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Toxicomics Report

Gene expression profiles of immortalized S1, S2, and S3 cells derived from each segment of mouse kidney proximal tubules

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ABSTRACT — Kidney proximal tubules are composed of S1, S2, and S3 segments having different properties of excretion and substance reabsorption. Since renal toxicants such as cadmium and cisplatin cause segment-specific toxicity, it is important to examine the segment-specific transport and detoxification systems of renal toxicants. Here, we investigated the gene expression profiles of immortalized S1, S2, and S3 cells derived from each segment of mouse kidney proximal tubules. Microarray analyses showed distinct expression of various genes in each cell line. We compared the expression levels of selected genes related to the transport and detoxification of renal toxicants. Some genes showed segment-specific expression patterns similar to those observed in *in vivo* studies. The gene expression profiles of each cell line shown in this study will provide a foundation for the future utilization of immortalized S1, S2, and S3 cells for toxicity screenings as well as for the elucidation of renal toxicity mechanisms.

Key words: Kidney, Proximal tubule, Segment, Microarray, Gene expression

INTRODUCTION

Kidney proximal tubules play important roles in the excretion and reabsorption of essential and non-essential substances. The proximal tubules, located just downstream of the glomerulus, are composed of the S1 and S2 segments and are also called convoluted proximal tubules. The S3 segment is straight and connects to Henle's loop. From a toxicological viewpoint, kidney is an important target organ for drugs, metals, and environmental pollutants. The impairment of functional excretion and reabsorption in the proximal tubules results in disturbed systemic pharmacokinetics of drugs and dysregulated homeostasis of essential nutrients. Some renal toxicants have been reported to show distinct renal accumulation and toxicity specific to the different proximal tubule segments. For example, cadmium (Cd) and potassium dichromate have been known to cause renal damage especially in segments S1 and S2 (Dorian *et al*., 1995; Cristofori *et al*., 2007), while cisplatin causes renal damage especially in segment S3 (Dobyan *et al*., 1980). However, because of the kidney's structural complexity, it is difficult to investigate segment-specific transport and toxicity of renal toxicants in an *in vivo* system. To understand the molecular mechanisms underlying segment-specific toxicity and transport of drugs and metals, cultured epithelial cells from each kidney proximal tubule segment are required.

In earlier studies, Dr. Endou and coworkers developed immortalized cell lines derived from several parts of mouse kidney (Hosoyamada *et al*., 1996; Takeda *et al*., 1995; Takeda *et al*., 1998). They established immortalized cells derived from the S1, S2, and S3 segments, hereafter called S1, S2, and S3 cells, and showed that S3 cells have a high sensitivity to cisplatin (Hosoyamada *et al*., 1996), suggesting that these cell lines may be useful for the study of segment-specific toxicity of renal toxicants. Until now, however, no systematic characterization of these cells has

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been performed. To elucidate the fundamental characteristics of these invaluable cell lines, here we investigated the gene expression profiles of S1, S2, and S3 cells.

MATERIALS AND METHODS

Cell culture

S1, S2, and S3 cells were kindly provided by Prof. Nobuhiko Anzai (Chiba University, Japan). The cells were cultured in Dulbecco's modified Eagle's medium/Ham's F12 Nutrient Mixture supplemented with 5% fetal bovine serum (FBS), 1 μg/mL insulin, 10 ng/mL epidermal growth factor, 10 μg/mL transferrin, penicillin, and streptomycin under 5% CO_2 at 37°C.

Microarray analysis

RNA samples for microarray analysis were prepared as reported previously (Matsumoto *et al*., 2010). Total RNA was extracted and purified from S1, S2, and S3 cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany). The RNA samples were submitted to the Agilent microarray analysis service (Hokkaido System Science, Sapporo, Japan). The array (SurePrint G3 Mouse, 8x60K, ver. 2.0, Agilent Technologies, Santa Clara, CA, USA) contained 27,122 genes.

RESULTS AND DISCUSSION

The results of microarray analyses showed distinct gene expression profiles among S1, S2, and S3 cells. When the expression levels of whole genes in S3 cells were compared with those of S1 cells, 146 genes showed > 50-fold higher expression while 156 genes showed < 1/50-fold lower expression. Similarly, the comparisons between S1 and S2 cells, and those between S2 and S3 cells, showed large differences in the expression of various genes. Therefore, we focused on selected genes that play important roles in the functions of proximal tubules and those that may affect the toxicity and transport of well-known renal toxicants. Table 1 shows the expression levels of several genes in S2 and S3 cells relative to those in S1 cells. The selected genes include those for major transporters and adhesion molecules, as well as those involved in kidney-specific functions such as megalin and cubilin for endocytosis, Na-dependent phosphate transporters (NPTs), vitamin D hydroxylase (CYP27B1), and sodium-dependent glucose transporter 2 (SGLT2).

Immunohistochemical studies in animal experiments have shown the segment-specific expression of some proteins in the kidney proximal tubules. For example, OCT1 (organic cation transporter 1) is expressed mainly in S1 and S2 segments (Karbach *et al*., 2000), while SNAT3 is highly expressed in the S3 segment (Cristofori *et al*., 2007). As shown in Table 1, the expression levels of OCT1 were higher in S1 and S2 cells than in S3 cells, and the expression level of SNAT3 was higher in S3 cells than in S1 and S2 cells. These data suggest that, as far as these marker proteins are concerned, S1, S2, and S3 cells may maintain the properties of the original segments. Western blot analyses are required in a future study to prove the segment-specific expression of these markers at the protein level.

Since proximal tubule epithelial cells maintain polarity *in vivo* and distinct segment-specific expression of claudin proteins has been reported (Kiuchi-Saishin *et al*., 2002), we examined the expression levels of some adhesion proteins for tight-junction formation. As shown in Table 1, the expression levels of E-cadherin, occludin, and ZO-1 were higher in S1 and S2 cells than in S3 cells (Table 1).

Recently, novel biomarkers for renal dysfunction, such as Kim-1 (kidney injury molecule-1) and clusterin, have been used to assess renal damage (Gautier *et al*., 2010; McDuffie *et al*., 2013). Since the expression levels of Kim-1 and clusterin in renal cells are enhanced upon renal injury, we examined the basal expression levels of Kim-1 and clusterin in these cells. As shown in Table 1, Kim-1 showed the highest expression in S3 cells at the basal level, while clusterin showed no clear differences among the three cells.

Megalin and cubilin play major roles in endocytosis especially in the S1 and S2 segments (Christensen *et al*., 2009). Unexpectedly, however, the expression level of megalin was highest in S3 cells, whereas cubilin did not show such a difference among the three cells. Western blot analyses are required in a future study to test whether the protein levels of these proteins reflect the mRNA levels.

Since the kidney is the target organ of several metals, including Cd, we examined the expression levels of metallothionein (MT) and metal transporters involved in Cd transport. The basal expression levels of MT1 and MT2 were higher in S3 cells than in S1 or S2 cells. Although the reason for the high expression of MTs in S3 cells is unknown, no report has compared the basal expression levels of MT among the proximal tubule segments. Among the zinc transporters involved in Cd transport, Zrt- and Irt-like protein 8 (ZIP8) showed higher expression in S3 cells than in S1 or S2 cells. We previously showed that ZIP8 is highly expressed in the proximal tubules of the outer stripe of the outer medulla, where the S3 segments of proximal tubules are located (Fujishiro *et al*., 2012). The expression levels of ZIP14 and DMT1 (divalent met-

Table 1. Expression levels of selected genes in S1, S2, and S3 cells.

			S1	S2	S3
Gene symbol	Synonyms	Accession number		ratio*	
Abcc1	MRP1	NM_008576	1.00	0.89	0.54
Abcc2	MRP2	NM_013806	1.00	0.90	0.85
Cdh1	E-Cadherin	NM_009864	1.00	0.68	0.00
Cldn16	PCLN1	NM_053241	1.00	0.23	0.16
Clu	clusterin	NM_013492	1.00	0.91	0.87
Cubn	cubilin	NM_001081084	1.00	0.70	0.91
Cyp27b1	CYP27B1	NM_010009	1.00	0.72	1.67
Havcr1	Kim1	NM_001166631	1.00	3.51	12.84
Lrp2	megalin	NM_001081088	1.00	0.59	7.71
Mt1	MT1	XM_006530752	1.00	1.42	6.48
Mt2	MT2	NM_008630	1.00	0.85	3.39
Ocln	occludin	NM_008756	1.00	0.75	0.02
Slc3a2	CD98	NM_001161413	1.00	1.01	0.82
Slc5a2	SGLT2	NM_133254	1.00	1.55	0.77
Slc7a11	xCT	NM_011990	1.00	0.50	1.14
Slc11a2	DMT1	NM_008732	1.00	0.99	1.06
Slc22a1	OCT1	NM_009202	1.00	1.09	0.64
Slc22a12	URAT1	NM_009203	1.00	0.94	0.39
Slc22a13	OAT3	NM_133980	1.00	1.37	1.17
Slc22a2	OCT2	NM_013667	1.00	0.90	0.85
Slc22a7	OAT2	NM_144856	1.00	0.90	0.86
Slc22a8	OAT1	NM_031194	1.00	0.90	1.39
Slc31a1	CTR1	NM_175090	1.00	1.20	1.49
Slc34a1	NPT2a	NM_011392	1.00	1.08	0.74
Slc34a2	NPT2b	NM_011402	1.00	0.65	0.62
Slc34a3	NPT2c	NM_080854	1.00	0.90	0.86
Slc38a3	SNAT3	NM_023805	1.00	1.10	4.89
Slc39a14	ZIP14	NM_001135151	1.00	0.56	0.50
Slc39a8	ZIP8	NM_026228	1.00	2.93	6.95
Slc47a1	MATE1	NM_026183	1.00	14.51	3.71
Tfrc	TfR1	NM_011638	1.00	0.54	0.41
Tjp1	ZO-1	NM_009386	1.00	1.06	0.77
Trpm7	TRPM7	NM_021450	1.00	1.06	1.17
Trpv6	CaT1	NM_022413	1.00	1.54	0.12

*Ratio of gene expression level to that in S1 cells.

al transporter 1) were also consistent with our previous observations in mouse kidney using *in situ* hybridization (Fujishiro *et al*., 2012).

Table 2 shows the expression levels of selected genes related to the transport of renal toxicants and the proteins related to protection against oxidative stress. Some genes for transporters and detoxification proteins show differences in expression levels among S1, S2, or S3 cells. Indeed, further studies are required to confirm the differences in expression levels at the protein level.

In the present study, we investigated the expression levels of selected genes among S1, S2, and S3 cells derived from each proximal tubule segment of mouse kidney. Several genes, such as OCT1, SNAT3, and ZIP8, clearly showed segment-specific expression patterns similar to those observed in *in vivo* histochemistry or *in situ* hybridization studies. However, further characterization of other genes is required. Several studies have attempted to utilize primary cultured cells derived from each segment (Helbert *et al*., 1997; Kamiyama *et al*., 2012). However, primary cultured segment-specific proximal tubule cells may not be suitable for toxicological studies because of the difficulty of ensuring the reproducibility of the experimental data. Due to their stability and data reproducibility, immortalized S1, S2, and S3 cells may comprise a useful tool for mechanistic studies to elucidate the segment-spe-

Table 2. Expression levels in S1, S2 and S3 cells of the genes related to toxicants transporters and oxidative stress.

			S1	S2	S3
Gene Symbol	Accession number	Definition		ratio*	
1. Transporters and Channels					
1-1. ZnT family					
Slc30a1	NM_009579	solute carrier family 30 (zinc transporter), member 1	1.00	0.53	2.41
Slc30a2	NM_001039677	solute carrier family 30 (zinc transporter), member 2	1.00	0.97	0.90
Slc30a3	NM_011773	solute carrier family 30 (zinc transporter), member 3	1.00	0.91	0.87
Slc30a4	NM_011774	solute carrier family 30 (zinc transporter), member 4	1.00	0.93	0.92
Slc30a5	NM_022885	solute carrier family 30 (zinc transporter), member 5	1.00	0.93	0.73
Slc30a6	NM_001252478	solute carrier family 30 (zinc transporter), member 6	1.00	0.98	0.92
Slc30a7	NM_023214	solute carrier family 30 (zinc transporter), member 7	1.00	0.86	1.34
Slc30a8	NM_172816	solute carrier family 30 (zinc transporter), member 8	1.00	1.42	0.89
Slc30a9	NM_178651	solute carrier family 30 (zinc transporter), member 9	1.00	1.17	1.14
Slc30a10	NM_001033286	solute carrier family 30, member 10	1.00	0.90	0.86
1-2. Na/phosphate cotransporter					
Slc34a1	NM_011392	solute carrier family 34 (sodium phosphate), member 1	1.00	1.08	0.74
Slc34a2	NM_011402	solute carrier family 34 (sodium phosphate), member 2	1.00	0.65	0.62
Slc34a3	NM_080854	solute carrier family 34 (sodium phosphate), member 3	1.00	0.90	0.86
1-3. ZIP family					
Slc39a1	NM_013901	solute carrier family 39 (zinc transporter), member 1	1.00	0.85	1.56
Slc39a2	NM_001039676	solute carrier family 39 (zinc transporter), member 2	1.00	0.97	1.28
Slc39a3	NM_134135	solute carrier family 39 (zinc transporter), member 3	1.00	1.14	1.07
Slc39a4	NM_028064	solute carrier family 39 (zinc transporter), member 4	1.00	6.58	1.52
Slc39a5	NM_028051	solute carrier family 39 (metal ion transporter), member 5	1.00	4.17	2.79
Slc39a6	NM_139143	solute carrier family 39 (metal ion transporter), member 6	1.00	0.66	1.51
Slc39a7	NM_008202	solute carrier family 39 (zinc transporter), member 7	1.00	1.00	0.75
Slc39a8	NM_026228	solute carrier family 39 (metal ion transporter), member 8	1.00	2.93	6.95
Slc39a9	AK014732	solute carrier family 30 (zinc transporter), member 9	1.00	0.96	0.86
Slc39a10	NM_172653	solute carrier family 39 (zinc transporter), member 10	1.00	0.82	1.22
Slc39a11	NM_027216	solute carrier family 39 (metal ion transporter), member 11	1.00	0.64	0.61
Slc39a12	NM_001012305	solute carrier family 39 (zinc transporter), member 12	1.00	0.90	0.85
Slc39a13	NM_026721	solute carrier family 39 (metal ion transporter), member 13	1.00	1.03	0.45
Slc39a14	NM_001135151	solute carrier family 39 (zinc transporter), member 14	1.00	0.56	0.50
1-4. ABC family					
Abcc1	NM_008576	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	1.00	0.89	0.54
Abcc2	NM_013806	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	1.00	0.90	0.85
Abcc3	NM_029600	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	1.00	59.85	34.83
Abcc4	NM_001033336	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	1.00	1.12	0.96
Abcc5	NM_013790	ATP-binding cassette, sub-family C (CFTR/MRP), member 5	1.00	1.31	2.01
Abcc6	NM_018795	ATP-binding cassette, sub-family C (CFTR/MRP), member 6	1.00	0.96	0.94
Abcc8	NM_011510	ATP-binding cassette, sub-family C (CFTR/MRP), member 8	1.00	0.90	0.86
Abcc9	BC094922	ATP-binding cassette, sub-family C (CFTR/MRP), member 9	1.00	0.91	0.87
Abcc10	NM_145140	ATP-binding cassette, sub-family C (CFTR/MRP), member 10	1.00	0.91	1.06
Abcc12	NM_172912	ATP-binding cassette, sub-family C (CFTR/MRP), member 12	1.00	0.91	0.85
2. Cell adhesion related					
Cdh1	NM_009864	cadherin 1	1.00	0.68	0.004
Cdh2	NM_007664	cadherin 2	1.00	0.54	0.41
Ocln	NM_008756	occludin	1.00	0.75	0.02
Tjp1	NM_009386	tight junction protein 1	1.00	1.06	0.77
Cldn1	NM_016674	claudin 1	1.00	0.71	1.17
Cldn2	NM_016675	claudin 2	1.00	1.12	8.45
Cldn3	NM_009902	claudin 3	1.00	0.90	0.49

Table 2. (Continued).

			S1	S2	S3
Gene Symbol	Accession number	Definition		ratio*	
Cldn4	NM_009903	claudin 4	1.00	0.97	0.03
Cldn5	NM_013805	claudin 5	1.00	1.47	0.49
Cldn6	NM_018777	claudin 6	1.00	262.86	1.99
Cldn7	NM_016887	claudin 7	1.00	1.43	0.02
Cldn8	NM_018778	claudin 8	1.00	10.90	0.85
Cldn9	NM_020293	claudin 9	1.00	2.70	1.15
Cldn10	NM_021386	claudin 10	1.00	3.05	3.41
Cldn11	NM_008770	claudin 11	1.00	2.38	1.34
Cldn12	NM_001193659	claudin 12	1.00	0.95	1.03
Cldn13	NM_020504	claudin 13	1.00	0.75	0.95
Cldn14	NM_019500	claudin 14	1.00	1.36	1.27
Cldn15	NM_021719	claudin 15	1.00	0.96	0.93
Cldn16	NM_053241	claudin 16	1.00	0.23	0.16
Cldn17	NM_181490	claudin 17	1.00	0.88	0.42
Cldn18	NM_001194922	claudin 18	1.00	0.93	1.00
Cldn19	NM_001038590	claudin 19	1.00	0.80	3.12
3. Oxidative stress protection					
Mt1	XM_006530752	metallothionein 1	1.00	1.42	6.48
Mt2	NM_008630	metallothionein 2	1.00	0.85	3.39
Nfe2l2	NM_010902	nuclear factor, erythroid derived 2, like 2	1.00	1.21	1.54
Keap1	NM_016679	kelch-like ECH-associated protein 1	1.00	1.16	0.96
Cul1	NM_012042	cullin 1	1.00	1.14	0.82
Hmox1	NM_010442	heme oxygenase 1	1.00	0.53	0.38
Sod1	NM_011434	superoxide dismutase 1, soluble	1.00	1.06	1.73
Sod2	NM_013671	superoxide dismutase 2, mitochondrial	1.00	1.09	0.94
Sod3	NM_011435	superoxide dismutase 3, extracellular	1.00	0.44	2.07
Gsta2	NM_008182	glutathione S-transferase, alpha 2 (Yc2)	1.00	0.53	0.13
Gsta3	NM_001077353	glutathione S-transferase, alpha 3	1.00	11.80	23.81
Gsta3	NM_001288617	glutathione S-transferase, alpha 3	1.00	4.85	9.21
Gsta4	NM_010357	glutathione S-transferase, alpha 4	1.00	1.46	5.93
Gstcd	NM_026231	glutathione S-transferase, C-terminal domain containing	1.00	0.89	0.92
Gstk1	NM_029555	glutathione S-transferase kappa 1	1.00	3.24	1.95
Gstm1	NM_010358	glutathione S-transferase, mu 1	1.00	1.81	5.68
Gstm2	NM_008183	glutathione S-transferase, mu 2	1.00	6.71	9.79
Gstm3	NM_010359	glutathione S-transferase, mu 3	1.00	1.72	5.41
Gstm4	NM_026764	glutathione S-transferase, mu 4	1.00	2.32	5.71
Gstm5	NM_010360	glutathione S-transferase, mu 5	1.00	0.62	2.42
Gstm5	NM_010360	glutathione S-transferase, mu 5	1.00	0.62	2.45
Gstm6	NM_008184	glutathione S-transferase, mu 6	1.00	3.58	6.37
Gstm7	NM_026672	glutathione S-transferase, mu 7	1.00	1.20	8.50
Gsto1	NM_010362	glutathione S-transferase omega 1	1.00	1.79	0.45
Gsto2	NM_026619	glutathione S-transferase omega 2	1.00	1.09	4.81
Gstp1	NM_013541	glutathione S-transferase, pi 1	1.00	1.43	1.74
Gstp2	NM_181796	glutathione S-transferase, pi 2	1.00	1.43	1.63
Gstt1	NM_008185	glutathione S-transferase, theta 1	1.00	62.48	24.14
Gstt2	NM_010361	glutathione S-transferase, theta 2	1.00	30.17	15.46
Gstt3	NM_133994	glutathione S-transferase, theta 3	1.00	39.30	7.15
Gstt4	NM_029472	glutathione S-transferase, theta 4	1.00	2.38	1.68
Gstz1	NM_010363	glutathione transferase zeta 1	1.00	0.92	0.79
Nqo1	NM_008706	NAD(P)H dehydrogenase, quinone 1	1.00	1.13	0.33

Table 2. (Continued).

			S1	S2	S3
Gene Symbol	Accession number	Definition		ratio*	
Nox1	NM_172203	NADPH oxidase 1	1.00	1.16	1.17
Noxo1	NM_027988	NADPH oxidase organizer 1	1.00	1.80	1.23
Rac1	NM_009007	RAS-related C3 botulinum substrate 1	1.00	0.81	0.87
Gpx1	NM_008160	glutathione peroxidase 1	1.00	0.56	0.54
Gpx2	NM_030677	glutathione peroxidase 2	1.00	1.27	0.86
Gpx3	NM_008161	glutathione peroxidase 3	1.00	0.27	971.56
Gpx4	NM_001037741	glutathione peroxidase 4	1.00	1.14	0.80
Gpx5	NM_010343	glutathione peroxidase 5	1.00	1.33	0.87
Gpx6	NM_145451	glutathione peroxidase 6	1.00	1.15	0.86
Gpx7	NM_024198	glutathione peroxidase 7	1.00	0.90	0.86
Gpx8	NM_027127	glutathione peroxidase 8	1.00	0.94	0.41
Glrx	NM_053108	glutaredoxin	1.00	1.17	1.20
Glrx2	NM_001038592	glutaredoxin 2	1.00	0.96	0.88
Glrx3	NM_023140	glutaredoxin 3	1.00	0.58	0.54
Glrx5	NM_028419	glutaredoxin 5	1.00	0.64	0.53
Cat	NM_009804	catalase	1.00	1.34	1.29
Txn1	NM_011660	thioredoxin 1	1.00	0.62	0.52
Txn2	NM_019913	thioredoxin 2	1.00	0.93	0.95
Txnrd1	NM_001042523	thioredoxin reductase 1	1.00	0.97	0.58
Prdx1	NM_011034	peroxiredoxin 1	1.00	0.81	0.92
Prdx1	NM_011034	peroxiredoxin 1	1.00	0.74	0.87
Prdx2	NM_011563	peroxiredoxin 2	1.00	0.96	0.65
Prdx3	NM_007452	peroxiredoxin 3	1.00	1.07	1.16
Prdx4	NM_016764	peroxiredoxin 4	1.00	0.41	0.89
Prdx5	NM_012021	peroxiredoxin 5	1.00	2.16	0.85
Prdx6	NM_001303408	peroxiredoxin 6	1.00	0.90	0.74
Prdx6b	NM_177256	peroxiredoxin 6B	1.00	1.81	1.13
Gclc	NM_010295	glutamate-cysteine ligase, catalytic subunit	1.00	1.45	3.12
Gclm	NM_008129	glutamate-cysteine ligase, modifier subunit	1.00	0.88	1.12

*Ratio of gene expression level to that in S1 cells.

cific toxicity and transport of various renal toxicants. The gene expression profiles shown in this study will help to validate these cells for toxicological studies.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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