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Two-dimensional electrophoretic profiling of normal human kidney:
differential protein expression in glomerulus, cortex and medullaBo Xu¹, Yutaka Yoshida¹, Ying Zhang¹, Eishin Yaoita¹, Tetsuo Osawa²
and Tadashi Yamamoto¹

SUMMARY

The diverse functions of the kidney are fundamentally fulfilled by the nephron, the functional unit of the kidney. The nephron consists of several functionally and structurally different segments, which spans the kidney from the cortex through the medulla. The functional specialization along the nephron and specific localization of the different segments should be reflected in the protein expression profiles of distinct anatomical compartments of the kidney. We performed a two-dimensional gel electrophoresis (2-DE)-based, quantitative, proteomic analysis to identify proteins differentially expressed in the cortex, medulla, and glomeruli purified from cortex of normal human kidney. The protein extracts of the three compartments obtained from 4 normal subjects were separated on 2-DE gels, and analyzed by using a 2-DE gel image analysis software. We detected 1,810 valid protein spots in the glomerulus, 1,758 in the cortex, and 1,275 spots in the medulla. Statistical analysis by one-way ANOVA followed by Tukey's post-hoc test revealed that 616 protein spots were differentially expressed in the glomeruli, 582 in the cortex, and 469 in the medulla. Among these protein spots, spots expressing in considerably higher relative amounts in one of the three compartments were selected and subjected to identification by peptide mass fingerprinting method with a MALDI-TOF mass spectrometer, which led to identification 19 protein spots preferentially expressed in the glomerulus, 35 in the cortex, and 21 in the medulla. The distinct protein expression profiles in different kidney compartments may serve as a resource for further study of physiology and pathophysiology of the human kidney.

Key words: human kidney, glomerulus, cortex, medulla, 2-DE, differential expression.

INTRODUCTION

The kidney is the major organ for the excretion of waste products such as urea, uric acid and creatinine. In the process of this activity, it carries out other functions, crucially important in homeostasis, the regulation of the electrolyte content, acid-base balance and the volume of the extracellular fluid by selective reabsorption of essential solutes, and the production of hormones that modulate blood pressure, hematopoiesis and calcium homeostasis. The diverse functions of the kidney are mainly fulfilled by the nephron, the

functional unit of the kidney. The nephron consists of several distinct specialized segments. The specialization in function along the nephron is reflected in the gross anatomy of the kidney: the cortex and the medulla. The former contains glomeruli, the juxtaglomerular apparatus, and the proximal, distal tubules and the part of collecting ducts, while the latter includes the loop of Henle and collecting ducts.

Proteomics can be defined as a systematic study of proteins including their expressions, functions and interactions to characterize biological processes under physiological and

¹ Department of Structural Pathology, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan.

² Department of Urology, Niigata City General Hospital, Niigata, Japan.

Correspondence address: Yutaka Yoshida, Department of Structural Pathology, Institute of Nephrology, Graduate School of Medical and Dental Sciences, Niigata University, 1-757 Asahimachi-dori, Niigata 951-8510, Japan.

Abbreviations: 2-DE, two-dimensional gel electrophoresis; MALDI-TOF MS, matrix-assisted laser desorption-time of flight mass spectrometer.

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pathophysiological conditions¹⁾. It also implies any large-scale or systematic characterization of proteins present in a cell, tissue, or organism²⁾. Proteomic approach thus may lead to discoveries relevant to physiological and pathophysiological processes, which have been veiled in the theory-driven, reductionistic approaches so far conducted in which one protein is studied at a time.

In this paper, we have analyzed differential expression of proteins in the glomeruli, cortex, and medulla of normal human kidneys by two-dimensional electrophoresis (2-DE) and identified differentially expressed proteins by peptide mass fingerprinting method with matrix-assisted laser desorption ionization-time of flight mass spectrometer (MALDI-TOF MS). This study was conducted to complement our previous proteomic analysis of the highly purified glomeruli of normal human kidneys in which 347 protein spots, representing 212 proteins, had been identified, and used for construction of an extensible markup language (XML)-based database⁴⁾.

MATERIALS AND METHODS

Materials

This study was approved by the Ethics Committees of Niigata University Faculty of Medicine and Niigata City General Hospital, and conducted in accordance with their ethical principles. Kidney tissues were obtained from patients under surgical nephrectomy due to renal tumor with their informed consent and permission. The pieces of tissue including cortex and medulla with normal appearance, far from the tumor mass, were excised, and part of the tissue was immediately sampled for histological examinations. The excised tissues were brought to our laboratory in ice-cold PBS within 30 min after removal. In this study we used kidney tissues with no apparent pathologic manifestations obtained from 4 male patients at the age of 38 to 64 years (51±11).

The kidney tissue was carefully dissected into cortex and medulla so as to exclude capsules, papillary tip, and adhering fat. Glomeruli were purified from the cortex by a standard sieving method and followed by picking-up into a glass micropipette under a phase-contrast microscope. The purified glomeruli were washed twice with PBS by suspending and centrifugation. The excised cortex and medulla, and purified glomeruli were stored at -80°C until use.

Two-dimensional gel electrophoresis (2-DE)

The tissue was homogenized with a Polytron homogenizer in 40–80 µl/mg wet weight of lysis solution (9.8 M urea, 2% NP-40, 2% Pharmalyte of pH 3–10 range, 100 mM DTT, 0.5 µg/ml E-64, 0.5 mM PMSF, 40 µg/ml TLCK, 1 µg/ml aprotinin, 10 µg/ml chymostatin, 0.5 mM EDTA, 0.01% bromophenol blue). The homogenate was incubated for 1 h at 37°C with occasional stirring, and then centrifuged in a microcentrifuge at 14,000 rpm for 20 min at room tem-

perature. The resultant supernatant was stored at -80°C until use. The protein concentration of 2-DE samples was determined by the method of Ramagli and Rodriguez³⁾ with human apo-transferrin as a standard.

Isoelectric focusing in the first dimension was carried out in a horizontal apparatus, IPGphor (Amersham Biosciences, Tokyo, Japan) using an 18 cm immobilized DryStrip (IPG strip) of pH 3–10 linear range (Amersham Biosciences). The IPG strips were rehydrated for 12 h at 20°C with 350 µl of rehydration solution containing sample protein (100 µg) and external standard proteins (2-D SDS-PAGE Standards, Bio-Rad, Tokyo, Japan). The composition of rehydration solution was the same as the lysis solution except that the concentration of Pharmalyte was reduced to 0.8%. After rehydration, the proteins were focused at 20°C for 1 h at 500 V, for next 1.25 h at 1,000 V, and finally at 8,000 V for time attaining to 63.3 KWh. The IPG strips after isoelectric focusing were equilibrated at room temperature by incubation for 20 min with 10 ml of SDS-equilibration buffer (6 M urea, 2% SDS, 50 mM Tris-HCl, pH 8.8, 30% glycerol, and 0.01% bromophenol blue) containing 100 mg DTT, followed by incubation for 20 min with 10 ml of SDS-equilibration buffer containing 250 mg iodoacetamide. The equilibrated IPG strips were then subjected to the second dimensional SDS-PAGE with 12.5% running gels (20×20×0.1 cm) without stacking gel in Dual-200 (Anatech, Tokyo, Japan). Electrophoresis was run at 30 mA/gel for 4.3 h in 25 mM Tris, 192 mM glycine and 0.1% SDS. Silver staining of 2-DE gels was carried out according to the MS analysis compatible staining protocol for PlusOne silver staining kit (Amersham Biosciences).

2-DE gel image analysis

All the silver-stained 2-DE gels were scanned at the resolution of 300 dpi with a flat-bed scanner (ES-2200, Seiko Epson Corp., Tokyo, Japan) to generate digitized images. The 2-DE gel images were analyzed by using PDQuest version 7.1, a 2-DE image analysis software (Bio-Rad, Tokyo, Japan). From the 2-DE gel images derived from the three different tissues of 4 normal subjects, 3 lower level matchsets were created. The same parameters for cropping gel area and automated spot detection were adopted for all the gel images. The spots detected by the software were validated by close examination of respective raw gel images, and missed spots and misidentified spots were corrected with manual spot editing tools. The valid spots thus identified on the respective member gel images comprising the corresponding match set were matched, and synthetic gel images representing 2-DE profiles of glomeruli, cortex and medulla were generated by merging spots from all the member gel images. Normalization for accurate comparison of expression levels of protein spots among the three compartments was then conducted in respective matchsets by using “total quantity in valid spots” method. In this normal-

ization method, the raw quantity of each spot in a member gel was divided by the total quantity of all the spots in that gel that had been included in the synthetic gel image of matchset. The three matchsets were then combined to create a high level matchset, and high level synthetic master gel image was generated by matching and merging valid spots included in the synthetic gel image of the respective 3 lower level matchsets. Any spot included in the master gel image of high level matchset contained all the normalized data of quantity of corresponding spots of all the individual member gel images of the 3 lower level matchsets. Statistical evaluation of expression levels of protein spots among the three compartments was then made by one-way ANOVA followed by Tukey's method to pick up proteins differentially expressed. Differences were considered significant at probability value less than 0.05.

In the present study we arbitrarily defined "preferentially" expressed proteins in each of the three tissues as follows. The proteins preferentially expressed in the glomerulus have spot density ratio of glomerulus to cortex more than 10 and that of glomerulus to medulla more than 5. The proteins preferentially expressed in the cortex have spot density ratio of cortex to glomerulus more than 10 and that of cortex to medulla more than 5. The proteins preferentially expressed in the medulla have spot density ratio of medulla to glomerulus more than 5 and that of medulla to cortex more than 5.

Protein identification by MALDI-TOF MS

Protein spots preferentially expressed in each of the three compartments of normal human kidney as defined above were manually excised with a scalpel from silver-stained 2-DE gels equilibrated with ultra pure water. The excised spots were cut into small pieces, and vigorously washed four times in 0.5 ml of 50 mM ammonium bicarbonate and 50% acetonitrile for 15 min. The gel pieces were shrunk in 0.1 ml of acetonitrile for 10 min, and air-dried. The dried gel pieces were swollen for 10 min in 0.1 ml of 10 mM DTT and 50 mM ammonium bicarbonate, and incubated for 1 hr at 56°C. They were dehydrated with acetonitrile as above, swollen in 50 mM iodoacetamide and 50 mM ammonium bicarbonate, and incubated at room temperature for 45 min. Excess DTT and iodoacetamide were washed out by washing twice in 0.1 ml of 50 mM ammonium bicarbonate, and dehydrated again with acetonitrile. To the dried gel pieces, 2 to 4 µl of 16 µg/ml trypsin (proteomics sequencing grade, Sigma-Aldrich, Tokyo, Japan) in 10 mM ammonium bicarbonate was added. After the trypsin solution were completely absorbed into gel pieces, minimum amount of 25 mM ammonium bicarbonate was added to cover the gel pieces, and the gel pieces were incubated for 16 hr at 37°C in an air incubator. The tryptic peptides were extracted twice with 40 µl of acetonitrile/water/trifluoroacetic acid (66:33:0.1, by volume) in a sonicator for 5 min. The gel

pieces were finally dehydrated with 30 µl of acetonitrile for 15 min, which was recovered and combined with the extract. The combined extracts were dried for 2 hr at 50°C in a vacuum centrifuge.

Mass spectra data of tryptic peptides was obtained by using a MALDI-TOF MS (AXIMA-CFR, Shimadzu Biotech, Kyoto, Japan) in the positive ion reflectron mode. μ -cyano-4-hydroxycinnamic acid (99% grade, Sigma-Aldrich) was used as a matrix throughout the experiment. The mass axis was calibrated using the following authentic peptides (Sigma-Aldrich) as external standards: bradykinin 1–7 with monoisotopic mass of 757.3997; angiotensin II of 1046.5423; P₁₄R (synthetic peptide) of 1533.8582; human ACTH (18–39) of 2465.1989. The mass spectra were processed with Kompact version 2.3.2 in order to obtain the accurate monoisotopic peaks. The blank gel experiments were performed to remove those masses derived from trypsin, matrix, known contaminants (typically keratins) and other unknown contaminants. The corrected mass lists were used as peptide mass fingerprinting data for search in databases. Mascot search engine (Matrix Science, Tokyo, Japan) was used to identify protein spots by searching in entries under the *Homo sapiens* category of SwissProt-nr and NCBI-nr databases using the assumptions that peptides are monoisotopic, partially oxidized at methionine residue, and carbamidomethylated at cysteine residues. Up to 1 missed trypsin cleavage was allowed. Mass tolerance of 0.2 to 0.4 Da for monoisotopic data was set to the window of error allowed for matching the peptide mass values. Protein matches with probability value less than 0.05 as indicated by probability-based MOWSE value provided from MASCOT were considered significant. Identities of protein spots that did not reach this significant level were not reported in this study.

RESULTS

We have performed a 2-DE-based proteomic analysis of normal human kidney tissues. The kidney tissues with normal appearance, dissected far from tumor mass, were subjected to histological examinations, and only those with no pathologic manifestation were used in the present study. For profiling and quantitative analysis of proteome of the cortex, medulla, and glomerulus, protein extract from respective compartments obtained from 4 normal subjects were subjected to a large format-2-DE separation followed by a 2-DE gel image analysis carried out with PDQuest, a 2-DE gel image analysis software.

With the assist of PDQuest, valid spots were first detected in 4 individual 2-DE gels and converged to create a synthetic 2-DE gel image by careful matching of valid spots. The synthetic 2-DE gel image of respective kidney tissues were shown in Figures, 1, 2, and 3. As shown in Table 1, we detected 1,810, 1,758, and 1,275 valid spots in the glomerulus, cortex, and medulla, respectively. The

higher number of valid spots in the synthetic 2-DE gel images of the glomerulus and the cortex than that of the medulla could be attributed to the increased number of valid spots both in the acidic and alkaline area and in the higher molecular area above 40 kDa (data not shown). Quantitative analysis of expression levels of valid spots revealed a great number of proteins differentially expressed among the three tissues: 616 protein spots in the glomerulus, 582 in the cortex, and 469 in the medulla were found to be expressed in significantly higher amounts in the respective compartments (Table 1).

Among those differentially expressed protein spots, we selected protein spots preferentially expressed in one of three compartments for identification by peptide mass fingerprinting method with a MALDI-TOF MS (see "Materials and Methods" section). In Table 2, summarized were identified protein spots preferentially expressed in each of the three compartments. Spot density values in the range of 7 to 15 ppm represented the background level of 2-DE gels indicating no valid spot or a trace amount of spot in the gel. Table 2, therefore, indicates that a considerable number of protein spots almost exclusively expressed in one of the

compartments are included in the identified protein list. Identified spots are marked in the corresponding synthetic gel image as shown in Figures, 1, 2, and 3.

DISCUSSION

Because of daunting difficulty in obtaining normal kidney tissues, we used tissues obtained from patients who underwent surgical nephrectomy due to renal tumor. We excised kidney tissues with normal appearance, far from tumor mass, and only the tissues with no pathologic lesions at light microscopic examinations were used for 2-DE analysis. It should be noted that the normal tissues as defined above were histologically normal, but showed minimal age-related changes due to the advanced age of patients ranging from 38 to 64 year-old. Thus, proteome of kidney tissues we analyzed obviously included age-related alterations. We are now analyzing proteins differentially expressed in the kidney tissues of aged subjects and will submit age-related changes to complement the results obtained in the present study.

Silver-staining has been widely used method for visualization of proteins separated on 2-DE gels, and still takes

Fig. 1. Synthetic 2-DE gel image of the glomerulus of normal human kidney.

The synthetic gel image was created from 2-DE gel images of protein extracts of highly purified glomeruli preparations obtained from 4 normal subjects by using a PDQuest 2-DE image analysis software as described in the "Materials and Methods" section. The protein spots preferentially expressed as defined in the "Materials and Methods" section are marked with crosshairs, among which spots with four digits number (SSP number assigned by PDQuest) are identified spots. Marker proteins used as external standard proteins are encircled.

Fig. 2. Synthetic 2-DE gel image of the cortex of normal human kidney.

The synthetic gel image was created from 2-DE gel images of protein extracts of cortex obtained from 4 normal subjects by using a PDQuest 2-DE image analysis software as described in the "Materials and Methods" section. The protein spots preferentially expressed (see the legend to Fig. 1) are marked with crosshairs, among which spots with four digits number (SSP number assigned by PDQuest) are identified spots. Marker proteins used as external standard proteins are encircled.

Table 1. Summary of 2-DE analysis of normal human kidney

Compartments	Valid spots	Protein spots differentially expressed ^a	Protein spots preferentially expressed ^b	Identified protein spots ^c
Glomerulus	1,810	616	204	19 (18)
Cortex	1,758	582	112	35 (29)
Medulla	1,275	469	80	21 (18)

^a Protein spots expressed in significantly higher amounts in one of the three kidney compartments.

^b Protein spots expressed in preferentially higher amounts in one of the three kidney compartments as defined in the "Materials and Methods" section.

^c Number of the identified protein spots of the protein spots preferentially expressed in the respective compartments. The number in the parenthesis indicate the number of unique proteins since some proteins are identified in multiple spots.

the major place in proteomics with the advent of silver-staining method compatible with MS analysis. It has high sensitivity enough to detect protein below ng order, but has several shortcomings; low dynamic range and preferential staining of proteins with some chemical features⁵). The quantitative data we obtained in the present study, thus, should be interpreted with cautions. With the low dynamic range and the relatively lower linearity of silver staining, large difference in quantity of a protein spot among differ-

ent tissues could lead to inaccurate estimate. Therefore, one should note that our quantitative data is not accurate estimate of difference in protein spot amount, but rather semi quantitative and rough estimate. In addition, several drawbacks intrinsic to 2-DE should be considered that hinder one for detecting certain types of proteins including proteins of low abundance, hydrophobic proteins, and proteins at the extreme of molecular size and acidic or basic isoelectric points.

Fig. 3. Synthetic 2-DE gel image of the medulla of normal human kidney.

The synthetic gel image was created from 2-DE gel images of protein extracts of medulla obtained from 4 normal subjects by using a PDQuest 2-DE image analysis software as described in the “Materials and methods” section. The protein spots preferentially expressed (see the legend to Fig. 1) are marked with crosshairs, among which spots with four digits number (SSP number assigned by PDQuest) are identified spots. Marker proteins used as external standard proteins are encircled.

Table 2. Identified protein spots

SSP No. ^b	Protein name	Accession No.	Expression level ^a (ppm)		
			Glomerulus ^c	Cortex ^c	Medulla ^c
<i>Protein spots preferentially expressed in the glomerulus^d</i>					
0112	Clathrin light chain A	P09496	9723.60 ± 6301.00	14.78 ± 6.53	14.13 ± 4.55
0117	Myosin light chain, alkali, non muscle form	P16475	12172.78 ± 6992.30	14.78 ± 6.53	14.13 ± 4.55
1003	Tumor rejection antigen 1	P14625	13402.50 ± 26771.10	14.78 ± 799.61	1865.28 ± 4.55
1029	Vinculin (Metavinculin)	P18206	3958.28 ± 3882.20	14.78 ± 6.53	14.13 ± 4.55
1424	Actin, cytoplasmic 1 (Beta-actin)	P60709	43871.00 ± 22537.40	3667.32 ± 2341.26	14.13 ± 4.55
1428	Actin, cytoplasmic 1 (Beta-actin)	P60709	26324.76 ± 13808.40	14.78 ± 6.53	14.13 ± 4.55
3104	ATP synthase D chain	O75947	132528.38 ± 37959.00	11288.98 ± 10986.12	12466.38 ± 18791.07
3106	USB/UBX 33.3 kDa protein	Q04323	64425.96 ± 61005.20	3983.90 ± 5281.31	14.13 ± 4.55
2112	Hypothetical protein FLJ13612	NP_079478	117296.06 ± 79492.30	3604.80 ± 3370.10	14.13 ± 4.55
2119	Alpha-actinin 4	O43707	6947.04 ± 4253.30	14.78 ± 6.50	14.13 ± 4.55
2229	Beta-actin	P02570	35661.52 ± 2458.50	1228.12 ± 1785.69	2164.63 ± 2351.24
2305	Alpha-actin 1	P02568	16581.72 ± 13764.80	14.78 ± 6.53	14.13 ± 4.55
3317	Gamma-actin	NP_001605	6267.44 ± 44628.80	14.78 ± 6.53	14.13 ± 4.55
4217	Protein disulfide isomerase A3	P30101	8788.00 ± 5637.20	14.78 ± 6.53	14.13 ± 4.55
4611	Proteasome activator complex subunit 1	Q06323	4115.70 ± 2761.90	14.78 ± 6.53	14.13 ± 4.55
5205	Gamma-actin	P02571	5166.06 ± 4790.50	14.78 ± 6.53	14.13 ± 4.55
7509	NG5 protein	NP_085154	14498.06 ± 5306.80	14.78 ± 6.53	14.13 ± 4.55

Table 2. (Continued)

SSP No. ^b	Protein name	Accession No.	Expression level ^a (ppm)		
			Glomerulus ^c	Cortex ^c	Medulla ^c
8015	Large conductance calcium-activated potassium channel	NP_002238	18253.22 ± 13475.00	14.78 ± 6.53	14.13 ± 4.55
8406	Peptidyl-prolyl cis-trans isomerase A	P05092	58127.32 ± 28648.70	1660.10 ± 2334.56	14.13 ± 4.55
<i>Protein spots preferentially expressed in the cortex^c</i>					
0218	Arginase 1 (Liver-type arginase).	P05089	7.18 ± 7.52	13432.30 ± 11378.83	14.13 ± 4.55
0603	Alpha-1-antitrypsin precursor (Alpha-1 protease inhibitor)	P01009	7.18 ± 7.52	13401.44 ± 9898.18	14.13 ± 4.55
1705	glucose-regulated protein (GRP 78)	P11021	7.18 ± 7.52	9307.16 ± 5988.69	14.13 ± 4.55
1803	Protein disulfide isomerase	P07237	7.18 ± 7.52	7929.76 ± 4757.84	14.13 ± 4.55
2007	1-acyl-sn-glycerol-3-phosphate acyltransferase alpha	Q99943	7.18 ± 7.52	6311.72 ± 4802.89	14.13 ± 4.55
2605	Acyl-CoA dehydrogenase family member 8, mitochondrial	Q9UKU7	7.18 ± 7.52	5736.22 ± 3690.90	14.13 ± 4.55
2716	Keratin, type I cytoskeletal 10 (Cytokeratin 10)	P13645	7.18 ± 7.52	5670.44 ± 1141.73	14.13 ± 4.55
2721	Serum albumin (ALBU)	P02768	7.18 ± 7.52	8838.30 ± 3295.74	14.13 ± 4.55
3806	Serum albumin (ALBU)	P02768	7.18 ± 7.52	36512.51 ± 8957.28	14.13 ± 4.55
3713	Serum albumin (ALBU)	P02768	7.18 ± 7.52	13763.48 ± 9983.66	14.13 ± 4.55
4709	Serum albumin (ALBU)	P02768	7.18 ± 7.52	44266.56 ± 42759.64	14.13 ± 4.55
2824	Vacuolar ATP synthase subunit B, kidney isoform	P15313	7.18 ± 7.52	12266.00 ± 12503.38	14.13 ± 4.55
2925	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	P28331	848.96 ± 1310.11	12086.26 ± 7046.78	14.13 ± 4.55
3128	Selenoprotein N	Q9NZV5	7.18 ± 7.52	3013.92 ± 2543.91	14.13 ± 4.55
3213	Keratin, type II cytoskeletal 1 (Cytokeratin 1) (K2C1)	P04264	7.18 ± 7.52	7625.74 ± 7492.97	14.13 ± 4.55
6609	Keratin, type II cytoskeletal 1 (Cytokeratin 1) (K2C1)	P04264	7.18 ± 7.52	13480.68 ± 10276.40	14.13 ± 4.55
7008	Keratin, type II cytoskeletal 1 (Cytokeratin 1)(K2C1)	P04264	7.18 ± 7.52	71087.48 ± 3322.72	14.13 ± 4.55
3214	Rho-GTPase-activating protein 6(RHG6)	O43182	7.18 ± 7.52	3263.76 ± 2139.35	14.13 ± 4.55
3516	Lipopolysaccharide-binding protein (LBP)	P18428	7.18 ± 7.52	48031.54 ± 3936.82	14.13 ± 4.55
3524	type I cytoskeletal 10 (K1CJ)	P13645	7.18 ± 7.52	5733.06 ± 2232.86	14.13 ± 4.55
3610	Kinesin light chain 1 (KLC 1)	Q07866	7.18 ± 7.52	32685.32 ± 3457.31	14.13 ± 4.55
3718	Delta-sarcoglycan (35 kDa dystrophin-associated glycoprotein)	Q92629	7.18 ± 7.52	24431.86 ± 15513.88	14.13 ± 4.55
3807	Variant glutamate formiminotransferase; Formimidoyltransferase-cyclodeamin	O95954	7.18 ± 7.52	8559.82 ± 8957.29	14.13 ± 4.55
4225	DNA mismatch repair protein Msh2	P43246	7.18 ± 7.52	5548.88 ± 3425.88	14.13 ± 4.55
4513	DNA mismatch repair protein Msh2	P43246	7.18 ± 7.52	5345.88 ± 4760.65	14.13 ± 4.55
4630	EF-hand domain-containing protein 2 (Swiprosin 1)	Q96C19	7.18 ± 7.52	5813.62 ± 4089.88	14.13 ± 4.55
5325	Myosin heavy chain, skeletal muscle, fetal (Myosin heavy chain IIb) (MyHC-IIb)	Q9Y623	7.18 ± 7.52	33622.76 ± 20998.59	14.13 ± 4.55
5721	Acyl-CoA dehydrogenase, medium-chain specific, mitochondrial	P11310	7.18 ± 7.52	13742.10 ± 6379.13	14.13 ± 4.55
6506	Upstream stimulatory factor 2 (Upstream transcription factor 2)	Q15853	7.18 ± 7.52	12313.50 ± 12494.17	14.13 ± 4.55
6719	Endonuclease III-like protein 1(NTH1_HUMAN)	P78549	7.18 ± 7.52	7574.12 ± 7434.17	14.13 ± 4.55
7430	Kelch-like ECH-associated protein 1 (Cytosolic inhibitor of Nrf2)	Q14145	7.18 ± 7.52	14039.14 ± 4791.08	14.13 ± 4.55
7610	Pantothenate kinase 2, mitochondrial	Q9BZ23	7.18 ± 7.52	15225.88 ± 4956.39	14.13 ± 4.55
7703	Argininosuccinate synthase (Citrulline— aspartate ligase)	P00966	7.18 ± 7.52	26613.54 ± 32643.66	14.13 ± 4.55
7610	Pantothenate kinase 2, mitochondrial	Q9BZ23	7.18 ± 7.52	15225.88 ± 4956.39	14.13 ± 4.55

Table 2. (Continued)

SSP No. ^b	Protein name	Accession No.	Expression level ^a (ppm)		
			Glomerulus ^c	Cortex ^c	Medulla ^c
7703	Argininosuccinate synthase(Citrulline— aspartate ligase).	P00966	7.18 ± 7.52	26613.54 ± 32643.66	14.13 ± 4.55
7824	Actin, cytoplasmic 2 (Gamma-actin)	P02571	774.7 ± 652.31	4820.12 ± 7644.96	14.13 ± 4.55
8417	Zinc finger protein 135	P52742	7.18 ± 7.52	10934.34 ± 8462.67	14.13 ± 4.55
<i>Protein spot preferentially expressed in the medulla^c</i>					
0719	Type I cytoskeletal 16	P08779	458.34 ± 628.38	14.78 ± 6.53	3804.50 ± 4015.80
0805	Protein disulfide isomerase (PDI)	P07237	3140.42 ± 2576.90	14.78 ± 6.53	16690.58 ± 9040.16
0808	COL3A1 protein	AAH28178	3816.74 ± 2640.92	4098.36 ± 6055.39	46474.75 ± 37051.82
1409	MHC class II antigen	AAB52237	7.18 ± 7.52	1698.80 ± 2395.05	14766.03 ± 11148.79
1619	Serine hydrolase-like protein (SEHL)	Q9H4I8	7.18 ± 7.52	14.78 ± 6.53	8704.40 ± 8222.71
1903	Brain-specific angiogenesis inhibitor 1 BAI1	O14514	995.88 ± 1066.24	14.78 ± 6.53	20009.55 ± 15713.90
7333	hypothetical protein	CAD97896	7.18 ± 7.52	14.78 ± 6.53	5274.10 ± 2133.79
4907	Serotransferrin (Transferrin)	P02787	3399.7 ± 4157.97	4525.72 ± 9444.80	24639.08 ± 5920.58
4913	Serotransferrin (Transferrin)	P02787	7147.14 ± 6833.37	14.78 ± 6.53	48411.68 ± 32499.32
4910	Serum albumin	P02768	158.34 ± 238.55	2357.80 ± 2949.65	14105.20 ± 6250.18
3908	Serum albumin	P02768	6232.64 ± 9431.45	14.78 ± 6.53	47971.73 ± 37315.47
3819	Wingless-type MMTV integration site family, member 11	NP_004617	7.18 ± 7.52	685.40 ± 1141.36	5300.05 ± 3664.88
2602	ACTB protein	AAH12854	7.18 ± 7.52	14.78 ± 6.53	87664.10 ± 69444.48
7904	Myeloid differentiation primary response gene	NP_002459	7.18 ± 7.52	3082.48 ± 4674.14	16401.10 ± 11915.57
1115	Tudor domain containing protein 4.	Q9NUY9	1232.98 ± 1866.36	14.78 ± 6.53	9836.63 ± 5533.87
2014	Chain A, crystal structure of the Apo Sh2 domains of Zap-70	1M61_A	7.18 ± 7.52	3428.78 ± 4883.77	22532.48 ± 23364.58
6509	Potassium voltage-gated channel subfamily KQT member 1(CIQ1)	P51787	222.38 ± 479.31	3873.20 ± 6256.83	32954.65 ± 33212.89
6705	Alpha enolase (Phosphopyruvate hydratase)	P06733	7.18 ± 7.52	14.78 ± 6.53	26380.78 ± 17401.59
6812	Spectrin beta chain, brain 3 (Spectrin, non-erythroid beta chain 3)	Q9H254	4025.88 ± 2299.23	14.78 ± 6.53	22324.98 ± 8283.20
7910	Protein-glutamine gamma-glutamyltrans- ferase 3-like	O95932	936.22 ± 1097.62	14.78 ± 6.53	7412.53 ± 6912.84
8338	Myosin heavy chain, cardiac muscle beta isoform	P12883	7.18 ± 7.52	14.78 ± 6.53	10318.88 ± 4323.08

^a Spot density measured by a 2-DE gel image analysis software PDQuest. The values represents the mean±S.D of 4 2-DE gels.

^b The spot number assigned automatically by the PDQuest.

^c Expression levels falling in the range of 7 to 15 ppm represented very weak or background levels of the spots in 2-DE gels of the corresponding tissues indicating that no valid spot or trace amount of spot were detected in the gels.

^d Protein spots expressed in considerably higher relative amounts in one of the three kidney compartments as defined in the “Materials and Methods” section.

Despite these limitations mentioned above, our quantitative proteomic analysis provided more detailed profile of proteins expressed in the three major functionally distinct compartments of normal human kidney. Witzman et al.⁶⁾ compared cytosolic proteins from the cortex and the medulla of rat kidney by 2-DE-based proteomic analysis. They resolved 727 protein spots in cortex, and 716 proteins in medulla, and found that 127 proteins were differentially expressed in the two tissues, of which 30 protein were found to be unique to one of the two tissues. They identified 20 protein spot representing 14 unique proteins. Arthur et al.⁷⁾ performed 2-DE based proteomic analysis on differentially protein expression in the whole extract of the cortex

and the medulla of normal rat kidney. They resolved a total of 1,095 protein spots in cortex and 885 in the medulla. They identified 72 protein spots representing 54 unique proteins, of which 16 protein spots were differentially expressed in the two kidney tissues. We have expanded these finding by using human kidney and large-format 2-DE separation, and more extensive quantitative analysis. Furthermore, our results would complement the comprehensive 2-DE-based database of proteome of the glomerulus of normal human kidney we have recently deposited to a Web site which will be soon available to researchers⁴⁾. The present result and the proteome database of the glomerulus, taken together, would contribute to understanding

physiological and pathophysiological processes of human kidney. Very recently, Higgins and his colleagues have conducted detailed analysis of differential gene expression in the glomerulus, inner and outer cortex, inner and outer medulla, papillary tips and renal pelvis of normal human kidney by using cDNA microarray with 41,859 unique cDNAs on it⁸⁾. They selected 1,548 genes on the basis of higher stringent algorithm and reported their expression variations among the different kidney tissues. The comprehensive dataset of regional differences in expression of mRNAs in couple with the comprehensive proteomic profiling of protein expression in the kidney would obviously serve as valuable resource for basic and clinical research of the kidney.

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