Article

Application of Two-Dimensional Electrophoresis in the Research of Retinal Proteins of Diabetic Rat

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Diabetes mellitus (DM) is a chronic disease which is associated with numerous serious health complications such as diabetic retinopathy, and is the leading cause of new cases of blindness in adults at the age of 20-74 years old. The aim of the study was to establish and optimize a two-dimensional polyacrylamide gel electrophoresis (2-DE) technique for retina proteomics to improve the resolution and reproducibility, and to observe the proteomic changes of retinal tissues in diabetic and normal rats. Proteins were extracted from retinal tissues of normal and 8 weeks diabetic SD rats and used in two-dimensional electrophoresis. Various conditions of retina proteomic 2-DE were adjusted, optimized and protein spots of differential expression were obtained through analysis of 2-DE images with PDQuest software. By choosing appropriate sample amount, using pre-cast IPG dry strips (pH 5-8) and casting 12% equal gel, satisfactory 2-DE images of retina were obtained and a steady 2-DE technique was established. In this way, we found 36 spots in 2-DE gel of diabetic retinas that exhibited statistically significant variations, including up-regulation of 5 proteins in diabetic rat retinas, down-regulation of 23, and disappearance of 8, in comparison with normal tissues. The differences of protein expression were observed in retinas between diabetic and normal rats. Our established 2-DE technique of retina proteins could be effectively applied in proteomics of retina diseases. *Cellular & Molecular Immunology*. 2007;4(1):65-70.

Key Words: diabetes, retinas, two-dimensional polyacrylamide gel electrophoresis

Introduction

Diabetes mellitus (DM) is a chronic medical disease caused by insufficient production of the hormone insulin by the pancreas, or insensitivity of cells to the effects of insulin, a hormone that allows blood glucose to enter the cells of the body and be used for energy. DM is the fifth-deadliest disease in the United States. In 1999-2002, more than 9 percent of persons 20 years of age and over and about one-fifth of adults 60 years and over had diabetes (1). There is a clear trend of increase in DM mortality in China (2). Diabetes is associated with numerous serious health complications. Eye disease such as diabetic retinopathy is a frequent complication of diabetes, for both type 1 and type 2. Some people with diabetes develop a serious eye disease called proliferative diabetic retinopathy, which is progressive damage to the eye's retina caused by long-term diabetes and often results in blindness. DM is responsible for 8% of legal blindness in the US, making it the leading cause of new cases of blindness in adults at the age of 20-74 years old. Each year, 12,000 to 24,000 people lose their sight because of diabetes. In the past few years, there exist ongoing progresses in researching of diabetic retina (3-10). However, the patho-genetic mechanism of retinal disease caused by DM is unclear and there is no cure for diabetes so far. For this reason, further comparative analyses of protein expression profiles in retinas of diabetic rats 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 electro-phoresis (2-DE) and matrix assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS), enable the study of disease-associated proteomics (11-14). 2-DE is a protein separation technique that separates proteins by charge (Isoelectric focusing, IEF) in the first dimension and by molecular mass (SDS-PAGE) in the second dimension (15). In spite of promising alternative or

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Received Jan 7, 2007. Accepted Feb 24, 2007.

complementary technologies (e.g., multidimensional protein identification technology, stable isotope labelling, protein or antibody arrays) that have emerged recently, 2-DE is currently the only technique that can be routinely applied for parallel quantitative expression profiling of large sets of complex protein mixtures such as whole cell lysates (16). Since the technique was pioneered in 1975, 2-D gel methods have undergone a series of enhancements to optimize resolution and reproducibility. The resolving power of 2-DE is superior to that of any other protein separation techniques. Using this technology, the changes of the protein expression in retinal tissue from diabetic rats in comparison with normal rats can also be demonstrated.

The complexity of diabetes and its complications need a unbiased and scientific method such as proteomics to study. At present many scholars have studied proteomic changes of the serum (17), vitreous body (18, 19), urine (20, 21) and kidneys organize (22) under the diabetic state. Now we apply this method in the retinal research in this study. Retinal proteomics to identify protein markers of retinal disease or related proteins may offer a new theoretical foundation for the pathogenesis, diagnosis and treatment of retinal disease. However, the reproducibility and stability of 2-DE is the technical key problem. Therefore, the conditions of the retinal protein 2-DE were adjusted and optimized as well as the expressional differences of the retinal proteins in the early diabetic rats were observed in this study, in the hope of setting up the stable retinal protein separating technique and establishing the foundation for the retinal proteomics.

Materials and Methods

Reagents

Alloxan Tetrahydrate, Acrylamide, Bis-Acrylamide, Coomassie Brilliant Blue R-250, Tris, Ammonium Persulfate, Glycine, EDTA, Sodium Dodecyl Sulfate (SDS), Bromophenol Blue, 2-Mercaptoethanol, Mineral Oil and TEMED were AMRESCO products (Solon, Ohio, USA). Dithiothreitol (DTT) was obtained from Merck (Darmstadt, Germany) and iodoacetamide from Acros (Geel, Belgium). Tributyl Phosphine (TBP), PMSF, Urea and Low-Melt Agarose were purchased from Sigma (St. Louis, MO, USA); CHAPS, ReadyStrip IPG Strip, 40% Bio-Lyte Ampholyte, Protein Assay kits were purchased from Bio-Rad (Hercules, CA, USA). All other chemicals were analytical grade and come from China. All solutions were prepared using Milli-Q water.

Apparatus

Low temperature supercentrifuge, circulating water bath box, Gel Cassettes, Model 550 Microplate Reader, PROTEAN IEF Cell and Protean II xi Cell were from Bio-Rad (Hercules, CA, USA). The transmission scanner is UMAX PowerLook.

Animals

The female Sprague-Dawley (SD) rats were provided by the Experimental Animal Center of North Sichuan Medical College.

Diabetic rat models

The study was approved by the Medical Ethical Committee of North Sichuan Medical College. Forty healthy adult female Sprague-Dawley (SD) rats weighing approximately 180-200 g were randomly divided into diabetic group and normal control group. Thirty rats of diabetic group received a single intra-peritoneal injection of Alloxan Tetrahydrate (200 mg/kg) in normal sodium. Ten rats of the control group received injection of an equal volume of normal sodium alone. Glucose levels in vena caudalis blood were measured with rapid glucose instrument (one Touch 6 type. Johnson Co., Ltd.) and Glucose levels in urine were measured with urine glucose test paper 72 hours after injection. Urine glucose levels of rats in diabetic group strongly positive (from +++ to ++++) and blood glucose levels higher than 16.7 mmol/L were deemed diabetes models. Then the urine glucose levels were measured once a day, and the blood glucose levels were measured once every week. Those rats whose urine glucose levels were negative or whose blood glucose levels were lower than 16.7 mmol/L were abandoned. All rats of experiment course drunk the water freely and fed the standard diet in an air-conditioned room where the ambient temperature is 20 ± 2°C and the relative humidity is 45-60%.

Rat retinal protein extraction and purification

After eight weeks, 20 rats including 11 diabetic rats and 9 non-diabetic control rats were sacrificed by cervical dislocation and the retinas were separated from retinal pigment epithelium (RPE), weighed immediately, and homogenized for 5 minutes on ice at a concentration of approximately 50 mg/ml in lysis buffer (ReadyPrep Sequential Extraction Kit, Reagent 3, Bio-Rad Laboratories, Inc, Hercules, CA) consisting of 5 M urea, 2 M thiourea, 2% (w/v) CHAPS, 2% (w/v) SB 3-10, 40 mM Tris, 0.2% (w/v) Bio-Lyte 3/10, 2 mM TBP, 1 mM PMSF, and 200 μg/ml DNase I. Sample was incubated at 4°C for 15 min and then centrifuged at 18°C for 30 min at 16,000 g 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 sample was measured on a Model 550 Microplate Reader using a RC DC Protein Assay Kit (Bio-Rad Laboratories) with BSA as standard. A liquots of the final protein solution of a rat was stored at -80℃ until further use.

Isoelectric focusing (IEF)

Appropriate amount of protein sample was mixed with a rehydration buffer containing 8 M urea, 4% (w/v) CHAPS, 65 mM DTT, 0.2% (v/v) Bio-Lytes and a trace of bromophenol blue, and loaded in the IEF focusing tray, then gently placed the IPG gel strip gel side down onto the rehydration solution and overlay each of the IPG strip with 3 ml of mineral oil to prevent evaporation during the rehydration process. Placed the IEF focusing tray in the PROTEAN IEF Cel and started the program. The strip after IEF was equilibrated for 15 min in the equilibrium buffer I

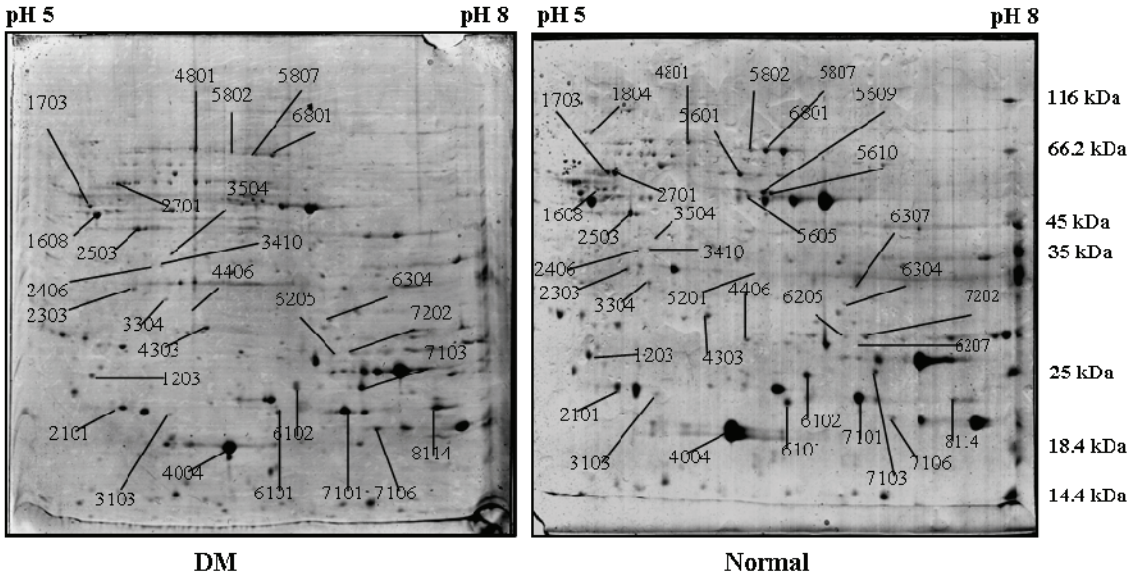

Figure 1. 2-DE images of retinal proteins in normal and 8 weeks diabetic (DM) rats. The proteins from retinal were extracted and separated on a pH 5-8 IPG strip, followed by a 12% SDS-PAGE and Coomassie Brilliant Blue R-250 staining. Spots that were found different expressed have been annotated.

including DTT, then for another 15 min in the equilibrium buffer II including iodoracetamide.

SDS-PAGE

After equilibrium, the gel strip was placed on the upper edge of the 12% separation gel with its back side pasted tightly on the long glass plate. Low molecular weight protein marker of 10 μl was added on one end of the IPG strip, then Low-Melt Agarose was used to fix the gel strip and to remove air bubbles. Gel was run first at voltage of 60 Volts, and then at 250 Volts when the bromophenol blue ran completely out of gel strip and condensed into one line. The electrophoresis was stopped when the bromophenol blue front had traversed the gel.

Gel staining and image acquisition and analysis

The completed 2-D gels were stained with silver nitrate or Coomassie Brilliant Blue R-250 and then scanned with an UMAX Scanner (Powerlook 1120, Taiwan). The image analysis, including image editing, spot finding, quantitation and matching, were carried out using the PDQuest 7.3.2 software package (Bio-Rad, Hercules, CA, USA). The PDQuest software calculated the 2-DE protein spot intensity by integrating the optical density over the spot area. Proteins separated by 2-DE gels were quantitated in terms of their normalized quantity. The individual protein spot quantity was normalized as a percentage of the total quantity of the valid spots present in the gel and expressed as percent. Comparison between two characteristics (normal *vs* diabetic tissue) was assessed using the Student's *t*-test and relationships were considered statistically significant when $p < 0.05$. The confidence level was 95% and the results were exported to Excel 2000 (Microsoft, Seattle, CA, USA) for further analysis.

Results

2-DE images of rat retinal proteins

Each protein sample from normal and diabetic rats groups was used in the 2-DE three times respectively. After the gels were stained and scanned, we got six images. Figure 1 shows the two-dimensional electrophoretic images of proteins in retinal tissues of 8 weeks diabetic and normal rats (17 cm pH 5-8 IPG gels, Coomassie Brilliant Blue R-250 staining). Image analysis found that there were about 600-950 protein spots in each image under the same parameter and the average match rate was above 70%, which shows that most proteins have expressed in the retinas both normal and diabetic rats. The relations between percentage of protein spots and molecular weights or isoelectric points were shown in Figure 2.

Reproducibility analysis

Three images selected from normal group and another 3 images from diabetic group were analyzed with PDQuest 7.3.2 software, then chose randomly some spots matched each other and distinguished clearly and outputted their information (distance of X axis direction means the distance of IEF direction, distance of Y axis direction means the distance of SDS-PAGE direction). The standard deviations of the distances on IEF direction and SDS-PAGE direction were calculated as the reproducibility index of position (23). We can see from Table 1 that there is a better position repeatability of proteins among these gels. Among them, the average deviation in SDS-PAGE direction is greater than the

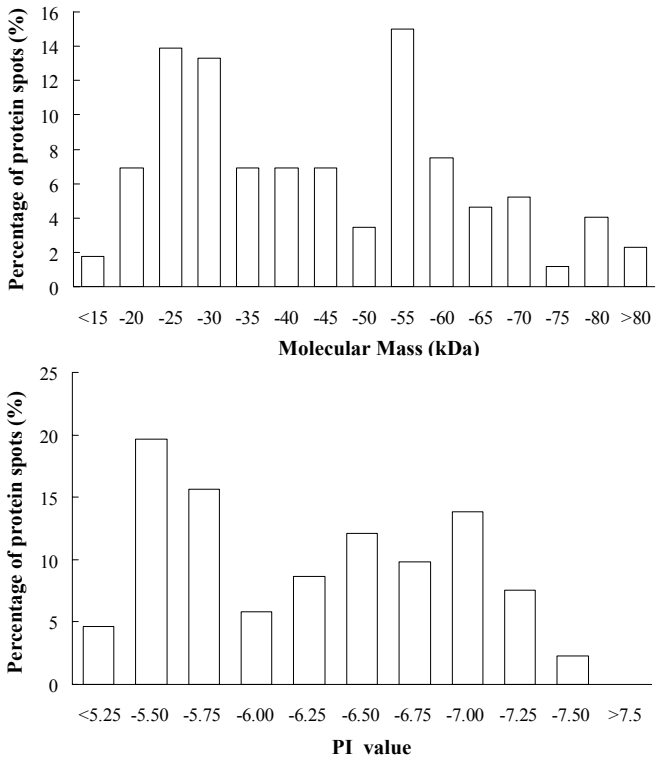

Figure 2. The relations between percentage of protein spots and molecular weights or isoelectric points.

Table 1. 2-DE profile reproducibility analysis of rat retinal proteins

SSP	SD on IEF (mm)	SD on SDS-PAGE (mm)
0102	0.845106	3.029941
1203	0.874572	2.994823
1503	0.917492	1.672424
2302	0.87075	2.606509
4004	2.343097	3.498848
5105	2.138766	1.783748
6204	1.224708	1.650521
6206	1.024025	1.767403
6506	0.593882	0.354556
7101	0.76362	2.008395
7103	0.676292	2.104465
7104	0.823447	2.16599
7106	0.675955	2.410811
7201	0.632254	1.864961
7206	0.819215	1.928831
8112	1.05563	2.036823
SD, mean	1.017 ± 0.506	2.117 ± 0.717
SD, range	0.594-2.343	0.355-3.499

SSP, standard spots number; SD, standard deviation.

average deviation in IEF direction, which proved that the position repetitiveness of IEF direction is superior to that of SDS-PAGE direction in this test.

Expression changes of rat retinal proteomics

Through image analysis with PDQuest 7.3.2 software and statistical analysis with Student *t*-test, these protein spots whose expression difference were statistical meaningful were detected. In this way, we found 36 spots in 2-D gels including up-regulation of 5 proteins in diabetic rat retinas, down-regulation of 23, and disappearance of 8, in comparison with that of normal rat retinas.

Discussion

Through the observation of the signs and symptoms and the detection of the glucose level in blood and urine, it could be concluded that the building of rat models was successful. The analysis result of 2-DE proved that there were obvious differences of the protein expressions in the retinas between early diabetic and normal rats. The meaning of differential expression and the protein roles in the pathomechanism of diabetic retina disease remain to be further studied.

Sample preparation is the key of the 2-DE. Successful 2-DE depends on efficient extraction and solubilization of proteins. The major goal of sample preparation is to solubilize as many proteins as possible and to maintain their solubility throughout the 2-DE process. There are many factors influencing protein extraction from tissues, compared with that from cells. Unlike the situation for DNA, there is no universal sample-preparation method suitable for all proteins. At present, many studies have focused on the increase of the protein solubility and the decrease of protein degradation and loss to improve 2-DE. We tried out many methods to break retinal tissues and chose ultimately the mechanical homogenate method and protein lysis buffer to clearage retinal tissues. As to the protein of different solubilities, we could also consider using the sequential extraction kit such as ReadyPrep Sequential Extraction Kit (Bio-Rad) to improve extraction result. Degeneration agent in sample lysate such as urea and thiourea can undermine the formation of hydrogen bonds between the protein molecules to prevent from the protein aggregation and the formation of secondary structures in the protein migration, and the high concentration urea is also beneficial to the thiourea resolving. So we chose 5 M Urea and 2 M Thiourea. Detergents such as CHAPS and SB 3-10 can dissolve hydrophobic groups, eliminate interaction between hydrophobic groups to enhance protein solubility at isoelectric point, and the concentrations of the CHAPS and SB 3-10 in our lysis buffer were 2% (w/v) respectively. Under the condition of united use of the degeneration agents and the detergents, reducing agent such as DTT and TBP contribute to open the disulfide bond of proteins so that the denatured protein can unfold and dissolve more completely. Carrier Ampholytes contribute to the ionic strength of the solution, to counteract a lack of salts in a sample, and can also raise the reproducibility of spot position (24). 2 mM

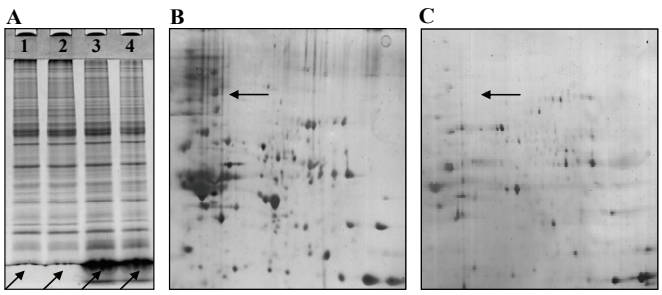

Figure 3. SDS-PAGE image and 2-DE images of normal rat retinal proteins pured with or without ReadyPrep 2-D Cleanup Kit. (A) SDS-PAGE image of normal rat retinal proteins (coomassie brilliant blue R-250 staining). Lane 1-2, proteins pured with ReadyPrep 2-D Cleanup Kit; Lane 3-4, proteins pured without ReadyPrep 2-D Cleanup Kit. (B) 2-DE image of normal rat retinal proteins pured without ReadyPrep 2-D Cleanup Kit (silver staining). (C) 2-DE image of normal rat retinal proteins pured with ReadyPrep 2-D Cleanup Kit (silver staining).

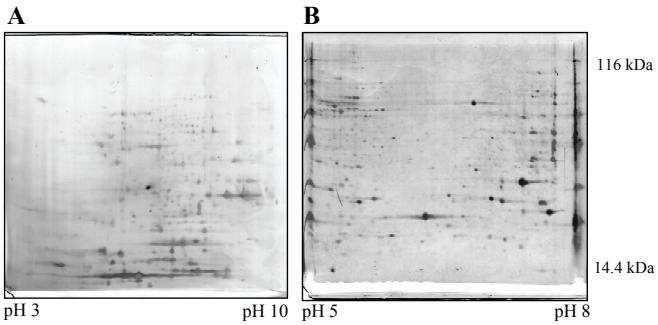

Figure 4. 2-DE images of retinal proteins of normal rats using pre-cast IPG dry strips with different pH ranges (17 cm gel strip, silver staining). (A) 2-DE image of proteins using pre-cast IPG dry strip with pH 3-10 range. (B) 2-DE image of proteins using pre-cast IPG dry strip with pH 5-8 range.

TBP and 0.2% (w/v) Bio-Lyte 3/10 were used in our lysis buffer.

Some studies reported that the strategy of putting protease inhibitor into lysis buffer before sample homogenate can obtain better result of protein extraction, while another authors supported that it was better to put protease inhibitor into lysis buffer before centrifugation. For the protein extraction of retinal tissue, we compared the extract results of adding PMSF in the lysis buffer at different time stages and found a good method to extract retinal proteins. That is, retina tissue was first homogenated mechanically, then added to the lysis buffer with PMSF (final concentration of 1 mmol/L) and continued to homogenate, at last centrifugated. We had also studied whether the nuclease was added to remove nucleinic acid. After several contrast, we believe that trace DNase I (final concentration of 600 μg/ml) could reduce obviously the sticky material in the homogenate while RNase A could not do it.

There are a lot of methods of protein purification, such as dialysis, ultrafiltration, chromatography, gel filtration, precipitation and some kits of removal impurity. We used ReadyPrep 2-D Cleanup Kit produced by Bio-Rad Company to remove various detergents, salt, polypeptide, nucleinic acid, lipide, phenol and some other small ions in sample, and got very good purification effect. There were brush strips in the SDS-PAGE image of normal rat retinal proteins pured without the kit (Figure 3A), and there were nucleic acids and other impurities in the second quadrant of 2-DE image (Figure 3B). The purification results of ReadyPrep 2-D Cleanup Kit were shown in Figures 3A and 3C. In addition, measuring of protein concentration showed that the use of ReadyPrep 2-D Cleanup Kit might result in the protein loss, and we may utilize ReadyPrep 2-D Cleanup Kit because of its protein concentrating function to raise protein concentration in the sample.

The total mount of protein to load per IPG strip will vary depending on the sample, the pH range and length of the IPG strip, and the detection system used. These proteins beyond the pH range of IPG strip is unable to be focused in the IPG strip. Therefore, with the IPG strip of same length, narrower pH range, the greater protein is load; with the IPG strip of same pH, longer IPG strip, the greater protein is load. The appropriate amount of protein sample results in the clear resolution of protein spots, and is good for the separation, detection, analysis and identification of the protein. Through repeated exploration, the suitable amount of rat retina protein to load per IPG strip of 17 cm pH 5-8 is 1,500 μg for coomassie brilliant blue R-250 staining and 300 μg for silver staining.

It is essential for IEF to keep stability, linear and repeatability of pH gradient, and immobility pH gradient (IPG) strips can meet this requirement to the greatest extent. We adopted 17 cm pH 3-10 IPG strip to analyze the population condition of protein and found the retinal proteins mainly concentrates on the section of pH 5-8 (Figure 4A) at first. Then we adopted the pH 5-8 IPG strip (Figure 4B) and further enhanced the protein resolution of 2-DE.

On the whole, we established and optimized a two-dimensional polyacrylamide gel electrophoresis technique for retina proteomics, and the established steady 2-DE technique of retina proteins could be effectively applied in proteomics of retina diseases. By choosing appropriate sample amount, using lysis buffer (consisting of 5 M urea, 2 M thiourea, 2% (w/v) CHAPS, 2% (w/v) SB 3-10, 40 mM Tris, 0.2% (w/v) Bio-Lyte 3/10, 2 mM TBP, 1mM PMSF, and 200 μg/ml DNase I), pre-cast IPG dry strips (17 cm, pH 5-8) and casting 12% equal gel, satisfactory 2-DE images of retina were obtained. The differences of protein expression were observed in retinas between diabetic and normal rats. Protein identification with mass spectrum and functional analysis would be done in the future study.

Acknowledgements

We thank Prof. Cheng-Jun Li, Prof. En-Jie Tang, Prof. Bin Weng, Prof. Ai-Dong Li, Prof. Bao-Qian Jing and Prof.

Zheng-Wei Yang for technical assistance.

The authors have declared that no competing interests exist.

References

1. American Diabetes Association: diabetes and retinopathy (Eye Complications). http://www.diabetes.org/diabetes-statistics/eye-complications.jsp. Accessed 1 January 2007.
2. Li R, Wang ZG. Analysis and forecast of the mortality of diabetes mellitus in China. J Environ Occup Mod. 2003:20: 168-171.
3. Bai NY, Tang SB, Ma J, et al. Increased expression of intercellular adhesion molecule-1, vascular cellular adhesion molecule-1 and leukocyte common antigen in diabetic rat retina. Eye Science. 2003;3:176-183.
4. Joussen AM, Huang S, Poulaki V, et al. *In vivo* retinal gene expression in early diabetes. Invest Ophthalmol Vis Sci. 2001; 12:3047-3057.
5. Nyengaard JR, Ido Y, Kilo C, Williamson JR. Interactions between hyperglycemia and hypoxia: implications for diabetic retinopathy. Diabetes. 2004;53:2931-2938.
6. Zheng L, Szabo C, Kern TS. Poly(ADP-Ribose) polymerase is involved in the development of diabetic retinopathy *via* regulation of nuclear factor-κB. Diabetes. 2004;53:2960-2966.
7. Klein R, Zinman B, Gardiner R, et al. The relationship of diabetic retinopathy to preclinical diabetic glomerulopathy lesions in type 1 diabetic patients the renin-angiotensin system study. Diabetes. 2005;54:527-533.
8. Wong TY, Shankar A, Klein R, Klein BE. Retinal vessel diameters and the incidence of gross proteinuria and renal insufficiency in people with type 1 diabetic. Diabetes. 2004;53: 179-184.
9. Nishida S, Sasaki T, Kimura H, et al. Immunization with ACE (angiotensin converting enzyme) develops diabetic changes in the kidney and retina in diabetogenic rats. Endocr J. 2003;50: 801-807.
10. Fong DS, Aiello LP, Ferris FL 3rd, Klein R. Diabetic retinopathy. Diabetes Care. 2004;27:2540-2553.
11. Stein RC, Zvelebil MJ. The application of 2D gel-based proteomics methods to the study of breast cancer. J Mammary Gland Biol Neoplasia. 2002;4:385-393.
12. Sarto C, Marocchi A, Sanchez JC, et al. Renal cell carcinoma and normal kidney protein expression. Electrophoresis. 1997;18: 599-604.
13. Qi YJ, Chiu JF, Wang LD, et al. Comparative proteomic analysis of esophageal squamous cell carcinoma. Proteomics. 2005;5:2960-2971.
14. Wu SL, Hancock WS, Goodrich GG, et al. An approach to the proteomic analysis of a breast cancer cell Line (SKBR-3). Proteomics. 2003;3:1037-1046.
15. Mary FL. Proteome analysis I. Gene products are where the biological action is. J Chromatogr B. 1999;722:191-202.
16. Angelika G, Walter W, Michael JD. Current two-dimensional electrophoresis technology for proteomics. Proteomics. 2004;4: 3665-3685.
17. Ahn BY, Song ES, Cho YJ, et al. Identification of an anti-aldolase autoantibody as a diagnostic marker for diabetic retinopathy by immunoproteomic analysis. Proteomics. 2006;6: 1200-1209.
18. Lee SH, Park KW, Kim CI, et al. Proteomics analysis of diabetic retinopathy vitreous body. Invest Ophthalmol Vis Sci. 2003;44: 2238-B77.
19. Ouchi M, West K, Crabb JW, et al. Proteomic analysis of vitreous from diabetic macular edema. Exp Eye Res. 2005;81: 176-182.
20. Jain S, Rajput A, Kumar Y, et al. Proteomic analysis of urinary protein markers for accurate prediction of diabetic kidney disorder. J Assoc Physicians India. 2005;53:513-520.
21. Meier M, Kaiser T, Herrmann A, et al. Identification of urinary protein pattern in type 1 diabetic adolescents with early diabetic nephropathy by a novel combined proteome analysis. J Diabetes Complications. 2005;19:223-232.
22. Thongboonkerd V, Barati MT, McLeish KR, et al. Alterations in the renal elastin-elastase system in type 1 diabetic nephropathy identified by proteomic analysis. J Am Soc Nephrol. 2004;15:650-662.
23. Huang ZY, Li JH, Wang S, et al. Proteome changes in chinese hamster ovary cells after DMSO treating. Fudan Da Xue Xue Bao. 2005;44:607-613.
24. Lopez MF, Patton WF. Reproducibility of polypeptide spot positions in two-dimensional gels run using carrier ampholytes in the isoelectric focusing dimension. Electrohoresis. 1997;18: 338-343.