· Basic Research ·

Analysis of proteomic differences between liquefied after-cataracts and normal lenses using two-dimensional gel electrophoresis and mass spectrometry

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Abstract

● AIM: To analyze and identify the proteomic differences between liquefied after-cataracts and normal lenses by means of liquefied chromatography-tandem mass spectrometry (LC-MS/MS).

● METHODS: Three normal lenses and three liquefied after-cataracts were exposed to depolymerizing reagents to extract the total proteins. Protein concentrations were separated using two-dimensional gel electrophoresis (2-DE). The digitized images obtained with a GS-800 scanner were then analyzed with PDQuest7.0 software to detect the differentially-expressed protein spots. These protein spots were cut from the gel using a proteome work spot cutter and subjected to in-gel digestion with trypsin. The digested peptide separation was conducted by LC-MS/MS.

● RESULTS: The 2-DE maps showed that lens proteins were in a pH range of 3-10 with a relative molecular weight of 21-70 kD. The relative molecular weight of the more abundant proteins was localized at 25-50 kD, and the isoelectric points were found to lie between PI 4-9. The maps also showed that the protein level within the liquefied after-cataracts was at 29 points and significantly lower than in normal lenses. The 29 points were identified by LC-MS/MS, and ten of these proteins were identified by mass spectrometry and database queries: beta-crystallin B1, glyceraldehyde-3-phosphate dehydrogenase, carbonyl reductase (NADPH) 1, cDNA FLJ55253, gamma-crystallin D, GAS2-like protein 3, sorbitol dehydrogenase, DNA FLJ60282, phosphoglycerate kinase, and filensin.

● CONCLUSION: The level of the ten proteins may play an important role in the development of liquefied after-cataracts.

● KEYWORDS: capsular block syndrome; liquefied after-cataract; liquid chromatography-tandem mass spectrometry

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INTRODUCTION

Capsular block syndrome (CBS) is a rare complication of phacoemulsification with continuous curvilinear capsulorhexis (CCC) and posterior chamber in-the-bag intraocular lens (IOL) implantation[1-3]. It is categorized into three types depending on the time of onset: intraoperative, early postoperative, and late postoperative[4]. Liquefied after-cataract (LAC) is a special type of late complication following standard surgery, without a shallow anterior chamber and secondary glaucoma. On average, late postoperative CBS occurs 3.8y after surgery and can be identified by white deposits behind the IOL inside the capsular bag[5]. Eifrig[6] showed that the white liquid contained high concentrations of alpha-crystallin and relatively low levels of albumin, suggesting that the liquid may originate from the epithelial cells of the cataract. However, the content of the white liquid is not fully understood. In this study, we adopted two-dimensional gel electrophoresis (2-DE) to investigate proteomic differences between LAC and normal lenses. We also explored the pathogenesis of LAC from the perspective of quantitative proteomics.

MATERIALS AND METHODS

Materials Ethical clearance for the study was obtained from the institutional review board according to the Declaration of Helsinki, and informed consent from all patients was obtained. Three fresh transparent lenses obtained from the donor eyes were provided by the Eye Bank of Shandong Eye Institute and used as controls (group A). The study enrolled three cases which presented with painless, gradual visual loss at 5-8y after uneventful cataract surgery and were diagnosed with LAC at the Qingdao Eye Hospital, Shandong Eye Institute (group B). The three patients in group B were aged 63, 69 and 72y respectively (Table 1). Standard coaxial ultrasonic phacoemulsification was performed in all of them, with capsulorhexis, hydrodissection, and enhanced cortical clean-up

Table 1 Clinical features of three cases

Case No.	Gender	Age (a)	Onset time after surgery (a)	Affected eye	IOP (mm Hg)
1	F	63	5	Left	15
2	M	69	7	Left	19
3	M	72	8	Left	18

and in-the-bag foldable IOL fixation with an anterior capsular overlap. Surgeries were performed by a single experienced surgeon with a superior corneal incision. The blurring of vision was gradual. On clinical examination, the intraocular pressure (IOP) was normal (ranging from 15 to 19 mm Hg). Anterior segment photographs showed fibrosis of CCC and anterior capsular opacity. The space between the IOL and the posterior capsule was filled with a milky opalescent fluid which in slim beam looked like a meniscus-shaped opaque space with concave anterior and convex posterior borders. The optical section appeared as though two lenses had been placed in the bag (Figure 1). Pentacam Scheimpflug examination of the anterior segment of the eye demonstrated normal anterior chamber depth. The milky white substance was located behind the IOL optic (Figure 2).

Surgical Technique Proper asepsis techniques were employed, and a blepharostat was placed in position. A clear corneal incision was made. A 27-gauge needle was inserted through the edge of the CCC into the capsular bag to extract aqueous humor from the anterior chamber along with the milky white substance for biochemical study (Figure 3). Then the liquid was centrifuged at 4℃, and the supernatant was drawn and preserved at -80℃. The fresh transparent lenses of the donor eyes were examined and shown to be without disease or signs of surgery or other trauma. The lenses were ground in liquid nitrogen and then dissolved in 1 mL lysis buffer to extract proteins before the supernatant was drawn and preserved at -80℃. The protein concentration of each sample was measured using a Bio-Rad protein assay method.

Reagents and Instruments The main reagents included isoelectric focusing (IEF) strip (18 cm, pH 3-10 linear range), dithiothreitol (DTT), 3-[(3-cholamidopropy)-dimethylammonio]-1-propane sulfonate (CHAPS), sodiumdodecylsulfate (SDS), iodoacetamide, urea, Tris (Bio-Rad, USA), coomassie blue, ammonium persulfate (Sigma, USA), and thiourea (Solarbio, China). The instruments were Protean IEF cell Isoelectric focusing system, Protean II xi cell Vertical electrophoresis tank, the Versa Doc 1000 gel imaging system, PDQuest7.0 image analysis software (Bio-Rad), Labofuge 400R High-speed refrigerated centrifuge (Heraeus, USA), and Biowave Ultraviolet spectrophotometer (Biochrom, Cambridge UK).

Two-dimensional Gel Electrophoresis According to the method of Gorg and the instructions of Protean IEF Cell Isoelectric Focusing System, each sample was joined with a loading buffer to 350 μL[7]. Hydration and isoelectric focusing were performed automatically on a Protean IEF cell. The program was set for the following intervals: 1) passive hydration for 12h; 2) at 250 V slow boost for 30min; 3) at 1000 V fast boost for 2h; 4) at 1000 V fast boost for 2h; 5) at 10000 V linear boost for 3h; and 6) at 10000 V holding to 6000 V for 1h. After IEF, the strips were balanced in a solution.

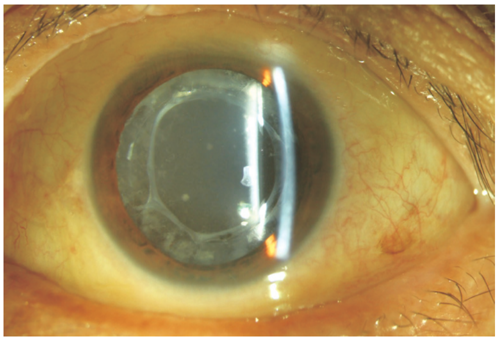

Figure 1 Slit-lamp photograph showing fibrosis of CCC, anterior capsular opacity, a backward extension of the posterior capsule, and the presence of milky-white fluid between IOL and the posterior capsule.

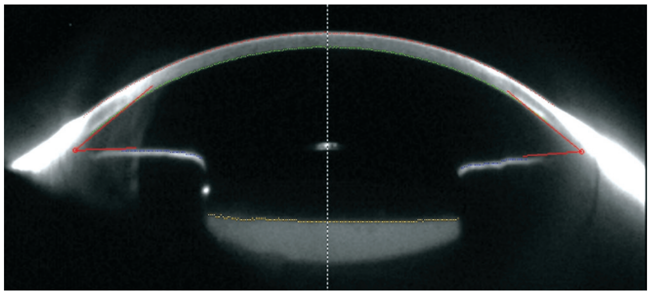

Figure 2 Scheimplug photography of the anterior segment of the eye showing normal anterior chamber depth and relative density of the milky white substance behind the IOL.

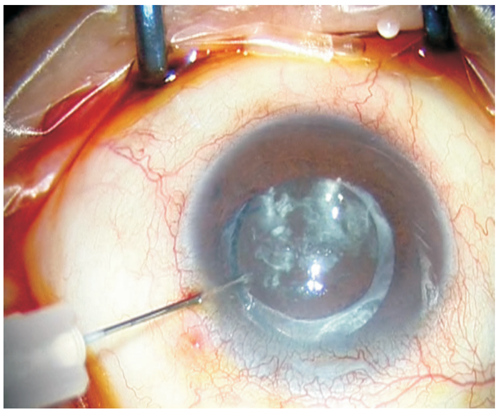

Figure 3 A 27-gauge needle was inserted through the edge of the CCC into the capsular bag to extract aqueous humor from the anterior chamber along with the milky white substance.

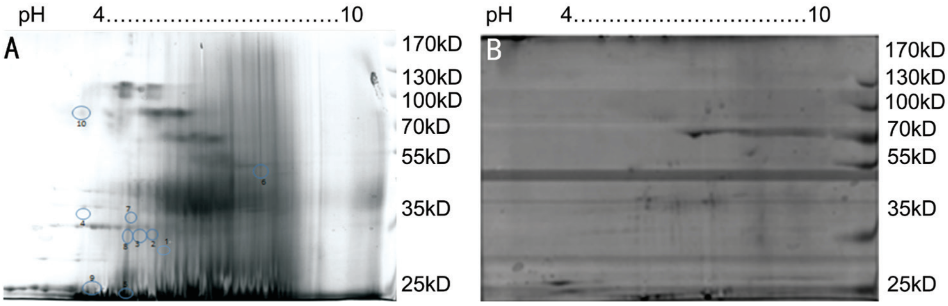

Figure 4 Two-dimensional electrophoresis of normal lenses (A) and liquefied after-cataract (B) 1: Beta-crystallin B; 2: Glyceraldehyde-3-phosphate dehydrogenase; 3: Carbonyl reductase (NADPH) 1; 4: cDNA FLJ55253; 5: Gamma-crystallin D; 6: GAS2-like protein 3; 7: Sorbitol dehydrogenase; 8: cDNA FLJ60282; 9: Phosphoglycerate kinase; 10: Filensin.

Table 2 Mass spectra results

Sample name	Protein ID	Description	Protein mass (Da)	Soelectric point	Coverage rate (%)
11	sp|P53674|CRBB1	Beta-crystallin B1	28062.91	8.88	56.35
7	sp|P04406|G3P	Glyceraldehyde-3-phosphate dehydrogenase	36201.46	8.73	50.75
2	sp|P16152|CBR1	Carbonyl reductase (NADPH) 1	30640.97	8.49	72.20
30	tr|B4DW52|B4DW52	cDNA FLJ55253	38950.3	5	61.96
23	sp|P07320|CRGD	Gamma-crystallin D	21066.96	7.46	83.33
27	tr|H0YIT6|H0YIT6_HUMAN	GAS2-like protein 3	41118.78	11.32	49.36
9	sp|Q00796|DHSO_HUMAN	Sorbitol dehydrogenase	38927.05	8.06	50.70
8	tr|B4DKI2|B4DKI2_HUMAN	cDNA FLJ60282	30314.58	7.89	55.76
10	tr|B4DHM5|B4DHM5_HUMAN	Phosphoglycerate kinase	25888.27	5.44	49.59
31	sp|Q12934|BFSP1_HUMAN	Filensin	74784.3	4.8	41.05

Mass spectrometer showing the 29 different protein spots belonging to 10 different proteins: 1, beta-crystallin B; 2, glyceraldehyde-3-phosphate dehydrogenase; 3, carbonyl reductase (NADPH) 1; 4, cDNA FLJ55253; 5, gamma-crystallin D; 6, GAS2-like protein 3; 7, sorbitol dehydrogenase; 8, cDNA FLJ60282; 9, phosphoglycerate kinase; 10, filensin.

Electrophoresis was performed at 15℃ on a 25% SDS-PAGE gel, before the electrophoresis gel was stained with coomassie blue for 50min, decolored, and finally stored in 7% acetic acid solution.

Image Acquisition and Fibrin Glue Point Identification We used the Versa Doc 1000 gel imaging system to obtain images after the gel was stained. PDQuest7.0 image analysis software was employed to analyze the results including tailoring, filtering, and matching. We repeated the test three times to ensure the reliability of the test results before filtering out the common different protein points and using the mass spectrometry analysis to identify the differential protein.

RESULTS

Two-dimensional Gel Electrophoresis Results and Analysis Using the method described above to carry out 2-DE of the two groups of proteins, we repeated the process three times, and the distribution of the protein was basically the same (Figure 4). Through PDQuest7.0 software analysis and statistical analysis, a total of 29 different points were found in all matching spots. Compared with group A (normal lens), the protein level in group B (LAC) was down-regulated.

Differences in Protein Mass Spectrum Identification Results A mass spectrometer successfully appraised the 29 different protein spots belonging to ten different proteins: filensin (2 spots), beta-crystallin B1 (2 spots), gamma-crystallin D (14 spots), glyceraldehyde-3-phosphate dehydrogenase (4 spots), carbonyl reductase (NADPH) 1 (1 spot), cDNA FLJ55253, highly similar to actin, cytoplasmic 1 (2 spots), GAS2-like protein 3 (fragment) (1 spot), sorbitol dehydrogenase (1 spot), cDNA FLJ60282, highly similar to sorbitol dehydrogenase (1 spot), and phosphoglycerate kinase (1 spot) (Table 2).

DISCUSSION

In recent years, the use of mass spectrometry techniques combined with 2-DE protein separation and identification has become an important means of protein research and has been widely used in various fields of life sciences. The crystalline lens contains high levels of proteins, which play an important part in maintaining transparency, normal morphology, and function of the lens. Any change in the structure or amount of specific crystallins can lead to cataract[8-12].

There have been few studies about the components of the white milky material in LAC. Our study aimed to apply protein research technology to investigate the pathogenesis of LAC from the molecular level.

Miyake postulated that the cortical cells underwent metaplastic changes and proliferated in the bag during the late postoperative period[13]. This may lead to posterior capsular opacification and cause occlusion of the capsular opening by sealing off the gap between the anterior capsule and the lens implant. These metaplastic cells can also lead to the release of a turbid fluid retained in the retro-lenticular space. Other related factors may be surgery-induced disturbance of blood ocular barriers that lead to free access of different molecules, growth factors, hormones, cells in the capsular bag or deposition of various cell types inside the capsule during and after surgery, and biocompatibility of IOL materials[14]. Accumulation of similar materials has been documented [15]. However, the components of the milky liquid are not clearly identified. The results of our study showed that filensin, beta-crystallin B1, gamma-crystallin D, glyceraldehyde-3-phosphate dehydrogenase, carbonyl reductase (NADPH) 1, cDNA FLJ55253, GAS2-like protein 3 (fragment), sorbitol dehydrogenase, cDNA FLJ60282, and phosphoglycerate kinase were all down-regulated when compared with normal lenses. Perhaps the ten proteins play a critical role in the formation process of the LAC.

The lenses of the eyes are composed of two types of cells: epithelial cells, which form a monolayer at the anterior surface of the lens, and lens fiber cells, which originate from epithelial cells and are highly differentiated. Lens fiber cells lack organelles, have lens-specific structures such as gap junctions and beaded filaments, and synthesize lens-specific proteins. Beaded filaments are lens fiber cell-specific intermediate filaments[16] composed of proteins of filensin and phakinin. Beaded filaments are 15-20 nm in diameter and consist of globular particles with a periodicity of 19-21 nm. Primary amino-acid sequence analysis showed that filensin and phakinin were members of the intermediate filament family of proteins[17]. Beaded filament proteins were found exclusively in the fiber cells of the lenses in all vertebrate orders examined[18], which suggests that beaded filaments play a critical role in lens function. Phakinin and filensin are expressed upon initiation of fiber cell differentiation, predominantly localizing to the fiber cell membrane in young fiber cells in the shallow cortex, and are proteolytically processed and become more cytoplasmic as the cells age and lose their organelles[19-20].

Previous studies[21-22] have shown that the deletion of filensin or phakinin expression in mice by gene targeting could cause cataracts and that some forms of hereditary cataracts in humans are caused by mutations of filensin or phakinin. The data suggest that beaded filaments are related to lens transparency. Our data proved that in the LAC, the filensin, beta-crystallin B1, and gamma-crystallin D were decreased, which may play an important part in the formation of cataracts.

The water-soluble protein of the lens is the main lens protein and is closely related to the transparency of the lens and diopter. The lens proteins mainly include alpha-crystallin, beta-crystallin, and gamma-crystallin. Our results showed that beta-crystallin B1 and gamma-crystallin D were reduced significantly in the LAC. Many human β- lens proteins occurred after they were translationally modified, including deamidation, protein truncation, and oxidation of methionine and tryptophan[23]. Accumulation of β-deamidation may damage lens protein interactions and reduce their stability. This in turn leads to the accumulation of insoluble beta-crystallin in the process of cataract development[24]. Gamma-crystallin is prone to deamidation[25]. Such modifications to lens proteins may lead to gamma-crystalline structural changes, and then aggregation occurs. Our 2-DE results showed that the content of gamma-crystallin D was reduced.

It should be noted that this study examined only three cases, but the saving grace was in three high consistency results. Despite its preliminary character, this study may have a certain representativeness. We will further expand the sample size to validate our results, and study the relationship between the specific content of each protein change and LAC, seeking solutions for LAC.

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