PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 April 14; 13(14): 2118-2124
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Differences in expression of retinal proteins between diabetic and normal rats

Shang-Qing Liu, Jian Kang, Cheng-Jun Li, En-Jie Tang, Bin Wen, Rong Cai, Hui-Jun Yang

Shang-Qing Liu, Hui-Jun Yang, Department of Anatomy, School of Preclinical and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan Province, China
Shang-Qing Liu, Jian Kang, Cheng-Jun Li, En-Jie Tang, Bin Wen, Rong Cai, North Sichuan Medical College, Nanchong 637007, Sichuan Province, China
Hui-Jun Yang, Chengdu Medical College, Chengdu 610083, Sichuan Province, China
Correspondence to: Dr. Hui-Jun Yang, Department of Anatomy, School of Preclinical and Forensic Medicine, Sichuan University, 17 Renming Nan Road, Chengdu 610041, Sichuan Province, China. proteomics@nsmc.edu.cn
Telephone: +86-817-8198242
Received: 2007-02-10 Accepted: 2007-03-28

Key words: Diabetes; Rat; Retina; Proteomics; Two-dimensional electrophoresis; Tandem mass spectrometry; Bioinformatics

Liu SQ, Kang J, Li CJ, Tang EJ, Wen B, Cai R, Yang HJ. Differences in expression of retinal proteins between diabetic and normal rats. *World J Gastroenterol* 2007; 13(14): 2118-2124

http://www.wjgnet.com/1007-9327/13/2118.asp

Abstract

AIM: To compare and identify the differences in expression of retinal proteins between normal and diabetic rats, and to analyze the molecular pathogenetic mechanisms of retinal diseases caused by diabetes.

METHODS: Changes in protein expression of retinal tissues from diabetic and normal rats were observed using 2-dimensional polyacrylamide gel electrophoresis (2-DE). Some protein spots exhibiting statistically significant variations ($P < 0.05$) were selected randomly and identified by tandem mass spectrometry and analyzed by bioinformatics.

RESULTS: 2-DE showed that the expression was up-regulated in 5 retinal proteins, down-regulated in 23 retinal proteins, and disappeared in 8 retinal proteins. Eight spots were identified from the 36 spots by tandem mass spectrometry (MS/MS) and analyzed by bioinformatics. Guanylate kinase 1, triosephosphate isomerase 1, ATP synthase subunit d, albumin and dimethylarginine dimethylaminohydrolase 2 played an important role in signal transduction. Triosephosphate isomerase 1, crystallin alpha B, ATP synthase subunit d and peroxiredoxin 6 were involved in energy metabolism of retinal tissues. Guanylate kinase 1 played an important role in photoexcitation of retinal rod photoreceptor cells. Whether crystallin beta A1 plays a role in diabetic retinas is unknown so far.

CONCLUSION: There are differences in expression of retinal proteins between diabetic and normal rats. These proteins may be involved in the mechanisms and prognosis of retinal diseases caused by diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a life-long disease characterized by high blood-sugar levels and is the fifth-deadliest disease in the United States. Retinal diseases such as diabetic retinopathy are a frequent complication of both type 1 and type 2 diabetes. DM is responsible for 8% of legal blindness in the USA, making it the leading cause of new cases of blindness in adults at 20-74 years of age[1]. However, the pathogenetic mechanism of retinal disease caused by DM is unclear and there is no satisfactory therapy for diabetes so far. The immediate goals of therapy are to stabilize blood sugar and eliminate its symptoms. The long-term goals of treatment are to prolong life, relieve symptoms, and prevent long-term complications such as retinal disease.

Some factors, such as vascular endothelial growth factor (VEGF), intercellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule-1 (VCAM-1), CD45 (leukocyte common antigen), angiopoietin-1, insulin-like growth factor- I , polymerase (ADP-ribose), hyperglycemia and hypoxia, are known to play an important role in retinal pathogenesis[2-8]. However, these changes do not reflect the biological nature of diabetic retina changes completely and precisely. Hence, expression profile analysis of genes and proteins in diabetic retina tissue is an essential step in understanding the pathogenetic mechanisms of DM. Joussen *et al*[9] have reported changes in mRNA expression of diabetic rat retinae by cDNA microarray. Mei *et al*[10] obtained a total of 3639 significant retinal transcriptional fragments by restriction fragment differential display-PCR (RFDD-PCR). Quin *et al*[11,12] have observed protein changes in rat retina induced by short-term diabetes. However, further comparative analyses of protein expre-

ssion profiles in diabetic rat retinae are needed.

Proteomics is defined as the characterization of all proteins encoded by the genome and allows identification of protein-protein interactions and disease-associated proteins. Tools, such as 2-dimensional polyacrylamide gel electrophoresis (2-DE) and matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), enable the study of disease-associated proteomics[13-15]. Using this technology, the changes of protein expression in retinal tissue from diabetic rats can also be demonstrated.

The present study was to compare the expression profile of retinal protein in diabetic and normal rats, and to analyze the molecular pathogenetic mechanisms of retinal diseases caused by diabetes.

MATERIALS AND METHODS

Reagents and apparatus

Alloxan tetrahydrate, tributyl phosphine (TBP), urea and low-melt agarose were from Sigma (St. Louis, MOU, SA). Acrylamide, bis-acrylamide, Coomassie brilliant blue R-250, Tris, ammonium persulfate, glycine, EDTA, SDS, bromophenol blue, 2-mercaptoethanol, endonuclease, mineral oil and TEMED were from Amersco (Solon, Ohio, USA). Dithiothreitol (DTT) was from Merck (Darmstadt, Germany). Iodoacetamide was from Acros (Geel, Belgium). ReadyStrip IPG strip, 40% bio-lyte ampholyte, 3-[(3-cholamidopropyl) dimethylammonio]-1 -propanesulphonate (CHAPS), protein assay kits, gel cassettes, model 550 microplate reader, protean IEF cell and protean Ⅱ xi cell were from Bio-Rad (Hercules, CA, USA). All other chemicals were of analytical grade.

Establishment of diabetic rat models

The study was approved by the Medical Ethical Committee of North Sichuan Medical College. Sixty healthy adult (eight-week-old) female Sprague-Dawley (SD) rats weighing 180-200 g (provided by Animal Experimental Center, North Sichuan Medical College) were randomly divided into diabetic group and normal control group. Forty rats in diabetic group received a single intra-peritoneal injection of alloxan tetrahydrate (200 mg/kg) in normal sodium, 20 rats in the control group received injection of an equal volume of normal sodium alone. Rats with positive urine glucose (measured by reagent papers) and blood glucose levels higher than 16.7 mmol/L 72 h (measured by blood glucose appearance) after injection served as diabetes models. Then the urine glucose levels were measured once a day and the blood glucose levels were measured once a week. The rats with negative urine glucose or with their blood glucose level lower than 16.7 mmol/L were excluded.

All rats were housed in an air-conditioned room with free access to standard laboratory chow and water in a 12-h light/dark cycle.

Extraction and purification of rat retinal protein

After eight weeks, the glucose levels in urine and blood and body weights of rats were measured. Rats were anesthetized with 2% soluble pentobarbitone (50 mg/kg) and retinal tissues of the two eyeballs were enucleated immediately(separated from RPE) and homogenized for 5 min on ice at a concentration of approximately 80 mg/mL in lysis buffer containing 5 mol/L urea, 2 mol/L thiourea, 2% (w/v) CHAPS, 2% (w/v) SB3-10, 40 mmol/L Tris, 0.2% (w/v) bio-lyte 3/10, 2 mmol/L TBP, 1 mmol/L PMSF, and 200 μg/mL DNase I . Samples were incubated at 4℃ for 15 min and then centrifuged at room temperature (18-20℃) for 30 min at 16 000 r/min to remove DNA, RNA and any particulate materials (tissue and cell debris). As extracted proteins, the supernatant was purified using ReadyPrep™ cleanup kit (Bio-Rad Laboratories). The protein concentration of samples was measured on a Model 550 microplate reader using a RC DC protein assay kit (Bio-Rad Laboratories) with BSA as standard. Liquor of the final protein solution was stored at -80℃ until further use.

First dimensional isoelectric focusing

2-DE was performed according to the manufacturer's instructions. The first dimensional isoelectric focusing (IEF) was performed on precast 17 cm immobilized pH 5-8 gradient (IPG) strips at 17℃ using a protean IEF cell. Protein (300 μg for analytical gels, 1500 μg for preparative gels) was utilized for IEF and subsequent second dimensional separation. Total proteins were mixed with a rehydration buffer containing 8 mol/L urea, 4% (w/v) CHAPS, 65 mmol/L DTT, 0.2% (v/v) bio-lytes, and a trace of bromophenol blue, to a total volume of 300 μL. The mixture was loaded onto the IPG strip. After rehydration for 12 h, IEF was carried out using the following conditions: (1) 250 V, slow, 25 min; (2) 1000 V, rapid, 2 h; (3) 8000 V, linear, 5 h; (4) 8000 V, rapid, 60 000 Vh; (5) 500 V, rapid, anytime. After IEF, the strips were stored at -80℃ until required for the second dimension.

Equilibraiton of IPGs using DTT and iodoacetamide

Following IEF, the gel strip was equilibrated for 15 min in the equilibration buffer containing 130 mmol/L DTT, 6 mol/L urea, 2% SDS, 0.375 mol/L Tris HCl, pH 8.8, and 20% glycerol. Then the gel strip was equilibrated for another 15 min in the same equilibration buffer except that DTT was replaced with 135 mmol/L iodoacetamide.

Second-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The second dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 12% acrylamide vertical slab gels using the protean Ⅱ xi cell system. The equilibrated strip was sealed at the top of the 1.0 mm thick second-dimensional gel with the help of 0.5% low-melting agarose in stacking buffer (0.1% SDS, 0.25 mol/L Tris, 0.192 mol/L glycine, supplemented with bromophenol blue as a tracking dye). Gels were run at constant voltage of 60 V for 20 min and then 250 V until the bromophenol blue front traversed the gel. Proteins were visualized by staining with Coomassie brilliant blue R-250 or silver nitrate. The completed analytical 2-D gels were stained with silver nitrate, the preparative gels were stained for 4 h in 0.1% w/v Coomassie brilliant blue R-250 in 45% v/v methanol,

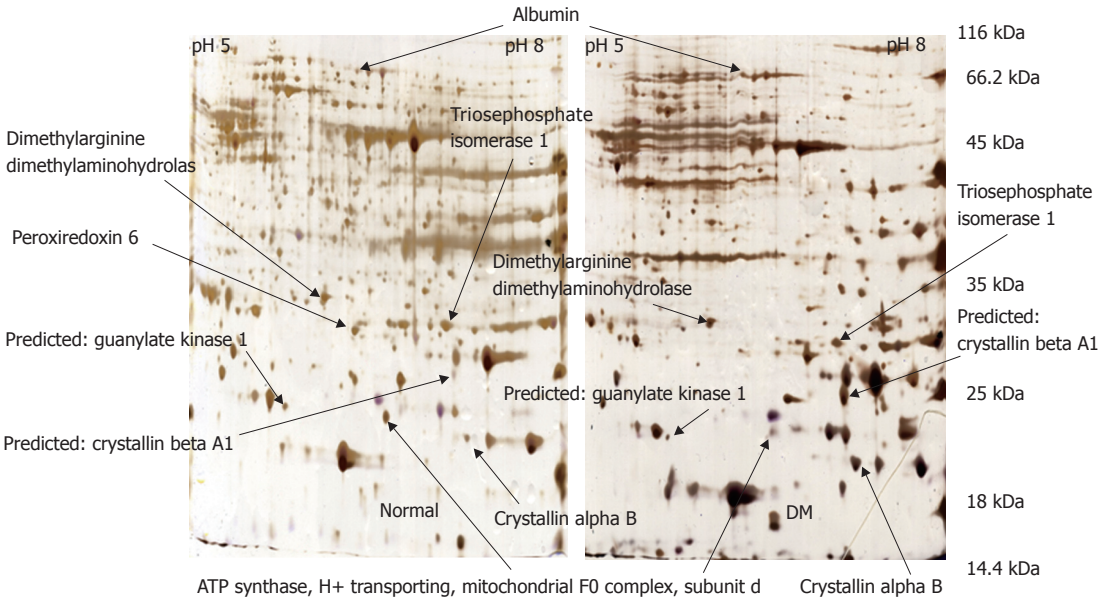

Figure 1 2-DE map of proteins in rat retinae. 2-DE was performed on an immobilized 17 cm pH 5-8 strip, followed by the second-dimensional separation on 12% polyacrylamide gels. The separated proteins were stained with silver nitrate.

10% v/v acetic acid, destained in 45% v/v methanol, 10% v/v acetic acid.

Image acquisition and analysis

The stained analytical gels were scanned by Umax Powerlook1120 scanner (UMAX, Taiwan, China), and the image analysis was carried out using the two-dimensional analysis software package PDQuest 7.2 (Bio-Rad, Hercules, CA, USA), including image editing, spot finding, quantitation and matching. The PDQuest software was used to calculate the 2-D spot intensity by integrating the optical density over the spot area. Proteins separated by 2-D gels were quantitated in terms of their normalized quantity. The individual protein spot quantity was normalized as a percentage of the total quantity of valid spots present in the gel. The two characteristics (normal *vs* diabetic tissue) were assessed using the Student's *t*-test and $P < 0.05$ was considered statistically significant. The confidence level was 95% and the results were exported to Excel 2000 (Microsoft, Seattle, CA, USA) for further analysis.

In-gel digestion by trypsin, tandem mass spectrometry and bioinformatics analysis

Differentially expressed protein spots were excised from the preparative gels according to the result of image analysis and transferred to a siliconized 1.5 mL Eppendorf tube (Sigma). Fifty mmol/L ammonium bicarbonate/50% acetonitrile (1:1) (Sigma) was added to the tube to cover the gel piece, and the tube was incubated at room temperature for 20 min. The liquid was discarded and washing was repeated. After shrunk by dehydration in 100 μL of 100% acetonitrile, which was then removed, the gel pieces were dried in a vacuum centrifuge. The gel pieces were swollen in 10 μL of 12.5 ng/μL trypsin (Roshe, Mannheim, Germany) and incubated at 4℃ for 30 min, then at 37℃ overnight. Mass spectra were obtained using 4700 proteomics analyzer (TOF/TOFTM) mass spectrometer (Applied Biosystems, USA). Database searches were carried out with GPS Explorer TM software (Applied Biosystems, USA)-MASCOT (Matrix Science, London, UK) against the NCBI nonredundant protein database (http://www.ncbi.nlm.nih.gov/).

RESULTS

Establishment of diabetic rat models

After eight weeks, the glucose levels in urine and blood and the body weights of 34 rats including 16 diabetic rats and 18 non-diabetic control rats were measured. The urine glucose levels in all normal rats were negative (-) while the urine glucose levels in all diabetic rats were strongly positive (from +++ to ++++). The blood glucose levels in all normal rats were much lower than 16.7 mmol/L (about 4-5 mmol/L), while the blood glucose levels in all diabetic rats were 19.2 mmol/L-31.05 mmol/L. The body weight was reduced in some diabetic rats but increased in normal rats.

2-DE protein separation and image analysis

Proteins obtained from normal and diabetic retinal tissues were applied to a 2-D gel. The protein spots were visualized by staining with Coomassie brilliant blue R-250 or silver nitrate. Examples of silver stained 2-D gels representing the proteome collected from diabetic and normal retinal tissues are shown in Figure 1. 2-DE was executed three times for protein sample from each rat and the best stained gel was picked out for image analysis. The normalized quantity of protein spots was compared between 3 normal *vs* 3 diabetic retinal tissue samples using the Student's *t*-test. Only the protein spots ($P < 0.05$) were selected for MS. The expression was up-regulated in 5 proteins, down-regulated in 23 proteins and disappeared in 8 proteins of diabetic rat tetinae. Figure 2 shows these 3 patterns of protein spots on a 2-D gel.

Protein identification by tandem mass spectrometry

Eight spots were selected randomly from 36 spots and identified by tandem mass spectrometry. The identified proteins are listed in Table 1 and Figure 2. The expression of crystallin alpha B and crystallin beta A1 increased in diabetic retinal tissues. The expression of albumin, guanylate kinase 1, dimethylarginine dimethylaminohydrolase 2,

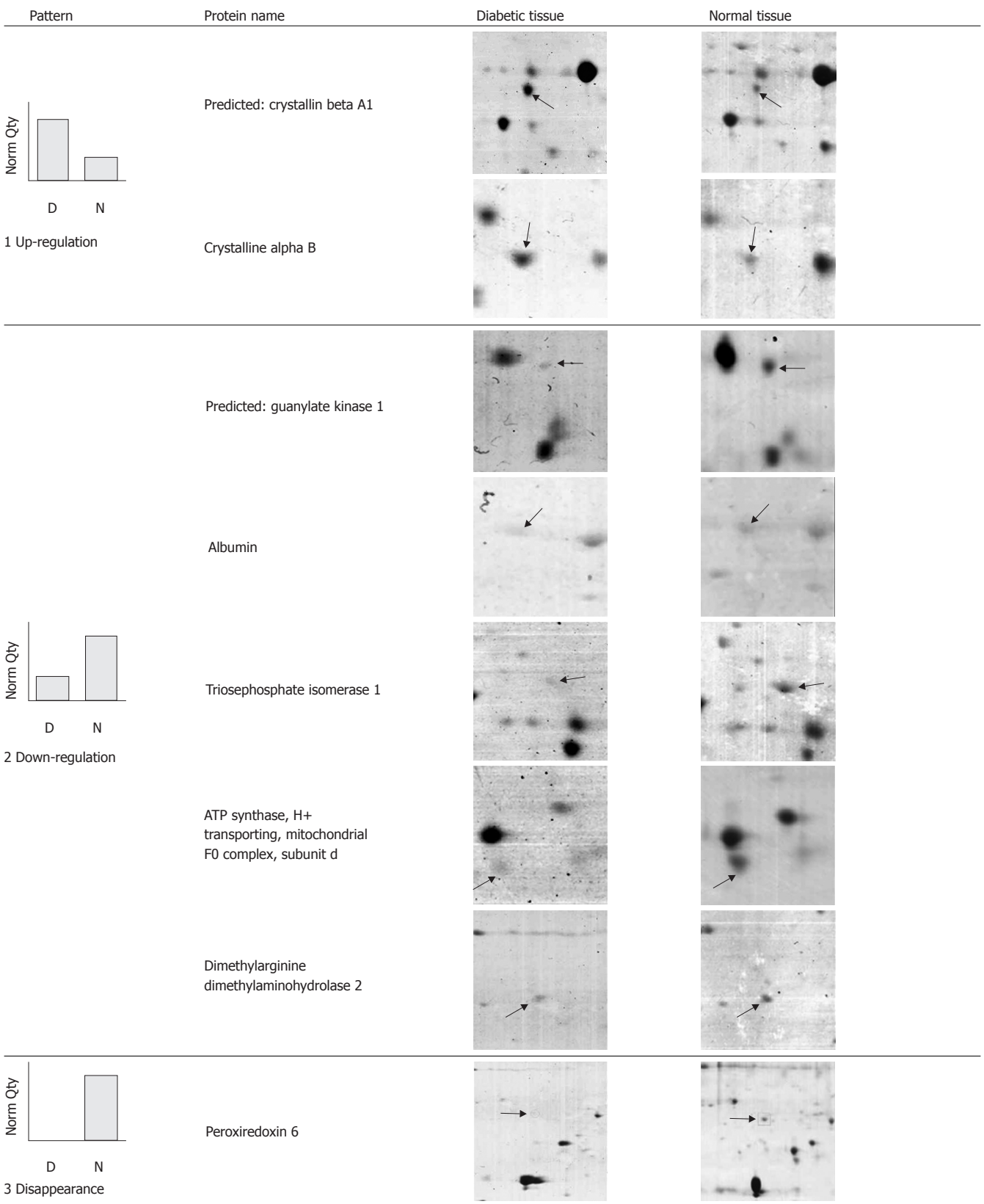

Figure 2 Types of protein expression patterns obtained from normal and diabetic retinal tissues and proteins pertaining to each pattern. Norm Qty on the Y-axis stands for normalized quantity, D and N on the X-axis stand for diabetic and normal retinal tissue, respectively. The 8 proteins visualized by MS/MS were divided by expression pattern into pattern 1 through 3 and displayed by protein spots in 2-D gel.

ATP synthase subunit d and triosephosphate isomerase 1 was down-regulated in diabetic retinal tissues. However, expression of peroxiredoxin 6 could hardly be found in diabetic retinal sample.

DISCUSSION

It is well known that retinal disease is the most common microvascular complication in patients with long-standing diabetes. However, the underlying molecular

Table 1 Identified proteins with different expressions in diabetic and normal retinae

SSP No.	Protein name	NCBI accession No.	Protein score	Best Ion score	Gene name	MW	pI	DM retina norm Qty (%)		Normal retina norm Qty (%)		*P*
								mean	SD	mean	SD	
7106	Crystallin alpha B	NP_037067	201	59	Cryab	20076.3	6.76	1.491	0.254	0.388	0.223	0.00483
7103	Predicted: crystallin, beta A1	XP_340847	480	132	Cryba1	25253.8	6.17	2.885	0.814	0.969	0.654	0.03358
3103	Predicted: guanylate kinase 1	NP_001013133	174	85	Guk1	21896.1	5.44	0.014	0.013	0.257	0.07	0.00146
4303	Dimethylarginine dimethylaminohydrolase 2	NP_997697	460	191	Ddah2	29669.4	5.66	0.16	0.141	0.829	0.312	0.02774
5802	Albumin	NP_599153	173	71	Alb	68674	6.09	0.024	0.031	0.275	0.105	0.0163
7202	Triosephosphate isomerase 1	NP_075211	326	128	Tpi1	26903.8	6.45	0.197	0.016	0.381	0.087	0.02275
6101	ATP synthase, H+ Transporting, mitochondrial F0 complex, subunit d	NP_062256	251	134	Atp5h	18751.6	6.17	0.005	0.0014	1.06	0.445	0.01482
5201	Peroxiredoxin 6	NP_446028	576	196	Prdx6	24803	5.64	n.d.	-	0.414	0.077	0.00078

SSP: standard spots number; MW: molecular weight; pI: isoelectric point; DM: diabetes mellitus; Norm Qty: normal quantity; SD: standard deviation.

pathways that lead to initiation and progression of this complication remain unresolved. There is little doubt that the pathogenesis of retinal disease is highly complicated and there is a pressing need to establish new therapeutic regimens that can effectively prevent or limit retinal microvascular cell dysfunction. Some investigations of functional changes in diabetic retina indicate that impairment of retinal function precedes the earliest signs of vascular complications[16,17]. The electroretinogram (ERG) responses of diabetic rats were reduced in amplitude compared to the responses of control rats 2 wk after onset of diabetes. Early functional changes of the inner retina are evident in diabetic patients before impairment of the outer retina is observed[18]. Joussen *et al*[9] and Mei *et al*[10] also reported that retinal gene expression is changed in early diabetes in the present study. These observations indicate that the functional and molecular retinal changes occur earlier than morphological change in experimental diabetic rats. The results of this study confirm this viewpoint.

Up-regulation of protein expression

Crystallin alpha B and crystallin beta A1 were over-expressed in diabetic tissues in comparison with normal tissues. Crystallin alpha B, one of the small heat-shock proteins (sHSPs), is the main structural protein of eye lens that contribute to the transparency and refractive properties of mammalian eye lens. It was recently reported that crystallin alpha B is highly expressed in multiple non-lenticular normal and diseased human tissues, such as brain, gliomas, renal carcinomas, breast carcinomas, having an extensive tissue distribution[19-22]. In the present study, a higher expression of crystallin alpha B was found in retinae of diabetic rats than in those of normal rats. Crystallin alpha B can effectively inhibit both formation of reactive oxygen species (ROS) and hyperglycemia-induced apoptosis, and has anti-apoptotic function and a cardioprotective role[23,24]. Crystallin alpha B also meets one of the "gold standards" for classification of oncogenic protein and may be a useful biochemical marker for studying the pathogenesis and prognosis of various human tumors[25,26]. In the present study, high blood glucose levels in diabetic rats (eight- week duration) enhanced the expression of crystallin alpha B in retinae, resulting in inhibition of apoptosis and ROS, suggesting that crystallin alpha B weakens the retinal tissue damage through cytoprotective action, which may provide a basis for future therapeutic interventions in diabetic vascular complications. In future study, we may compare the expression of crystallin alpha B between diabetic and normal rats to determine if it may be regarded as a powerful biochemical marker for diagnosis, treatment and prognosis of diabetes. Beta-crystallins have previously been regarded as lens-specific proteins[27]. However, beta B2-and beta A3/A1-crystallin RNAs are transcribed and beta-crystallin polypeptides are detectable in developing chick retinae[28]. In our study, crystallin beta A1 was over-expressed in diabetic rat retinae in comparison with normal retinal tissues, implying a function for extra-lenticular beta-crystallins. However, the function of crystallin beta A1 in non-lenticular tissues remains equivocal so far.

Down-regulation of protein expression

In this study, five proteins were down-regulated in diabetic retinae, including albumin, dimethylarginine dimethylaminohydrolase 2, triosephosphate isomerase 1, ATP synthase subunit d and guanylate kinase 1. Albumin may regulate the colloidal osmotic pressure, increase the internal diameter of blood vessels, especially arterioles or capillaries, and also play a role in nitric oxide signaling by mediating formation of S-nitrosothiols (RS-NO)[29,30]. Albumin has antioxidant activity, which may inhibit the reactions induced by dioxygen (O_2) or peroxides. One of the most common characteristics of inflammatory diseases is a severe reduction in plasma albumin concentration and diabetic retinopathy is now recognized as an inflammatory disease in which albumin reduction is associated with physiological disorders, such as modification of the intravascular oncotic pressure or alterations in the transport of exogenous and endogenous substrates[31]. The result of our study indicate that diabetes results in the low-expression of albumin in retinae, which affects blood colloidal osmotic pressure and nitric oxide signaling, increases vascular resistance and permeability, and aggravates retinal cell death and causes blindness. Dimethylarginine dimethylaminohydrolase 2 (DDAH Ⅱ) belongs to the DDAH family and has dimethylargininase activity which hydrolyzes N (G), N (G)-dimethyl-L-arginine (asymmetrical dimethylarginine; ADMA) and N (G)-monomethyl-L-arginine (MMA). Since ADMA and MMA

act as inhibitors of NO synthases (NOS), DDAH Ⅱ plays an important role in nitric oxide generation. DDAH Ⅱ has a more widespread distribution and its expression correlates with the distribution of eNOS[32]. According to the present study, down-regulated expression of DDAH Ⅱ in diabetic rat retinae increases plasma ADMA and decreases NO synthesis and may protect retinal cells from injury induced by hyperglycemia. Therapeutic modulation of ADMA levels, possibly *via* pharmacological or genetic modification of DDAH expression or activity, may represent a new strategy for the treatment of diabetes.

Triosephosphate isomerase 1 (EC 5.3.1.1, TIM1) belongs to the triosephosphate isomerase family, plays an important role in glycolysis by catalysing the reversible interconversion of D-glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP)[33]. Glucose is broken down to pyruvate *via* a chemical pathway that involves 10 enzymes and TIM1 is the fifth enzyme in this reaction chain that converts DHAP into GAP. High blood glucose level in diabetic rats may increase nitration of TIM1, leading to low expression of TIM1[34], which is consistent with the results of the present study. Low level of TIM1 decreases the synthesis of ATP. Further studies are needed to determine the feasibility of TIM1 replacement therapies. Guanylate kinase 1 (predicted) (EC: 2.7.4.8, GK) catalyzes the ATP-dependent phosphorylation of GMP into GDP, and is essential for recycling GMP and indirectly, cGMP. Photoexcitation of retinal rod photoreceptor cells involves the activation of cGMP enzyme cascade[35]. In the retinae of diabetic rats, low expression of GK reduces the phosphorylation of GMP into GDP, thus increasing GMP level. The increased GMP level inhibits the hydrolyzation of cGMP to GMP, resulting in accumulation of cGMP, which does not close the cation-specific channels. As a result, the increased cytosolic calcium level interrupts hyperpolarization of plasma membrane and generation of neural signals. Maybe, it is one of the mechanisms of blindness in diabetic patients with retinopathy. ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d (ATP synthase subunit d) are the subunit d of mitochondrial H (+) -ATP synthase, which catalyzes ATP synthesis from ADP and phosphate utilizing the energy derived from an electrochemical gradient of protons across the inner membrane during oxidative phosphorylation. However, the exact function of ATP synthase subunit d is unknown. The data obtained in this study revealed that expression of ATP synthase subunit d was scarcely found in the retinae of 8-wk old diabetic rats, suggesting that reduced ATP synthesis in retinae affects the function of retinal cells.

Disappeared expression of proteins

Peroxiredoxin 6 matches pattern 3. In this pattern, peroxiredoxin 6 is acarcely expressed in the diabetic samples. Peroxiredoxin 6 (Prdx6, EC 1.11.1.15) is a unique, bifunctional protein with both GSH peroxidase and phospholipase A2 activities, and is the only mammalian 1-Cys member of the peroxiredoxin family of antioxidant proteins which protect cells from oxidative damage by reducing cellular peroxides[36]. Knockout studies and transgenic over-expression of Prdx6 in mice have demonstrated an important role of this protein in protection against oxidative stress which contributes to the pathogenesis of the long-term complications of diabetes[37]. Disappearance of peroxiredoxin 6 in the retinae of diabetic rats may cause excess reactive oxygen species (ROS), such as hydrogen peroxide, superoxide anion and hydroxyl radicals, contributing to accumulation of cellular peroxides-convert, and accentuating tissue oxidative injury.

In conclusion, there are differences in expression of retinal protein between diabetic and normal rats. These proteins, up-regulated or down-regulated, may be involved in the pathogenetic mechanisms and prognosis of retinal diseases caused by diabetes, or in the maintenance of survival of retinal cells. Of these proteins, guanylate kinase 1, TIM1, ATP synthase subunit d, albumin and DDAH Ⅱ may play an important role in signal transduction. TIM1, crystallin alpha B, ATP synthase subunit d and peroxiredoxin 6 are involved in energy metabolism of retinal tissues. The role of crystallin beta A1 in diabetic retinae has not yet been found. Further study is necessary to determine if some proteins such as crystallin alpha B, DDAH Ⅱ and TIM1 can be regarded as candidates of biochemical markers for diagnosis and treatment of diabetes. Our findings provide direct and visible evidence for analyzing the molecular pathogenetic mechanisms of retinal diseases caused by diabetes.

ACKNOWLEDGMENTS

The authors thank Professor Ai-Dong Li, Dr. Wei-Min Hu, Professor Bao-Qian Jing and Dr. Guang Pan for their technical assistance, as well as Professor Zheng-Wei Yang for his critical reading of the manuscript.

REFERENCES

1 **American Diabetes Association: Diabetes and Retinopathy (Eye Complications) (webpage)**. Available from URL: http://www.diabetes.org/diabetes-statistics/eye-complications.jsp. Accessed 16 December 2006
2 **Bai N**, Tang S, Ma J, Luo Y, Lin S. Increased expression of intercellular adhesion molecule-1, vascular cellular adhesion molecule-1 and leukocyte common antigen in diabetic rat retina. *Yanke Xuebao* 2003; **19**: 176-183
3 **Ishida S**, Usui T, Yamashiro K, Kaji Y, Ahmed E, Carrasquillo KG, Amano S, Hida T, Oguchi Y, Adamis AP. VEGF164 is proinflammatory in the diabetic retina. *Invest Ophthalmol Vis Sci* 2003; **44**: 2155-2162
4 **Joussen AM**, Poulaki V, Qin W, Kirchhof B, Mitsiades N, Wiegand SJ, Rudge J, Yancopoulos GD, Adamis AP. Retinal vascular endothelial growth factor induces intercellular adhesion molecule-1 and endothelial nitric oxide synthase expression and initiates early diabetic retinal leukocyte adhesion in vivo. *Am J Pathol* 2002; **160**: 501-509
5 **Joussen AM**, Poulaki V, Tsujikawa A, Qin W, Qaum T, Xu Q, Moromizato Y, Bursell SE, Wiegand SJ, Rudge J, Ioffe E, Yancopoulos GD, Adamis AP. Suppression of diabetic retinopathy with angiopoietin-1. *Am J Pathol* 2002; **160**: 1683-1693
6 **Poulaki V**, Joussen AM, Mitsiades N, Mitsiades CS, Iliaki EF, Adamis AP. Insulin-like growth factor-I plays a pathogenetic role in diabetic retinopathy. *Am J Pathol* 2004; **165**: 457-469
7 **Zheng L**, Szabó C, Kern TS. Poly(ADP-ribose) polymerase is involved in the development of diabetic retinopathy via regulation of nuclear factor-kappaB. *Diabetes* 2004; **53**: 2960-2967
8 **Nyengaard JR**, Ido Y, Kilo C, Williamson JR. Interactions

between hyperglycemia and hypoxia: implications for diabetic retinopathy. *Diabetes* 2004; **53**: 2931-2938
9 **Joussen AM**, Huang S, Poulaki V, Camphausen K, Beecken WD, Kirchhof B, Adamis AP. In vivo retinal gene expression in early diabetes. *Invest Ophthalmol Vis Sci* 2001; **42**: 3047-3057
10 **Mei Y**, Zhou HY, Xiang T, Lu YG, Li AD, Tang EJ, Yang HJ. Preliminary analysis of retinal gene expression profile of diabetic rat. *Zhonghua Yixue Yichuanxue Zazhi* 2005; **22**: 563-565
11 **Quin GJ**, Gillies MC. Proteomic comparison of diabetic versus non–diabetic rat retinae. Invest Ophthalmol Vis Sci 2004; 45: E-Abstract 3247-B882. Available from URL: http://abstracts.iovs.org/cgi/content/abstract/45/5/3247
12 **Quin GJ**, Gillies MC. Proteomic analysis of diabetic and non –diabetic rat eetinae. Invest Ophthalmol Vis Sci 2005; 46: E-Abstract 406-B380 Available from URL: http://abstracts.iovs.org/cgi/content/abstract/46/5/406
13 **Stein RC**, Zvelebil MJ. The application of 2D gel-based proteomics methods to the study of breast cancer. *J Mammary Gland Biol Neoplasia* 2002; **7**: 385-393
14 **Qi Y**, Chiu JF, Wang L, Kwong DL, He QY. Comparative proteomic analysis of esophageal squamous cell carcinoma. *Proteomics* 2005; **5**: 2960-2971
15 **Wu SL**, Hancock WS, Goodrich GG, Kunitake ST. An approach to the proteomic analysis of a breast cancer cell line (SKBR-3). *Proteomics* 2003; **3**: 1037-1046
16 **Li Q**, Zemel E, Miller B, Perlman I. Early retinal damage in experimental diabetes: electroretinographical and morphological observations. *Exp Eye Res* 2002; **74**: 615-625
17 **Park SH**, Park JW, Park SJ, Kim KY, Chung JW, Chun MH, Oh SJ. Apoptotic death of photoreceptors in the streptozotocin-induced diabetic rat retina. *Diabetologia* 2003; **46**: 1260-1268
18 **Palmowski AM**, Sutter EE, Bearse MA, Fung W. Mapping of retinal function in diabetic retinopathy using the multifocal electroretinogram. *Invest Ophthalmol Vis Sci* 1997; **38**: 2586-2596
19 **Wang J**, Xu G, Li H, Gonzales V, Fromholt D, Karch C, Copeland NG, Jenkins NA, Borchelt DR. Somatodendritic accumulation of misfolded SOD1-L126Z in motor neurons mediates degeneration: alphaB-crystallin modulates aggregation. *Hum Mol Genet* 2005; **14**: 2335-2347
20 **Horwitz J**. Alpha-crystallin. *Exp Eye Res* 2003; **76**: 145-153
21 **Dahlman JM**, Margot KL, Ding L, Horwitz J, Posner M. Zebrafish alpha-crystallins: protein structure and chaperone-like activity compared to their mammalian orthologs. *Mol Vis* 2005; **11**: 88-96
22 **Fujita Y**, Ohto E, Katayama E, Atomi Y. alphaB-Crystallin-coated MAP microtubule resists nocodazole and calcium-induced disassembly. *J Cell Sci* 2004; **117**: 1719-1726
23 **Shu E**, Matsuno H, Akamastu S, Kanno Y, Suga H, Nakajima K, Ishisaki A, Takai S, Kato K, Kitajima Y, Kozawa O. alphaB-crystallin is phosphorylated during myocardial infarction: involvement of platelet-derived growth factor-BB. *Arch Biochem Biophys* 2005; **438**: 111-118
24 **Li DW**, Liu JP, Mao YW, Xiang H, Wang J, Ma WY, Dong Z, Pike HM, Brown RE, Reed JC. Calcium-activated RAF/MEK/ERK signaling pathway mediates p53-dependent apoptosis and is abrogated by alpha B-crystallin through inhibition of RAS activation. *Mol Biol Cell* 2005; **16**: 4437-4453
25 **Gruvberger-Saal SK**, Parsons R. Is the small heat shock protein alphaB-crystallin an oncogene? *J Clin Invest* 2006; **116**: 30-32
26 **Moyano JV**, Evans JR, Chen F, Lu M, Werner ME, Yehiely F, Diaz LK, Turbin D, Karaca G, Wiley E, Nielsen TO, Perou CM, Cryns VL. AlphaB-crystallin is a novel oncoprotein that predicts poor clinical outcome in breast cancer. *J Clin Invest* 2006; **116**: 261-270
27 **Fedtsova NG**, Minina TA, Barabanov VM. Synthesis of a lens-specific antigen (delta-crystallin) in rudimentary chicken adenohypophysis. *Biull Eksp Biol Med* 1981; **92**: 314-316
28 **Head MW**, Peter A, Clayton RM. Evidence for the extralenticular expression of members of the beta-crystallin gene family in the chick and a comparison with delta-crystallin during differentiation and transdifferentiation. *Differentiation* 1991; **48**: 147-156
29 **Rafikova O**, Rafikov R, Nudler E. Catalysis of S-nitrosothiols formation by serum albumin: the mechanism and implication in vascular control. *Proc Natl Acad Sci USA* 2002; **99**: 5913-5918
30 **Fujioka S**, Karashima K, Nishikawa N, Saito Y. Optic disk manifestation in diabetic eyes with low serum albumin: late fluorescein staining and high blood flow velocities in the optic disk. *Jpn J Ophthalmol* 2004; **48**: 59-64
31 **Ruot B**, Papet I, Bechereau F, Denis P, Buffiere C, Gimonet J, Glomot F, Elyousfi M, Breuille D, Obled C. Increased albumin plasma efflux contributes to hypoalbuminemia only during early phase of sepsis in rats. *Am J Physiol Regul Integr Comp Physiol* 2003; **284**: R707-R713
32 **Rossiter S**, Smith CL, Malaki M, Nandi M, Gill H, Leiper JM, Vallance P, Selwood DL. Selective substrate-based inhibitors of mammalian dimethylarginine dimethylaminohydrolase. *J Med Chem* 2005; **48**: 4670-4678
33 **Kursula I**, Partanen S, Lambeir AM, Antonov DM, Augustyns K, Wierenga RK. Structural determinants for ligand binding and catalysis of triosephosphate isomerase. *Eur J Biochem* 2001; **268**: 5189-5196
34 **Du Y**, Miyagi M, West K, Crabb JW, Kern TS. Identification of retinal proteins that are nitrated in elevated glucose concentration. Invest Ophthalmol Vis Sci 2003 44: E-Abstract 3901-B604. Available from URL: http://abstracts.iovs.org/cgi/content/abstract/44/5/3901
35 **Stolworthy TS**, Black ME. The mouse guanylate kinase double mutant E72Q/D103N is a functional adenylate kinase. *Protein Eng* 2001; **14**: 903-909
36 **Simeone M**, Phelan SA. Transcripts associated with Prdx6 (peroxiredoxin 6) and related genes in mouse. *Mamm Genome* 2005; **16**: 103-111
37 **Hodgkinson AD**, Bartlett T, Oates PJ, Millward BA, Demaine AG. The response of antioxidant genes to hyperglycemia is abnormal in patients with type 1 diabetes and diabetic nephropathy. *Diabetes* 2003; **52**: 846-851

S- Editor Liu Y **L- Editor** Wang XL **E- Editor** Liu Y