity. Conversely, the application of IOP-lowering medications resulted in the downregulation of these proteins (Fig. 3d). KEGG pathway analysis identified that FDM modeling upregulated metabolic pathways, the HIF-1 signaling pathway, and notably, pathways linked to cardiac dilation, such as cardiac muscle contraction, dilated cardiomyopathy, and hypertrophic cardiomyopathy. In contrast, the other three groups treated with IOP-lowering medications exhibited a downregulation of these pathways (Fig. 3f). Regarding clusters 1, 3, and 4, GO analysis suggested a downregulation of proteolysis, extracellular region involvement, fibrinogen complex, protein and metal ion binding in the FDM group compared to the control group, while these aspects were upregulated in the IOP-lowering groups (Fig. 3e). KEGG pathway analysis indicated that complement and coagulation cascades, metabolic pathways, the PI3K-Akt signaling pathway, regulation of actin cytoskeleton, and the HIF-1 signaling pathway were downregulated in the FDM group but exhibited an upregulation after the application of IOP-lowering medications (Fig. 3g).

By other means, the comparison among the protein profiles of FDM_A group vs. FDM group, FDM_B group vs. FDM group, and FDM_AB group vs. FDM group unveiled 14 proteins that were consistently identified across all three profiles (Fig. 4). Subsequent literature review highlighted beta-2-microglobulin (B2M) and CD109 as proteins potentially associated with glaucoma, displaying connections with factors such as strain, stretch, pressure, or force. This revelation suggests a plausible link between reducing IOP and potential mechanisms involved in scleral remodeling.

#### Bioinformatic subgroup analysis of differential proteins

To determine the key contributors to scleral elongation at the molecular level, we focused on the identification of differentially expressed genes through bioinformatic subgroup analysis, and further mapped their respective differential proteins onto the PPI network. Utilizing the GO database and UniProt knowledgebase (http://www.uniprot.org/), we elucidated the subcellular localization and functional attributes of the identified proteins. As depicted in the heatmap, we investigated the target proteins and genes involved in the upregulated and downregulated pathways across various groups (Fig. 5a). This investigation spotlighted genes and proteins—such as DES, ACTN2, MYOT, TPM3, and TCAP—as being upregulated in the FDM group, while downregulated in the three IOP-lowering groups. These proteins, associated with intermediate filaments or Z-disc structures, demonstrated connections with cytoskeletal protein binding, actin binding, and cardiac muscle hypertrophy in response to stress (Fig. 5b).

In addition to the proteins overexpressed in the FDM group yet underexpressed in the IOP-lowering groups—such as COPS6, HDAC1, NUCB1, MTMR3, ZFPL1, and B2M, which were associated with functions like metallopeptidase activity, metal ion binding and homeostasis (calcium ion, zinc ion, iron ion). Notably, proteins involved in the fibrinogen complex (FGG, FGB, FGA), F11, and KLKB1 played roles in the regulation of fibrinolysis and exhibited downregulation in the IOP-lowering groups when compared to the FDM group. Moreover, proteins linked to the vitamin D metabolic process and vitamin transport (GC, APOA1), protein ubiquitination and phosphorylation (HSP90A, RBX1, and PRKAR2), as well as keratinocyte proliferation and differentiation (CD109), all participated in the FDM modeling process but displayed downregulation in the IOP-lowering groups (Fig. 5b). PPI networks were established utilizing differential proteins associated with targeted KEGG and GO terms (Fig. 5c, d).

## Discussion

Previously, we have proposed a perspective that lowering IOP plays an important role in controlling high myopia progression [34]. In the current investigation, we observed variable IOP reductions with different IOP-lowering medications in a guinea pig model of FDM. Notably, the FDM_AB group exhibited the greatest decrease in IOP, correlating with the most substantial delay in axial elongation. Lowering IOP also corresponded to increased ChT and modifications in the structure of scleral collagen fibrils. Our proteomic analyses exhibit, for the first time, scleral proteomic alterations of lowering IOP in myopic guinea pigs. These findings provide compelling evidence supporting the potential clinical utility of strategies aimed at controlling myopia progression.

As is known, various IOP-lowering medications exhibit differences in their capacity for IOP reduction and duration of efficacy. Besides, the sensitivity and pharmacological response to these medications vary among different species [35]. Compared to human eyes, guinea pigs exhibit more stable IOP and are less responsive to IOP-lowering medications [36]. Previous

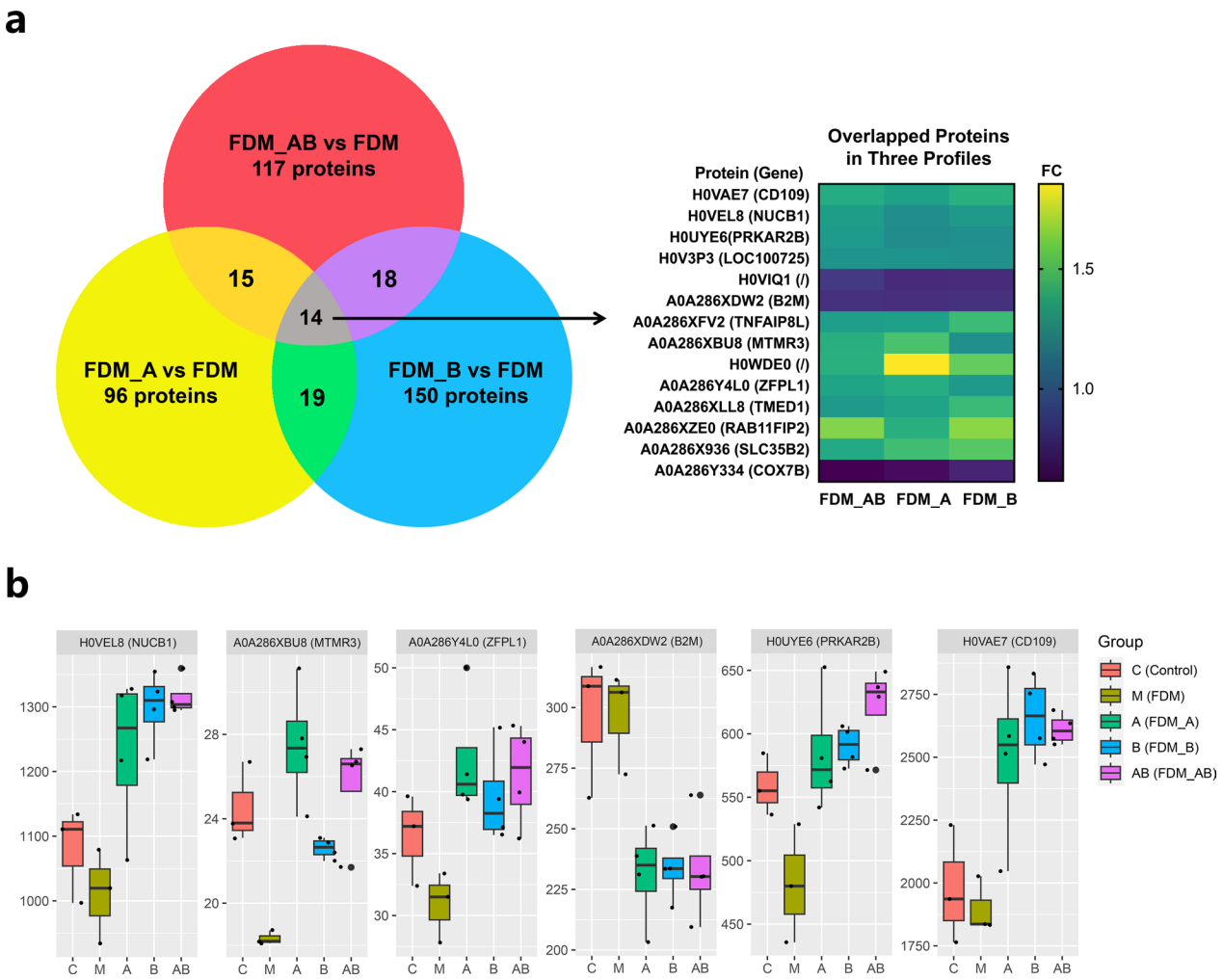


**Fig. 4** Overlapped proteins among differential protein profiles and select proteins. **a** The protein profiles of FDM_A vs FDM groups and FDM_B vs FDM groups, FDM_AB vs FDM groups and FDM_B vs FDM groups, FDM_A vs FDM groups and FDM_B vs FDM groups exhibited overlaps with 15, 18, and 19 differential proteins, respectively. A total of 14 proteins were found to be overlapped across all three profiles, and their details and fold changes are presented in the heatmap. **b** Relative quantitative values of selected labeled proteins

researches have reported that prostaglandin and α-adrenergic agonist eye drops significantly inhibit axial elongation in myopic guinea pigs, while β-adrenoceptor antagonists primarily reduce IOP without effectively influencing axial elongation [24–27, 31]. These findings are influenced by several factors including animal age, tonometry methods, the extent of IOP reduction, and specific pharmacological receptor profiles. Consequently, the uncertainty persists regarding the efficacy of IOP reduction in managing the progression of myopia due to the lack of direct comparability among findings from different studies. Similar to research in glaucoma, considering the impact of 24-h IOP fluctuations is crucial when investigating risk factors for the development of myopia [37]. Previous studies relying on weekly IOP measurements have proven inadequate in accurately capturing the dynamic IOP changes. In the present study, we observed that the mean 24-h IOP fluctuations in the FDM_AB group were significantly lower than those in the FDM_A and FDM_B groups. These findings are in line with a prior study exploring the impacts of four IOP-lowering agents (carteolol, bromonidine, brinzolamide, latanoprost) on 24-h IOP in guinea pigs [36]. Collectively, it can be inferred that the combination of medications (FDM_AB group) led to a more substantial reduction in IOP compared to the use of either medication alone. Crucially, this decrease in IOP corresponded to myopic progression, implying that a greater reduction in IOP correlates with a slower rate of AL growth.

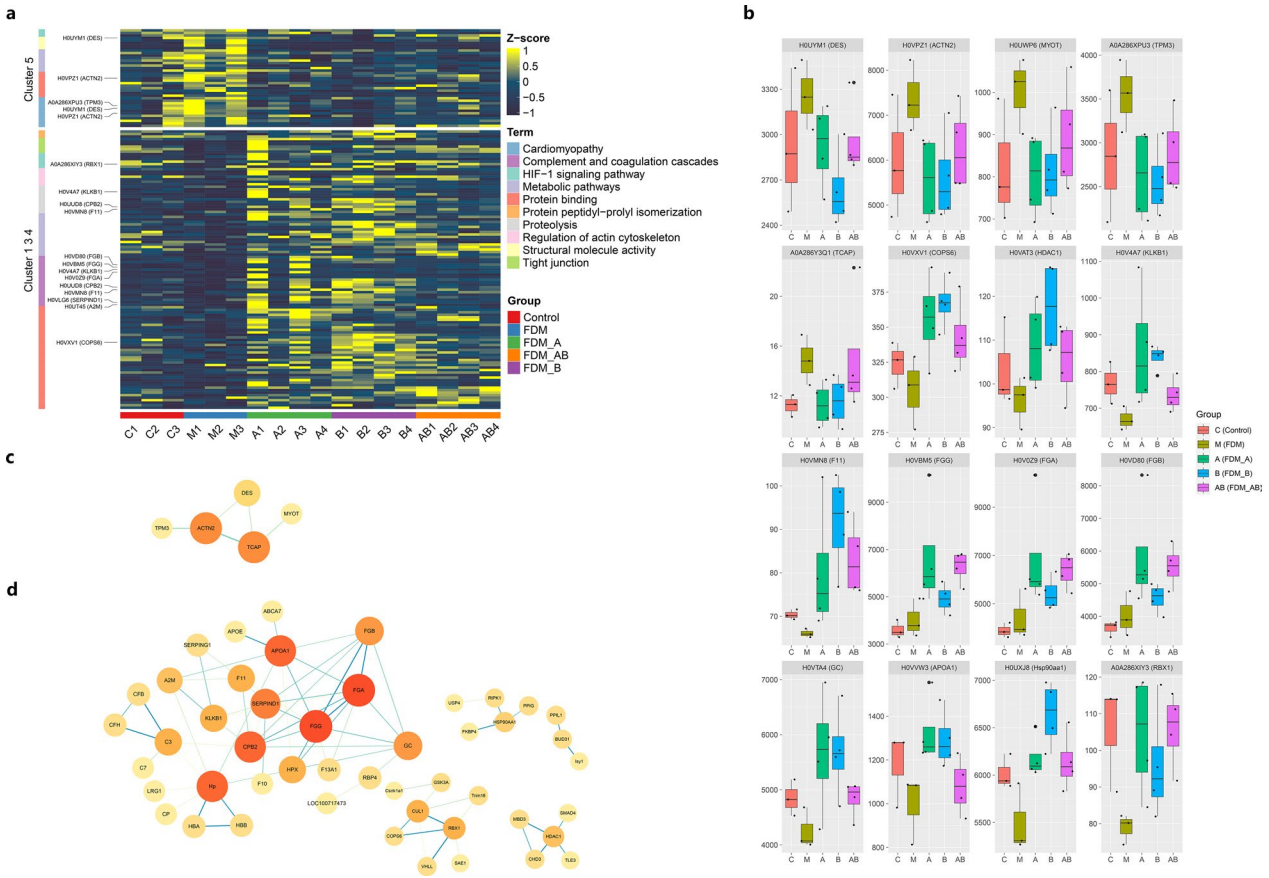


**Fig. 5** Subgroup analysis of differential proteins within targeted GO and KEGG terms from clusters. **a** Heatmap depicting differential proteins associated with targeted GO and KEGG terms from Cluster 5 and Clusters 1, 3, and 4. **b** Relative quantitative values of select labeled proteins. **c** Protein–protein interaction (PPI) analysis of differential proteins linked to targeted GO and KEGG terms from Cluster 5. **d** PPI analysis of differential proteins linked to targeted GO and KEGG terms from Clusters 1, 3, and 4

The FDM group exhibited significantly thinner ChT than the control group, while the FDM_AB group demonstrated significantly thicker ChT compared to the FDM group. These findings suggest that the reduction of IOP might regulate the progression of myopia by enhancing choroidal blood perfusion. Notably, the FDM_B group, the least change over time in terms of IOP profile, AL elongation, and SE change among the three IOP lowering groups, exhibited no significant difference in ChT compared with FDM group. Thus, we infer that changes in ChT, akin to axial elongation, may be correlated with the degree of IOP reduction. Some studies have additionally emphasized the role of choroidal blood flow in myopia progression, proposing ChT as a potential marker for monitoring myopia progression [33, 38–40]. Further research is necessary for a comprehensive understanding of the mechanisms connecting reduced IOP, ChT, and axial growth in myopia.

Excessive axial elongation and scleral thinning are prominent pathological features in myopia. In this study, the elevated IOP and axial elongation in the FDM group resulted in disorganized alignment and modified diameter of posterior scleral collagen fibrils, with an average distribution spanning from 60 to 150 nm. These findings contrast with those reported in the myopia model of tree shrews, where short-term myopia induction did not significantly affect the diameter of scleral collagen fibrils, whereas long-term myopia induction resulted in a significant thinning of collagen fibrils in the posterior sclera [41]. The disparity in outcomes might be ascribed to the duration of FDM modeling. In the three IOP-lowering medication groups, the distribution of scleral collagen fibril diameters fell between that of the control group and the FDM group, indicating that IOP might impact the structure of scleral collagen fibrils and potentially impede the alteration of collagen fibrils from a regular form to an FDM-like configuration. Additionally, when compared with the FDM group, only the FDM_AB group exhibited a significant difference, which suggests that the alterations in scleral collagen fibrils were also correlated

with the degree of IOP reduction. Further investigation is required to clarify the influence of these structural alterations on the biomechanical characteristics of the sclera.

The investigation into scleral proteins and their interactions presents an opportunity to comprehend cell function regulation, biological process mechanisms, and advancements in drug design and disease treatments. Previous studies have explored the impact of myopia induction and axial elongation on guinea pig scleral proteomics [42–44]. However, the specific response of scleral proteomics to IOP reduction remains unknown, which is crucial for elucidating how IOP reduction influences axial elongation and scleral remodeling. In our study, we conducted screening and visualization of significant differences in protein expression among five groups using fuzzy c-means clustering, and also compared their overlapped proteins. Our findings demonstrated that the upregulated expression proteins in IOP-lowering medication groups are mainly associated with proteolysis, fibrinolysis, metal ion binding, vitamin metabolism, and protein ubiquitination. Conversely, downregulated proteins are associated with intermediate filament organization, cytoskeletal protein binding, and alterations resembling cardiac dilation. Furthermore, the KEGG pathway analysis revealed their involvement in pathways such as complement and coagulation cascades, the HIF-1 signaling pathway, and the PI3K-Akt signaling pathway. Srinivasalu et al. demonstrated that prostaglandin receptor activation enhances cAMP and HIF-1α signaling pathways, reducing scleral fibrosis and increasing myopia in guinea pigs [45]. Additionally, Tong et al. reported that dibazole inhibits myopia progression in FDM rabbits by down-regulating HIF-1α expression in the retina and sclera [46]. Moreover, Yu et al. found that inhibiting the PI3K-Akt signaling pathway suppresses fibroblast proliferation and reduces collagen deposition [47]. Based on these collective findings, we hypothesize that dysregulated proteins associated with IOP reduction may promote scleral fibroblast proliferation and induce scleral fibrosis, consequently delaying axial elongation and myopia development.

Among the 14 overlapped proteins identified in the three IOP-lowering groups and the FDM group, B2M and CD109 genes were notably included. Lee et al. demonstrated a significant correlation between B2M and parameters such as mean ganglion-internal plexus thickness, blood vessel density, and fundus blood perfusion in individuals diagnosed with normal-tension glaucoma [48]. Elevated IOP can induce increased B2M expression and trigger acute immune response [49]. Additionally, Man et al. [50] observed that CD109 inhibits the TGF-β signaling pathway, effectively reducing excessive extracellular matrix production in fibroblasts of patients diagnosed with systemic sclerosis. In light of these findings, our study hypothesizes that the reduction of IOP may modulate the expression of B2M and CD109 genes, potentially enhancing fundus blood perfusion and regulating the production of extracellular matrix by scleral fibroblasts. Consequently, this modulation may contribute to altering scleral stiffness and impeding the progression of myopia. However, further research and validation are imperative to investigate the specific pathways involved in the modulation of scleral protein expression and gene changes induced by IOP reduction.

Limitations of this study should be noted. Firstly, the guinea pig sample size was relatively modest, and the duration of IOP-lowering treatment was limited to four weeks. The potential for myopia rebound following the cessation of IOP-lowering treatment has not been investigated. Secondly, although we initially identified differences in protein expression and gene regulation in the sclera following IOP reduction, further investigations are imperative to elucidate the determinative signaling pathways and underlying mechanisms associated with scleral gene and protein expression, changes in ChT, scleral collagen remodeling, and alterations in scleral biomechanics. Furthermore, the translation of these experimental results into clinical implications requires additional exploration. Our ongoing randomized controlled trial is expected to provide additional insights into the clinical significance of IOP reduction in delaying axial elongation in myopic patients (*Clinicaltrials.gov*, identifier: NCT06201455).

## Conclusions

In conclusion, our findings suggest that interventions aimed at reducing IOP reduction hold promise as an effective strategy for managing myopia. The use of IOP-lowering medications demonstrated efficacy in delaying AL growth, with a greater reduction in IOP resulting in a decelerated rate of axial elongation. These effects were concomitant with increased ChT, alterations in scleral collagen fibril structure, and changes in scleral protein expression involving critical processes like proteolysis, fibrinolysis, and metal ion binding. Consequently, our study provides novel perspectives and potential biomarkers for myopia treatment, and lays a robust foundation for future research exploring the use of IOP reduction as a viable approach to impede the progression of myopia.

**Abbreviations**

| | |
|---|---|
| AL | Axial length |
| IOP | Intraocular pressure |
| ChT | Choroidal thickness |
| D | Diopters |
| FDM | Form-deprivation myopia |
| FDM_A | FDM and twice-daily instillations of Brinzolamide |
| FDM_AB | FDM and instillations of Brinzolamide and Latanoprost |

| | |
|---|---|
| FDM_B | FDM and daily instillations of Latanoprost |
| HIF-1α | Hypoxia-inducible factor-1α |
| MMPs | Matrix metalloproteinases |
| SD | Standard deviation |
| SE | Spherical equivalent |
| TGF-β | Transforming growth factor-beta |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06546-8.

Additional file 1.

**Acknowledgements**
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**Funding**
The National Key Research and Development Program of China (2022YFC2502800); the National Natural Science Foundation of China (82070955); the Natural Science Foundation of Guangdong Province (2020A1515011282); the Science and Technology Program of Guangzhou, China (202201020362, 2024A03J0257); the High-level Hospital Construction Project, Zhongshan Ophthalmic Center, Sun Yat-sen University (303020104); and the Medical Scientific Research Foundation of Guangdong Province of China (A2025291).

**Data availability**
Data are available from the corresponding author upon reasonable request.

## Declarations

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no completing of interests.

Received: 30 January 2024 Accepted: 29 April 2025
Published online: 16 May 2025

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