Aqueous Lumican Correlates with Central Retinal Thickness in Patients with Idiopathic Epiretinal Membrane: A Proteome Study

Wei-Cheng Chang,1,2 Cho-Hao Lee,3 Shih-Hwa Chiu,4,5,6,7 Chen-Chung Liao,8 and Chao-Wen Cheng1,9,10

1Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei 11031, Taiwan
2Department of Ophthalmology, Taoyuan General Hospital, Ministry of Health and Welfare, Taoyuan 33004, Taiwan
3Division of Hematology and Oncology, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei 114202, Taiwan
4Department of Medical Research, Taipei Veterans General Hospital, Taipei 11217, Taiwan
5School of Medicine, National Yang Ming Chiao Tung University, Taipei 11211, Taiwan
6Institute of Pharmacology, National Yang Ming Chiao Tung University, Taipei 11221, Taiwan
7Genomic Research Center, Academia Sinica, Taipei 11529, Taiwan
8Metabolomics-Proteomics Research Center, National Yang Ming Chiao Tung University, Taipei 11221, Taiwan
9Traditional Herbal Medicine Research Center, Taipei Medical University Hospital, Taipei Medical University, Taipei 11031, Taiwan
10Cell Physiology and Molecular Image Research Center, Wan Fang Hospital, Taipei Medical University, Taipei 11031, Taiwan

Correspondence should be addressed to Chen-Chung Liao; ccliao@ym.edu.tw and Chao-Wen Cheng; ccheng@tmu.edu.tw

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Idiopathic epiretinal membrane (iERM) is a pathological fibrocellular change in the vitreoretinal junction over the macular area; however, possible pathogenic mechanisms remain unclear. Changes in the differential protein composition of the aqueous humor (AH) may represent potential molecular changes associated with iERM. To gain new insights into the molecular mechanisms of iERM pathology, a sensitive label-free proteomics analysis was performed to compare AH protein expressions in patients with cataracts with or without iERM. This study employed nanoflow ultra-high-performance liquid chromatography-tandem mass spectrometry to investigate protein compositions of the AH obtained from individual human cataract eyes from 10 patients with iERM and 10 age-matched controls without iERM. Eight proteins were differentially expressed between the iERM and control samples, among which six proteins were upregulated and two were downregulated. A gene ontology (GO) analysis revealed that iERM was closely associated with several biological processes, such as immunity interactions, cell proliferation, and remodeling of the extracellular matrix. Additionally, multiple proteins, including lumican, cyclin-dependent kinase 13, and collagen alpha-3(VI) chain, were correlated with the central retinal thickness, indicating a multifactorial response in the pathogenic process of iERM. Changes in the AH level of lumican between iERM and control samples were also confirmed by an enzyme-linked immunosorbent assay. In conclusion, several pathological pathways involved in iERM were identified in the AH by a proteomic analysis, including immune reactions, cell proliferation, and remodeling of the extracellular matrix. Lumican is a potential aqueous biomarker for predicting iERM development and monitoring its progression. More clinical parameters also need to be identified to complete the analysis, and those could provide additional targets for treating and preventing iERM.
1. Introduction

By definition, idiopathic epiretinal membrane (ERM; iERM) is a pathological proliferative fibrocellular change in the vitreoretinal junction over the macular area with no other ocular disease or secondary to any other intraocular surgery, intraocular disease, or ocular trauma [1]. It can reduce vision, cause metamorphopsia, and require vitreoretinal surgery to peel off the membrane [2]. Two main components, noncellular and cellular, of the ERM are often discussed as extracellular matrix (ECM) structures like fibronectin, vitronectin, and collagen and various cell types of retinal and extraretinal origin, such as glial cells, astrocytes, Muller cells, retinal pigment epithelial cells, and myofibroblasts [3–8]. Some studies also reported that specific cytokines are expressed by iERMs, such as vascular endothelial growth factor, interleukin-6 (IL-6), transforming growth factor-beta, and connective tissue growth factor [9–11]. Although the true pathogenic mechanisms are not well known, changes in various cell types of retinal and extraretinal origin, such as glial cells, astrocytes, Muller cells, retinal pigment epithelial cells, and myofibroblasts [3–8].

A proteomics analysis is a valuable approach to define changes in protein levels in tissues and cells. It has been used to examine biofluids such as the aqueous humor (AH), vitreous humor (VH), tears, and serum to find many biomarkers in diabetic retinopathies [12]. The emergence of liquid biopsies for diabetic retinopathies and other vitreoretinal diseases was also noted for precision medicine [13, 14]. Recent proteomics studies of human AH revealed many proteins in patients with intraocular disease [13, 15–19]. Pollreisz et al. [20] first performed isobaric tags for relative and absolute quantitation (iTRAQ) 4-plex quantitative protein analysis of the aqueous and vitreous fluids from human eyes with iERM. They found 323 proteins in the aqueous and vitreous fluids from eyes with iERM, and only 3.96% of the identified proteins exhibited significant differential expression between the aqueous and vitreous fluids. Yu et al. [21] first examined the vitreous proteome from iERMs and donor controls in a differential analysis by reversed-phase high-performance liquid chromatography. It revealed 226 significant protein changes in the vitreous proteome of iERM patients compared to the vitreous of normal control subjects. Mandal et al. [22] analyzed vitreous samples from patients with iERM and idiopathic macular hole (iMH) with two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), and Christakopoulos et al. [23] compared iERM samples to internal limiting membrane samples from patients with iMH with label-free quantitative nanoflow-liquid chromatography-tandem mass spectrometry (n-LC-MS/MS). However, until now, no study has reported differential changes in the protein composition of the AH from individual patients and controls with iERM.

So far, there are no proteomics studies that focused on analyzing aqueous fluids from eyes with iERM without a pooling method. In this study, we employed nanoflow ultra-high-performance (UHP) LC-MS/MS to investigate the protein composition of the aqueous obtained from individual human cataract eyes with iERM and aged-matched controls. This sensitive proteomics approach should help elucidate the underlying pathophysiology of iERM disease using a relatively small amount of aqueous sample and thus was the favored methodological approach selected for this investigation. This research may reveal valuable insights into molecular changes in the AH of patients with iERM.

2. Materials and Methods

2.1. Subjects. The Medical Ethics and the Institutional Review Board of Taoyuan General Hospital (TYGH), Ministry of Health and Welfare, approved the study protocol (TYGH109009), and the study was conducted according to the tenets of the Declaration of Helsinki. All study participants provided written informed consent before their enrollment, and the nature and possible consequences of the study were fully explained to them. Human AH samples from treatment-naïve patients with iERM (n = 10) and age-matched controls (n = 10) were collected when the subjects were undergoing cataract surgery at TYGH (Taoyuan, Taiwan). The diagnostic criterion for iERM was defined by optical coherence tomography (OCT) without other ocular diseases, trauma, or a previous intraocular operative history. Control eyes belonged to senile cataract patients free from other ocular or systemic diseases. In both groups, the inclusion criteria were cataract patients aged older than 55 years. The exclusion criteria were a history of any systemic disease including hypothyroidism or ocular disorders including glaucoma, previous ocular infectious diseases, retinal vascular disorders, ocular surgery, or trauma. The best-corrected visual acuity was measured as the logarithm of the minimum angle of resolution (logMAR). Spectral-domain- (SD-) OCT (Heidelberg Engineering, Germany) was used for the OCT examination (Figure 1). The central retinal thickness was measured with a caliper tool of Heidelberg OCT software.

2.2. AH Sample Collection. AH samples were collected from patients during the implantation of phakic intraocular lenses. The sample was collected using a 1 ml tuberculin syringe with a 30-gauge needle at the limbus under a surgical microscope before any other entry into the eye to avoid hemorrhaging and other ocular surface contaminants. Approximately 50–100 μL of AH was collected from each patient by anterior chamber paracentesis. Undiluted AH samples were collected and stored at −80°C until preparation was initiated within 24 h.

2.3. n-UPLC-MS/MS. Protein concentrations of AH samples were determined by a dye-binding method based on the Bradford assay (Bio-Rad Laboratories, Richmond, CA, USA) (Table 1), and samples were further diluted in 1x phosphate-buffered saline to a final concentration of 0.1 μg/μL. Samples were prepared according to the SMART digestion kit protocol from Thermo Fisher Scientific (Waltham, MA, USA) and cleaned up by solid-phase extraction plates from Thermo Fisher Scientific. The resulting peptides collected from the filters were dried in a vacuum centrifuge and stored at −80°C. Thereafter, 50 μL of diluted AH samples was resuspended in 0.1% formic acid and analyzed by n-UPLC-MS/MS. Tryptic peptides were loaded into an Elite
mass spectrometer with a nanoelectrospray ionization source (Thermo Electron, MA, USA) connected to a nanoACQUITY UPLC system (Waters, MA, USA). Peptide samples were separated by a 25 cm × 75 μm BEH130 C18 column (Waters) with a 0%~95% segmented gradient of 3%~40% B for 168 min, 40%~95% B for 2 min, and 95% B for 10 min at a flow rate of 0.5 μL/min. Mobile phase A was 0.1% formic acid in water, while mobile phase B was 0.1% formic acid in acetonitrile. The mass spectrometer was based on the data-dependent acquisition method (isolation width: 1.5 Da). According to the data-dependent acquisition method, the first ten most intensively charged peptide ions were selected and fragmented by the collision-induced dissociation method.

2.4. Protein Identification. The acquired MS/MS raw data files were then applied to search against a UniProt human protein database (containing 20,387 protein sequences; released April 2021) with PEAKS Studio 7.5 (Bioinformatic Solution, Ontario, CA). PEAKS Studio 7.5 was combined with UniProt’s protein database search settings as follows: the enzyme was set to trypsin; it is up to two missing cutting sites; precursor and fragment mass tolerances were 20 ppm and 0.8 Da, respectively; and the false discovery rate (FDR) was <1%, as obtained from a search of the decoy database. Furthermore, each identified protein had to contain at least one unique peptide, and the protein quantification method was based on a label-free quantitative analysis. Additionally, spectral counts were normalized to the total identification spectrum of each biological sample.

2.5. Enzyme-Linked Immunosorbent Assay (ELISA). According to the manufacturer’s instructions, the ELISA was performed to measure lumican concentrations in iERM and control AH samples with a Human Lumican ELISA Kit (EH310RB, Thermo Fisher Scientific).

2.6. Statistical Analysis. Clinical data were analyzed using Stata (vers. 16.1, StataCorp, College Station, TX, USA) to define the statistical significance between groups by a t-test or chi-squared test, and p < 0.05 was considered statistically significant. Statistical analysis by Fisher’s exact test was used to verify that there was no statistically significant difference in age between the iERM and age-matched control groups (Table 1).

Table 1: Samples for the proteomic analysis.

| Variable         | Control group | ERM group | p value |
|------------------|---------------|-----------|---------|
| ERM              | 254.20 ± 12.97| 319.50 ± 65.70| 0.004^# |
| Gender           |               |           | 0.007   |
| Female           | 8 (80.0%)     | 2 (20.0%) |         |
| Male             | 2 (20.0%)     | 8 (80.0%) |         |
| Age              | 73.90 ± 5.72  | 74.00 ± 5.23 | 0.675^# |
| VA               | 0.42 ± 0.10   | 0.29 ± 0.20 | 0.128^# |
| LogMAR           | 0.39 ± 0.11   | 0.79 ± 0.67 | 0.128^# |
| AXL              | 23.83 ± 1.17  | 24.26 ± 2.01 | 0.631^# |
| Protein concentration | 0.22 ± 0.06 | 0.36 ± 0.16 | 0.029^# |
| Side             |               |           | 1.000^# |
| Left             | 4 (40.0%)     | 5 (50.0%) |         |
| Right            | 6 (60.0%)     | 5 (50.0%) |         |

^# Analyzed by Fisher’s exact test. *Data are expressed as the mean ± standard deviation. ERM: epiretinal membrane; VA: visual acuity; MAR: minimal angle of resolution; AXL: axial length.

Figure 1: Optical coherence tomography (OCT) of (a) a healthy retina and (b) an idiopathic epiretinal membrane (ERM; iERM). The cross-sectional B-scan was obtained with a spectral-domain OCT device in (a) a healthy eye and (b) an eye with iERM. (b) shows the retina of an eye with an ERM.
3. Results

Demographic data of the iERM and control groups are shown in Table 1 and Table S1. The mean age of patients with iERM was 74.00 ± 5.23 years, and that of control individuals was 73.90 ± 5.72 years. All patients with iERM revealed an ERM by an OCT examination. The mean protein concentration of the AH was 0.36 ± 0.16 μg/μL in the iERM group and 0.22 ± 0.06 μg/μL in the control group. There were statistical differences between total protein contents in these groups (p = 0.029; Fisher’s exact test, Wilcoxon test, or Kruskal-Wallis test) and no statistical differences between ages in these groups (p = 0.675; Fisher’s exact test, Wilcoxon test, or Kruskal-Wallis test).

In total, 405 proteins were successfully identified by LC-ESI MS/MS in iERM and control AH samples (Fig. S1). Compared to the control proteome, 189 proteins were present at

| Table 2: Pathway analysis of aqueous humor (AH) proteins using Ingenuity Pathway Analysis tools. |
| --- |
| Canonical pathway | Overlapping proteins in the iERM and control groups |
| Interleukin-15 signaling | 3 |
| B-cell receptor signaling | 3 |
| Systemic lupus erythematosus in B-cell signaling pathway | 3 |
| Communication between innate and adaptive immune cells | 3 |
| Complement system | 1 |

| Protein ID | Protein name | Gene name | ERM group | Control group | p value | Multiple of change ERM/control |
| --- | --- | --- | --- | --- | --- | --- |
| P51884 | Lumican | LUM | 2.51 ± 1.19 | 1.47 ± 0.84 | 0.023* | 1.70748299 |
| P05543 | Thyroxine-binding globulin | THBG | 1.10 ± 0.57 | 0.39 ± 0.50 | 0.004* | 2.82051282 |
| A0A0J9 | Immunoglobulin heavy variable 5-10-1 | HV5X1 | 0.11 ± 0.34 | 1.05 ± 1.23 | 0.027* | 0.1047619 |
| P17050 | Alpha-N-acetylgalactosaminidase | NAGAB | 0.00 ± 0.00 | 0.38 ± 0.48 | 0.031* | NA |
| P78563 | Double-stranded RNA-specific editase 1 | RED1 | 0.39 ± 0.51 | 0.00 ± 0.00 | 0.031* | NA |
| Q14004 | Cyclin-dependent kinase 13 | CDK13 | 0.41 ± 0.53 | 0.00 ± 0.00 | 0.031* | NA |
| P12111 | Collagen alpha-3(VI) chain | CO6A3 | 0.70 ± 0.48 | 0.20 ± 0.42 | 0.039* | 3.5 |
| Q7Z7A3 | Cytoplasmic tRNA 2-thiolation protein 1 | Q7Z7A3 | 0.52 ± 0.54 | 0.10 ± 0.32 | 0.045* | 5.2 |

*Tested by Fisher’s exact test.

| Table 3: Statistically significantly regulated proteins in patients with epiretinal membrane (ERM) versus controls in a proteomics analysis. |
| --- | --- | --- | --- | --- | --- |
| P51884 | Lumican | LUM | 2.51 ± 1.19 | 1.47 ± 0.84 | 0.023* |
| P05543 | Thyroxine-binding globulin | THBG | 1.10 ± 0.57 | 0.39 ± 0.50 | 0.004* |
| A0A0J9 | Immunoglobulin heavy variable 5-10-1 | HV5X1 | 0.11 ± 0.34 | 1.05 ± 1.23 | 0.027* |
| P17050 | Alpha-N-acetylgalactosaminidase | NAGAB | 0.00 ± 0.00 | 0.38 ± 0.48 | 0.031* |
| P78563 | Double-stranded RNA-specific editase 1 | RED1 | 0.39 ± 0.51 | 0.00 ± 0.00 | 0.031* |
| Q14004 | Cyclin-dependent kinase 13 | CDK13 | 0.41 ± 0.53 | 0.00 ± 0.00 | 0.031* |
| P12111 | Collagen alpha-3(VI) chain | CO6A3 | 0.70 ± 0.48 | 0.20 ± 0.42 | 0.039* |
| Q7Z7A3 | Cytoplasmic tRNA 2-thiolation protein 1 | Q7Z7A3 | 0.52 ± 0.54 | 0.10 ± 0.32 | 0.045* |

*Tested by Fisher’s exact test.

| Table 4: Correlations between proteomics data and the central retinal thickness. |
| --- | --- | --- | --- | --- |
| Protein ID | Protein name | Gene name | Correlation, r | p value |
| P51884 | Lumican | LUM | 0.65573455 | 0.002 |
| P01042 | Kininogen-1 | KNG1 | 0.50337911 | 0.024 |
| P05543 | Thyroxine-binding globulin | THBG | 0.38883989 | 0.049 |
| Q86YZ3 | Hornerin | HORN | 0.5426932 | 0.013 |
| Q02487 | Desmocollin-2 | DSC2 | 0.5640969 | 0.013 |
| P61769 | Beta-2-microglobulin | B2MG | 0.48090844 | 0.032 |
| P12111 | Collagen alpha-3(VI) chain | CO6A3 | 0.12831005 | 0.032 |
| Q6UX71 | Plexin domain-containing protein 2 | PXDC2 | 0.52355015 | 0.018 |
| Q14574 | Desmocollin-3 | DSC3 | 0.46152818 | 0.041 |
| P29622 | Kallistatin | KAIN | 0.55527954 | 0.011 |
| O43827 | Angiopoietin-related protein 7 | ANGL7 | 0.48941527 | 0.029 |
| Q96JP9 | Cell adhesion molecule 1 | CADM1 | 0.47306149 | 0.035 |
| Q14004 | Cyclin-dependent kinase 13 | CDK13 | 0.59142594 | 0.006 |
| O75592 | E3 ubiquitin-protein ligase MYCBP2 | MYCB2 | 0.44938655 | 0.047 |
| Q9UBP4 | Dickkopf-related protein 3 | DKK3 | 0.78550025 | 0.001 |
| Q96JP9 | Cadherin-related family member 1 | CDHR1 | 0.6932842 | 0.001 |
| O95274 | Ly6/PLAUR domain-containing protein 3 | LYPD3 | 0.78550025 | 0.0001 |

Tested by Pearson’s correlation coefficients. *Proteins statistically significantly differentially regulated in epiretinal membrane patients.

3. Results
higher levels, and 216 proteins were present at lower levels in the iERM proteome. To understand the biological meanings of the changes in protein expressions observed in iERM, differentially expressed proteins were analyzed in terms of "molecular functions," "biological processes," and "cellular components" by gene ontology (GO) annotations. Our results indicated that differentially expressed proteins in iERM and controls were associated with molecular functions, biological processes, and cellular components. The most important biological processes for downregulated proteins were adaptive immune responses, carbohydrate catabolic processes, glycolipid catabolic processes, and glycoside catabolic processes. In contrast, biological processes of upregulated proteins were strongly associated with ECM organization, negative regulation of endopeptidase activity, positive regulation of cell proliferation, negative regulation of cell proliferation, axon guidance, and cell adhesion. In terms of molecular functions, results revealed that collagen binding, ECM structural constituents, serine-type endopeptidase inhibitor activity, ATP binding, and cyclin binding were elevated. In contrast, antigen binding, alpha-N-acetylgalactosaminidase activity, alpha-galactosidase activity, and protein homodimerization activity showed decreased levels.

We used the Ingenuity Pathway Analysis (IPA, Qiagen) to show canonical pathways that were potentially involved in iERM pathogenesis. Table 2 lists pathways that were associated with AH proteins from patients with iERM and the controls. The top canonical pathways, included interleukin-(IL-) 15 signaling, B-cell receptor signaling, systemic lupus erythematosus in the B-cell signaling pathway, and communication between innate and adaptive immune cells, and significant associations with AH proteins were demonstrated.

A statistical analysis was performed on these 405 proteins, and eight proteins statistically significantly differed in content in iERM samples compared to the controls (Table 3). Among these eight proteins whose contents had changed, six proteins were higher in the iERM group, including lumican, thyroxine-binding globulin (TBG), double-stranded (ds)RNA-specific editase 1, cyclin-dependent kinase 13, collagen alpha-3(VI) chain, and cytoplasmic transfer (t)RNA 2-thiolation protein 1. Another two proteins, viz., immunoglobulin (Ig) heavy variable 5-10-1 and alpha-N-acetylgalactosaminidase, exhibited lower contents in the iERM group (Table 3).

Among the 405 proteins, 17 proteins were positively correlated with the central retina thickness (CRT) (Table 4, Figure 2). Proteins that were positively correlated with the CRT, which significantly differed in iERM samples compared to the controls, included lumican, TBG, cyclin-dependent kinase (CDK) 13, and collagen alpha-3(VI) chain (Figure 3). Additionally, the proteomics analysis revealed an increased level of lumican in iERM samples compared to the controls with the most significant correlation to the CRT among the eight proteins. The peptide sequence of lumican is shown in Figure 4. Associations and relative levels of lumican and the other seven major proteins are also shown in Figure S2. We then chose lumican for further ELISA
Figure 3: Continued.
verification (multiple of change = 1.70, \( p = 0.023 \)) (Table 4; Figure 3). We performed an ELISA to determine the concentration of lumican, and the average concentration of lumican was significantly (\( p = 0.0058 \)) elevated in patients with iERM (4.172 μg/mL) compared to control subjects (2.006 μg/mL) (Figure 5).

4. Discussion

This is the first study to use label-free nanoflow UHP LC-MS/MS quantitation to analyze the human AH proteome in iERM disease compared to controls. We found increased total protein levels in the AH and changes in the AH protein profile in iERM subjects, which differed from controls. Eight aqueous proteins were significantly higher in the AH of patients with iERM. Notably, lumican was positively correlated with the CRT, which is a useful measurement of iERM progression. ELISA confirmation of the evaluation of aqueous lumican levels further validated the proteomics findings. The increase in the AH lumican concentration may reflect an increased risk of pathogenic features of iERM. However, we have a limited understanding of the exact molecular events that regulate these eight significantly changed protein expressions in retinal disease.

Relationships between proteome parameters and eye diseases were previously studied. A similar technique was recently used to analyze changes in the AH of patients with keratoconus (KC) and patients with myopia [24, 25]. Yu et al. [21] performed similar research analyzing the vitreous proteome of patients with iERM and healthy donor controls.
Figure 4: Continued.
and concluded that iERM involves a complicated pathological process including inflammation, immune responses, and cytoskeletal remodeling, with similar results as this study. The proteome of the AH was proven to be closely associated with fundal diseases [19, 20]. Although the aqueous protein does not come into direct contact with the retina, the ECM and secreted products may leak into intraocular fluids of the eye. Pollreisz et al. showed that only 3.96% of identified proteins exhibited significant differential expression between the aqueous and vitreous fluids from human eyes with iERM [20].

Furthermore, this leakage can increase the total protein level in bodily fluids. The increase in the total protein concentration in fluids can be used as a marker of the severity of fibrocellular changes. Similarly, increases in protein levels were observed in both the VH and AH in patients with diabetes mellitus retinopathy [16, 26, 27]. Our data showed that total protein levels in the AH were higher in iERM patients. Both the iERM and control groups had the same background conditions of cataracts; thus, we assumed that dysregulated proteins we found in the AH were related to the iERM pathogenesis. This finding assumes that elevated total protein levels may also serve as a biomarker for pathogenic changes in iERM.

Lumican is a keratan sulfate proteoglycan widely expressed in connective tissues, and it is critical in

![Figure 4: Correlations between aqueous proteins and the central retinal thickness (CRT). Vertical values represent the CRT (µm) measured by OCT. Horizontal values denote the contents of the proteins measured by mass spectrometry (spectral count (SpC)). Correlations were calculated as Pearson’s correlation coefficients (r). Proteins were significantly positively correlated with the CRT (a–d).](image-url)
maintaining corneal transparency [28]. It is also a component of the sclera, and thinning of the sclera was found in Lum−/− knockout mice [29]. A proteomics study of postmortem human eyes revealed that lumican was also found in Bruch’s membrane, the choroid, and neurosensory retinae [30], where it was highly associated with ERM’s origin. Due to being a member of the class II small leucine-rich proteoglycan (SLRP) family, lumican can maintain retinal homeostasis, is involved in collagen fibrillogenesis, and regulates proinflammatory responses [31]. Animal studies also proved increased expression of lumican along with age and modifications of lumican by macrophages, neutrophils, and polymorphonuclear neutrophils in immune responses [32–34]. A possible pathogenesis of iERM was shown by Bu et al. to be associated with age-related accumulation of advanced glycation end products which possibly contributes to anomalous posterior vitreous detachment. Remodeling of the ECM at the vitreoretinal interface by aging and fibrotic changes contributes to iERM pathogenesis [35]. ERM was postulated to involve the proliferation of fibroblasts, glial cells, and astrocytes after ILM disruption, and ECM structures where lumican is located and in which it is involved were discussed. Extracellular microstructures formed by cytoskeletal and ECM proteins contribute to maintaining retinal integrity and visual functions [36–38]. Genetic studies of gene polymorphisms in humans also support a role for lumican in scleral involvement of myopia which is closely associated with fibromodulin [39–42]. Alterations in lumican may thus contribute to myopia and various retinal diseases. Another recent study showed that lumican was negatively associated with mean defect (MD) values of a visual field test in patients with glaucoma, influencing the aqueous outflow resistance by trabecular meshwork reformation [39]. Considering the hypothesis of lumican’s location and close association with ERM pathogenesis, all previous studies provided hints that lumican may contribute to iERM formation with scleral and ECM involvement and to maintaining the ocular immunologic status in iERM. Its expression may be identified as one of the hallmarks for ERM.

Furthermore, the increase in lumican levels in patients with iERM was also associated with an advanced status of iERM. Our finding of significant changes in lumican expression indicates that it could serve as a potential biomarker in managing iERM; however, further investigation is needed to elucidate the roles of lumican in the iERM pathogenesis. Another microstructural protein, collagen alpha-3(VI) chain, binds ECM proteins that aid in microfibril formation [40]. It was associated with diseases related to muscles and connective tissues [41, 42]. The appearance of this structural protein in the AH may indicate a possible loss of integrity of the retina.

Levels of three cell proliferation-related proteins, dsRNA-specific editase 1, CDK 13, and cytoplasmic tRNA 2-thiolation protein 1, were altered in the aqueous of patients with iERM. dsRNA-specific editase 1 catalyzes the hydrolytic deamination of adenosine to inosine in dsRNA [43] in nucleoli. It is expressed by the ADARB1 gene, which is highly expressed in the brain and heart [44]. It was reported to be involved in neurodevelopmental disorders of hypotonia, microcephaly, and seizures (NEDHYMS) [45]. CDK 13 is responsible for RNA splicing [46] in nucleoli and is highly expressed in the fetal brain, liver, muscles, and adult brain. Dysregulation of CDK 13 is a factor leading to congenital heart defects, dysmorphic facial features, and intellectual developmental disorder (CHDFIDD) [47]. Cytoplasmic tRNA 2-thiolation protein 1 directly binds tRNAs and probably acts by catalyzing adenylation of tRNAs, an intermediate step required for 2-thiolation in the cytoplasm [48]. In terms of the eye, overexpressions of dsRNA-specific
editase 1, CDK 13, and cytoplasmic tRNA 2-thiolation protein 1 are unknown in retinal disease based on past studies. We suspect that these three proteins are involved in cell proliferation processes in the formation of fibroproliferative iERM. This finding related to iERM may provide new investigation targets for future research.

TBG is well known in the serum and binds thyroid hormones in the blood circulation to regulate thyroid function. Upregulation of TBG leads to more binding of the thyroid hormone, which decreases the amount of free hormone available in the blood. This leads to stimulation of the thyroid-stimulating hormone and the production of more thyroid hormone. Upregulation of TBG levels may be caused by hypothyroidism, liver disease, pregnancy, acute intermittent porphyria, or genetics [49]. In this study, a significant change in TBG was found in patients with iERM; however, we did not determine serum levels in these patients with iERM. Further surveys can be arranged to compare serum and aqueous fluid TBG levels in concert with TBG or even a thyroid function test, which could be an early diagnostic biomarker.

Two downregulated proteins, Ig heavy variable 5-10-1 and alpha-N-acetylgalactosaminidase, were also found in aqueous changes in patients with iERM. Alpha-N-acetylgalactosaminidase is an isoenzyme of alpha-galactosidases that functions in the lysosome [50] and is related to Schindler disease [51] and Kanzaki disease [52] with variable neuroaxonal dystrophy and neurological signs. Therefore, we considered lysosome function to be involved in the iERM formation process. Ig heavy variable 5-10-1 is in the V region of the variable domain of Ig heavy chains that participate in antigen recognition [53]. Additionally, B lymphocytes secrete Igs, also known as antibodies that mediate the effector phase of humoral immunity [54]. Recently, some research showed significant changes in Igs in tear protein profiles in specific diseases like diabetic retinopathy, primary open-angle glaucoma, and postrefractive surgery [55, 56]. In our study, we found a decrease in Ig heavy variable 5-10-1 in the iERM group. This indicated some immune interactions in iERM formation. These findings provide us with more information on the pathogenic process of iERM formation.

In this study, we investigated the protein composition of the AH obtained from human cataract eyes with iERM and aged-matched controls without iERM. To the best of our knowledge, this is the first study to individually examine differential changes in the protein composition of the AH in iERM. However, several limitations should be specified. First, only 10 samples in each group were investigated, and future larger-scale studies could help verify our results. Second, only a small amount of AH (50–100 μL) could be obtained due to anatomical features, which limited our ability to conduct subsequent validation assays. Third, the development of multiplex immunoassays can be improved. Finally, we can only provide associations of proteomic data with clinical presentations due to a lack of essential evidentiary support. Thus, the exact pathway by which lumican is involved in the iERM pathogenesis remains unknown. More studies to analyze the lumican levels in iERM patients are needed, and further serum and vitreous analyses of iERM patients should be conducted. More future investigations of molecular pathways are also needed to discuss how and why the proteomics data varied with the CRT and ultimately supply better knowledge of iERM for the whole of humanity.

5. Conclusions

In conclusion, iERM involves a complicated pathological process, including several proteins that participate in immune reactions, cell proliferation, and remodeling of the ECM, which were identified in the AH by a proteomics analysis. Thus, lumican could be a potential aqueous biomarker for predicting iERM development and monitoring its progression. However, more clinical parameters also need to be identified for a more-comprehensive analysis and to provide additional targets for treating and preventing iERM.

Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| AH           | Aqueous humor |
| AXL          | Axial length |
| CDK          | Cyclin-dependent kinase |
| CRT          | Central retinal thickness |
| ECM          | Extracellular matrix |
| ELISA        | Enzyme-linked immunosorbent assay |
| ERM          | Epiretinal membrane |
| GO           | Gene ontology |
| iERM         | Idiopathic epiretinal membrane |
| Ig           | Immunoglobulin |
| IPA          | Ingenuity Pathway Analysis |
| LASIK        | Laser-assisted in situ keratomileusis |
| MAR          | Minimal angle of resolution |
| MD           | Mean defect |
| OCT          | Optical coherence tomography |
| RNA          | Ribonucleic acid |
| SMILE        | Small incision lenticule extraction |
| TGB          | Thyroxine-binding globulin |
| VA           | Visual acuity |
| VH           | Vitreous humor |

Data Availability

All data generated or analyzed during this study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors have declared that no competing interest exists.

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Supplementary Materials

Table S1: demographic characteristics of enrolled patients. Fig. S1: protein expression distributions of the idiopathic
epiretinal membrane (iERM) and control groups. In total, 405 proteins were identified by LC-ESI MS/MS in iERM and control aqueous humor (AH) samples; 344 proteins were detected in the iERM group, and 350 proteins were detected in the control group. Fig. S2: relative levels of lumican and its associations with other major proteins. (Supplementary Materials)

References

[1] S. Fraser-Bell, M. Guzowski, E. Rochtchina, J. J. Wang, and P. Mitchell, “Five-year cumulative incidence and progression of epiretinal membranes: the Blue Mountains Eye Study,” *Ophthalmology*, vol. 110, no. 1, pp. 34–40, 2003.

[2] F. S. Ting and A. K. Kwok, “Treatment of epiretinal membrane: an update,” *Hong Kong Medical Journal*, vol. 11, no. 6, pp. 496–502, 2005.

[3] W. E. Smiddy, W. R. Green, R. G. Michels, and P. K. Nase, “Ultrastructural features of progressive idiopathic epiretinal membrane removed by vitreous surgery,” *American Journal of Ophthalmology*, vol. 90, no. 6, pp. 797–809, 1980.

[4] A. Kampik, W. R. Green, R. G. Michels, and P. K. Nase, “Ultrastructural characteristics and clinicopathologic correlation,” *Ophthalmology*, vol. 96, no. 6, pp. 811–821, 1989.

[5] P. S. Hiscott, I. Grierson, C. J. Trombetta, A. H. Rahi, J. Marshall, and D. McLeod, “Retinal and epiretinal glia—an immunohistochemical study,” *The British Journal of Ophthalmology*, vol. 68, no. 10, pp. 698–707, 1984.

[6] S. Y. Oberstein, J. Byun, D. Herrera, E. A. Chapin, S. K. Fisher, and G. P. Lewis, “Cell proliferation in human epiretinal membranes: characterization of cell types and correlation with disease condition and duration,” *Molecular Vision*, vol. 17, pp. 1794–1805, 2011.

[7] S. de Bustrós, J. T. Thompson, R. G. Michels, T. A. Rice, and B. M. Glaser, “Vitrectomy for idiopathic epiretinal membranes causing macular pucker,” *The British Journal of Ophthalmology*, vol. 72, no. 9, pp. 692–695, 1988.

[8] M. B. Bellhorn, A. H. Friedman, G. N. Wise, and P. Henkind, “Ultrastructure and clinicopathologic correlation of idiopathic preretinal macular fibrosis,” *American Journal of Ophthalmology*, vol. 79, no. 3, pp. 366–373, 1975.

[9] E. Mandelcorn, Y. Khan, L. Javorska, J. Cohen, D. Howarth, and M. Mandelcorn, “Idiopathic epiretinal membranes: cell type, growth factor expression, and fluorescein angiographic and retinal photographic correlations,” *Canadian Journal of Ophthalmology*, vol. 38, no. 6, pp. 457–463, 2003.

[10] T. Yamamoto, N. Akabane, and S. Takeuchi, “Vitrectomy for diabetic macular edema: the role of posterior vitreous detachement and epimacular membrane,” *American Journal of Ophthalmology*, vol. 132, no. 3, pp. 369–377, 2001.

[11] E. J. Kuiper, F. A. van Nieuwenhoven, M. D. de Smet et al., “The angio-fibrotic switch of VEGF and CTGF in proliferative diabetic retinopathy,” *PloS One*, vol. 3, no. 7, article e2675, 2008.

[12] H. Youngblood, R. Robinson, A. Sharma, and S. Sharma, “Proteomic biomarkers of retinal inflammation in diabetic retinopathy,” *International Journal of Molecular Sciences*, vol. 20, no. 19, p. 4755, 2019.

[13] G. Velez, P. H. Tang, T. Cabral et al., “Personalized proteomics for precision health: identifying biomarkers of vitreoretinal disease,” *Translational Vision Science & Technology*, vol. 7, no. 5, p. 12, 2018.

[14] E. Midena, L. Frizziro, G. Midena, and E. Pilotto, “Intraocular fluid biomarkers (liquid biopsy) in human diabetic retinopathy,” *Graefe’s Archive for Clinical and Experimental Ophthalmology*, vol. 259, no. 12, pp. 3549–3560, 2021.

[15] Y. Ji, J. Rao, X. Song, S. Lou, Z. Zheng, and Y. Lu, “Metabolic characterization of human aqueous humor in relation to high myopia,” *Experimental Eye Research*, vol. 159, pp. 147–155, 2017.

[16] S. Y. Chiang, M. L. Tsai, C. Y. Wang et al., “Proteomic analysis and identification of aqueous humor proteins with a pathophysiological role in diabetic retinopathy,” *Journal of Proteomics*, vol. 75, no. 10, pp. 2950–2959, 2012.

[17] S. C. Qu, D. Xu, T. T. Li, J. F. Zhang, and F. Liu, “iTRAQ-based proteomics analysis of aqueous humor in patients with dry age-related macular degeneration,” *International Journal of Ophthalmology*, vol. 12, no. 11, pp. 1758–1766, 2019.

[18] A. Pollreisz, M. Funk, F. P. Breitwieser et al., “Quantitative proteomics of aqueous and vitreous fluid from patients with idiopathic epiretinal membranes,” *Experimental Eye Research*, vol. 108, pp. 48–58, 2013.

[19] J. Yu, L. Feng, Y. Wu et al., “Vitreous proteomic analysis of idiopathic epiretinal membranes,” *Molecular BioSystems*, vol. 10, no. 10, pp. 2558–2566, 2014.

[20] N. Mandal, M. Kofod, H. Vorum et al., “Proteomic analysis of human vitreous associated with idiopathic epiretinal membrane,” *Acta Ophthalmologica*, vol. 91, no. 4, pp. e333–e334, 2013.

[21] C. Christakopoulos, L. J. Cehofski, S. R. Christensen, H. Vorum, and B. Honoré, “Proteomics reveals a set of highly enriched proteins in epiretinal membrane compared with inner limiting membrane,” *Experimental Eye Research*, vol. 186, article 107722, 2019.

[22] M. Xue, Y. Ke, X. Ren et al., “Proteomic analysis of aqueous humor in patients with pathologic myopia,” *Journal of Proteomics*, vol. 234, article 104088, 2021.

[23] J. Soria, A. Villarrubia, J. Merayo-Lloves et al., “Label-free LC-MS/MS quantitative analysis of aqueous humor from keratoconic and normal eyes,” *Molecular Vision*, vol. 21, pp. 451–460, 2015.

[24] D. S. Fosmark, R. Bragadóttir, I. Stene-Johansen et al., “Increased vitreous levels of hydroimidazolone in type 2 diabetes patients are associated with retinopathy: a case-control study,” *Acta Ophthalmologica Scandinavica*, vol. 85, no. 6, pp. 618–622, 2007.

[25] S. Kato, S. Takahashi, O. Ikawa et al., “Aqueous protein concentration in diabetics. Report 5. Relationship between aqueous humor protein concentration and pathological findings of iridial vessels,” *Nippon Ganka Gakkai Zasshi*, vol. 96, no. 8, pp. 1000–1006, 1992.
[28] S. Amjadi, K. Mai, P. McCluskey, and D. Wakefield, “The role of lumican in ocular disease,” International Scholarly Research Notices, vol. 2013, Article ID 632302, 2013.

[29] S. Chakravarti, “Functions of lumican and fibromodulin: lessons from knockout mice,” Glycoconjuate Journal, vol. 19, no. 4, pp. 287–293, 2002.

[30] T. D. Keenan, S. J. Clark, R. D. Unwin, L. A. Ridge, A. J. Day, and P. N. Bishop, “Mapping the differential distribution of proteoglycan core proteins in the adult human retina, choroid, and sclera,” Investigative Ophthalmology & Visual Science, vol. 53, no. 12, pp. 7528–7538, 2012.

[31] S. W. Y. Low, T. B. Connor, I. S. Kassem, D. M. Costakos, and S. S. Chaurasia, “Small leucine-rich proteoglycans (SLRPs) in the retina,” International Journal of Molecular Sciences, vol. 22, no. 14, p. 7293, 2021.

[32] J. L. Funderburgh, R. R. Mitschler, M. L. Funderburgh, M. R. Roth, S. K. Chapes, and G. W. Conrad, “Macrophage receptors for lumican. A corneal keratan sulfate proteoglycan,” Investigative Ophthalmology & Visual Science, vol. 38, no. 6, pp. 1159–1167, 1997.

[33] E. C. Carlson, M. Lin, C. Y. Liu, W. W. Y. Kao, V. L. Perez, and E. Pearlman, “Keratocon and lumican regulate neutrophil infiltration and corneal clarity in lipopolysaccharide-induced keratitis by direct interaction with CXCL1,” The Journal of Biological Chemistry, vol. 282, no. 49, pp. 35502–35509, 2007.

[34] Y. Hayashi, M. K. Call, T. I. Chikama et al., “Lumican is required for neutrophil extravasation following corneal injury and wound healing,” Journal of Cell Science, vol. 123, no. 17, pp. 2987–2995, 2010.

[35] S. C. Bu, R. Kuijer, X. R. Li, J. M. M. Hooymans, and L. I. Los, “Idiopathic epiretinal membrane,” Retina, vol. 34, no. 12, pp. 2317–2335, 2014.

[36] B. K. Chauhan, A. Disanza, S. Y. Choi et al., “Cdc42- and IRSp53-dependent contractile filopodia tether presumptive lens and retina to coordinate epithelial invagination,” Development, vol. 136, no. 21, pp. 3657–3667, 2009.

[37] B. B. Boycott and J. M. Hopkins, “A neurofibrillar method stains solitary (primary) cilia in the mammalian retina: their distribution and age-related changes,” Journal of Cell Science, vol. 66, no. 1, pp. 95–118, 1984.

[38] T. Evans, D. X. Deng, S. Chen, and S. Chakrabarti, “Endothelin receptor blockade prevents augmented extracellular matrix component mRNA expression and capillary basement membrane thickening in the retina of diabetic and galactose-fed rats,” Diabetes, vol. 49, no. 4, pp. 662–666, 2000.

[39] S. K. Kodeboyina, T. J. Lee, K. Bollinger et al., “Aqueous humor proteomic alterations associated with visual field index parameters in glaucoma patients: a pilot study,” Journal of Clinical Medicine, vol. 10, no. 6, p. 1180, 2021.

[40] S. R. Lamanse, M. Morgelin, N. E. Adams, C. Selan, and J. M. Allen, “The C5 domain of the collagen VI α3(VI) chain is critical for extracellular microfibril formation and is present in the extracellular matrix of cultured cells,” The Journal of Biological Chemistry, vol. 281, no. 24, pp. 16607–16614, 2006.

[41] K. M. Bushby, J. Collins, and D. Hicks, “Collagen type VI myopathies,” Advances in Experimental Medicine and Biology, vol. 802, pp. 185–199, 2014.

[42] Y. Z. Zhang, D. H. Zhao, H. P. Yang et al., “Novel collagen VI mutations identified in Chinese patients with Ulrich congenital muscular dystrophy,” World Journal of Pediatrics, vol. 10, no. 2, pp. 126–132, 2014.

[43] C. Cenci, R. Barzotti, F. Galeano et al., “Down-regulation of RNA editing in pediatric astrocytomas,” The Journal of Biological Chemistry, vol. 283, no. 11, pp. 7251–7260, 2008.

[44] A. Gerber, M. A. O’Connell, and W. Keller, "Two forms of human double-stranded RNA-specific editase 1 (hRED1) generated by the insertion of an Alu cassette," RNA, vol. 3, no. 5, pp. 453–463, 1997.

[45] T. Y. Tan, J. Sedmík, M. P. Fitzgerald et al., “Bi-allelic ADAR1 variants associated with microcephaly, intellectual disability, and seizures,” American Journal of Human Genetics, vol. 106, no. 4, pp. 467–483, 2020.

[46] R. Berro, C. Pedati, K. Kehn-Hall et al., “CDK13, a new potential human immunodeficiency virus type 1 inhibitor factor regulating viral mRNA splicing,” Journal of Virology, vol. 82, no. 14, pp. 7155–7166, 2008.

[47] A. Sifrim, M. P. Hitz, A. Wilsdon et al., “Distinct genetic architectures for syndromic and nonsyndromic congenital heart defects identified by exome sequencing,” Nature Genetics, vol. 48, no. 9, pp. 1060–1065, 2016.

[48] C. D. Schlieker, A. G. van der Veen, J. R. Damon, E. Spooner, and H. L. Ploegh, “A functional proteomics approach links the ubiquitin-related modifier Urm1 to a tRNA modification pathway,” Proceedings of the National Academy of Sciences of the United States of America, vol. 105, no. 47, pp. 18255–18260, 2008.

[49] G. C. Schussler, “The thyroxine-binding proteins,” Thyroid, vol. 10, no. 2, pp. 141–149, 2000.

[50] B. Asfaw, D. Schindler, J. Ledvinová, B. Černý, F. Šmid, and E. Conzelmann, “Degradation of blood group a glycolipid A-6-2 by normal and mutant human skin fibroblasts,” Journal of Lipid Research, vol. 39, no. 9, pp. 1768–1780, 1998.

[51] A. M. Wang, D. Schindler, and R. Desnick, “Schindler disease: the molecular lesion in the alpha-N-acetylgalactosaminidase gene that causes an infantile neuronal dystrophy,” The Journal of Clinical Investigation, vol. 86, no. 5, pp. 1752–1756, 1990.

[52] K. Kodama, H. Kobayashi, R. Abe et al., “A new case of alpha-N-acetylgalactosaminidase deficiency with angiokeratoma corporis diffusum, with Meniere's syndrome and without mental retardation,” The British Journal of Dermatology, vol. 144, no. 2, pp. 363–368, 2001.

[53] M. P. Lefranc, “Immunoglobulin and T cell receptor genes: IMGT((R)) and the birth and rise of immunoinformatics,” Frontiers in Immunology, vol. 5, p. 22, 2014.

[54] H. W. Schroeder Jr and L. Cavacini, “Structure and function of immunoglobulins,” The Journal of Allergy and Clinical Immunology, vol. 125, no. 2, pp. S41–S52, 2010.

[55] Y. C. Liu, G. H. F. Yam, M. T. Y. Lin et al., “Comparison of tear proteomic and neuromediator profiles changes between small incision lenticule extraction (SMILE) and femtosecond laser-assisted in-situ keratomileusis (LASIK),” Journal of Advanced Research, vol. 29, pp. 67–81, 2021.

[56] J. Y. W. Ma, Y. H. Sze, J. F. Bhat, and T. C. Lam, “Critical role of mass spectrometry proteomics in tear biomarker 486 discovery for multifactorial ocular diseases (Review),” International Journal of Molecular Medicine, vol. 47, no. 5, 2021.