Global Gene Expression Profiling in PPAR-γ Agonist-Treated Kidneys in an Orthologous Rat Model of Human Autosomal Recessive Polycystic Kidney Disease

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Kidneys are enlarged by aberrant proliferation of tubule epithelial cells leading to the formation of numerous cysts, nephron loss, and interstitial fibrosis in polycystic kidney disease (PKD). Pioglitazone (PIO), a PPAR-γ agonist, decreased cell proliferation, interstitial fibrosis, and inflammation, and ameliorated PKD progression in PCK rats (Am. J. Physiol.-Renal, 2011). To explore genetic mechanisms involved, changes in global gene expression were analyzed. By Gene Set Enrichment Analysis of 30655 genes, 13 of the top 20 downregulated gene ontology biological process gene sets and six of the top 20 curated gene set canonical pathways identified to be downregulated by PIO treatment were related to cell cycle and proliferation, including EGF, PDGF and JNK pathways. Their relevant pathways were identified using the Kyoto Encyclopedia of Gene and Genomes database. Stearoyl-coenzyme A desaturase 1 is a key enzyme in fatty acid metabolism found in the top 5 genes downregulated by PIO treatment. Immunohistochemical analysis revealed that the gene product of this enzyme was highly expressed in PCK kidneys and decreased by PIO. These data show that PIO alters the expression of genes involved in cell cycle progression, cell proliferation, and fatty acid metabolism.

1. Introduction

Polycystic kidney diseases (PKD) are characterized by progressive enlargement of numerous fluid-filled cysts in both kidneys, often leading to chronic kidney disease (CKD). Autosomal dominant PKD (ADPKD) is one of the most common hereditary disorders in humans with an incidence of 1:500–1,000, caused by mutations in the PKD1 or PKD2 gene. Progressive kidney enlargement is due to aberrant proliferation of the cystic epithelia, together with an accumulation of fluid into the cyst cavities due to transepithelial chloride (Cl⁻) and fluid secretion [1–3]. Autosomal recessive PKD (ARPKD) is known as a juvenile-type cystic disease with an incidence of 1:20,000 [3]. Kidneys in ARPKD patients are characterized by cystic fusiform dilations of the collecting ducts accompanied by increased cell proliferation and fluid secretion, leading to massive kidney enlargement and renal failure occurring in the first few years after birth [4]. Increased cell proliferation, stimulated fluid secretion, and interstitial fibrosis are often observed in cystic liver disease in ARPKD as well [5].
Peroxisome proliferator-activated receptors (PPARs) belong to a nuclear receptor superfamily of ligand-activated transcription factors with subtypes α, β/δ, and γ. PPAR-γ is widely expressed in several organs including kidneys and known to be activated by fatty acids [6, 7]. Antidiabetic agents, pioglitazone (PIO), troglitazone, ciglitazone, and rosiglitazone, are used to control blood sugar levels in patients with diabetes mellitus. These PPAR-γ agonists also have important roles in regulation of cell cycle, inhibition of fibrosis, infiltration and metastasis of cancer cells, and modulation of inflammatory cytokines.

Treatment with PIO improved survival and ameliorated cardiac defects and the degree of renal cystogenesis in embryos of Pkd1−/− mice in a previous study [8]. In addition, long-term treatment of this agonist improved endothelial function by increasing production of nitric oxide in adult heterozygous Pkd1+/− mice [8]. Another PPAR-γ agonist, rosiglitazone attenuated PKD progression and prolonged survival of Han:SPRD Cy rats [9]. In our recent study, daily treatment of PIO ameliorated polycystic kidney disease through inhibiting Raf/MEK/ERK and AKT/mTOR/S6 signaling cascades in the PCK rat, an orthologous model of human ARPKD [10]. These findings suggest that PPAR-γ agonists may have therapeutic value in ARPKD via altering several cellular signaling pathways. In the current study, we applied global gene expression profiling to explore novel cellular signaling pathways potentially related to the ameliorating effects of PIO in PCK rat kidneys.

2. Methods

2.1. PCK Rat and Study Design. PCK rats were originally derived from a strain of Sprague-Dawley rats in Japan and descendants of this colony have been maintained at the Education and Research Center of Animal Models for Human Diseases, Fujita Health University. PCK rats and normal Sprague Dawley rats (+/+; Charles River Japan Inc., Kanagawa, Japan) were allowed free access to water and food throughout the study. Female PCK and +/+ rats, aged 4–20 weeks (n = 10 per gender) were randomly assigned to one of two groups: treatment with 10 mg/kg PIO (Takeda Pharmaceutical Company Limited, Osaka, Japan) or vehicle control (0.5% DMSO) by gavage every day as previously reported [10]. The protocol for the ethics and use of these animals was approved by the Animal Care and Use Committee at Fujita Health University.

At 20 weeks of age, rats were anesthetized with sodium pentobarbital (Schering-Plough Corp., Kenilworth, NJ), and the kidneys were removed rapidly, causing lethal exsanguination. Half of the left kidney was frozen in liquid nitrogen for RNA extraction. Half of the right kidney was immersed in 4% paraformaldehyde, embedded in paraffin, and sectioned for immunohistochemistry.

2.2. RNA Extraction. RNA was extracted from kidneys of rats with or without PIO treatment using a monophase solution of phenol/guanidine isothiocyanate and TRIzol reagent (Invitrogen Co., Carlsbad, CA, USA) in accordance with their manual, and the samples were incubated with RNase-free DNase I (Ambion, TX, USA). The quality and concentration of each sample was confirmed by spectrophotometry (NanoDrop ND-1000; Asahi glass Co. Ltd., Tokyo, Japan). Total RNA obtained from three females was pooled in each PIO-treated or control vehicle-treated (CONT) group in accordance with our previous report [11].

2.3. Microarrays. DNA microarray experiments were performed essentially as described previously [11]. Briefly, 500 ng aliquots of total RNA obtained from kidneys of five rats were labeled using a Quick Amp Labeling Kit, one-color (Agilent Technologies, Inc., Santa Clara, CA, USA), according to the manufacturer’s instructions. The pooled renal RNA of PIO- or vehicle-treated PCK rats were labeled with the Cy3-fluorescence dye. After determination of labeling efficiency, 1.65 µg aliquots of Cy3-labeled RNA were hybridized using the Gene Expression hybridization kit (Agilent Technologies) onto Rat Oligo Microarrays (Agilent Technologies, product no. G4130A) according to the manufacturer’s hybridization protocol. The microarray slides were examined with an Agilent microarray scanner and software. Data analysis was performed with Agilent Feature Extraction software (version A.7.1.1).

Data from microarray experiments of PIO- or vehicle-treated rats were analyzed independently. Primary microarray data are available from the Gene Expression Omnibus (GEO) (accession number GSE00000). Evaluation of signal intensity was divided into three classes, {0: nondetected, {1: weakly detected, and {2: strongly detected} transcription product. Gene ontology analysis of biological process (C5BP) and curated gene sets of canonical pathways (C2CP) were analyzed by importing the data into Gene Set Enrichment Analysis (GSEA version 2, the Broad Institute/Massachusetts Institute of technology, USA) [12].

Using the GeneSpring software, the changed probes were listed as “Log2 ratio was over 1 (over 2-fold) or less than −1 (less than 1/2-fold) between PIO group and CONT group” and “the signal evaluation was {2} (strongly detected) in both groups”. In the changed genes, Kyoto Encyclopedia of Gene and Genomes (KEGG) analysis was used [13].

2.4. Real-Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). cDNA was produced from total RNA by reverse transcriptase using random hexamer primers (SuperScript II First Strand Synthesis System; Invitrogen Co., Carlsbad, CA, USA). To compare gene expression patterns of PCK kidneys with PIO or vehicle treatment, we selected a key enzyme in fatty acid metabolism, stearoyl-coenzyme A desaturase 1 (Scd1), and uncoupling protein 1 (Ucp1). Gene expression was detected by real-time RT-PCR (ABI 7300 real-time PCR system; Applied Biosystems, Foster City, CA, USA) using the TaqMan reagent-based chemistry protocol. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a housekeeping gene was used for data normalization. The probes of Scd1, UCP-1, and GAPDH were CCCCATGCTCCTAAGAGATCTCCAG, CTCTCTAGGAGAGAAACGCTGCC, and AACCCAT-CACCCTCTCCAGAGCG, respectively (TaqMan Gene
Table 1: (a) Top 20 downregulated gene ontology biological process gene sets in PIO-treated compared with vehicle-treated kidneys. The name of biological process is listed in order of nominal P value. (b) Upregulated gene ontology biological process gene sets in PIO-treated compared with vehicle-treated kidneys. The name of biological process is listed in order of nominal P value.

(a) Name of biological process gene sets | Number of genes in the gene set | Nominal P value
---|---|---
Defense response | 98 | 0.000
Regulation of cell proliferation | 136 | 0.000
Cell cycle phase | 53 | 0.000
Positive regulation of cell proliferation | 64 | 0.000
Cell cycle process | 61 | 0.000
Positive regulation of cellular process | 258 | 0.000
Cellular morphogenesis during differentiation | 22 | 0.001
Positive regulation of developmental process | 91 | 0.001
Immune system process | 128 | 0.001
Cellular defense response | 19 | 0.003
Neuron differentiation | 35 | 0.004
Negative regulation of cell proliferation | 73 | 0.004
Neurite development | 27 | 0.005
G Protein signaling coupled to ip3 second messenger phospholipase C activating | 22 | 0.005
Inflammatory response | 56 | 0.005
Regulation of response to stimulus | 15 | 0.006
Neuron development | 30 | 0.007
M phase | 27 | 0.007
Interphase | 29 | 0.008
Axonogenesis | 21 | 0.009

(b) Name of biological process gene sets | Number of genes in the gene set | Nominal P value
---|---|---
Nitrogen compound catabolic process | 17 | 0.000
Amine catabolic process | 15 | 0.000
Amino acid metabolic process | 46 | 0.000
Amino acid and derivative metabolic process | 58 | 0.000
Organic acid metabolic process | 106 | 0.000
Carboxylic acid metabolic process | 104 | 0.022

Expression Assays; Applied Biosystems). Relative quantification of gene expression was compared to one in SD control vehicle-treated (CONT) kidneys (set to 1.0).

2.5. Immunohistochemistry: Kidney sections were fixed, embedded, and sectioned for immunoreaction as described previously [10, 11]. Sections were incubated with Scd1 antibody (1:250 ab19862 Abcam, Cambridge, UK) in PBS containing 1% BSA plus 0.05% NaN₃ overnight at 4°C. To test for a specific Scd1 immunoreaction in the kidney, mouse IgG2b, κ isotype control antibody (1:200 400323 BioLegend, San Diego, CA), was used. Sections were incubated with secondary antibody Histofine MAX-PO (MULTI: for anti-mouse/rabbit IgG, IgA, and IgM) obtained from Nichirei Biosciences (Tokyo, Japan). Immune reaction products were developed using 3,3′-diaminobenzidine (ENVISION kit HRP Dako Cytomation K3466, Dako Japan Inc., Tokyo, Japan).

2.6. Statistical Analysis. Results are expressed as the arithmetic mean ± standard error. Statistical comparisons between groups were performed by Student’s t-test and two-way analysis of variance, and differences were considered to be significant at P < 0.05.

3. Results

3.1. Identification of Differentially Expressed Genes by Expression Profiling. Previous report indicates that PPAR-γ agonistic action decreases expression of endothelin receptor type A (EDNRA) [14], suggesting that EDNRA is one of the downstream target gene of PPAR-γ agonists. In our current study, expression of Ednra was also downregulated in PIO-treated kidneys (Log2 ratio = −1.30). EDNRA expression is increased in human ADPKD, and overexpression of Ednra causes cyst formation in transgenic mouse kidneys [15]. Because not only EDNRA but also various genes
may be influenced by PPAR-γ agonistic actions, it became intriguing to determine the expression of other potential gene targets of PIO in PCK rat kidneys. 30,655 of 43,379 probes yielded detectable signals in both PIO- and vehicle-treated kidneys of PCK rats. The 11,809 genes represented by these 30,655 probes were analyzed by GSEA. In gene ontology analysis of biological process (C5BP) gene sets, 334 were formed from these 11,809 genes. 293 of those 334 gene sets were downregulated in PIO-treated kidneys compared with vehicle-treated kidneys, of which 77 were significantly different ($P < 0.05$, Table 1(a)). In the top 20 downregulated C5BP gene sets with the greatest significant differences, 13 were related to cell proliferation, cell cycle, morphogenesis, differentiation, and development, and 4 gene sets were related to cellular defense and inflammation. On the other hand, 41 of the 334 gene sets were upregulated in PIO-treated

Table 2: (a) Downregulated gene ontology biological process gene sets from genes with greater than 1.25-fold changes in PIO-treated compared with vehicle-treated kidneys. The name of biological process is listed in order of nominal $P$ value. (b) Common genes found in cell cycle or cell proliferation gene sets downregulated by PIO treatment. ✓: listed gene in the gene set, —: non-listed gene in the gene set. (c) Upregulated gene ontology biological process gene sets with greater than 1.25-fold changes in PIO-treated compared with vehicle-treated kidneys.

(a)

| Name of biological process gene sets                                           | Number of genes in the gene set | Nominal $P$ value |
|-------------------------------------------------------------------------------|---------------------------------|-----------------|
| Carbohydrate METABOLIC PROCESS                                               | 16                              | 0.019           |
| Cell proliferation GO 0008283                                                 | 70                              | 0.024           |
| Organelle organization and biogenesis                                         | 34                              | 0.025           |
| Cell cycle GO 0007049                                                        | 44                              | 0.027           |
| Negative regulation of cell proliferation                                     | 28                              | 0.032           |
| Cell cycle process                                                            | 31                              | 0.037           |

(b)

| Gene symbol | Description                                      | Cell cycle GO 0007049 | Negative regulation of cell proliferation | Cell cycle process | Cell proliferation GO 0008283 |
|-------------|--------------------------------------------------|-----------------------|------------------------------------------|-------------------|-----------------------------|
| Brc2        | Breast cancer 2                                  | ✓                     | ✓                                        | ✓                 | ✓                           |
| Cdkn2b      | Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4) | ✓                     | ✓                                        | ✓                 | ✓                           |
| Chek1       | CHK1 checkpoint homolog                          | ✓                     | ✓                                        | ✓                 | —                           |
| BUB1B       | Cell cycle checkpoint protein kinase Bub1 fragment | ✓                     | ✓                                        | —                 | ✓                           |
| PLK1        | Polo-like kinase 1                               | ✓                     | ✓                                        | —                 | ✓                           |
| Cdkn1c      | Cyclin-dependent kinase inhibitor 1C             | ✓                     | ✓                                        | ✓                 | —                           |
| Cul5        | Cullin 5                                         | ✓                     | ✓                                        | ✓                 | ✓                           |
| Tgfβ2       | Transforming growth factor, beta 2               | ✓                     | —                                        | ✓                 | ✓                           |
| Bcat1       | Branched chain aminotransferase 1               | ✓                     | ✓                                        | —                 | ✓                           |
| PTPRC       | Protein tyrosine phosphatase, receptor type, C   | ✓                     | ✓                                        | —                 | ✓                           |
| POLA1       | Polymerase (DNA directed), alpha 1               | ✓                     | ✓                                        | —                 | ✓                           |

(c)

| Name of biological process gene sets                                           | Number of genes in the gene set | Nominal $P$ value |
|-------------------------------------------------------------------------------|---------------------------------|-----------------|
| Neurological System Process                                                  | 40                              | 0.032           |
kidneys compared with vehicle-treated kidneys, of which 6 were significantly different \((P < 0.05, \text{Table 1}(b))\). These gene sets were related to catabolic and metabolic processes.

To examine the gene sets with the greatest changes, only 2,611 genes, which changed more than 1.25-fold in PIO-treated kidneys compared to vehicle-treated kidneys, were analyzed. 141 gene sets were formed from these 2,611 genes. 112 of those 141 gene sets were downregulated in PIO-treated kidneys compared with vehicle-treated kidneys, only one, related to neurological system processes, was significantly elevated \((P < 0.05, \text{Table 2}(c))\). In curated gene sets of canonical pathways (C2CP), 257 were formed from the 11,809 genes detected. 201 of these 257 gene sets were downregulated in PIO-treated kidneys compared with vehicle-treated kidneys, of which 33 were significantly lower \((P < 0.05, \text{Table 2}(a))\). 4 of these 6 gene sets are related to cell cycle and cell proliferation \((P < 0.05, \text{Table 2}(a))\). Common genes in these gene sets include G1/S or G2/M checkpoint related genes, breast cancer 2 \((\text{Brc}a2)\), cyclin-dependent kinase inhibitor 2B \((\text{Cdkn}2b)\), CHK1 checkpoint homolog \((\text{Chek}1)\), cell cycle checkpoint protein kinase Bub1 fragment \((\text{Bub}1\text{B})\), pololike kinase 1 \((\text{PLK}1)\), and cyclin-dependent kinase inhibitor 1C \((\text{Cdkn}1c)\) \((P < 0.05)\) \((\text{Table 2}(b))\). Of the remaining 29 of the 141 gene sets that were upregulated in PIO-treated kidneys compared with vehicle-treated kidneys, only one, related to neurological system processes, was significantly elevated \((P < 0.05)\) \((\text{Table 2}(c))\).

In curated gene sets of canonical pathways (C2CP), 257 were formed from the 11,809 genes detected. 201 of these 257 gene sets were downregulated in PIO-treated kidneys compared with vehicle-treated kidneys, of which 33 were significantly lower \((P < 0.05)\). From the 20 downregulated C2CP gene sets with the highest significant differences \((P < 0.05)\), 6 gene sets were related to cell cycle and cell proliferation including c-Jun N-terminal kinase \((\text{JNK})\), epidermal growth factor \((\text{EGF})\), and platelet-derived growth factor \((\text{PDGF})\) pathways, and 3 gene sets were related to inflammatory signals including interleukin-1 receptor \((\text{IL1R})\) and interleukin-6 \((\text{IL6})\) pathways \((\text{Table 3}(a))\). One gene set, extracellular matrix (ECM) receptor interaction, also was in the top 20 downregulated in C2CP. On the other hand, 56 of 257 gene sets were upregulated in PIO-treated kidneys compared with vehicle-treated kidneys, of which 5 gene sets were significantly higher \((P < 0.05, \text{Table 3}(b))\). 3 of these 5 gene sets are related to glutamate, alanine, and aspartate metabolism.

**Table 3:** (a) Top 20 downregulated curated gene sets of canonical pathways in PIO-treated compared with vehicle-treated kidneys. The name of biological process is listed in order of nominal \(P\) value. (b) Upregulated curated gene sets of canonical pathways in PIO-treated compared with vehicle-treated kidneys. The name of biological process is listed in order of nominal \(P\) value.

| Name of biological process gene sets | Number of genes in the gene set | Nominal \(P\) value |
|-------------------------------------|-------------------------------|------------------|
| HSA04640 hematopoietic cell lineage  | 32                            | 0.000            |
| HSA04610 complement and coagulation cascades | 37 | 0.000 |
| HSA04510 focal adhesion             | 110                           | 0.001            |
| Breast cancer estrogen signaling    | 60                            | 0.001            |
| HSA04060 cytokine cytokine receptor interaction | 99 | 0.002 |
| HSA04912 GNRH Signaling Pathway     | 64                            | 0.002            |
| HSA04110 cell cycle                 | 44                            | 0.002            |
| HSA01430 cell communication         | 39                            | 0.004            |
| IL1R pathway                        | 15                            | 0.007            |
| Eicosanoid synthesis                | 15                            | 0.009            |
| HSA04512 ECM receptor interaction   | 41                            | 0.009            |
| Cell cycle KEGG                     | 34                            | 0.012            |
| ST JNK MAPK pathway                 | 17                            | 0.017            |
| EGF pathway                         | 23                            | 0.023            |
| PDGF pathway                        | 23                            | 0.028            |
| FCER1 pathway                       | 26                            | 0.029            |
| GSK3 pathway                        | 18                            | 0.029            |
| Prostaglandin and leukotriene metabolism | 19 | 0.032 |
| IL6 pathway                         | 17                            | 0.032            |
| HSA02010 ABC transporters general  | 21                            | 0.033            |

| Name of biological process gene sets | Number of genes in the gene set | Nominal \(P\) value |
|-------------------------------------|-------------------------------|------------------|
| HSA00190 oxidative phosphorylation | 37                            | 0.000            |
| Glutamate metabolism                | 15                            | 0.000            |
| HSA00252 alanine and aspartate metabolism | 17 | 0.010 |
| HSA00710 carbon fixation            | 15                            | 0.019            |
| HSA00251 glutamate metabolism       | 17                            | 0.019            |

### 3.2. Cellular Expression and Distribution of Scd1 in Rodent Polycystic Kidneys

For Scd1 and Ucp1, in order to confirm the mRNA expression by DNA microarray screening above, real-time RT-PCR analysis was performed. The mRNA level of Scd1 in the kidney was increased in PCK rats compared...
Table 4: (a) Downregulated genes in PIO-treated compared with vehicle-treated (CONT) kidneys of PCK rats. The name of gene symbol was listed in order of PIO/CONT Log2 ratio. (b) Upregulated genes in PIO-treated compared with vehicle-treated (CONT) kidneys of PCK rats. The name of gene symbol was listed in order of PIO/CONT Log2 ratio.

| Gene symbol | Description | KEGG pathway | PIO/CONT Log2 ratio |
|-------------|-------------|--------------|---------------------|
| *Olr1436*   | Olfactory receptor 1436 | Olfactory transduction | −3.27 |
| *Xylt1*     | Xylosyltransferase 1 | Glycosaminoglycan biosynthesis-chondroitin sulfate/glycosaminoglycan biosynthesis-heparan sulfate/metabolic pathways | −3.03 |
| *Map3k10*   | Mixed-lineage kinase 2 | MAPK signaling pathway | −2.24 |
| *Icosl1*    | — | Cell adhesion molecules (CAMs)/intestinal immune network for IgA production | −2.15 |
| *Scl1*      | Stearoyl-coenzyme A desaturase 1 | Biosynthesis of unsaturated fatty acids/PPAR signaling pathway | −2.01 |
| *Ucp1*      | Uncoupling protein 1 | PPAR signaling pathway/Huntington’s disease | −1.91 |
| *Oxt*       | Oxytocin, prepropeptide | Neuroactive ligand-receptor interaction | −1.81 |
| *Chrm1*     | Cholinergic receptor, muscarinic 1 | Calcium signaling pathway/Neuroactive ligand-receptor interaction/regulation of actin cytoskeleton | −1.75 |
| *Avp*       | Arginine vasopressin | Neuroactive ligand-receptor interaction/vascular smooth muscle contraction/vasopressin-regulated water reabsorption | −1.58 |
| *Lpcat2*    | Lysophosphatidylcholine acyltransferase 2 | Glycerophospholipid metabolism/ether lipid metabolism/metabolic pathways | −1.37 |
| *Il12rb1*   | Interleukin 12 receptor, beta 1 | Cytokine-cytokine receptor interaction/jak-STAT signaling pathway | −1.34 |
| *EDNRA*     | Endothelin receptor type A | Calcium signaling pathway/neuroactive ligand-receptor interaction/vascular smooth muscle contraction | −1.30 |
| *Cfd*       | Complement factor D (adipsin) | Complement and coagulation cascades | −1.20 |
| *Serpinb5*  | Serine (or cysteine) peptidase inhibitor, clade B, member 5 | p53 signaling pathway | −1.19 |
| *Htr2b*     | 5-Hydroxytryptamine (serotonin) receptor 2B | Calcium signaling pathway/neuroactive ligand-receptor interaction/gap junction | −1.19 |
| *Cox8b*     | Cytochrome c oxidase, subunit VIIIb | Oxidative phosphorylation/metabolic pathways/cardiac muscle contraction/Alzheimer’s disease/Parkinson’s disease/Huntington’s disease | −1.17 |
| *Peg12*     | Paternally expressed 12 | Wnt signaling pathway | −1.11 |
| *Sema3d*    | Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3D | Axon guidance | −1.07 |
| *Atp1a2*    | ATPase, Na+/K+ transporting, alpha 2 polypeptide | Cardiac muscle contraction/aldosterone-regulated sodium reabsorption/proximal tubule bicarbonate reclamation/salivary secretion/gastric acid secretion | −1.05 |
| *Dll3*      | Delta-like 3 | Notch signaling pathway | −1.05 |
| *Bra2*      | Breast cancer 2 | Homologous recombination/pathways in cancer/pancreatic cancer | −1.04 |
| *Aqp4*      | Aquaporin 4 (Aqp4), transcript variant 2 | Vasopressin-regulated water reabsorption | −1.02 |
| *Gys2*      | Glycogen synthase 2 | Starch and sucrose metabolism/insulin signaling pathway | −1.01 |

KEGG pathway: Koto Encyclopedia of Gene and Genomes pathway.
### Gene symbol Description KEGG pathway P/O CONT Log 2 ratio

| Gene symbol | Description | KEGG pathway | P/O CONT Log 2 ratio |
|-------------|-------------|--------------|----------------------|
| GuC2d       | Guanylate cyclase 2d (Gucy2d) | Purine metabolism/olfactory transduction/phototransduction | 1.59 |
| Cyp2b1      | Cytochrome P450, family 2, subfamily b, polypeptide 1 (Cyp2b1), mRNA | Arachidonic acid metabolism/retinol metabolism/metabolism of xenobiotics by cytochrome P450/drug metabolism-cytochrome P450/metabolic pathways | 1.45 |
| Cyp2d3      | Cytochrome P450, family 2, subfamily d, polypeptide 3 (Cyp2d3) | Drug metabolism-cytochrome P450 | 1.20 |
| Tarsl2      | Threonyl-tRNA synthetase-like 2 (Tarsl2), mRNA | Aminoacyl-tRNA biosynthesis | 1.17 |
| Prl         | Prolactin (Prl), mRNA | Cytokine-cytokine receptor interaction/neuroactive ligand-receptor interaction/jak-STAT signaling pathway | 1.17 |
| Olr1331     | Olfactory receptor 1331 (Olr1331), mRNA | Olfactory transduction | 1.17 |
| Dynclh1     | Dynein cytoplasmic 1 heavy chain 1 (Dynclh1), mRNA | Phagosome/vasopressin-regulated water reabsorption | 1.11 |
| Olr297      | Olfactory receptor 297 (Olr297) | Olfactory transduction | 1.06 |

KEGG pathway: Koto Encyclopedia of Gene and Genomes pathway.

### 4. Discussion

In our previous report, we demonstrated that PIO treatment in PCK rats inhibited renal Raf/MEK/ERK and AKT/mTOR/S6 activity and reduced proliferation of diseased renal cells [10]. In the current study, we analyzed DNA microarray using GSEA and KEGG pathway analysis in order to detect gene-based effects of PIO treatment [12, 13]. The results of GSEA analysis of CSBP and C2CP are consistent with our previous findings, as a number of gene sets related to cell cycle and cell proliferation are downregulated in kidneys of PIO-treated PCK rats.

Both EGF and PDGF pathways were downregulated by PIO treatment (Table 3(a)). In PKD cystic epithelial cells, growth factors such as EGF and PDGF activate the Raf/MEK/ERK pathway via receptor binding and tyrosine kinase activation [19–21]. Therefore, PIO may ameliorate PKD in PCK rats by inhibiting cell proliferation through suppression of the activity of EGF and PDGF pathways. Further, in PKD patients, several reports show that cystic kidneys have significant levels of apoptosis [22, 23]. The JNK pathway is known to have critical roles in cell apoptosis, and JNK is overexpressed in cystic epithelial cells in Pkd1 conditional knockout mice [23, 24]. In the current study, the JNK MAPK pathway also was downregulated by PIO treatment. Therefore, PIO may have antiapoptotic effects via inactivation of the JNK pathway.

PIO, as well as other PPAR-γ agonists rosiglitazone and troglitazone, is known to induce cell cycle arrest and cell apoptosis in human cancer cells [25–27]. Although it has recently been reported that rosiglitazone inhibits cell proliferation by inducing G1 cell cycle arrest in ADPKD cyst-lining epithelial cells [28], the inhibitory mechanism of PIO is under studied in PKD. In the current analysis, Brca2, Bub1b, Cdkn1c, Cdkn2b, Chek1, and PLK1 were downregulated. These genes are involved in cell cycle regulation, G0/G1, G1/S and/or G2/M checkpoints [29–35], suggesting that the antiproliferative effect of PIO may be related to cell cycle arrest.

After searching each gene expression with significant change by PIO treatment, we then focused on Scd1 because it is known to stimulate cell proliferation in cancer cells through phosphorylation of AKT [16–18, one of the responsible kinases in cystic cell proliferation in PKD [10, 36]. Immunohistochemical analysis demonstrated that Scd1 expression was increased in noncystic tubules in PCK kidneys, and PIO treatment reduced its overexpression, suggesting that Scd1 may relate to the onset of cyst formation through phosphorylation of AKT. In addition, activation of the cell cycle increases syntheses of phospholipids and cholesterol [37–39], and Scd1 controls the balance of saturated and monounsaturated fatty acids,
Figure 1: Cellular expression and distribution of Scd1 in rodent polycystic kidneys. (a) Relative gene expression levels for Scd1. mRNA expression levels are shown for vehicle-treated (CONT) or PIO-treated SD and PCK kidneys as compared to vehicle-treated (CONT) SD kidneys (set to 1.0) (*P < 0.05 SD (CONT) versus PCK (CONT), #P < 0.05 PCK (CONT) versus PCK (PIO)). Expression levels were normalized to GAPDH. (b) Renal Scd1 distribution in vehicle-treated (CONT) or PIO-treated SD and PCK rats. Representative kidney sections from vehicle-treated (CONT) or PIO-treated SD and PCK rats were stained with an antibody to Scd1. Mouse IgG2b, κ isotype control antibody, did not show any reaction in the kidney. (c) Ratio of Scd1-positive cysts or noncystic tubules in kidney sections. Positive-stained cysts or non-cystic tubules were counted in five random fields of kidney sections obtained from five rats in each group by a naive observer using a 20x objective. (**P < 0.01 PCK (CONT) versus PCK (PIO) in noncystic tubules in the kidney section).

Regulating the composition of cholesterol esters and phospholipids in cell membrane structure [16]. Therefore, PIO may reduce cell proliferation by the downregulation of Scd1 gene expression not only through reducing AKT signaling activity but also through altering fatty acid synthesis. In abnormal cell proliferation in cancer, Scd1 expression is increased, and the cell proliferation is suppressed by treatment with PPAR-γ agonists, although the changes in Scd1 expression are not always consistent [16, 40, 41]. On the other hand, in diabetes mellitus with insulin resistance,
adipose tissue or skeletal muscle Scd1 expression is decreased and increased by PPAR-γ agonists [42–44]. Therefore, the expression level of Scd1 and the effect of PPAR-γ agonists may depend on the disease and/or the state of cell proliferation.

Clinically, increased body weight, oedema, and urinary bladder tumors are concerned as possible side effects of PPAR-γ agonists. Although those phenomena were not observed in both genders of PCK rats in the current PIO treatment, the effect of longer term treatment with different doses will need to be studied carefully. Since ameliorative effects are reported in several animal models of PKD [8–10, 45], PPAR-γ agonists are thought to be a potential candidate for therapeutic interventions in both ARPKD and ADPKD patients.

5. Conclusions

In the current study, PIO reduced PKD progression and altered the expression of renal genes involved in cell proliferation, cell cycle progression, and fatty acid metabolism in an orthologous rat model of human ARPKD. In addition to the previously demonstrated inhibition of Raf/MEK/ERK and AKT/mTOR/S6 signaling pathways by treatment of PCK rats with 10 mg/kg PIO for 16 weeks [10], suppression of cell proliferation may also be related to reductions in EGF, PDGF, and JNK pathways, cell cycle arrest related to Brca2, BUB1B, Cdkn1c, Cdkn2b, Chek1, and PLK1 genes, and alteration of fatty acid metabolism related to Scd1.

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References

[1] J. J. Grantham, S. Mulamalla, and K. I. Swenson-Fields, "Why kidneys fail in autosomal dominant polycystic kidney disease," Nature Reviews Nephrology, vol. 7, no. 10, pp. 556–566, 2011.
[2] V. E. Torres, P. C. Harris, and Y. Pirson, "Autosomal dominant polycystic kidney disease," Lancet, vol. 369, no. 9569, pp. 1287–1301, 2007.
[3] P. C. Harris, "2008 Homer W. Smith award: insights into the pathogenesis of polycystic kidney disease from gene discovery," Journal of the American Society of Nephrology, vol. 20, no. 6, pp. 1188–1198, 2009.
[4] W. E. Sweeney Jr and E. D. Avner, "Diagnosis and management of childhood polycystic kidney disease," Pediatric Nephrology, vol. 26, no. 5, pp. 675–692, 2011.
[5] B. Turkbey, I. Ocak, K. Daryanani et al., "Autosomal recessive polycystic kidney disease and congenital hepatic fibrosis (ARPKD/CHF)," Pediatric Radiology, vol. 39, no. 2, pp. 100–111, 2009.
[6] A. Yessoufou and W. Wahl, "Multifaceted roles of peroxisome proliferator-activated receptors (PPARs) at the cellular and whole organism levels," Swiss Medical Weekly, vol. 140, no. 13071, pp. 4–10, 2010.
[7] C. Noziger and B. L. Blazer-Yost, "PPARγ agonists, modulation of ion transporters, and fluid retention," Journal of the American Society of Nephrology, vol. 20, no. 12, pp. 2481–2483, 2009.
[8] S. Muto, A. Aiba, Y. Saito et al., "Pioglitazone improves the phenotype and molecular defects of a targeted Pkd1 mutant," Human Molecular Genetics, vol. 11, no. 15, pp. 1731–1742, 2002.
[9] B. Dai, Y. Liu, C. Mei et al., "Rosiglitazone attenuates development of polycystic kidney disease and prolongs survival in Han:SPRD rats," Clinical Science, vol. 119, no. 8, pp. 323–333, 2010.
[10] D. Yoshihara, H. Kurahashi, M. Morita et al., "PPAR-γ agonist ameliorates kidney and liver disease in an orthologous rat model of human autosomal recessive polycystic kidney disease," American Journal of Physiology, vol. 300, no. 2, pp. F465–F474, 2011.
[11] M. Kugita, K. Nishii, M. Morita et al., "Global gene expression profiling in early-stage polycystic kidney disease in the Han:SPRD Cyt rat identifies a role for RXR signaling," American Journal of Physiology, vol. 300, no. 1, pp. F177–F188, 2011.
[12] A. Subramanian, P. Tamayo, V. K. Mootha et al., "Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles," Proceedings of the National Academy of Sciences of the United States of America, vol. 102, no. 43, pp. 15545–15550, 2005.
[13] H. Ogata, S. Goto, K. Sato, W. Fujibuchi, H. Bono, and M. Kanehisa, "KEGG: kyoto encyclopedia of genes and genomes," Nucleic Acids Research, vol. 27, no. 1, pp. 29–34, 1999.
[14] W. Arozal, K. Watanabe, P. T. Veeraveedu et al., "Telmisartan prevents the progression of renal injury in daunorubicin rats with the alteration of angiotensin II and endothelin-1 receptor expression associated with its PPAR-γ agonist actions," Toxicology, vol. 279, no. 1–3, pp. 91–99, 2011.
[15] M. Y. Chang and A. C. Ong, "Endothelin in polycystic kidney disease," Contributions to Nephrology, vol. 172, pp. 200–209, 2011.
[16] R. A. Igal, "Stearoyl-coa desaturase-1: a novel key player in the mechanisms of cell proliferation, programmed cell death and transformation to cancer," Carcinogenesis, vol. 31, no. 9, pp. 1509–1515, 2010.
[17] D. Hess, J. W. Chisholm, and R. A. Igal, "Inhibition of stearoylCoA desaturase activity blocks cell cycle progression and induces programmed cell death in lung cancer cells," PloS One, vol. 5, no. 6, p. e11394, 2010.
[18] N. Scaglia, J. W. Chisholm, and R. A. Igal, "Inhibition of stearoylCoA desaturase-1 inactivates acetyl-CoA carboxylase and impairs proliferation in cancer cells: role of AMPK," PloS ONE, vol. 4, no. 8, Article ID e6812, 2009.
[19] T. Yamaguchi, S. Nagao, D. P. Wallace et al., "Cyclic AMP activates B-Raf and ERK in cyst epithelial cells from autosomal-dominant polycystic kidneys," Kidney International, vol. 63, no. 6, pp. 1983–1994, 2003.
[20] T. Nakamura, I. Ebihara, I. Nagaoka et al., "Growth factor gene expression in kidney of murine polycystic kidney disease," Kidney International, vol. 63, no. 2, pp. 427–437, 2003.
[22] O. Ibragimov-Beskrovnaya, “Targeting dysregulated cell cycle and apoptosis for polycystic kidney disease therapy,” Cell Cycle, vol. 6, no. 7, pp. 776–779, 2007.

[23] S. Nishio, M. Hatano, M. Nagata et al., “Pkd1 regulates immortalized proliferation of renal tubular epithelial cells through p53 induction and INK apoptosis pathway,” Journal of Clinical Investigation, vol. 115, no. 4, pp. 910–918, 2005.

[24] W. Yu, T. Kong, S. Beaudry et al., “Polycystin-1 protein level determines activity of the Gα-12/13/INK apoptosis pathway,” Journal of Biological Chemistry, vol. 285, no. 14, pp. 10243–10251, 2010.

[25] K. Yoshizawa, D. P. Cioca, S. Kawa, E. Tanaka, and K. Kiyosawa, “Peroxisome proliferator-activated receptor γ ligand troglitazone induces cell cycle arrest and apoptosis of hepatocellular carcinoma cell lines,” Cancer, vol. 95, no. 10, pp. 2243–2251, 2002.

[26] M. Ming, J. P. Yu, X. Z. Meng, Y. H. Zhou, H. G. Yu, and H. S. Luo, “Effect of ligand troglitazone on peroxisome proliferator-activated receptor γ expression and cellular growth in human colon cancer cells,” World Journal of Gastroenterology, vol. 12, no. 45, pp. 7263–7270, 2006.

[27] L. Cerqueti, C. Sampao, D. Amendola et al., “Rosiglitazone induces autophagy in H295R and cell cycle deregulation in SW13 adrenocortical cancer cells,” Experimental Cell Research, vol. 317, no. 10, pp. 1397–1410, 2011.

[28] Y. Liu, B. Dai, L. Fu, J. Jia, and C. Mei, “Rosiglitazone inhibits cell proliferation by inducing g1 cell cycle arrest and apoptosis in ADPKD cyst-lining epithelia cells,” Basic and Clinical Pharmacology and Toxicology, vol. 106, no. 6, pp. 523–530, 2010.

[29] E. Sagulenko, L. Savelyeva, V. Ehemann et al., “Suppression of polyploidy by the BRCA2 protein,” Cancer Letters, vol. 257, no. 1, pp. 65–72, 2007.

[30] J. W. Davenport, E. R. Fernandes, L. D. Harris, G. A. M. Neale, and R. Goorha, “The mouse mitotic checkpoint gene Bub1b, a novel Bub1 family member, is expressed in a cell cycle-dependent manner,” Genomics, vol. 35, no. 1, pp. 113–117, 1999.

[31] E. Myslinski, M. A. Gérard, A. Krol, and P. Carbon, “Transcription of the human cell cycle regulated BUB1B gene requires hStaf/ZNF143,” Nucleic Acids Research, vol. 35, no. 10, pp. 3453–3464, 2007.

[32] S. Sengupta, J. Nie, R. J. Wagner, C. Yang, R. Stewart, and J. A. Thomson, “MicroRNA 92b controls the G1/S checkpoint gene p57 in human embryonic stem cells,” Stem Cells, vol. 27, no. 7, pp. 1524–1528, 2009.

[33] Z. Ullah, C. de Renty, and M. L. DePamphilis, “Checkpoint kinase 1 prevents cell cycle exit linked to terminal cell differentiation,” Molecular and Cellular Biology, vol. 31, no. 19, pp. 4129–4143, 2011.

[34] D. T. Wu, M. Bitzer, W. Ju, P. Mundel, and E. P. Böttinger, “TGF-β concentration specifies differential signaling profiles of growth arrest/differentiation and apoptosis in podocytes,” Journal of the American Society of Nephrology, vol. 16, no. 11, pp. 3211–3221, 2005.

[35] Y. Degenhardt and T. Lampkin, “Targeting polo-like kinase in cancer therapy,” Clinical Cancer Research, vol. 16, no. 2, pp. 384–389, 2010.

[36] P. R. Wahl, M. L. Hir, A. Vogtseder et al., “Mitotic activation of Akt signalling pathway in HanSPRD rats with polycystic kidney disease,” Nephrology, vol. 12, no. 4, pp. 357–363, 2007.

[37] Z. Ullah, C. de Renty, and M. L. DePamphilis, “Cell cycle regulation of membrane phospholipid metabolism,” Journal of Biological Chemistry, vol. 271, no. 34, pp. 20219–20222, 1996.

[38] M. D. Siperstein, “Role of cholesterogenesis and isoprenoid synthesis in DNA replication and cell growth,” Journal of Lipid Research, vol. 25, no. 13, pp. 1462–1468, 1984.

[39] L. S. Goolman, M. Bakovic, and D. E. Vance, “Transcription of the CTP:Phosphocholine Cytidyltransferase α Gene Is Enhanced during the S Phase of the Cell Cycle,” Journal of Biological Chemistry, vol. 276, no. 47, pp. 43688–43692, 2001.

[40] H. Koga, K. Selvendiran, R. Sivakumar et al., “PPARγ potentiates anticancer effects of gemcitabine on human pancreatic cancer cells,” International Journal of Oncology, vol. 40, no. 3, pp. 679–685, 2012.

[41] S. Takano, T. Kubota, H. Nishibori et al., “Pioglitazone, a ligand for peroxisome proliferator-activated receptor-γ acts as an inhibitor of colon cancer liver metastasis,” Anticancer Research, vol. 28, no. 6, pp. 3593–3599, 2008.

[42] A. Yao-Borengasser, N. Rassouli, V. Varma et al., “Stearoyl-coenzyme A desaturase 1 gene expression increases after pioglitazone treatment and is associated with peroxisomal proliferator-activated receptor-γ responsiveness,” Journal of Clinical Endocrinology and Metabolism, vol. 93, no. 11, pp. 4431–4439, 2008.

[43] K. Mai, J. Andres, T. Bobbert et al., “Rosiglitazone increases fatty acid Δ9-desaturation and decreases elongase activity index in human skeletal muscle in vivo,” Metabolism, vol. 61, no. 1, pp. 108–116, 2012.

[44] J. Song, H. Liu, H. W. Ressom, S. Tiwari, and C. M. Ecelbarger, “Chronic rosiglitazone therapy normalizes expression of ACE1, SCD1 and other genes in the kidney of obese Zucker rats as determined by microarray analysis,” Experimental and Clinical Endocrinology and Diabetes, vol. 116, no. 6, pp. 315–325, 2008.

[45] B. L. Blazer-Yost, J. Haydon, T. Eggleston-Gulyas et al., “Pioglitazone attenuates cystic burden in the PCK rodent model of polycystic kidney disease,” PPAR Research, vol. 2010, Article ID 274376, 8 pages, 2010.