Clinical Proteomics

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Comparative proteomic analysis of human vitreous in rhegmatogenous retinal detachment and diabetic retinopathy reveals a common pathway and potential therapeutic target

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Abstract

Background The vitreous humor serves as a window into the physiological and pathological processes of the eye, particularly the retina. Diabetic retinopathy (DR), a leading cause of blindness, involves hyperglycemia-induced damage to retinal cells, leading to ischemia and elevated nitric oxide levels, culminating in vascular proliferation. Rhegmatogenous retinal detachment (RD) results from a break in the neuroretina, triggering ischemia, photoreceptor death, and cellular proliferation. Proliferative vitreoretinopathy (PVR) further complicates these conditions through fibrous proliferation. Despite their prevalence and potential for blindness, our understanding of the molecular mechanisms underlying these vitreoretinal diseases is incomplete.

Methods and results To elucidate disease mechanisms and identify potential therapeutic targets, we conducted a comparative proteomic analysis of vitreous samples from DR, RD, and macular pucker (P) patients, which were chosen as controls. LC–MS analysis identified 988 quantifiable proteins, with distinct clustering observed among disease groups. Differential expression analysis revealed 202 proteins in RD vs. P and 167 in DR vs. P, highlighting distinct proteomic signatures. Enrichment analysis identified glucose metabolism as an altered process in both diseases, suggesting common pathways despite differing etiologies. Notably, aldo–keto reductase family 1 member B1 (AKR1B1) has emerged as a potential key player in both DR and RD, indicating its role in glucose metabolism and inflammation. In silico drug screening identified diclofenac, an approved ophthalmic non-steroidal anti-inflammatory drug (NSAID), as a potential therapeutic agent targeting AKR1B1.

Conclusion Our study revealed distinct proteomic signatures and common pathways in vitreoretinal diseases, highlighting AKR1B1 as a potential therapeutic target. Using diclofenac during diagnosis and postoperative care for diabetic retinopathy or rhegmatogenous retinal detachment may reduce complications, lower costs, and improve

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quality of life. Future research will focus on confirming AKR1B1's role in vitreoretinal diseases and understanding diclofenac's mechanism of action.

Keywords Vitreoretinal diseases, Proteomics signature, Therapeutic target, AKR1B1, Glucose metabolism, Inflammation

Introduction

The vitreous humor can provide important information about physiological and pathophysiological processes within the eye, particularly concerning the retina of various vitreoretinal diseases that can ultimately lead to blindness. The vitreous body not only has a structural role but also physiological functions, as it serves as a repository and facilitates the diffusion of substances involved in eye metabolism. Due to its close contact with the inner retina and ciliary body, it reflects both physiological and pathological conditions of the eye [10]. Currently, there is no clear understanding of many of the molecular processes underlying vitreoretinal diseases.

Diabetic retinopathy (DR) is the leading cause of blindness among working-age adults [3]. It can result in macular edema and tractional retinal detachment (TRD). Hyperglycemia can induce damage to retinal cells and capillary closure, leading to retinal ischemia and elevated levels of nitric oxide (NO). This, in turn, increases the activity of protein kinase C and the levels of different growth factors, including vascular endothelial growth factor (VEGF). These events can stimulate the development of new blood vessels from existing matured vessels, which are subsequently encompassed by glial cells and fibrous proliferation, potentially leading to TRD [25]. Moreover, DR is characterized by a severe inflammatory state due to an impairment of glucose metabolism, leading to ROS accumulation [2]. In this context, the aldo–keto reductase (AKR1) family has drawn much interest in recent decades because of its involvement in diabetes complications such as TRD due—among other mechanisms—to the activation of NF-Kb signaling [30].

Rhegmatogenous retinal detachment (RD) is the separation of the neural retinal layers from the retinal pigment epithelium (RPE). It is induced by a break in the neuroretina that allows the influx of liquefied vitreous. In the detached retina, the outer layers become ischemic, and photoreceptors undergo cell death, mainly through apoptosis. Moreover, 3–4 days after retinal detachment, a peak of proliferation of nonneuronal cells, such as astrocytes, endothelial cells, pericytes, and microglia, can be observed [13]. A possible complication of RD is proliferative vitreoretinopathy (PVR), a condition defined by the growth and contraction of cellular membranes within the vitreous cavity. Fibroblasts or fibrocytes have been detected in epiretinal membranes in PVR, but the origin of these cells is debated. Different authors have suggested that these cells could be transformed RPE cells or metaplastic vascular endothelial cells, glial cells, macrophages, or hyalocytes [18]. Inflammation was found to be a significantly altered process among the pathogenic mechanisms of PVR in RD patients [36, 37].

Macular pucker (P) is a condition defined by an epiretinal membrane, described as a semitranslucent non-vascularized membrane over the internal limiting membrane (ILM) on the surface of the retina. It can be idiopathic or secondary to various conditions, such as trauma and intraocular inflammation. The initial event that can induce idiopathic pucker syndrome is posterior vitreous detachment (PVD), which can cause dehiscence of the internal limiting membrane. Through this opening, microglial cells move to the preretinal surface, where they can interact with hyalocytes and laminocytes. Subsequent trans-differentiation to fibroblast-like cells leads to the development of an epiretinal membrane [20].

These diseases can be surgically treated, albeit with a profound disparity in the results. The impact of the illness and other elements, such as age, ethnicity, and other—yet unknown—factors, play a role in the observed differences in surgical outcomes. This means that a portion of the patient population continues to show limited response to existing medical and surgical interventions, presenting an opportunity for potential benefits from innovative therapeutics. Despite the pressing demand for novel treatments, identifying disease targets and developing therapeutic compounds for these targets are both time-consuming and costly.

Cellular alterations that could be causative or correlated factors are at the core of the different medical and surgical responses. Thus, utilizing early and minimally invasive techniques to observe changes in the eye could offer practical and straightforward means to guide the treatment of high-risk patients and enhance our understanding of molecular and cellular alterations associated with vitreoretinal diseases. Vitrectomy allows vitreous fluid to be obtained for subsequent analyses in a straightforward and almost riskless way, compared to obtaining retinal tissue. Among the informative analyses to be performed, liquid chromatography-mass spectrometry (LC–MS)-based proteomics has become the most widely used method for unbiased and large-scale analysis of complex samples, such as clinical tissues and biofluids. Compared

to traditional immunoassays, this high-throughput technique increases the depth of proteome coverage and enables the accurate identification and quantification of thousands of proteins without prior knowledge. Thus, comparative proteomic studies can be designed without a specific biological hypothesis, allowing the formulation of new research questions and the detection of potential biomarkers. In light of these considerations, the LC–MS method is a powerful tool for investigating the involvement of specific molecular and cellular alterations in vitreoretinal diseases [22]. However, most existing proteomics studies only analyze single diseases (for example, see [17, 24, 29]).

Here, we compared the human vitreous proteomic profiles of patients with either diabetic retinopathy or rhegmatogenous retinal detachment with those of patients with macular pucker, which composed the control group [14, 17].

We aimed to identify specific and common proteomic patterns for each pathology to deepen our understanding of physio-pathological processes and delineate potential novel therapeutic targets.

Materials & methods

Sample collection

Approval from the Azienda Provinciale per I Servizi Sanitari—Trento Institutional Review Board was obtained (APSS Protocol 7.2.4—n. 1684|2021), and written informed consent was acquired in accordance with the Declaration of Helsinki on Biomedical Research Involving Human Subjects. Considering the different timings of the onset of the diseases investigated, we selected groups of patients aged 20–70 years. We excluded patients with hereditary retinal diseases, optic nerve diseases, glaucoma, para-oncologic exudative retinopathies, melanoma, hypertensive retinopathy, vascular occlusions, and prior/current intravitreal therapies with anti-VEGF.

We collected vitreous samples from patients undergoing vitrectomy for rhegmatogenous retinal detachment (RD, n = 8, three females and five males, mean age 55 years, mean HbA1c 34 mmol/mol), diabetic tractional retinal detachment (DR, n = 8, four females and four males, mean age 58 years, mean HbA1c 70 mmol/mol), and macular pucker (P, n = 7, four females and three males, mean age 65 years, mean HbA1c 36 mmol/mol) at "Azienda Provinciale per i Servizi Sanitari" of Trento. In the operating theatre, anesthesia was administered, and the sterile operating field was prepared. Subsequently, three 23 G trocars were inserted, and 0.3 mL of vitreous fluid was aspirated into a tuberculin syringe using a vitrector with infusion off and 7500 cuts per minute. The samples were labeled and associated with each patient and immediately stored at −80 °C.

Sample preparation for proteomic analysis

Vitreous samples were centrifuged at 15000 × *g* for 15 min at 4 °C to remove cell debris and impurities. The supernatants were transferred to a clean tube and supplemented with a complete protease inhibitor cocktail. To increase proteome coverage, immunodepletion of highly abundant proteins was carried out. Briefly, 125 µl of the vitreous sample was loaded onto High Select Top14 Abundant Protein Depletion Mini Spin (Thermo Scientific) and incubated for 60 min at room temperature with gentle rotation. Depleted flowthroughs were recovered by centrifugation. Subsequently, the samples were adjusted to a final concentration of 2% SDS and 50 mM HEPES (pH8) and sonicated at 4 °C for 10 min (30 s on and 30 s off) to promote an effective protein extraction and denaturation. The protein concentration was determined using a bicinchoninic acid (BCA) protein assay (Thermo Scientific). Afterward, 30 µg of protein from each sample was reduced with 10 mM dithiothreitol (DTT) at 56 °C for 30 min and alkylated with 22.5 mM iodoacetamide (IAA) at room temperature for 30 min.

As previously described, clean-up and protein digestion were performed using the single-pot solid-phase-enhanced sample preparation (SP3) method [16]. SP3 method is a broadly used approach for proteomic samples because of its high sensitivity, scalability, and cost-effectiveness, allowing rapid and efficient detergent removal [28]. Briefly, 6 µL of carboxylate magnetic beads (50 µg/µL) was added to the samples. Afterward, acetonitrile (ACN) was added to a final concentration of 70% (v/v), and the samples were mixed at room temperature for 20 min. Subsequently, the beads were immobilized by incubation on a magnetic rack for 2 min. The supernatant was discarded, and the beads were rinsed twice with 70% (v/v) EtOH and 100% ACN. The beads were resuspended in 40 µL of 100 mM ammonium bicarbonate supplemented with trypsin at an enzyme-to-protein ratio of 1:20 (w/w). After overnight digestion at 37 °C, the peptide mixture was collected by incubation on a magnetic rack. Peptides were then acidified by adding trifluoroacetic acid (TFA) to a final concentration of 0.1% and desalted using a C18 stage tip. For LC–MS analysis, desalted tryptic peptides were lyophilized and resolubilized in 0.1% formic acid (FA).

LC–MS analysis

One µL of resolubilized peptides was injected into an Easy-nLC 1200 HPLC system (Thermo Scientific) and separated on a 75 µm × 30 cm C18 reversed-phase nanocolumn (EASY-Spray™ HPLC Columns) with a two-component mobile phase (A = 0.1% formic acid in water, and B = 0.1% formic acid in acetonitrile). The peptides were

then eluted using a gradient from 5 to 25% over 120 min, followed by 25–40% over 10 min, and 40–98% over 10 min, for a total gradient of 155 min at a flow rate of 300 nL/min. The nano-column was heated to 40 °C.

The peptides were analyzed on an Orbitrap Fusion Tribrid mass spectrometer (Thermo Scientific) in data-dependent mode. The temperature of the ion transfer tube was set to 275 °C. Full scans were performed at a resolving power of 120.000 FWHM and voltage of 2100 (mass range: 350–1100 m/z, AGC target value: 10e6 ions, maximum injection time: 50 ms). Each full scan was followed by a set of (HCD) MS/MS scans within a 3-s cycle, with a collision energy of 30%. Fragment data were acquired in an ion trap (AGC target: 10e4 ions, maximum injection time: 150 ms). The ion transfer tube temperature was set at 200 °C. Dynamic exclusion was enabled and set to 50 s, with a mass tolerance of 5 ppm.

The data were acquired using Xcalibur 4.3 and Tune 3.3 software (Thermo Scientific). Quality control standards utilizing QCloud [5] were applied to all acquisitions to monitor long-term instrumental performance during the project.

Proteomics data analysis

All proteomic data were searched against the in silico digested Human database (UniProt), which includes a list of major known contaminants and reversed versions of each sequence. Proteome Discoverer v.2.2.0 (Thermo Scientific) and the MASCOT search engine (v.2.6.2, MatrixScience) were used for protein identification, with a precursor mass tolerance of 10 ppm and a product mass tolerance of 0.6 Da. Trypsin was selected as the enzyme, allowing for five missed cleavages. The search incorporated static modification of carbamidomethyl (C) and variable modification of oxidation (M) and N-terminal protein acetylation. Contaminants were filtered out, and a false discovery rate (FDR) of < 0.01 was applied for both peptides and proteins. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [31] partner repository with the dataset identifiers PXD050611 and https://doi.org/10.6019/PXD050611.

MS downstream analysis was performed using the ProTN proteomics pipeline {ProTN, manuscript in preparation, www.github.com/TebaldiLab/ProTN and www.rdds.it/ProTN). In summary, peptide intensities were log_2-transformed, normalized (median normalization), and summarized into proteins (median sweeping) using functions in the DEqMS Bioconductor package [39]. Missing intensities were imputed using the PhosR package [21]. Differential analysis was performed with the DEqMS package, considering proteins with an absolute log_2-fold change (FC) > 0.75 and $p < 0.05$ as significant. Thresholds were applied to p-values and log2 fold changes to identify differentially expressed proteins with both statistical and biological significance. We set cutoff values of 0.05 for p-values (< 5% probability of a type I error) and 0.75 for log2 fold changes (> 68% increases or decreases in abundance). Hierarchical clustering analysis was performed using the pheatmap R package version 1.0.12 (https://CRAN.R-project.org/package=pheatmap).

Enrichment analysis

Differentially expressed proteins (DEPs) were filtered using a cutoff of $p < 0,05$ (statistically significant results) and an absolute log_2 fold change (FC) > 0.75. UniProt IDs of DEPs were converted to Entrez IDs by UniProt ID mapping tools, and redundancies were corrected manually. DEP Entrez IDs were used to perform GO enrichment analysis by using the Bioconductor (Bioconductor version: Release 3.18) clusterProfiler R package v. 4.5.2 [35–37]. Quantitative GO overrepresentation analyses (ORA) were performed [4]. The ORA results were graphically represented using the cnetplot function of the clusterProfiler R package.

In silico drug screening

We performed an in silico drug screening by exploiting the "target data fetcher" tool from MolBook software. This tool allowed us to identify compounds showing activity on selected targets, as reported in the ChEMBL database [12], by entering the UniProt ID of the ten most differentially regulated proteins for each comparison: 9 upregulated and 1 downregulated protein for RD vs. P,8 upregulated and 2 downregulated proteins for DR vs P. The rationale of this choice was that the highest fold change of protein expression between pathological condition and control would represent the effect of key regulators of those processes. Moreover, to find a common signature between the two contrasts, we performed the same analysis on the 33 differentially expressed proteins common to the two comparisons: 26 upregulated and 7 downregulated (Fig. 7).

The identified small molecules were combined with the approved small molecules list in the chEMBL database (https://www.ebi.ac.uk/chembl/; [8, 38]). Subsequently, to refine the analysis, we restricted our search to the small molecules in the chEMBL database already approved for an eye-specific use.

Results

Comparative proteomics analysis of human retinopathies

During ocular and retinal diseases, the disruption of retinal tissue could release proteins into the vitreous humor. Therefore, studying the proteomic profile of vitreous humor could be a valuable tool to elucidate

differences among different diseases. Vitreous samples were collected from patients undergoing vitrectomy for rhegmatogenous retinal detachment (RD, n = 8), from those with diabetic retinopathy (DR, n = 8), and from patients with macular pucker syndrome (P, n = 7), without distinction of sex.

To elucidate the biological processes underlying these pathologies, we conducted a proteomic analysis by depleting the samples of abundant proteins (see Methods) and profiling them using a label-free quantitative proteomics approach (LFQ), as shown in Fig. 1. Through MS/MS spectrum database search analysis, 988 quantifiable proteins were identified. Multidimensional scaling analysis using all identified proteins revealed distinct clustering of the RD, DR, and P groups (Fig. 2). A comparative analysis of the differentially expressed proteins (DEPs) in DR and RD samples, respectively, compared to those in P samples, was performed to explore the signature of diseased individuals. Following filtering based on a threshold set to $p < 0.05$ and $\log_2$-fold changes > 0.75, 202 proteins were detected as DEPs in RD compared to the control, including 95 upregulated and 107 downregulated proteins; 167 proteins were detected as DEPs in DR compared to the control, including 58 upregulated and 109 downregulated proteins (Fig. 3). Complete lists of differentially expressed proteins between sample groups are provided in the supplemental data (Supplementary Table 1).

Retinopathies exhibit distinct proteomic signatures

We conducted a hierarchical clustering analysis on the protein signatures obtained from our samples. The raw data were normalized to protein abundances filtered by using DEPs ($p < 0.05$); we utilized a complete linkage method for hierarchical clustering, defining the distance between two clusters as the maximum distance between their individual components. We constructed a heatmap in which each protein is represented by a single row of colored boxes and columns representing different patient samples. The protein abundance is represented by different colors (Fig. 4). The analysis revealed differences in the proteomic signature of the three experimental groups, highlighting the differences between the protein expression profiles of DR and RD patients. This result suggested the differential expression of proteins that collectively contribute to the onset and progression of each pathology.

Different proteomics signatures are enriched in similar biological processes

To further investigate the differences between these two clinical conditions, we performed an overrepresentation analysis (ORA), a statistical method to determine whether genes from preexisting sets (e.g., genes in GO terms) were present more often than expected (overrepresented) in a subset of the data of interest. We conducted an ORA using the enrichGO function of the ClusterProfiler R package on the DEPs obtained from

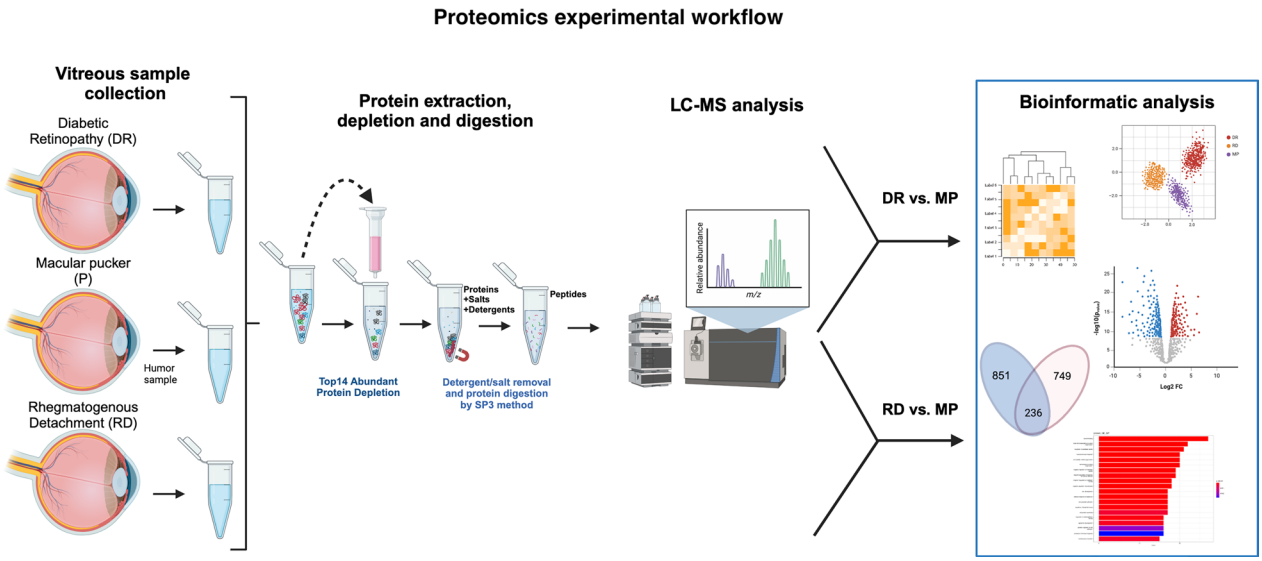


Fig. 1 Experimental workflow for the identification and quantification of vitreous proteins from patients with diabetic retinopathy (DR), rhegmatogenous detachment (RD), or macular pucker (P). Vitreous samples were collected during vitrectomy, proteins were extracted, highly abundant proteins were depleted, and the samples were digested with trypsin using the single-pot, solid-phase-enhanced sample preparation (SP3) method. The resulting peptides were desalted and profiled by LC–ESI–MS/MS on a Thermo Fisher Orbitrap Fusion mass spectrometer, followed by bioinformatics and statistical analysis. Images were created using BioRender

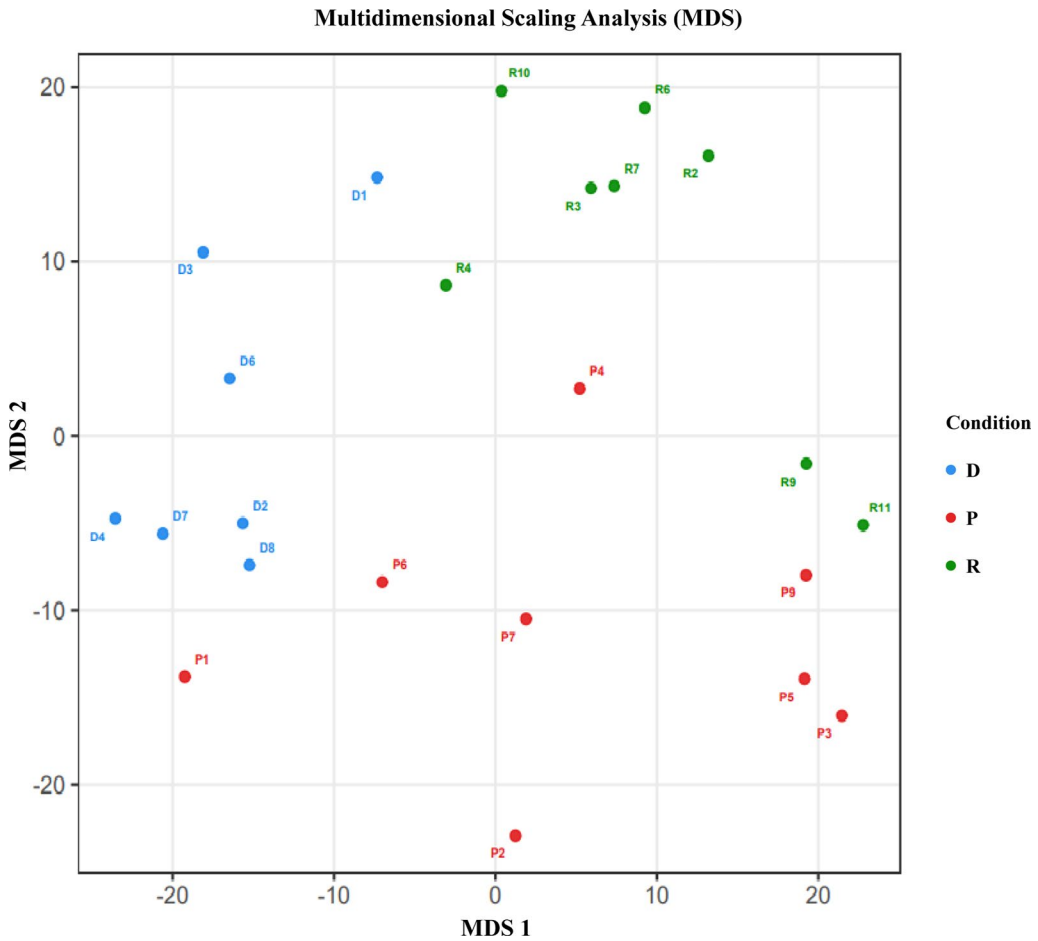


Fig. 2 Multidimensional scaling analysis (MDS) based on normalized and scaled levels of 988 quantified proteins. The samples are color-coded and display good separation. D: diabetic retinopathy, R: rhegmatogenous detachment, P: macular pucker

the bioinformatic analysis, distinguishing for each experimental comparison the upregulated proteins with a $\log_2$-fold change > 0.75 from the downregulated proteins with a $\log_2$-fold change < 0.75. In the case of DR, as expected, biological process (BP) terms such as "glucose catabolic process" and "carbohydrate biosynthetic process" were enriched in the upregulated proteins (Supplementary Table 2). Moreover, in this subset of DEPs, BPs such as "wound healing," "negative regulation of wound healing," and "regulation of complement activation" were enriched, collectively highlighting an inflammatory response during DR. Some of the most represented enriched BPs are shown in Fig. 5. The downregulated proteins in the DR-enriched group were involved in cell–cell and cell-ECM adhesion, keratinocyte differentiation, and negative regulation of peptidase and proteolysis. These results suggest that the ECM is rearranged as cells move toward a fibrotic state (Fig. 5). Clinically, these processes reflect the proliferative phase of diabetic retinopathy, which leads to a tractional retinal detachment due to fibrovascular proliferation. Unexpectedly, proteins upregulated in RD formed a cluster of carbohydrate/glucose metabolic-enriched biological processes similar to those in DR, probably because in both conditions, there is retinal ischemic damage: in diabetes, due to the nature of the disease and in rhegmatogenous retinal detachment, due to ischemic damage to the outer layers. Additionally, upregulated DEPs in RD (i.e., TMSB10, SFRP1, and PFN1) were found in enriched processes related to actin polymerization/depolymerization. In contrast, the downregulation of proteins such as ROBO4, GFAP, and SEMA3A plays an essential role in the negative regulation of axon genesis (Fig. 6). Clinically, these processes may be associated with the epithelial-mesenchymal transition, which initiates vitreoretinal proliferation and the progression of this pathological condition. Taken together, these data show that, even though with different quantitative proteomic profiles, these two retinopathies exhibit carbohydrate/glucose metabolism dysregulation.

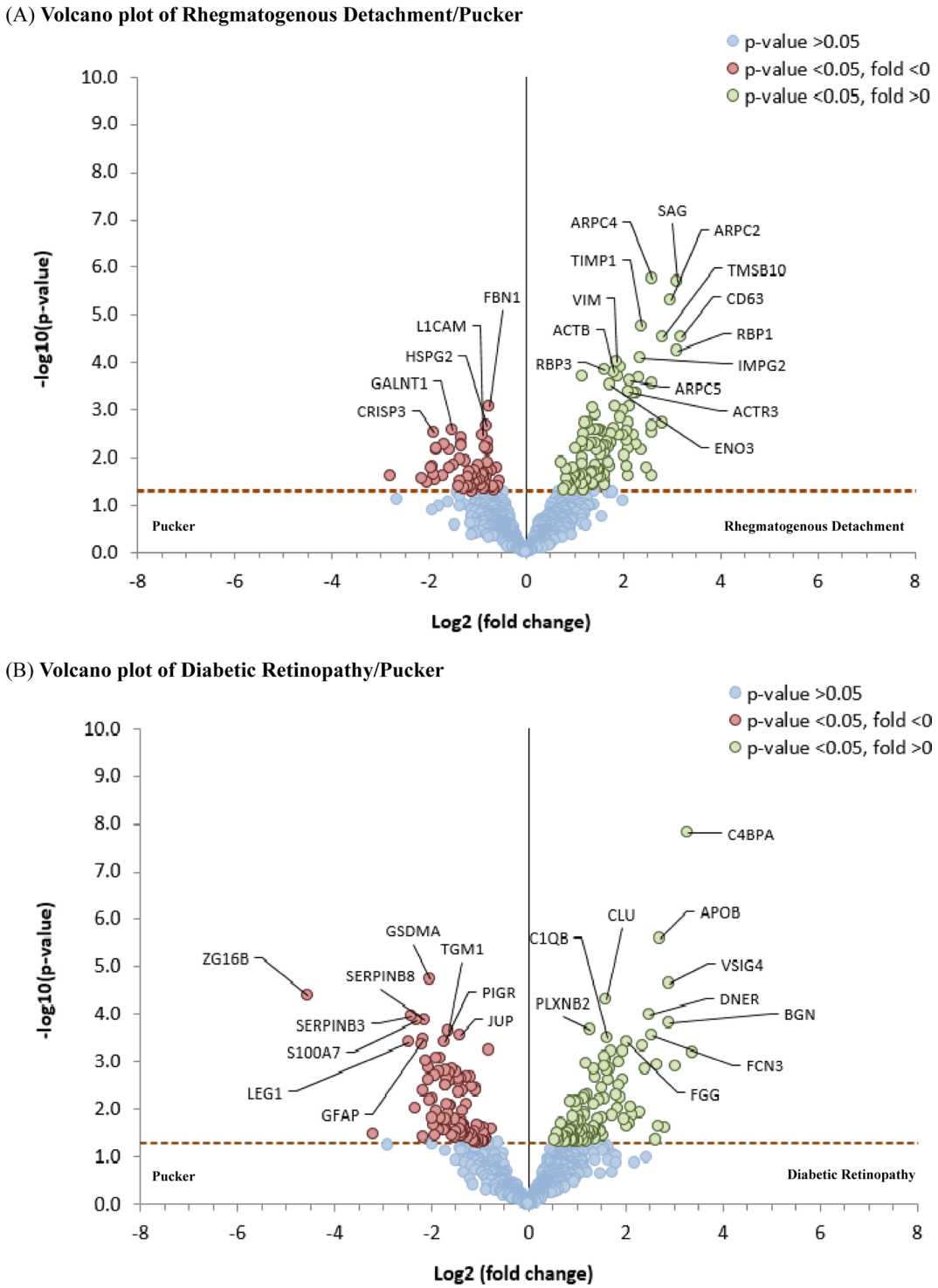


Fig. 3 Volcano plots showing the up and downregulated proteins in the experimental comparisons. **A** rhegmatogenous detachment vs. macular pucker, **B** diabetic retinopathy vs. macular pucker. The blue dots represent non-significantly differentially expressed proteins ($p > 0.05$), and the red and green dots represent significantly differentially expressed proteins ($p < 0.05$) that were downregulated and upregulated, respectively, compared to those in the control condition

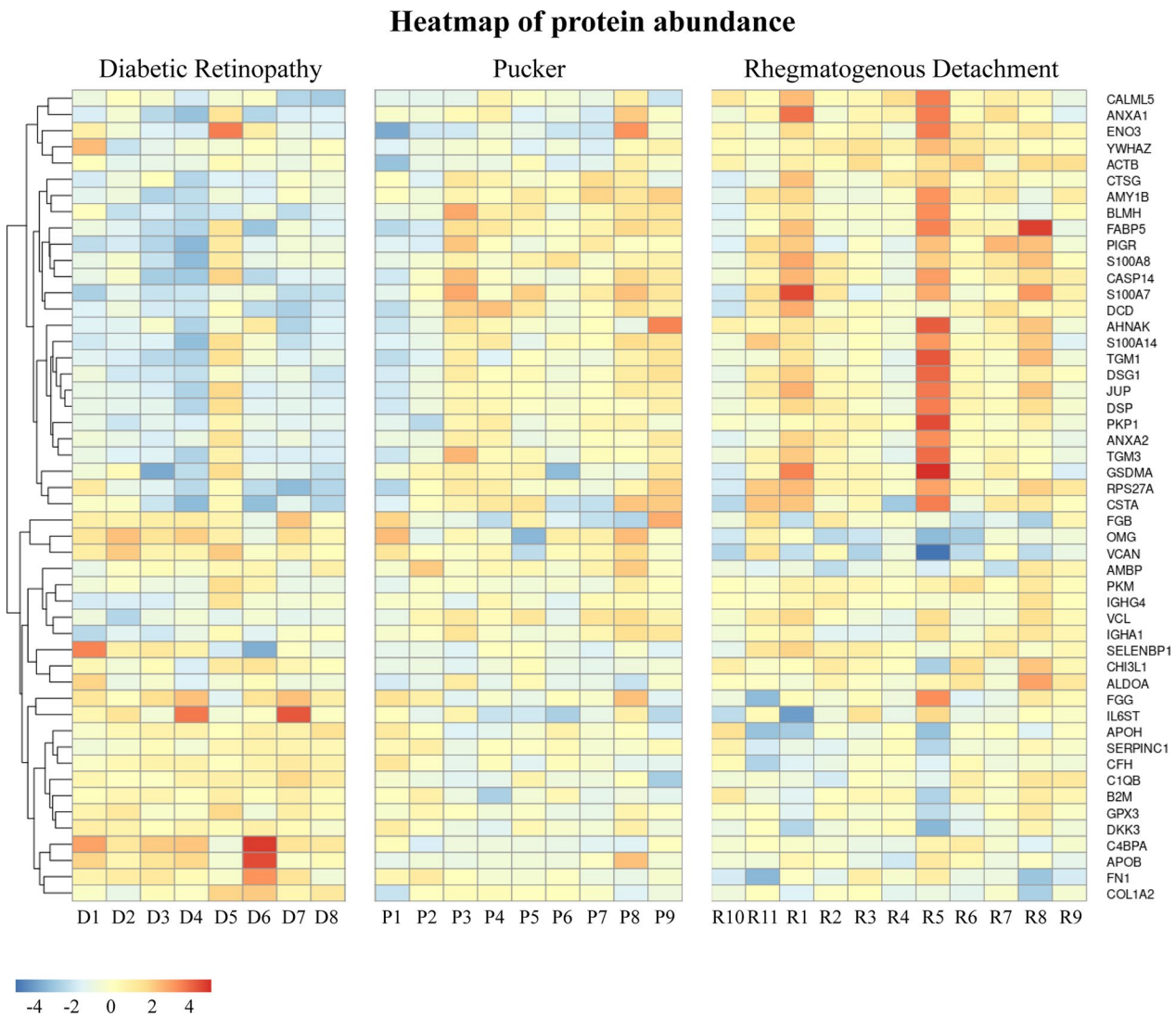


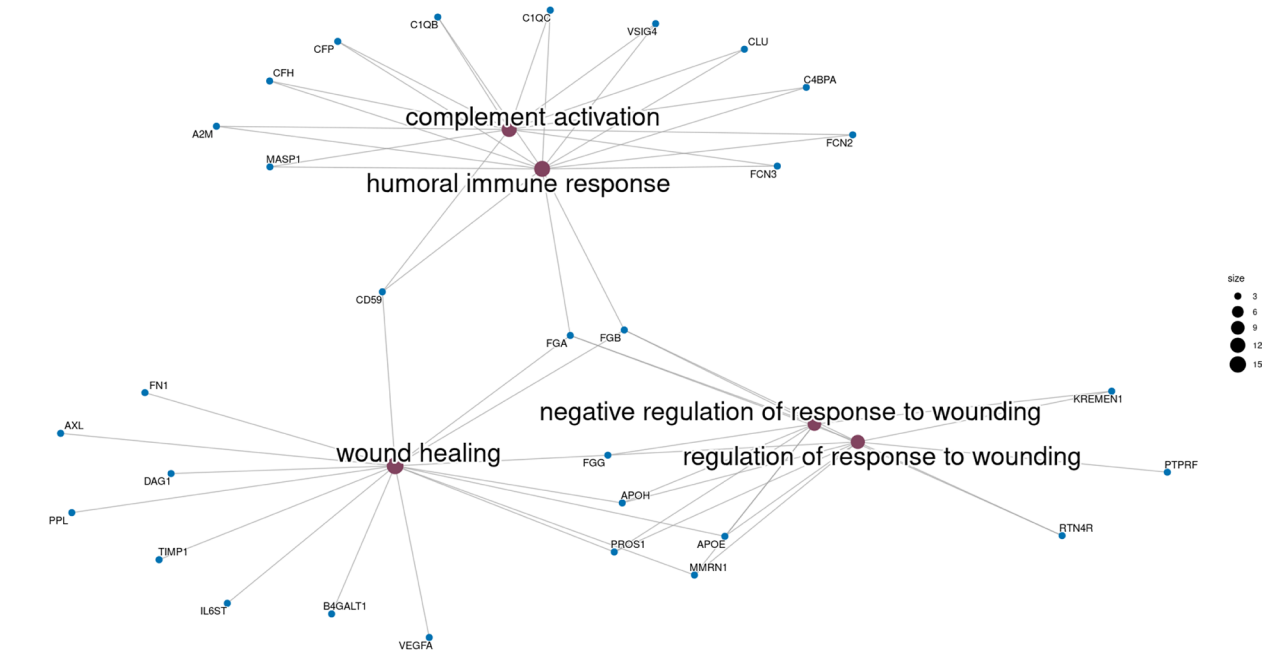
Fig. 4 Hierarchical clustering analysis of the protein abundance of the most differentially expressed proteins ($p < 0.05$). The values are normalized across each row, where red and blue represent up and downregulation, respectively. The analysis revealed a clear separation between the diabetic retinopathy (D) and the rhegmatogenous detachment (R) groups. P: macular pucker

Aldo–keto reductase family 1 member B1 (AKR1B1) is commonly upregulated in both retinopathies

In agreement with our results, previous metabolomics analysis of similar patient groups [14] suggested that glucose metabolism is a central pathway involved in retinopathies such as RD and DR, possibly as a cause of DR or a consequence. To identify possible common proteins involved in glucose metabolism and altered in both diseases, we intersected the DEPs of the two groups to detect similarities in protein expression (Fig. 7 and Supplementary Table 3). We obtained two common protein pools from the intersections: 26 upregulated proteins (Fig. 7A) and 7 downregulated proteins (Fig. 7B). Among these common proteins, AKR1B1 stands out not only for its enrichment in biological processes related to glucose metabolism but also for its retina-specific expression and involvement in eye diseases such as DR and PHACE syndrome [15, 26].

AKR1B1 is an aldo–keto reductase that catalyzes the NADPH-dependent reduction of various carbonyl-containing compounds. Moreover, it displays enzymatic activity toward endogenous metabolites and plays a vital role in the polyol pathway, catalyzing glucose reduction to sorbitol during hyperglycemia [15]. Our results, in accordance with published data [7, 9], confirm the involvement of AKR1B1 in diabetic retinopathy but also suggest a potential yet undiscovered role for AKR1B1 in a broader group of retinal diseases. Our data indicate that

(A) **Diabetic Retinopathy/Pucker Up Regulated Biologial Processes**



(B) **Diabetic Retinopathy/Pucker Down Regulated Biologial Processes**

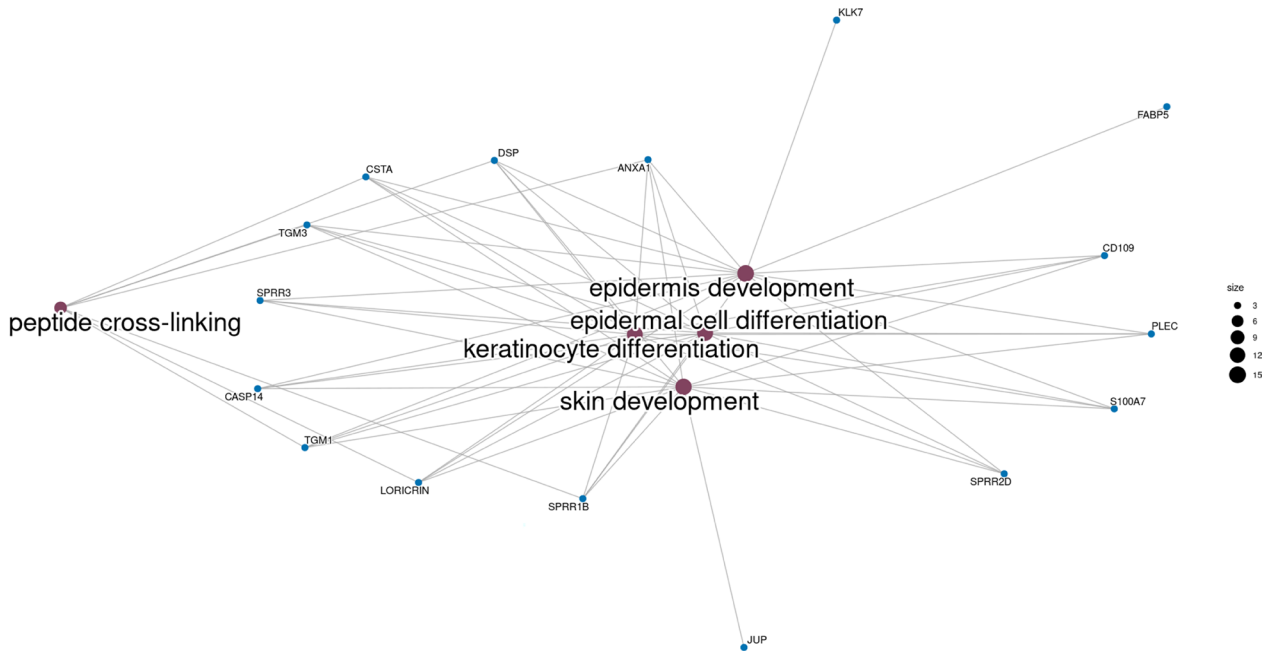
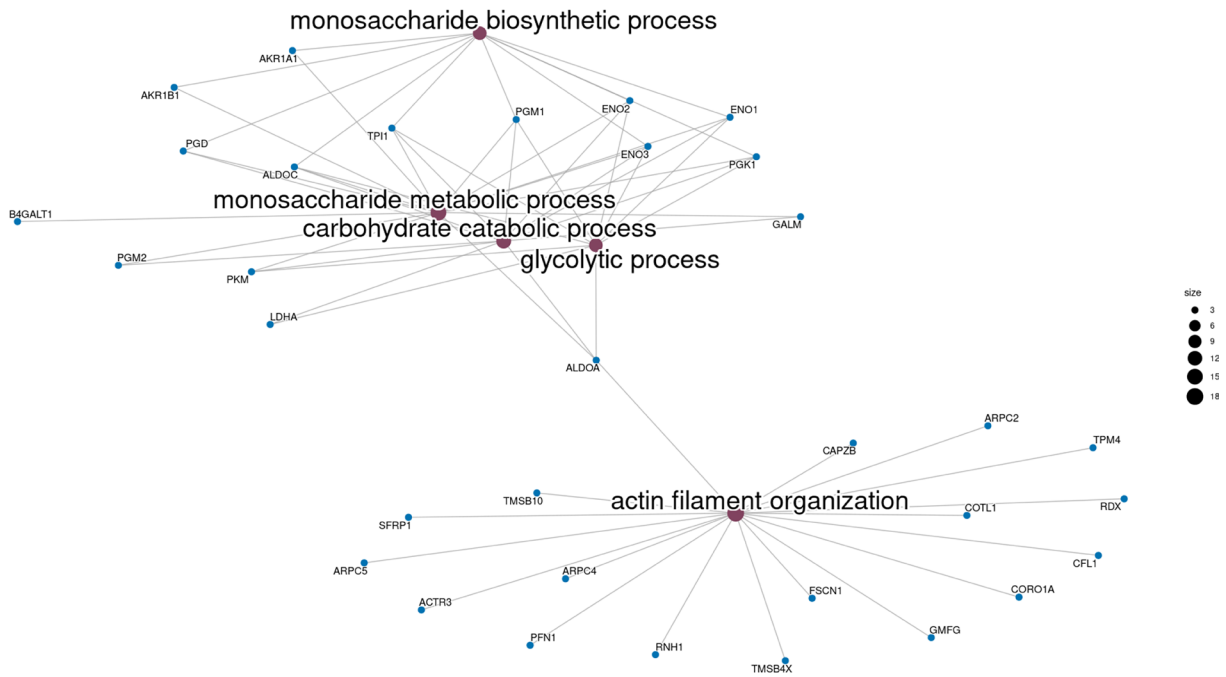


Fig. 5 Cnet plot of enriched biological processes (BP) in diabetic retinopathy against macular pucker syndrome. The plots were produced in ClusterProfiler based on the results of the "enrichGO" function and show the five most enriched BPs resulting from the overrepresentation analysis (ORA) of upregulated (**A**) and downregulated (**B**) differentially expressed proteins. The dot size represents the number of differentially expressed genes that belong to the enriched BP

(A) **Rhegmatogenous Detachment/Pucker Up Regulated Biological Processes**



(B) **Rhegmatogenous Detachment/Pucker Down Regulated Biological Processes**

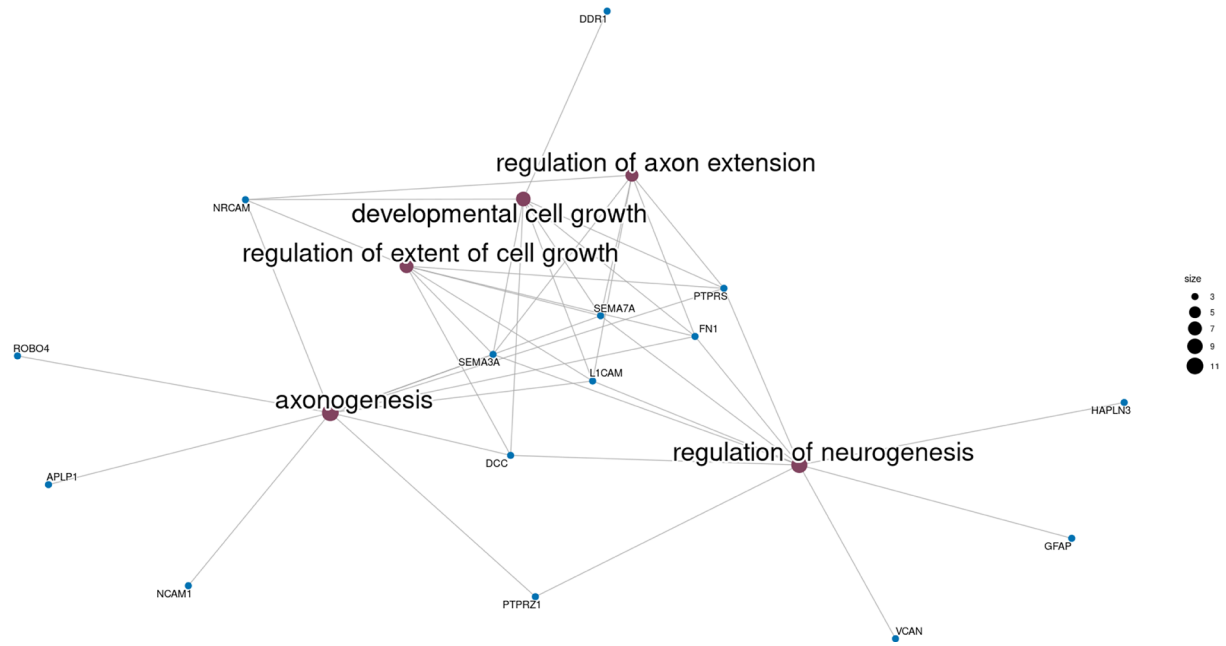


Fig. 6 Cnet plot of enriched biological processes (BP) in rhegmatogenous detachment against macular pucker. The plots were produced in ClusterProfiler based on the results of the "enrichGO" function and show the five most enriched BPs resulting from the overrepresentation analysis (ORA) of upregulated (**A**) and downregulated (**B**) differentially expressed proteins. The dot size represents the number of differentially expressed genes that belong to the enriched BP

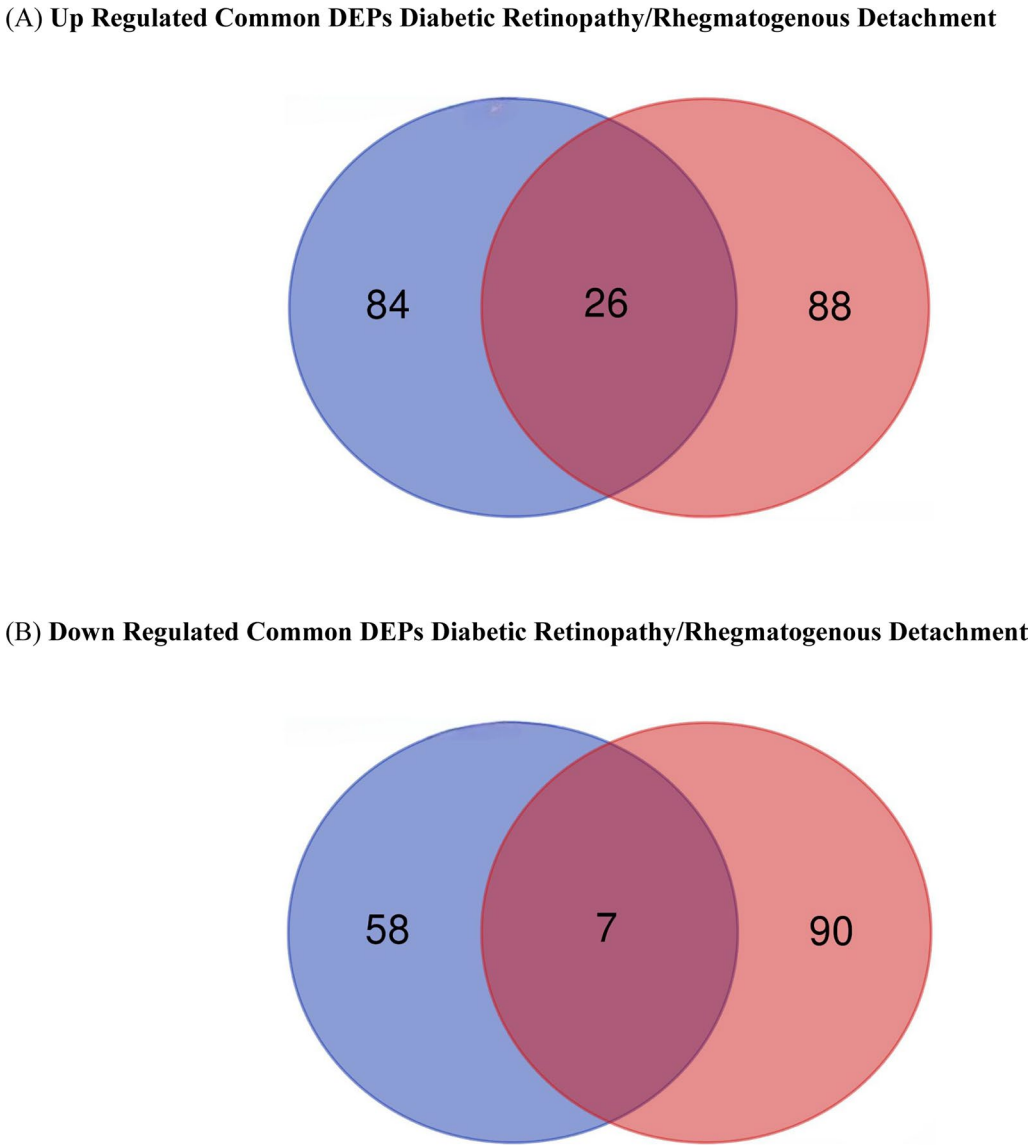


Fig. 7 Venn diagrams showing the upregulated (**A**) and downregulated (**B**) differentially expressed proteins common to both comparisons. DR vs. P: blue set; RD vs. P: orange set

AKR1B1 plays a pivotal role in both DR and RD, where this protein may act as a molecular metabolic switch and contribute to retinal degeneration by promoting a proinflammatory phenotype.

In silico small molecule screening revealed a potential shared role for diclofenac in DR and RD

The previous results emphasized that, despite distinct etiopathologies, the presence of a pool of common proteins—mainly upregulated proteins—might drive the deregulation of carbohydrate metabolism in both pathologies. For this reason, to identify putative small molecules targeting our DEPs, we decided to perform an in silico drug screening on the 10 most regulated proteins for each comparison and, in parallel, all the differentially expressed proteins common to the two comparisons (Fig. 7) to detect approved small molecules of pharmacological interest. For this purpose, we utilized the "target data fetcher" tool on the "MolBook" platform, which allowed us to detect small molecules targeting any protein of interest. Performing this analysis for the two conditions (RD vs. P and DR vs. P) yielded non-significant findings. Conversely, after screening the 33 common DEPs, we identified 1340 small molecules that may target ARK1B1. We then intersected our list with all the approved small molecules and with those specific to the

eye present on chEMBL, a manually curated database of bioactive molecules with drug-like properties (Figure S1A). We obtained a group of thirteen approved small molecules, putative AKR1B1 interactors (Figure S2B). However, only one small compound matches all three criteria: diclofenac, a small molecule already approved and used in the ophthalmic field. Diclofenac belongs to the class of nonsteroidal anti-inflammatory drugs (NSAIDs) used to treat inflammatory processes and reduce pain, as well as in the treatment of retinal cataract surgery [32, 34]. These data point to the inhibition of AKR1B1 using Diclofenac as a novel potential treatment for eye diseases affecting retinal integrity, where cellular carbohydrate metabolism is altered and a proinflammatory phenotype manifests.

Discussion

Our data show that altered glucose metabolism is a common feature of both rhegmatogenous retinal detachment (RD) and diabetic retinopathy (DR).

In RD, the upregulation of glucose metabolic processes could be explained by the ischemia of the retinal outer layers [27]. The outer retinal layers experience ischemic damage due to the lack of oxygen and nutrients from the choriocapillaris, leading to alterations in glucose metabolism, photoreceptor death, and the creation of a proinflammatory environment. In photoreceptors, glucose is also used to produce metabolites necessary for outer segment renewal, as well as to generate reducing power through the pentose phosphate pathway, to protect photoreceptors against oxidative damage that can increase the pro-inflammatory environment [14, 17, 18, 23]. The altered milieu observed in RD justifies the peak of proliferation of nonneuronal cells, such as astrocytes, endothelial cells, pericytes, and microglia, which can be observed 3–4 days after retinal detachment, and the epithelial-mesenchymal transition of retinal pigment epithelium marks the beginning of proliferative vitreoretinopathy [6, 13, 17–19]. These observations can explain the upregulation of actin organization and the downregulation of axonal proteins observed in our datasets.

Altered glucose metabolism is the basis of diabetes. In the development of diabetic retinopathy (RD), this dysregulation can be exacerbated by increasing ischemic and inflammatory processes, as shown by our data where we find an enrichment of complement activation and humoral immune response pathways—some proinflammatory molecules could also be synthesized by Müller cells [25, 33]. An altered environment can induce fibrovascular proliferation with increased internal-retinal-blood barrier damage, which boosts inflammation, ischemic damage, and impaired glucose metabolism, constituting a vicious cycle [25]. Fibrovascular proliferation is characterized by the reorganization of vascular and extracellular matrix (ECM) components, which, of course, are linked. Vascular cells interact with the ECM through mechanosensitive integrins and the associated cytoskeleton, producing matricellular proteins such as CCN1 and CTGF. Moreover, matrix stiffness independently regulates cell migration, differentiation, and proliferation. The basement membrane (BM) in DR typically thickens, influencing ECM–cell signaling, cell–cell communication, vascular cell survival, and barrier functions. Alterations in the extracellular milieu can ultimately lead to tractional retinal detachment [33]. Consequently, avoiding the onset of the vicious cycle or the ability to interrupt it could be a significant therapeutic goal, as this could prevent diabetes-related vision loss and its serious complications, such as tractional retinal detachment. Thus, disrupted glucose metabolism, a proinflammatory milieu, and impaired cellular metabolism are implicated in both proliferative vitreoretinopathy – a possible consequence of RD—and DR.

We found that the enzyme AKR1B1 was overexpressed in both DR and RD patients compared with controls, indicating the potential involvement of AKR1B1 in these pathological processes. AKR1B1 is the initial enzyme in the polyol pathway that allows the conversion of glucose to sorbitol. Sorbitol is subsequently oxidized into fructose by sorbitol dehydrogenase. The activation of the polyol pathway under hyperglycemic conditions is recognized as a possible cause of various long-term metabolic alterations leading to cell damage, increased oxidative stress and inflammation, vascular damage, and ischemia [2]. This finding suggests that the inhibition of AKR1B1 could prevent the development of proliferative vitreoretinopathy, diabetic retinopathy, and their aggravations [1, 2]. Furthermore, aldose reductases such as AKR1B1 have been implicated in retinal complications of diabetes and shown to have a pro-inflammatory role by microglia activation [7]. We identified diclofenac, which is already approved for use in ocular diseases and is inexpensive, as a potential new inhibitor of AKR1B1. Diclofenac, a nonsteroidal anti-inflammatory drug (NSAID), has been reported as a selective inhibitory on ARK1B10 and AKR1B1. Diclofenac, GA, N-phenyl-anthranilic acids, and flufenamic acid are solid and specific competitive inhibitors of AKR1B10 because of a selective interaction between drugs and specific aminoacidic residue of the enzyme [11].

The validation of this new finding could lead to an important therapeutic and economic goal, using a cheap and already established drug. Indeed, diabetic retinopathy is the leading cause of blindness in the developed world, and avoiding its complications would enable many working-age adults to continue contributing to society,

thereby reducing the socioeconomic costs of diabetes [3]. Furthermore, reducing the occurrence of vitreoretinal proliferation and tractional retinal detachment would optimize healthcare costs, decreasing the number of necessary surgical interventions. Lastly, these actions have an essential effect on patients' quality of life.

When analyzing this comparative data, it's essential to consider that macular pucker is itself a condition, so using vitreous from pucker patients may introduce bias. However, because vitrectomy is invasive, obtaining vitreous humor from healthy controls is not ethically feasible. Consequently, international researchers have used vitreous from patients with epiretinal membranes and macular holes as the gold standard non-inflammatory control for comparisons with vitreoretinal diseases. Finally, it's important to remember that the proteome represents a set of proteins interacting in the patient's disease state at a specific moment, which may indicate both causes and effects. This makes it challenging to determine if a particular protein contributes to the disease's development or is simply a result of it. Nevertheless, all these proteins need to be studied—if they are part of the cause, they could serve as drug targets; if they are a result of the disease, they could be developed as biomarkers. In either case, a thorough investigation of the different stages of the disease is essential.

Conclusion

In conclusion, our experimental workflow offers a low-impact, bioethical research approach suitable for identifying small molecules that can be repurposed as drugs. We identified altered metabolic pathways in both diabetic retinopathy (DR) and complications of rhegmatogenous retinal detachment (RD). Some of these pathways, such as those related to glucose metabolism and inflammatory response (upregulated in DR) and cytoskeletal reorganization, which was upregulated in RD, were expected and aligned with the clinical manifestations of these diseases. Notably, we found that glucose/carbohydrate metabolism was upregulated in both diseases and identified a specific enzyme—AKR1B1—as a potential new therapeutic target. This insight could deepen our understanding of vitreoretinal disease pathophysiology and support the development of new, low-cost treatments with significant patient benefits and efficient resource use.

Using diclofenac at diagnosis and postoperatively for DR or RD may reduce complications, lower costs, and improve quality of life. Future research will focus on confirming the role of AKR1B1 in DR and RD, clarifying diclofenac's mechanism of action, and exploring other NSAIDs for treating vitreoretinal diseases.

Abbreviations

AKR1B1	Aldo–keto reductase family 1 member B1
BP	Biological process
DEPs	Differential expressed proteins
DR	Diabetic retinopathy
ECM	Extracellular matrix
ILM	Internal limiting membrane
LC–MS	Liquid chromatography-mass spectrometry
NSAID	Nonsteroidal anti-inflammatory drugs
ORA	Overrepresentation analysis
P	Macular pucker
PVD	Posterior vitreous detachment
PVR	Proliferative vitreoretinopathy
RD	Rhegmatogenous retinal detachment
RPE	Retinal pigment epithelium
TRD	Tractional retinal detachment
VEGF	Vascular endothelial growth factor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12014-024-09515-3.

Additional file1.

Acknowledgements

Not applicable

Author contributions

F.R. M.S. and S.C. conceived the experimental design. F.C., M.S. and E.Z. obtained patients samples. D.P. and R.B. treated patients samples and performed LC–MS. G.N, G.T., T.T., U.B. performed proteomics analysis. T.B., G.N., M.M., G.T., T.T., U.B., S.C. analyzed the data. G.N, T.B., U.B., S.C. wrote the manuscript text. G.T., D.P., R.B., T.T. prepared Figs. 1–3. G.N. and S.C. prepared Figs. 1, 4–7. F.R. and S.C. provided financial support. S.C. supervised the project. All authors reviewed the manuscript. All authors contributed to the article and approved the submitted and revised versions. T.B. and G.N contributed equally to this work.

Funding

Not applicable.

Availability of data and materials

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifiers PXD050611 and https://doi.org/10.6019/PXD050611.

Declarations

Ethics approval and consent to participate

Approval from the Azienda Provinciale per I Servizi Sanitari—Trento Institutional Review Board was obtained (APSS Protocol 7.2.4—n. 1684|2021), and written informed consent was acquired in accordance with the Declaration of Helsinki on Biomedical Research Involving Human Subjects.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 1 July 2024 Accepted: 16 November 2024
Published online: 28 November 2024

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