Original Article

Differential Protein Expression between Type 1 Diabetic Cataract and Age-Related Cataract Patients

(proteomics / crystallin / diabetic cataract / age-related cataract / spectrometry)

Y. QIANQIAN[1], Y. YONG[1], C. ZHAODONG[1], T. YONGHUI[2], S. JUN[1], H. YUZHENG[2]

[1]Department of Ophthalmology, Affiliated Wuxi People's Hospital of Nanjing Medical University, Wuxi, China
[2]The Key Lab of Technology on Parasitic Diseases Prevent and Control, Ministry of Health; Jiangsu Institute of Parasitic Diseases, Wuxi, China

Abstract. Diabetes has become one of the major diseases affecting human health. Diabetic cataracts (DCs) are considered a common complication in diabetic patients. The present study investigated differences in lens proteomic profiles between DCs and age-related cataracts (ACs) to determine the mechanism underlying the formation of DCs. Intrasurgical samples were collected from eight DC patients and 12 AC patients, and lens proteins were extracted by lysis and separated using two-dimensional gel electrophoresis (2-DE). The electrophoretic bands were analysed using PD-Quest software 8.0.1. Differentially expressed proteins were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and peptide mass fingerprinting combined with protein database searching. In the 2-DE maps, the DC and AC lens proteins migrated in the region of pH 5–9 with a relative molecular weight (RMW) of 14–97 kDa, whereas the RMW of more abundant crystallin was 20–31 kDa. Approximately three protein spots with differential intensity were detected. Two crystallin proteins (αB and βB1) were identified using MALDI-TOF-MS. Proteomic analysis of the crystalline humour is feasible, and the proteins can be well separated; moreover, differentially expressed lens proteins can be analysed using 2-DE and mass spectrometry to compare DC and AC. The present results indicate that the αB and βB1 crystallins may accelerate the development of DCs. These techniques offer new avenues for mechanistic evaluation and future prevention or therapy of DCs.

Introduction

More than 285 million people are affected by diabetes mellitus worldwide, and this number is expected to increase to 439 million by 2030 (Pollreisz and Schmidt-Erfurth, 2010), indicating a rapid growth in incidence. Diabetic cataracts (DCs) are considered a common complication in diabetic patients that manifest as fibrosis and opacity of the anterior and posterior subcapsular cortex in the crystalline humour (Obrosova et al., 2010). Compared with age-related cataracts (ACs), DCs typically present a younger age of onset, and faster progression and invasion of the binoculus. Many patients experience a loss of physical working capacity because of blindness occurring in the prime of life (Shah and Chen, 2010). In addition, severe inflammatory reactions, posterior capsular opacity, and poorer prognosis occur in post-operative DC patients (Afshari, 2011). Thus, it is important to study the pathogenesis and potential drug therapies for DCs, and to reduce its incidence from the source.

The factors that are significantly associated with cataract formation include longer duration of diabetes, older age at examination, increased severity of retinopathy, diuretic usage, and higher levels of glycosylated haemoglobin in individuals with a younger onset, in addition to age at examination, increased severity of retinopathy, diuretic usage, lower intraocular pressure, smoking, and lower diastolic blood pressure in individuals with older onset (Hermans et al., 2011). Growing evidence indicates that the duration of diabetes and quality of glycaemic control are the most important risk factors for DC formation.

Comprehensive analysis of the proteome between DC and AC has not been performed to date but has the po-

Received February 6, 2014. Accepted March 2, 2015.

This work was supported by the Affiliated Wuxi People's Hospital of Nanjing Medical University and the Key Lab of Technology on Parasitic Diseases Prevention and Control of the Ministry of Health, in addition to the Jiangsu Institute of Parasitic Diseases.

Corresponding author: Yao Yong, Department of Ophthalmology, Affiliated Wuxi People's Hospital of Nanjing Medical University, Qingyang Road 299, Wuxi, Jiangsu, 214023, China. Phone: +86-(0510)-85351885; e-mail: yaoyongmeta@sina.com

Abbreviations: 2-DE – two-dimensional gel electrophoresis, ACN – acetonitrile, ACs – age-related cataracts, DCs – diabetic cataracts, HSP – heat-shock protein, IEF – isoelectric focusing, IPG – immobilized pH-gradient gel, MALDI-TOF-MS – matrix-assisted laser desorption ionization time-of-flight mass spectrometry, RMW – relative molecular weight.

tential to reveal important information regarding the pathogenesis of DCs. In the present study, we examined proteomic changes in DCs and ACs by two-dimensional gel electrophoresis (2-DE) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) to identify potential key lens proteins. It is difficult to identify the type of cataract (DC or AC) in type 2 diabetic patients; therefore, several younger type 1 diabetic patients who were diagnosed with DC, not AC, were evaluated. The aim of our study was to demonstrate the biochemical differences between two types of lenses.

Material and Methods

Patient information and sample selection

The study included eight patients with DC and 12 patients with AC. The mean age ± standard deviation was 37 ± 3.7 years (age range: 33–43 years) in the DC patient group. The selection criteria included type 1 diabetes without systemic disease, blurry vision, visual acuity ≤ 0.3, obviously reduced lens opacity on slit lamp examination, and definite cataracts. Further, only subjects < 50 years were included. The AC patient group (age range: 65–83 years, mean age: 73 ± 5.7 years) was selected using similar selection criteria; the diagnostic criteria matched those used for the DC patient group except for the age restriction. The study was approved by the Ethics Committee of Nanjing Medical University, and informed consent was collected and permission was obtained from the study patients and their families. The lens without the capsule was aspirated using a 50-ml syringe needle before phacoemulsification under topical anaesthesia (hydrochloric acid oxybuprocaine) in patients with DC. The same technique was used for AC patients, where the lens was split using ultrasound, and the lens without the capsule was removed by snare. All operations were performed by the same surgeon. The lens was immediately preserved at –80 °C.

Protein extraction

AC or DC lens tissue without the capsule from each patient was placed into a pre-cooled homogenizer and homogenized in a lysis buffer (7 M urea, 2 M thiourea, 1% DTT, 4% [w/v] CHAPS, 2% [w/v] Bio-Lyte, PMSF [1 : 100]), and the mixture was then ground with ice. The samples were separated at 160 W (3 s × 20 times, with an interval of 30 s). The insoluble molecules were removed by centrifugation at 15,000 × *g* and 4 °C for 30 min, and the supernatant was collected. The supernatant was treated with trichloroacetic acid-acetone to remove polysaccharides, salts, and other interfering substances, and was then resuspended in rehydration buffer (8 M urea, 4% CHAPS, 65 mM DTT, 0.2% Bio-Lyte, 0.001% bromochlorophenol blue). The protein concentration in each sample was determined using the BCA protein quantification kit (Thermo Fisher Scientific Co., Waltham, MA), with bovine serum albumin used as the standard. The protein amounts were equalized in the DC and AC samples prior to the subsequent experiments.

Two-dimensional gel electrophoresis (2-DE)

The AC and DC lens proteins were dissolved in rehydration buffer to a final volume of 150 µl and used for 2-DE as previously described (Li et al., 2008). First-dimension isoelectric focusing (IEF) involved the use of a 7-cm, pH 3–10, linear-immobilized pH-gradient gel (IPG) strips (Bio-Rad, Hercules, CA). The IPG strips were rehydrated for 12–16 h at 20 °C by placing the strips gel-side down and then covering the strips with mineral oil. IEF was performed at 20 °C in the following steps: 250 V linear for 0.5 h, 500 V gradient for 0.5 h, 4000 V linear for 3 h, 4000 V gradient until 20,000 Vh, and 500 V gradient as needed. After IEF, the IPG strips were equilibrated in buffer I containing 6 M urea, 20% glycerol, 0.375 M Tris-HCl (pH 8.8), 2% SDS and 2% DTT for 15 min, followed by 15 min in buffer II containing the same buffer I with 25 mg/ml iodoacetamide replacing DTT. The second dimension of gel electrophoresis was performed in 12% polyacrylamide gels in an ETTAN-DALTSIX (GE) device at 5 W per gel for 15 min, and then at 17 W per gel until the dye front reached the bottom of the gel.

Staining and destaining

Analytical gels were stained in Coomassie Blue Brilliant R-250 (40% colonial spirit, 10% glacial acetic acid, and 0.25% R-250) (Biotechnology, Shanghai, China), and then incubated at room temperature for 1 h. After incubation, the staining solution was removed, and the gel was incubated overnight at room temperature in the destaining solution (40% colonial spirit, 10% glacial acetic acid, and Milli-Q water).

Image analysis

Images were analysed using PD-Quest software 8.0.1 (Bio-Rad) for spot detection, spot editing, background subtraction, spot matching, and normalization. The selection criteria for the candidate spots were as follows: 3.0-fold increases or decreases in spot intensity between AC and DC lens proteins under the same experimental conditions, no intensity changes among time points, and a new spot detected at least at two time points.

In-gel digestion

1. Enzymolysis: Selected protein spots in the gel lanes were excised and washed in 50 µl of 50% acetonitrile (ACN) (Sigma-Aldrich, St. Louis, MO)/50% distilled water twice for 20 min each. The pieces were destained using 50 µl of 30% ACN/100 mM NH_4HCO_3, 50% ACN/50 mM NH_4HCO_3, and 50% ACN/25 mM NH_4HCO_3 three times at room temperature, and then dried in a Hetovac vacuum centrifuge (Heto, Allerod, Denmark).

2. Enzymatic digestion: Dried pieces of gel were rehydrated for 30 min at 4 °C in 3 µl of 10 ng/µl trypsin

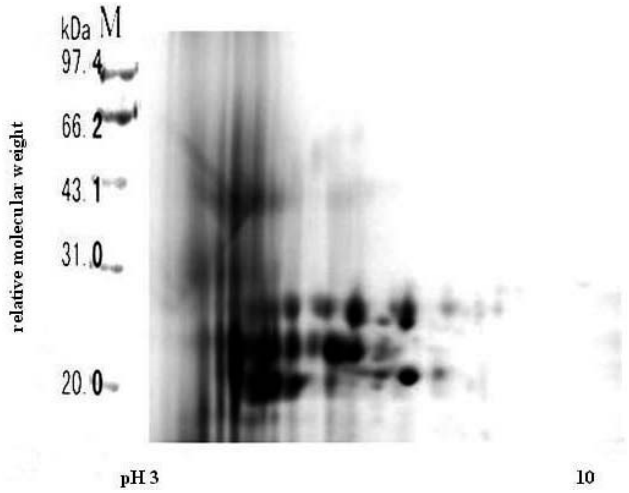

Fig. 1. Age-related cataract lens protein 2D gel image

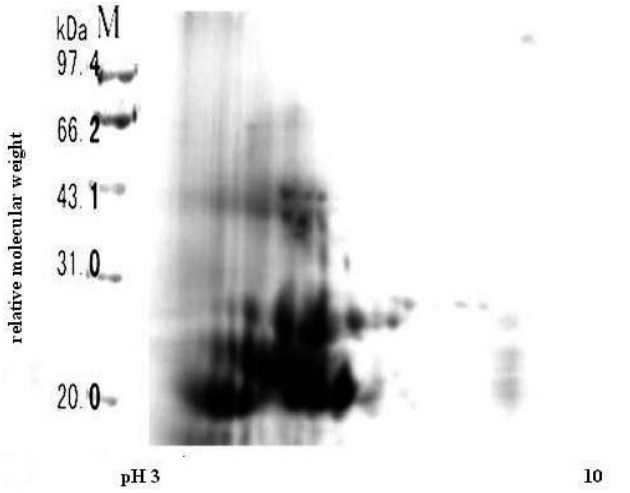

Fig. 2. Diabetic cataract lens protein 2D gel image

(Promega, Southampton, UK), and then 10 µl of 25 mM NH_4HCO_3 was added.

3. Peptide extraction: After overnight incubation at 37 °C, the peptides were eluted in 20 µl of 50% ACN/0.1% trifluoroacetic acid for 30 min at room temperature with occasional shaking, and the hydrolysates were collected by centrifugation, after which the above-described process was repeated twice.

Protein identification by MALDI-TOF-MS

MALDI-TOF-MS (Bruker Daltonics, Billerica, MA) was performed to identify the selected spots. The obtained peptide mass fingerprints (FlexAnalysis software) were used for protein identification. The non-redundant NCBI or SwissProt database was searched using the MASCOT search engine (http://www.matrixscience.com/ and http://www.uniprot.org/).

Results

Repeatability analysis of 2-DE for protein samples

Electrophoresis of each sample was repeated three times, and according to the image contrast observed for the same sample, the reproducibility ranged from 86 % to 94 %, which demonstrates that our results were valid and not spurious.

2-DE analysis of protein between DC and AC

To examine the differences in protein expression between DC and AC, whole-tissue extracts were prepared and analysed by 2-DE electrophoresis. Representative gels for DC and AC tissue extracts are shown in Figs. 1, 2. 2-DE with loading of an equal amount of total protein (1.0 mg/gel) revealed approximately 76 protein spots in DC samples and 68 protein spots in AC samples in each 2-D gel. Lens protein spots in both sample types were distributed in the following region: isoelectric point (PI) (pH = 5–9), relative molecular weight (RMW) 14–97 kDa, with an RMW of 20–31 kDa for high-abundance proteins (Figs. 1, 2). All gel spots showing significant changes in abundance were highlighted and checked manually to eliminate any artefacts generated by gel distortion, inappropriate matching, or incorrect detection. Three protein spots with at least 3-fold difference in abundance between DC and AC were detected (Fig. 3). Moreover, three protein spots demonstrated higher abundance in the DC group than in the AC group.

Spot analysis and mass spectrometry identification of DC and AC lens proteins

To identify the proteins whose spots indicated changed expression, the differentially expressed spots were excised from the gels and subjected to in-gel digestion with trypsin, and the resulting peptides were identified by MALDI-TOF-MS as described in Material and Methods. From a total of three differentially expressed protein spots identified by MS, two were identified as components of crystallin: the protein in spot 1 was hydrolysed into 26 peptides, of which 12 were identical with segments of α-crystallin B chain (αB, http://www.uniprot.org/uniprot/P02511, Fig. 4), with the matched sequence coverage of 46 %; the protein in spot 3 was hydrolysed into 10 peptides, and six were identical with segments of β-crystallin B1 (βB1, http://www.uniprot.org/uniprot/P53674, Fig. 5), with the matched sequence coverage of 60 %. However, the remaining spot (spot 2) was not identified because of interference by the dopant (maybe). Moreover, the expression of the two identified proteins was consistently increased in DCs relative to ACs.

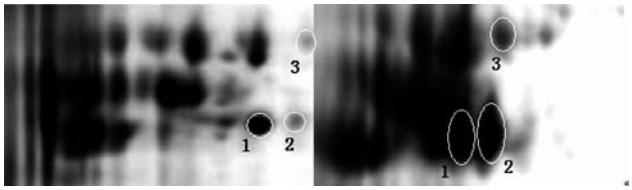

Fig. 3. Age-related cataract and diabetic cataract lens protein localized on two-dimensional gel map comparison chart

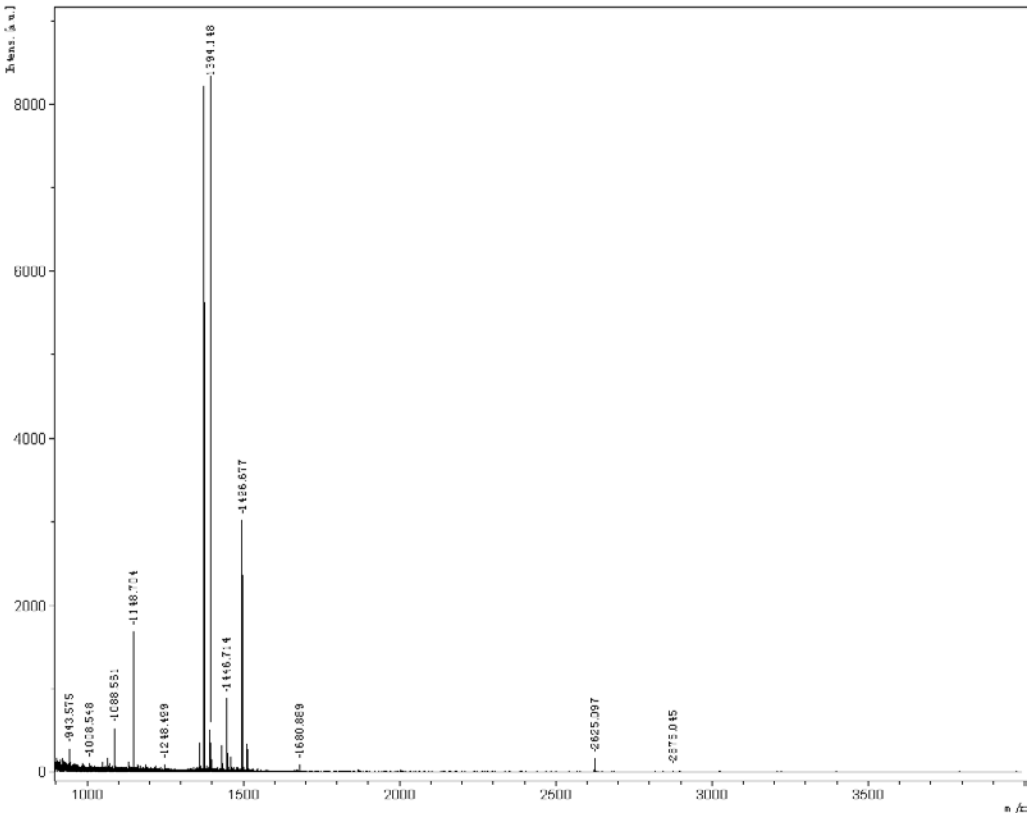

Fig. 4. The protein extracted from spot 1 was digested with trypsin and 26 peptides were identified by MALDI-TOF-MS. The protein was then identified as α-crystallin B chain with 12 matched peptides, with the matched sequence coverage of 46 %. http://www.uniprot.org/uniprot/P02511.

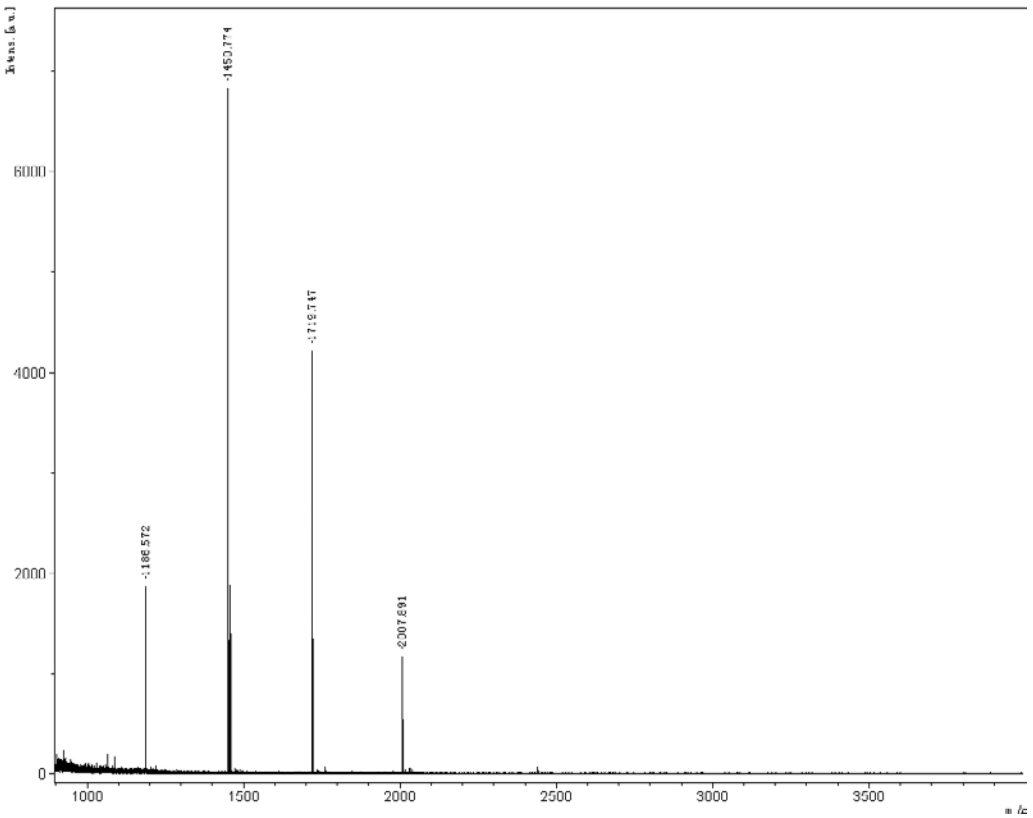

Fig. 5. The protein extracted from spot 3 was digested with trypsin and 10 peptides were identified by MALDI-TOF-MS. The protein was then identified as β-crystallin B1 with six matched peptides, with the matched sequence coverage of 60 %. http://www.uniprot.org/uniprot/P53674.

Discussion

The physiological activity of the lens depends on crystallin, and the lens has the highest crystallin content compared to other organs in the body (Harding 1991). The lens is composed primarily of α-, β- and γ-crystallins, which play important roles in lens clarity (Bloemendal, 1981). Abnormal expression of these proteins or weakened molecular chaperone activity may be associated with stress states or lenticular opacity (Humphery-Smith et al., 1997; Neal et al., 2005). The appropriate concentration and well-regulated alignment of lens protein plays an important role in maintaining the clarity of the crystalline humour (Chiou and Wu, 1999). Cataracts are the leading cause of blindness worldwide. With aging, proteins in the lens are damaged by oxidation, deamination, or truncation, thus resulting in unstable proteins that have a higher propensity to aggregate and thus promote cataract formation (Bloemendal et al., 2004). Saxenaet al. (2000) and Shamsi et al. (2000) both showed that lens crystallin modifications by oxidative processes and carbonyl agents were dramatically increased in aging crystalline humour and age-matched cataracts relative to normal crystalline humour and that a breakdown of the permeability barrier of fibre cells plays a key role in cataractogenesis.

We found that the concentration of αB-crystallin was significantly higher in DC lenses, thus suggesting that αB-crystallin plays an important role in the formation of non-water-soluble aggregates of lens protein in DC. A-crystallin, containing αA- and αB-crystallins, is a member of the small heat-shock protein (HSP) family and has chaperone-like activity that suppresses aggregation of other lenticular proteins, thus delaying the onset of cataracts (Reddy et al., 2000; Horwitz 2003). Molecular chaperones (also known as chaperone proteins) not only promote the correct folding of newly synthesized peptides, but also mediate the refolding of degenerated proteins for recovery of the natural spatial conformation (Clark et al., 2012). α-Crystallin is necessary for differentiation and clearing of the normal crystalline humour. α-Crystallin, after glycosylation, can acquire conformational changes and alterations of physical chemistry and function such as decreased molecular chaperone activity (Kumar et al., 2009). Molecular chaperones can reduce the level of oxygen radicals and simultaneously ameliorate reduction in glutathione levels (Mehlen et al., 1996; Wyttenbach et al., 2002). In mature crystalline cells, molecular chaperones initially act as refolding proteins. When proteins are present at elevated concentrations, molecular chaperones interact with the aberrantly expressed protein to form a large polymer that can be more easily degraded by proteasomes. Phosphorylation of α-crystallin and cleavage in the carboxyl terminus may degrade the chaperone activity, thus leading to cataracts (Kamei et al., 2004). In our study, the activity of αB-crystallin in DC was reduced because of structural changes arising from glycosylation and other modifications. Compensatory increased transcription of αB-crystallin results in a higher density of αB-crystallin in the gelatum. At the same time, production of αB-crystallin is increased but its activity is decreased, which may result in functional deficiency of molecular chaperones that in turn results in increased cloudiness of crystalline fibres.

It is thought that most β-crystallin becomes insoluble during the formation of cataracts (Wang et al., 2011). We observed that the concentration of βB1-crystallin was higher in DCs than in ACs, which suggests that βB1-crystallin quickly and easily forms insoluble polymers. βB1-crystallin easily forms a dipolymer, which is suitable for forming a more highly ordered structure that results in lens opacity. Mutations in residues in the catenating domain affect βB1 catenation (Harms et al., 2004). Extension of the N-terminal in mammalian β-crystallin may be reduced in adults and with aging (David et al., 1994; Lampi et al., 1998; Shih et al., 1998; Werten et al., 1999). Non-prognostic truncation of β-crystallin extension in rodents was previously associated with the formation of cataracts. Crystallins αA and αB show molecular chaperone activity and extensive phosphorylation, and αB1-crystallin and non-crystallin proteins, such as β-enolase, HSP27, and glucose-6-phosphatase, have been found to be phosphorylated at some special sites (Chiou et al., 2012). Diabetes mellitus may promote dimerization of βB1-crystallin and accelerate excision of the N-terminal extension, which eventually accelerates the development of cloudiness.

Many improvements have been achieved in proteomics (Klose, 1999; Wolters et al., 2001). Two-dimensional gel electrophoresis technology provides the core and foundation for proteomics, which can segregate thousands of proteins based on different electric charges and molecular weights (Benedek, 1983). Protein extraction is critical for two-dimensional electrophoresis, whose principle requires extraction of as much protein as possible from cells. Proteins should first be dissolved in the lysate as much as possible, and degradation and loss in the extraction process should be minimized. It is also necessary to ensure that the protein cannot be modified. Our image analyses showed that the number of protein points in the gel were different in the two groups; however, this result may have been influenced by differences in the collection and management of the proteins. Moreover, in cataract surgery, it is unclear whether ultrasound, heat, and oxygenation affect the solubility and structure of proteins; however, the operation is known to affect sodium hyaluronate, which is a polysaccharide that can cross-link lens protein and thus make separation difficult, as indicated by the tailing in the gel images in our study. In our study, positive results were not obtained for protein point 2 because of the combination of two or more proteins with the same isoelectric point and molecular weight but different structure.

The high abundance of some lens proteins in the crystalline humour can prevent separation of low-abundance lens proteins to a great extent (Datiles et al., 1992; Fountoulakis and Takacs, 2001), thus affecting detec-

tion of these proteins. Non-lens proteins may have specific electrophoresis transport ratios and are thus invisible in 2-D electrophoresis. All known non-lens proteins play key roles in maintaining the function of the crystalline humour, and it is thus necessary to further investigate these proteins to probe the pathogenesis of DCs. Some of these proteins are structural proteins such as actin, microtubular proteins, vimentin, and spectrin, which are necessary to maintain the structure of transparent cells. To further investigate the pathogenesis of diabetic cataracts, we should address the solution and segregation of proteins. In the present study, we only identified a few proteins, and it is necessary to increase the number of proteins identified. However, some limitations should be mentioned in our study. First, quantitation of the two differential expressions should be calculated. Second, we did not research the function of two proteins.

In summary, our study found that type 1 DC lenses may contain higher abundance of αB and βB1 crystallins than AC patients. We wish future researches should focus on the protein function, and finally could offer directions for prevention, drug treatment, and reduction in incidence of cataracts.

Declaration of interest

The authors have no proprietary, commercial, or financial interests in any of the products described in this study.

References

Afshari, N. (2011) Cataract surgery: from couching to femtosecond, look how far we have come! *Curr. Opin. Ophthalmol*. **22,** 1.

Benedek, G. (1983) Why the eye lens is transparent. *Nature* **302,** 383-384.

Bloemendal, H. (1981) The lens proteins. In: *Molecular and Cellular Biology of the Eye Lens,* pp. 1-14, John Wiley & Sons, New York.

Bloemendal, H., De Jong, W., Jaenicke, R., Lubsen, N. H., Slingsby, C., Tardieu, A. (2004) Ageing and vision: structure, stability and function of lens crystallins. *Prog. Biophys. Mol. Biol*. **86,** 407-485.

Chiou, S. H., Wu, S. H. (1999) Evaluation of commonly used electrophoretic methods for the analysis of proteins and peptides and their application to biotechnology. *Anal. Chim. Acta* **383,** 47-60.

Chiou, S. H., Huang, C. H., Lee, I. L., Wang, Y. T., Liu, N. Y., Tsay, Y. G., Chen, Y. J. (2012) Identification of in vivo phosphorylation sites of lens proteins from porcine eye lenses by a gel-free phosphoproteomics approach. *Mol. Vis*. **16,** 294-302.

Clark, A. R., Lubsen, N. H., Slingsby, C. (2012) sHSP in the eye lens: crystallin mutations, cataract and proteostasis. *Int. J. Biochem. Cell Biol*. **44,** 1687-1697.

Datiles, M. B., Schumer, D. J., Zigler J. S., Jr., Russell, P., Anderson, L., Garland, D. (1992) Two-dimensional gel electrophoretic analysis of human lens proteins. *Curr. Eye Res.* **11,** 669-677.

David, L. L., Azuma, M., Shearer, T. R. (1994) Cataract and the acceleration of calpain-induced β-crystallin insolubilization occurring during normal maturation of rat lens. *Invest. Ophthalmol. Vis. Sci*. **35,** 785-793.

Fountoulakis, M., Takacs, B. (2001) Effect of strong detergents and chaotropes on the detection of proteins in two-dimensional gels. *Electrophoresis* **22,** 1593-1602.

Harding, J. J. (1991) Aspirin and cataract. *Arch. Ophthalmol*. **109,** 1344-1345.

Harms, M. J., Wilmarth, P. A., Kapfer, D. M., Steel, E. A., David, L. L., Bächinger, H. P., Lampi, K. J. (2004) Laser light scattering evidence for an altered association of βB1-crystallin deamidated in the connecting peptide. *Protein Sci.* **13,** 678-686.

Hermans, M. P., Ahn, S. A., Rousseau, M. F. (2011) Statin therapy and cataract in type 2 diabetes. *Diabetes Metab*. **37,** 139-143.

Horwitz, J. (2003) Alpha-crystallin. *Exp. Eye Res.* **76,** 145-153.

Humphery-Smith, I., Cordwell, S. J., Blackstock, W. P. (1997) Proteome research: complementarity and limitations with respect to the RNA and DNA worlds. *Electrophoresis* **18,** 1217-1242.

Kamei, A., Takamura, S., Nagai, M., Takeuchi, N. (2004) Phosphoproteome analysis of hereditary cataractous rat lens α-crystallin. *Biol. Pharm. Bull*. **27,** 1923-1931.

Klose, J. (1999) Large-gel 2-D electrophoresis. *Methods Mol. Biol*. **112,** 147-172.

Kumar, P. A., Reddy, P. Y., Srinivas, P. N., Reddy, G. B. (2009) Delay of diabetic cataract in rats by the antiglycating potential of cumin through modulation of α-crystallin chaperone activity. *J. Nutr. Biochem*. **20,** 553-562.

Lampi, K. J., Ma, Z., Hanson, S. R., Azuma, M., Shih, M., Shearer, T. R., Smith, D.L., Smith, J. B., David, L. L. (1998) Age-related changes in human lens crystallins identified by two-dimensional electrophoresis and mass spectrometry. *Exp. Eye Res*. **67,** 31-43.

Li, Y. B., Wang, R., Wu, H. L., Li, Y. H., Zhong, L. J., Yu, H. M., Li, X. J. (2008) Serum amyloid A mediates the inhibitory effect of Ganoderma lucidum polysaccharides on tumor cell adhesion to endothelial cells. *Oncol. Rep*. **20,** 549-556.

Mehlen, P., Kretz-Remy, C., Préville, X., Arrigo, A. P. (1996) Human hsp27, Drosophila hsp27 and human αB-crystallin expression-mediated increase in glutathione is essential for the protective activity of these proteins against TNFα-induced cell death. *EMBO J*. **15,** 2695-2706.

Neal, R., Aykin-Burns, N., Ercal, N., Zigler, J. S., Jr. (2005) Pb^{2+} exposure alters the lens αA-crystallin protein profile in vivo and induces cataract formation in lens organ culture. *Toxicology* **212,** 1-9.

Obrosova, I. G., Chung, S. S., Kador, P. F. (2010) Diabetic cataracts: mechanisms and management. *Diabetes Metab. Res. Rev*. **26,** 172-180.

Pollreisz, A., Schmidt-Erfurth, U. (2010) Diabetic cataract – pathogenesis, epidemiology and treatment. *J. Ophthalmol*. **2010,** 608751.

Reddy, G. B., Das, K. P., Petrash, J. M., Surewicz, W. K. (2000) Temperature-dependent chaperone activity and structural properties of human αA- and αB-crystallins. *J. Biol. Chem*. **275,** 4565-4570.

Saxena, P., Saxena, A. K., Cui, X. L., Obrenovich, M., Gudipaty, K., Monnier, V. M. (2000) Transition metal-catalyzed oxidation of ascorbate in human cataract extracts: possible role of advanced glycation end products. *Invest. Ophthalmol. Vis. Sci*. **41,** 1473-1481.

Shah, A. S., Chen, S. H. (2010) Cataract surgery and diabetes. *Curr. Opin. Ophthalmol*. **21,** 4-9.

Shamsi, F. A., Sharkey, E., Creighton, D. (2000) Maillard reactions in lens proteins: methylglyoxal-mediated modifications in the rat lens. *Exp. Eye Res*. **70,** 369-380.

Shih, M., Lampi, K. J., Shearer, T. R., David, L. L. (1998) Cleavage of β-crystallins during maturation of bovine lens. *Mol. Vis*. **4,** 4.

Wang, K. J., Wang, S., Cao, N. Q., Yan, Y. B., Zhu, S. Q. (2011) A novel mutation in CRYBB1 associated with congenital cataract-microcornea syndrome: the p.Ser129Arg mutation destabilizes the βB1/βA3-crystallin heteromer but not the βB1-crystallin homomer. *Hum. Mutat*. **32,** E2050-2060.

Werten, P. J., Vos, E., De Jong, W.W. (1999) Truncation of βA3/A1-crystallin during aging of the bovine lens; possible implications for lens optical quality. *Exp. Eye Res.* **68,** 99-103.

Wolters, D. A., Washburn, M. P., Yates, J. R., 3rd. (2001) An automated multidimensional protein identification technology for shotgun proteomics. *Anal. Chem.* **73,** 5683-5690.

Wyttenbach, A., Sauvageot, O., Carmichael, J., Diaz-Latoud, C., Arrigo, A. P., Rubinsztein, D. C. (2002) Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin. *Hum. Mol. Genet*. **11,** 1137-1151.