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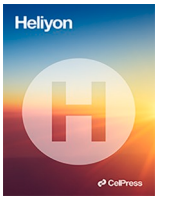

Research article

Interplay of endothelial-mesenchymal transition, inflammation, and autophagy in proliferative diabetic retinopathy pathogenesis

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ABSTRACT

Background: Assessment and validation of endothelial-mesenchymal transition (EndoMT) in the retinal endothelium of patients with proliferative diabetic retinopathy (PDR) at the level of retinal and vitreous specimens, and preliminary discussion of its regulatory mechanisms.
Methods: Transcriptome sequencing profiles of $CD31^{+}$ cells from 9 retinal fibrovascular membranes (FVMs) and 4 postmortem retinas were downloaded from GEO databases to analyze EndoMT-related differentially expressed genes (DEGs). Then, 42 PDR patients and 34 idiopathic macular holes (IMH) patients were enrolled as the PDR and control groups, respectively. Vitreous humor (VH) samples were collected, and the expression of EndoMT-related proteins was quantified by enzyme-linked immunosorbent assay.
Results: A total of 5845 DEGs were identified, and we subsequently focused on the analysis of 24 EndoMT-related marker genes, including the trigger of EndoMT, endothelial genes, mesenchymal genes, transcription factors, inflammatory factors, and autophagy markers. Six of these genes were selected for protein assay validation in VH, showing increased mesenchymal marker (type I collagen α 2 chain, COL1A2) and decreased endothelial marker (VE-cadherin, CDH5) accompanied by increased TGFβ, IL-1β, LC3B and P62 in PDR patients. In addition, anti-VEGF therapy could enhance EndoMT-related phenotypes.
Conclusions: EndoMT may underlie the pathogenesis of PDR, and the induction and regulation correlate with autophagy defects and the inflammatory response.

1. Introduction

Diabetic retinopathy is a common eye complication of diabetes and one of the leading global causes of irreversible blindness in working-age adults [1,2]. In 2020, the overall prevalence of DR in the global diabetic population was approximately 22.27 %, with 6.17 % for sight-threatening DR (STDR) [3]. Proliferative diabetic retinopathy (PDR) is a severe stage of DR and the main type of STDR [4]. As the key pathological hallmarks of PDR, vascular endothelial growth factor (VEGF)-associated retinal angiogenesis and fibrosis are the main reasons patients undergo vitrectomy and may cause irreversible visual impairment [5]. Endothelial cell dysfunction is directly responsible for such pathological changes, and although anti-VEGF therapy can improve outcomes of some PDR cases, it may

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lead to undesirable adverse effects such as Anti-VEGF crunch syndrome [6]. Thus, exploring the mechanisms that underlie vascular endothelial abnormalities other than VEGF is urgently needed.

Endothelial-mesenchymal transition (EndoMT), a pathophysiological process classified as a specialized form of Epithelial-to-Mesenchymal Transition (EMT) in which endothelial cells lose their epithelial characteristics and differentiate into mesenchymal cells, occurs in many pathological states, especially, in vascular endothelial cells with various pathological conditions [7,8]. EndoMT may be associated with the pathological process of PDR. Firstly, during EndoMT, the phenotypic alterations of endothelial cells are similar to the process of neovascularization and FVM formation in PDR [9]. In vivo and in vitro studies have demonstrated that the high glucose milieu induces EndoMT in human retinal microvascular endothelial cells (HRMECs) and mouse retina [10,11]. Moreover, inflammatory responses and autophagy defects are critical contributors to DR pathogenesis which is also involved in EndoMT regulation [12].

To date, few studies have reported EndoMT in PDR from a clinical validation per-spective. Analyses of retinal and vitreous specimens directly investigate the pathways of retinal metabolism and reflect changes in intraretinal homeostasis [13]. Accordingly, we hypothesize that EndoMT associated with autophagy and inflammation contributes to vascular endothelial dysfunction in PDR.

In the current study, we extracted and analyzed the high-throughput transcriptome sequencing data of the expression profile of retinas and FVMs from the Gene Expression Omnibus (GEO) database as a means to identify gene expression differences associated with EndoMT, autophagy, and inflammation. Also, the protein level variations of corresponding genes in the vitreous humor of PDR patients were evaluated to provide new therapeutic ideas for PDR treatment, such as the maintenance and protection of endothelials function through inhibition of EndoMT and the reduction of the adverse effects of anti-VEGF therapy.

2. Materials and methods

2.1. Participants

This retrospective study recruited 42 PDR inpatients and 34 idiopathic macular hole (IMH) inpatients from the First Affiliated Hospital of Soochow University between March 2021 and December 2021. Patients with the following were excluded: vitreoretinal diseases except for PDR, previous history of vitrectomy surgery, vitreous hemorrhage less than one month (avoid possible interactions between haemoglobin and factors to be measured), a severe systemic disease other than diabetes, such as benign and malignant tumors, blood diseases, renal insufficiency and hypertension, or any other surgical contraindications. Control patients did not have any ocular disease other than IMH and also did not have any serious systemic health problems other than diabetes mellitus. To investigate the effect of anti-VEGF treatment on EndoMT, we performed a subgroup analysis, dividing 42 PDR patients were divided into two groups: patients without prior anti-VEGF treatment (untreated group) and patients with a history of intravitreal injection (IVI) with ranibizumab or conbercept before vitrectomy (IVI group). The preoperative assessment, including an eye exam, relevant medical history, physiology, laboratory blood tests, and intraoperative data, were collected. The collection and use of the samples was reviewed and approved by the Institutional Ethics Committee of the First Affiliated Hospital of Soochow University.

2.2. Bioinformatic analyses

High-throughput sequencing datasets (GSE94019) downloaded from GEO databases (GEO, http://www.ncbi.nlm.nih.gov/geo/) included 13 samples of 9 proliferative diabetic retinal fibrovascular membrane and 4 normal retinal samples. CD31 was the endothelial marker. To better study the endothelial cell changes in PDR, the $CD31^{+}$ cells of normal retinal samples were isolated and subjected to transcriptome analysis using GPL11154 lllumina HiSeq2000. Differentially expressed genes (DEGs) were analyzed using the R language limma package (version 3.48.0, https://bioconductor.org/packages/release/bioc/html/limma.html) with a P-value < 0.05 and log fold change (FC) > 2.5 to filter differential genes. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyzes were performed using the cluster Profiles package (version 4.0.5, https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html). Through bioinformatic analyses of PDR endothelial-related databases, we initially explored the changes in the expression of endothelial, mesenchymal, inflammatory and autophagic genes involved in the present study.

2.3. Human vitreous humor tissue collection

The vitreous humor samples were collected from patients who underwent a 23-gauge standard three-port pars plana vitrectomy without an infusion of artificial fluid using the CONSTELLATION Vision System (Alcon Laboratories, Inc., Fort Worth, TX, USA). The vitreous humor (0.5–0.6 mL) was collected by manual aspiration into a syringe via the vitrectomy with the cutting function activated. Vitreous humor samples were immediately centrifuged at 13,000 rpm for 5 min at 4 °C in sterile 1.5 mL Eppendorf tubes. Supernatants were rapidly collected and stored at −80 °C until further analysis. Standard aseptic procedures were used throughout.

2.4. Enzyme-linked immunosorbent assay (ELISA) for target proteins

The commercially available ELISA kits for TGFβ, CDH5, and COL1A2 (Jianglai Biological, Shanghai, China), IL1β (R&D Systems, Minneapolis, MN, USA), P62 and LC3B (Cusabio, Wuhan, China) were used according to the manufacturer's instructions and in a blinded manner (samples were anonymised and recoded) by an experienced technician. Multi-well measurements, positive controls, and standard curves were utilized as quality control measures. Through pre-experimentation we confirmed the approximate

concentration ranges of the individual factors, To cover the ELISA kit's range, the vitreous humor samples were diluted five times for CDH5 and four times for TGFβ.

2.5. Statistical analysis

In this study, the sample size of each group was calculation and validation by PASS 11. GraphPad Prism 9.0 Software (GraphPad, San Diego, CA, USA) and the R Programming Language were used for data analysis and drawing graphs. The patient clinical data and vitreous test results are presented as mean ± SD. Since there was a non-normal distribution of our data, the Mann-Whitney *U* test was employed to compare two groups. Categorical variables such as age, gender was assessed using the chi-square test. Spearman correlation coefficients were used to determine the correlations of the proteins in the vitreous humor. Correlations do not imply causation, and other confounding factors might influence the results. We used the Gpower software (www.gpower.hhu.de) for the calculation of effect size. For all comparisons, a value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Patient characteristics

The patients were randomly assigned to a PDR group and a control group (IMH group), and their basic characteristics are provided in Table 1. The history of anti-VEGF agent therapy, as well as the diagnosis of tractional retinal detachment (TRD), were also recorded in the ocular characteristic column. There was no significant difference in age, gender between groups. The systolic blood pressure of patients, mean fasting plasma glucose (FBG) levels and BMI in the PDR group were significantly higher than in the control group. Although FBG was higher in PDR patients than in the control group, the glycaemia of PDR patients was effectively controlled numerically. Some of the PDR patients had comorbid hypertension, which resulted in higher systolic blood pressure than the control group.

The PDR group was further divided into two groups, the untreated and IVI groups (Table 2). In total, 15 PDR patients who received anti-VEGF IVI 3–5 days (mean: 3.67 days) before the vitrectomy were enrolled in the IVI group. For safety, only two cases with slight macula-off TRD were included in the IVI group. A shorter surgical time, lower probability of intraoperative bleeding marked neovascular attenuation, and some degree of FVM proliferation were observed in the IVI group during vitrectomy. The above differences suggest that anti-VEGF drugs on the one hand reduced bleeding and made surgery less difficult, and on the other hand may have aggravated FVM.

3.2. Identification and analyses of DEGs in PDR

The GSE94019 dataset was screened using bioinformatics methods to obtain 25,835 genes and 5845 differential genes, including 744 upregulated genes and 5101 down-regulated genes (Fig. 1A). Although we increased the threshold for differential genes (log FC > 2.5), the number of differential genes was still significant enough for subsequent analyses compared to similar studies. The gene markers of EndoMT summarized by Yoshimatsu et al. [12,14]between DEGs were used to further screen for DEGs (Supplementary Table S1). Among them, 24 EndoMT-related genes were selected for visualization (Fig. 1B), including EndoMT trigger (TGFβ1), transcription factors (SNAIL1, SNAIL2, HEY1, HEY2), inflammation markers (IL6, IL1β, TNF, CCL2, CXCL2, ICAM1), endothelial cell markers (CDH5, ERG, APLN, FLT4, FSTL3, PECAM1), mesenchymal cell markers (COL1A2, MMP9, MMP2, MMP14, α-SMA, FN1) and autophagy markers (LC3B, ATG5, ATG16L1, P62). Then, six of these genes were selected to evaluate the protein expression in the vitreous humor, and their average expression is illustrated in Fig. 1C, showing significantly increased levels of EndoMT trigger,

Table 1
Systemic and ocular characteristics of controls (IMH) and PDR group.

	Control (n = 34)	PDR (n = 42)	*P*-value
Patient characteristics			
Age (years)	64.65 ± 5.83	54.79 ± 10.84	<0.0001
Female/male (n)	18/16	19/23	0.6448
Duration of diabetes (years)	–	10.19 ± 5.28	
FBG (mmol/L)	4.98 ± 0.44	6.72 ± 2.50	0.0012
Body mass index (kg/m^2)	22.17 ± 2.33	23.65 ± 3.08	0.0302
Hypertension			
Systolic BP (mmHg)	130.70 ± 10.12	139.50 ± 15.84	0.0054
Diastolic BP (mmHg)	81.09 ± 9.314	85.05 ± 10.61	0.1177
Ocular characteristics			
Operated eye (right/left)	9/25	30/12	0.0002
Previous anti-VEGF treatment history (NO/YES)	–	27/15	–
Diagnosis of tractional retinal detachment/total patients	–	7/42	–

Data are displayed either as mean ± SD or numbers of subjects. *P*-value ≤0.05 was considered statistically significant. PDR, proliferative diabetic retinopathy; FBG, fasting plasma glucose; BP: blood pressure.

Table 2
Baseline characteristics and intraoperative findings of the PDR subgroup.

	IVI (n = 15)	Untreated (n = 27)	*P*-value
Patient characteristics			
Age (years)	53.47 ± 14.82	55.52 ± 8.10	0.8710
Female/male (n)	8/7	11/16	0.5249
Duration of diabetes (years)	11.40 ± 6.36	9.52 ± 4.57	0.2916
Ranibizumab/Conbercept	6/9	–	–
TRD	2	5	–
Days between IVI and surgery (mean, range)	3.67, 3 to 5	–	–
Intraoperative bleeding	3	16	–
Surgical time (min)	51.40 ± 10.22	73.15 ± 15.20	P < 0.0001

Data are depicted either as mean ± SD or numbers of subjects. *P*-value ≤0.05 was considered statistically significant. TRD, tractional retinal detachment. IVI, intravitreal injection.

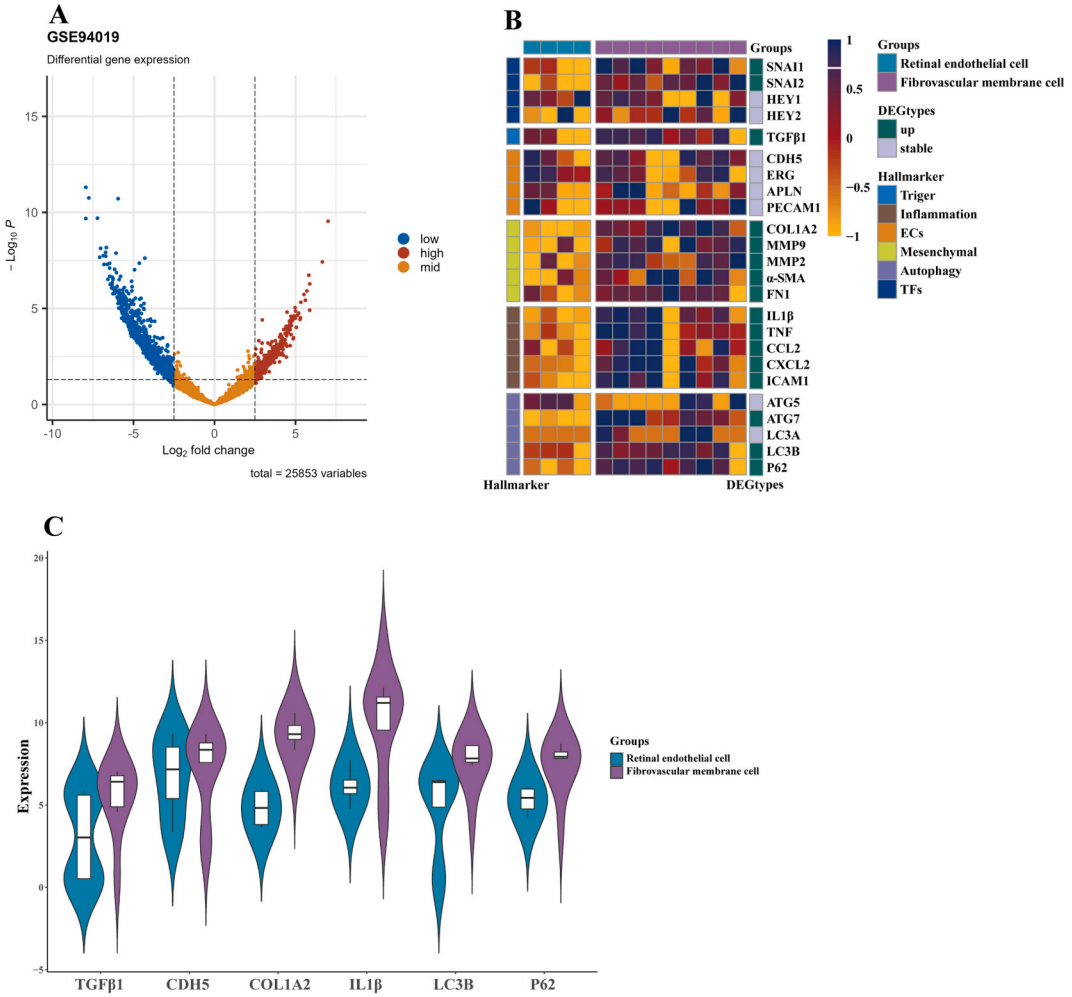

Fig. 1. Transcriptomic analysis of CD31$^+$ cells from the fibrovascular membrane and CD31$^+$ cells from control retinas (GSE94019). (A) Volcano plot of differentially expressed genes. Red for upregulated and blue for downregulated DEGs). (B) A heatmap of the expression of 24 EndoMT-related genes in all samples across different groups. (C) The violin plot displayed the average expression of 6 selected genes (TGFβ, CDH5, COL1A2, IL-1β, LC3B, P62). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

transcription factors and mesenchymal cell marker genes. Yet, no noticeable decrease in endothelial marker genes was shown. In addition, there was a significant increase in inflammation markers, and autophagy-related genes.

3.3. GO and KEGG analyses

The cluster profile package was used to further explore the biological significance of DEGs and performed an overrepresentation analysis of the above EndoMT-related genes to analyze possible biological functions and pathways. The ten most enriched GO terms and the KEGG pathways are depicted in the Supplementary materials (Supplementary Figs. S1 and S2, respectively). In the MF category, the upregulated DEGs were involved in cytokine activity, cytokine receptor binding, signaling receptor activator activity, chemokine activity, and collagen binding (Supplementary Fig. S1A). In the BP category, the upregulated DEGs were involved in mesenchyme development, negative regulation of apoptotic signaling pathways, and vitamin D biosynthetic process (Supplementary Fig. S1B). In the CC category, the upregulated DEGs were involved in the collagen-containing extracellular matrix, autophagosome, and contractile fibers (Supplementary Fig. S1C). According to enrichment analysis results, some typical pathways of EndoMT were involved. We present the top 10 KEGG pathways, including AGE-RAGE signaling pathway, TNF signaling pathway and IL-17 signaling pathway (Supplementary Fig. S2).

3.4. Protein expression in vitreous humor samples

The protein assay results were in line with the bioinformatics analyses, with higher levels of TGFβ and COL1A2 in the vitreous humor samples of the PDR group and significantly reduced CDH5 compared to the control group (Table 3, Fig. 2A). Likewise, IL-1β, LC3B, and P62 increased in the PDR group. In the subgroup analysis, there was a higher level of COL1A2 as well as a lower level of CDH5 in the IVI group compared to the untreated group. (Table 4, Fig. 2B).

3.5. Correlation of vitreous IL1-β levels with P62 and TGFβ levels

The Spearman correlation analysis revealed that in the PDR group, IL-1β strongly positively correlated with TGFβ ($R = 0.6574$, $P < 0.001$, Fig. 3A) and slightly less positively with P62 ($R = 0.6821$, $P < 0.001$, Fig. 3B). Conversely, these aforementioned correlations were diminished in the control group (Fig. 3C–D).

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

4. Discussion

The present study demonstrated, for the first time, the differential expression of EndoMT-related markers in the vitreous humor of PDR patients. The visualization of the 24 EndoMT-related genes from transcriptomic analysis showed that EndoMT may occur in patients with PDR and is associated with inflammation and autophagy. Specifically, a decrease in EC marker and an increase in mesenchymal cell marker were observed in PDR vitreous humor compared to the control group. In parallel, as the EndoMT trigger, the level of TGF-β was significantly increased. Also, the inflammatory factor (IL-1β), autophagosomal marker (LC3-B), and autophagy substrate (P62) were upregulated in the PDR group. These findings are consistent with the transcriptomic analysis of the endothelial cells from fibrovascular membranes in PDR. Thus, in addition to important mechanisms such as chronic inflammation and oxidative stress, EndoMT may also be involved in the pathological process of PDR, and the underlying mechanism may be mediated by autophagy defects and inflammation.

The most intuitive characteristics of this transition are an increase in EndoMT-related trigger, a downregulation of endothelial markers, and an upregulation of mesenchymal markers. The transcriptome analyses were largely in line with the abovementioned trends, and the protein expression in the vitreous humor confirmed those tendencies. It is well established that angiogenesis and fibrous proliferation are critical features of PDR, which can eventually lead to severe visual impairment [15,16]. On the one hand, the complex process of angiogenesis during PDR involves remodeling the vascular endothelial extracellular matrix, degradation of the basement membrane, and enhancement of endothelial cell invasion/migration [17], culminating in the formation of immature capillaries. This process is similar to the initial stages of EndoMT, whereby intercellular adhesion is weakened, then endothelial cells acquire mesenchymal properties, such as elevated migration and invasive capacity [12]. On the other hand, the FVM formation is accompanied by an accumulation of extracellular matrix and characterized by migration and proliferation of various cells. Among

Table 3
Comparison of the vitreous humor protein concentrations between the PDR and control groups.

	TGFβ (pg/ml)	CDH5 (ng/ml)	COL1A2 (ng/ml)	IL-1β (pg/ml)	LC3B (pg/ml)	P62 (ng/ml)
PDR	784.10 ± 152.00	108.50 ± 15.73	1.68 ± 0.65	26.19 ± 6.57	86.42 ± 26.80	16.96 ± 6.55
Control	213.3 ± 12.87	233.1 ± 31.16	1.09 ± 0.40	7.74 ± 3.65	55.21 ± 19.48	4.15 ± 0.66
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Effect size d	5.2918	5.0482	1.0932	3.4716	1.3221	2.7518

PDR, proliferative diabetic retinopathy.

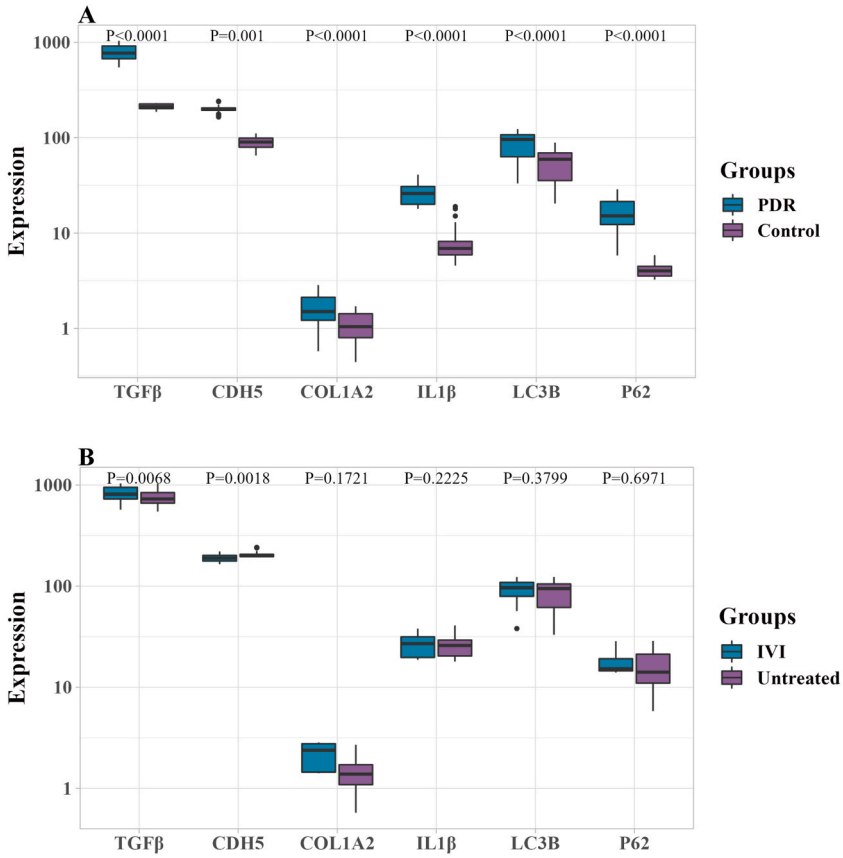

Fig. 2. Comparison of the vitreous humor protein concentrations. (A) The concentrations of selected proteins in the PDR and control groups. (B) The concentrations of selected proteins in the IVI and untreated groups.

Table 4
Subgroup analysis of protein concentrations between IVI and untreated groups.

	TGFβ (pg/ml)	CDH5 (ng/ml)	COL1A2 (ng/ml)	IL-1β (pg/ml)	LC3B (pg/ml)	P62 (ng/ml)
IVI	825.50 ± 152.20	99.35 ± 17.36	2.12 ± 0.66	26.69 ± 6.96	92.02 ± 25.28	18.12 ± 5.53
Untreated	761.20 ± 149.70	113.7 ± 12.33	1.43 ± 0.50	25.91 ± 6.47	83.30 ± 27.58	16.31 ± 7.07
P value	0.2225	0.0068	0.0018	0.6971	0.3799	0.1721
Effect size d	2.7518	0.9530	1.1784	0.1160	0.3296	0.2851

IVI: intravitreal injection.

them, myofibroblasts positive for α-smooth muscle actin (α-SMA) derived from fibrocytes have a major role and may cause contraction of the FVM [18]. EndoMT might be an important source of such cells, and during the latter stage of EndoMT, ECs express typical markers of myofibroblastic differentiation such as α-SMA and collagens and might transform into mesenchymal cells, which subsequently generate activated myofibroblasts, thereby promoting the progression of fibrotic diseases [19]. Taken together, EndoMT is likely to be one of the major pathological changes occurring in PDR.

In addition to EndoMT markers, our study also revealed significant differences in EndoMT trigger (TGF-β), inflammatory factor (IL-1β), and autophagy indicators (LC3B, P62). TGF-β signaling is the most well-characterized pathway of EndoMT, which regulates the expression of various transcription factors, intracellular messengers, and cellular components that confer ECs with mesenchymal characteristics. The TGF-β superfamily, including three isoforms (TGF-β1–3), is strongly associated with EndoMT in different diseases [20]. In DR, previous in vitro and in vivo studies confirmed the existence of EndoMT mediated by TGF-β [11,21], and it can be prevented by inhibition of TGF-β. Recent lines of evidence highlighted the effects of the inflammatory pathway and autophagic impairment on EndoMT. In human primary aortic endothelial cells, the pro-inflammatory cytokines TNF-α and IL-1β induce EndoMT through an interplay with BMPs, a subfamily of the TGF-β family [22]. EndoMT was considered to be the main manifestation of vascular inflammation in atherosclerosis. The co-treatment of TNF-α and IL-1β induced EndoMT in HUVECs [23]. Another key role of EndoMT induction together with TGF-β is autophagy [24]. Yuta Takagaki et al. found that EndoMT was induced in autophagy-deficient human microvascular ECs (HMVECs), which cannot be prevented by TGF-β-neutralizing anti-body; rather, this EndoMT was

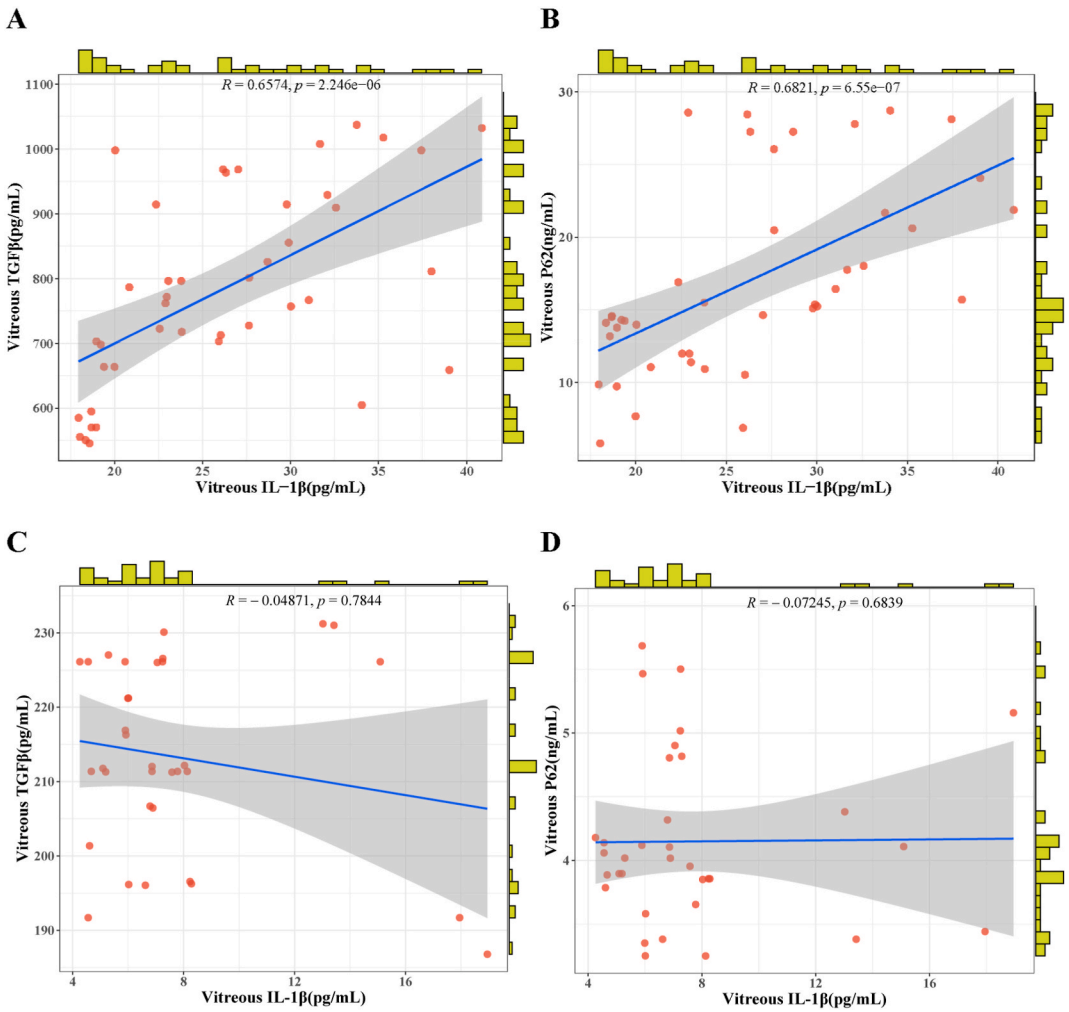

Fig. 3. Correlation analysis of the proteins in the vitreous humor. Spearman's correlation tests presented positive correlations in the PDR group (A) between vitreous IL-1β and vitreous TFGβ and (B) between vitreous IL-1β and vitreous P62. Spearman's correlation tests presented exhibited no significant correlations in the control group (C) between vitreous IL-1β and vitreous TFGβ and (D) between vitreous IL-1β and vitreous P62.

IL-6-dependent [25]. Gui et al. found that the knockdown of the autophagy-related 16-like gene (ATG16L) inhibited autophagy characterized by a decrease in autophagosome formation, enhancing the process of EndoMT in human renal glomerular endothelial cells (HRGECs) [26]. Simultaneously, ATG16L knockdown promoted the excretion of inflammatory cytokines (IL-1β, IL-6 and TNF-α), suggesting that autophagy may be upstream of inflammation in the regulation of EndoMT.

Remarkably, endothelial inflammation and autophagy defects play important roles in DR pathogenesis. Higher IL-1β and NLRP3 inflammasome vitreous levels in PDR patients were observed in our present and previous studies [27]. Elevated LC3B levels, accompanied by an increase of P62 in this study, revealed the incomplete status of autophagic flux [28]. P62 is an autophagy substrate protein that is degraded by the autophagic system, whereas p62 accumulation may indicate autophagic inhibition [29]. Our additional correlation analyses also revealed significant positive correlations between IL-1β and TGFβ as well as P62. Thus, autophagy defects and inflammatory response might be contributing factors in the EndoMT during PDR.

Besides retinal capillary endothelial cells, alterations in retinal pigment epithelial (RPE) cells are also involved in the development of DR. According to another hypothesis, disruption of the blood retinal barrier in DR can activate retinal pigment epithelium cells, which initiating EMT, termed RPE-EMT [30]. In an in vitro study, Ding et al. found that 25 mM glucose-treated retinal pigment epithelial (RPE) cells underwent EMT, which was characterized by a down-regulation of *E*-Cadherin and a concomitant up-regulation of α-SMA [31]. A recent in vivo study has also demonstrated that the mRNA levels of EMT markers Snai1, Snai2, and Twist1 were significantly elevated in the RPE cells from diabetic mice [32]. Meanwhile, RPE has been reported to be directly involved in proliferative vitreoretinopathy (PVR) membrane formation [33]. Several studies indicated the RPE-EMT is a critical mechanism for membrane development in ocular diseases such as PVR [34–36].

The subgroup analyses revealed that patients in the IVI group had higher levels of mesenchymal cell marker (COL1A2) and lower EC marker (CDH5) than patients in the untreated group, which reflected a higher degree of EndoMT. In previous study, DR patients

who received intravitreal injections of bevacizumab one week before vitrectomy were found to have significantly reduced levels of Annexin V and CD144 (CDH5). This is consistent with our conclusion [37]. In addition, the FVM fibroplasia and the regression of neovascularization were observed to varying degrees in most cases during vitrectomy. Fortunately, no new-onset tractional retinal detachment (TRD) was found. Recently, several clinical studies in PDR patients demonstrated that anti-VEGF IVI before vitrectomy not only can reduce intraoperative bleeding from immature new vessels and facilitate the peeling of fibrovascular membranes but also contribute to reducing vitreous hemorrhage recurrence and improvement of postoperative BCVA [38]. However, preoperative IVI is also reported to result in adverse reactions such as increasing the severity of fibrosis and more severe anti-VEGF crunch syndrome [6], which is similar to the pathological change of EndoMT. Consequently, regulation of endothelial EndoMT status may be a new strategy to prevent adverse effects of anti-VEGF therapy. Some patients (with TRD), for example, receive therapy with anti-VEGF treatment combined with a dose of a collagen inhibitor.

5. Conclusion

The present study demonstrated differentially expressed EndoMT-related marker genes between PDR FVMs and normal retinal endothelial cells. The trends in expression suggested the occurrence of EndoMT in PDR patients. Subsequently, the protein expression of some representative genes was verified in the vitreous humor of PDR patients, confirming the presence of EndoMT. Most of these proteins were quantified for the first time in the vitreous humor. At the same time, the transcriptome sequencing data and protein expression demonstrated increased inflammatory signing and autophagy impairment. In particular, more significant EndoMT-related protein expression profiles were observed in the IVI group of PDR patients, which also coincided with the retinal neovascularization changes observed during vitrectomy. Therefore, taken together, these results may indicate the possible underlying influence of EndoMT in PDR pathogenesis. The modulation towards autophagy and inflammation and the interaction between them may be contributing factors of EndoMT signaling, offering novel potential strategies for the prevention and treatment of DR as well as directions for the improvement of existing anti-VEGF therapy. Firstly, a combination of inflammation-controlling and autophagy-activating drugs can be considered to block EndoMT. Secondly, attempts to reverse endothelial cells that undergo partial EndoMT. Limited by the volume of vitreous samples, only some EndoMT-related proteins were detected in this study. Transcriptomic comparisons between normal retinal $CD31^{+}$ cells and PDR fvm are also only partially representative of the pathological state of PDR. The heterogeneity of the PDR may also influence our results. EndoMT is an extremely complex process in which numerous factors may be involved, such as the extracellular matrix components and multiple growth factors. Further in vivo and in vitro studies are underway to explore the underlying molecular mechanisms of EndoMT, inflammatory signing and autophagy impairment.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the First Affiliated Hospital of Soochow University (Approval ID: 2023-No.154).

Data availability statement

High-throughput sequencing datasets (GSE94019) are available from GEO databases (GEO, http://www.ncbi.nlm.nih.gov/geo/). Other data related to patient privacy, if necessary, please obtaining from the corresponding author of reasonable request.

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CRediT authorship contribution statement

Gaocheng Zou: Writing – original draft, Visualization, Methodology. **Lijuan Que:** Investigation, Funding acquisition. **Yaping Liu:** Supervision, Methodology, Investigation, Data curation. **Qianyi Lu:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25166.

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