Activation of the JAK1 / STAT1 Signaling Pathway is Associated with Prdx6 Expression Levels in Human Epididymis Epithelial Cells

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Abstract

I. Background: Peroxiredoxin 6 (Prdx6) is widely expressed in mammalian tissues. Our previous study demonstrated that Prdx6 was expressed in human epididymis and spermatozoa, and the protective role of Prdx6 in human spermatozoa was also reported. In this study, we demonstrate the potential role and mechanism of Prdx6 in human epididymis epithelial cells (HEECs).

II. Methods and Results: Western blotting was used to measure expression levels of key proteins in the JAK / STAT signaling pathway. Digital gene expression analysis (DGE) was used to identify gene expression patterns in control HECs and in HECs after Prdx6-RNA interference (P6-RNAi). The DGE analysis identified 589 up-regulated and 314 down-regulated genes (including Prdx6) in Prdx6-RNAi (P6-RNAi) HEECs. Thirteen significantly different pathways were identified between the two groups, with the majority different expressed genes belonging to the *CCL*, *CXCL*, *IL*, and *IFIT* families. In particular, the expression levels of *IL6*, *IL6ST*, and eighteen *IFN* related genes were significantly increased in the condition of the down-regulated expression of *Prdx6*. Compared to control HEECs, the expression levels of JAK1, STAT1, phosphorylated JAK1 and STAT1 were significantly increased, while the expression levels of SOCS3 was significantly decreased in P6-RNAi HEECs. The Malondialdehyde (MDA) level and total [antioxidant](#) capacity in P6-RNAi HEECs were significantly increased and decreased compared to that of control, respectively.

III. Conclusions: We speculated that knockdown of *Prdx6* resulted in higher levels of ROS in HEECs, which in turn, activated the JAK1 / STAT1 signaling pathway induced by *IL-6* receptor and *IFN*.

Background

Peroxiredoxins (Prdxs) are antioxidant enzymes and are composed of six family members (Prdx1-6)[1]. Prdx1-5 contains 2-Cys, while Prdx6 contains only 1-Cys[2]. Prdx6 is an abundant cellular protein with peroxidase, phospholipase A2 (PLA2), and lysophospholipid acyl transferase (LPCAT) activity[3].

The primary function of Prdxs is to prevent oxidative damage induced by reactive oxygen species (ROS) [4]. The relationship between Prdxs, inflammation, and immunity has also been widely reported. Prdx1 act as an immunomodulator in macrophages and endothelial cells[5], and has been associated with the occurrence of atherosclerosis and rheumatoid arthritis[6]. Moon et al. (2006) demonstrated the inhibitory function of Prdx2 in immune cell responsiveness[7], while Szabó-Taylor et al. (2012) demonstrated the role of Prdx2 in chronic inflammation and the relationship between Prdx2 and the occurrence of rheumatoid arthritis[8]. Prdx3 is the only member that lacks an immunomodulatory function, and its deficiency has been shown to increase abnormal lipid accumulation in adipose tissue[9]. Prdx4 inhibits inflammation induced by oxidative stress in various tissues[10], and its molecular mechanism may be via the regulation of NF- κ B, which is an important pro-inflammatory transcription factor[11]. Prdx4 expression has also been shown to be associated with rheumatoid arthritis[12]. Prdx5 regulates inflammatory processes through the Trx system[13], while Prdx6 has been shown to be involved in

regulating cell proliferation, apoptosis, embryonic development, lipid metabolism, immune response, and osteogenic differentiation[14-21]. Furthermore, Prdx6 has been shown to activate the NF- κ B / AP-1 and JAK pathways necessary for the development of rheumatoid arthritis[22]. In brief, the Prdxs family, especially Prdx6, is associated with immunity.

Multiple developmental and immunological processes are regulated by the JAK / STAT signaling pathway[23]. There are four JAKs and seven STATs found in mammals. The JAK / STAT signaling pathway is triggered by the binding of extracellular ligands to cell surface receptors to activate receptor-associated JAKs; JAKs phosphorylate and activate cytoplasmic STAT dimers; phosphorylated STAT translocate to the nucleus and regulates target gene expression[24]. The suppressor of cytokine signaling (SOCS) proteins provides selective negative feedback to STAT activation to prevent over-stimulation of the immune system. Of the eight members, only SOCS1 and SOCS3 have been well studied. SOCS1 and SOCS3 have been shown to inhibit components of the JAK/STAT and other cell signaling pathways in a highly cell-type-specific manner[25-27].

Mammalian epididymis provides the critical environment for spermatozoa to develop motility and fertilization ability[28]. Our previous study showed that Prdx6 was expressed in the human epididymis[29], while Fernandez et al (2018) demonstrated that Prdx6 functioned in antioxidant defense in human spermatozoa[30]. In the current study, based on the role of Prdx6 in the immune response and the regulatory role of the JAK / STAT signaling pathway during the immune response, we aimed to discuss whether there was relationship between Prdx6 and the JAK / STAT signaling pathway in the human epididymis epithelial cells (HEECs), and which member of JAK / STAT families participated in the process.

Materials And Methods

Culturing HEECs

HEECs[31] were kindly provided by Daniel G. Cyr (INRS-Institut Armand Frappier, University of Quebec, Laval, Quebec, Canada). HEECs were cultured in DMEM/HAM F12 media with penicillin (50 U/ml), streptomycin (50 lg/ml), L-glutamine (2 mM), insulin (10 lg/ml), transferrin (10 lg/ml of), hydrocortisone (80 ng/ml), testosterone (5 nM), epidermal growth factor (10 ng/ml), cAMP (10 ng/ml), sodium selenium (2 ng/ml), tocopherol (200 ng/ml), retinol (200 ng/ml), and 10% fetal bovine serum [FBS] [Sigma-Aldrich]. Cells were seeded in culture plates coated with collagen IV (BD Biosciences, Mississauga, Canada) and incubated in a humidified chamber at 32°C with 5% CO₂.

Construction of the Prdx6 shRNA expression plasmid

Short hairpin RNA (shRNA) sequences targeting the mRNA of the human Prdx6 gene (Gens ID: 9588) were designed using the online tool ([http://www.genesil.com/siRNA design.asp](http://www.genesil.com/siRNA%20design.asp))[32] (Table 1). The Prdx6 shRNA sequences were then inserted into the endonuclease loci of *Bam*H I and *Hind* III in the pGenesil-1

vector (Wuhan GeneSil Biotechnology, Wuhan, China). The shRNA sequences were specific and did not target mRNA sequences of other known human genes (Table 5).

Plasmid Transfection

HEECs at 80% confluency were transfected with the Prdx6 shRNA expressing construct using the FuGENE HD Transfection Reagent (Roche, Mannheim, Germany). In addition, HEECs were transfected with non-targeting shRNA constructs to be used as the control. Transfections were performed in triplicate.

Protein extractions and Western blot analysis

Total proteins were extracted from 48hr post-transfected HEECs using RIPA lysis buffer (P0013B, Beyotime Biotechnology, China) supplemented with PMSF (ST506, Beyotime Biotechnology) and protease phosphatase inhibitors (P1050, Beyotime Biotechnology). Total proteins were then separated on a 10% (w/v) SDS-PAGE for JAK1 and STAT1 detection and 12% (w/v) SDS-PAGE for Prdx6, SOCS3 and GAPDH detection. After electrophoresis, transferred and blocked membrane, the membrane was were incubated with primary antibodies overnight at 4°C. Anti-JAK1 antibody (ab133666, Abcam, UK) was used at 1:800; anti-JAK1 (phospho Y1022 + Y1023) (ab138005, Abcam) at 1:500; anti-STAT1 (ab47425, Abcam) at 1:500; anti-STAT1 (phospho Y701) (ab29045, Abcam) at 1:500; anti-Prdx6 (ab92322, Abcam) at 1:1000; anti-SOCS3 (ab16030, Abcam) at 1:1000; and anti-GAPDH (ab181602, Abcam) at 1:2000. After incubation, the membranes were washed with PBS-Tween (1000:1) and then incubated with the relevant HRP-labeled secondary antibody for 2h at room temperature. X-ray film (Bio-Rad, Hercules, CA) was used for chemiluminescent detection of target proteins. Experiments were performed in biological triplicates.

Detection of MDA levels and **total antioxidant capacity** of HEECs

The MDA levels and **total antioxidant capacity** in HEECs of control and Prdx6-RNAi were detected with Commercial kits (S0131S, Beyotime Biotechnology; A015-2-1, Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's protocol.

Sample preparation and RNA isolation

Total RNA was extracted from 48 hr post-transfected cells using TRIZOL (Invitrogen, Carlsbad, CA, USA). RNA quality was measured using ultraviolet spectrophotometry and denaturing agarose gel electrophoresis.

DGE library preparation and sequencing

Library preparation and sequencing were performed by BGI using the Illumina Gene Expression Sample Prep Kit and Solexa Sequencing Chip (flowcell) on the Illumina Cluster Station and Illumina HiSeq™ 2000 System.

Data transformation and gene annotation

Raw solexa sequences were transformed using the following steps: 1) removal of the 3' adaptor sequence, empty reads, and low-quality tags; 2) selection of 21nt read length tags; 3) removal of single copy tags; 4) generation of Clean Tags. After quality assessment, the clean tags were used to generate alignment statistics between the P6-RNAi and control cells.

All clean tags were mapped to the reference sequence and only 1bp mismatch was considered during alignment. The number of unambiguous clean tags for each gene was calculated and then normalized to TPM (number of transcripts per million clean tags)[33-34].

Detection of differential gen expression

Differentially expressed genes between the two groups of transfected HEECs were determined using the Audic-Claverie method (1997)[35]. The threshold used to determine significant differences in gene expression was based on False Discovery Rate (FDR) ≤ 0.001 and the absolute value of $\log_2 \text{Ratio} \geq 1$, as described in Benjamini, Yekutieli (2001)[36].

Gene ontology functional enrichment analysis for DEGs

Gene Ontology (GO) functional enrichment and Genomes (KEGG) pathway enrichment analysis were performed to uncover biological function and metabolic pathways of the differentially expressed genes, respectively. GO analysis was performed based on the methods used by Ye et al. (2006)[37]. Significantly enriched metabolic and signal transduction pathways were identified using the KEGG public database[38].

Pathway enrichment analysis for DEGs

Pathway enrichment analysis was performed similarly to that of GO analysis.

Real-time PCR

Total RNA was extracted using TRIZOL (Invitrogen, Carlsbad, CA, USA) based on the manufacturer's instructions. The first strand of cDNA was generated with ReverTra Ace (MMLV Reverse Transcriptase RNase H-) (TRT-101, Toyobo Co., Ltd., Osaka, Japan). Real-time PCR was performed with EvaGreen 2x

qPCR Master Mix (Applied Biological Materials, Inc., Vancouver, Canada) with Rotor-Gene Q (QIAGEN, Hilden, Germany). The results were expressed as the real-time quantity of the target gene / GAPDH. Gene-specific primer sequences and PCR conditions are shown in Table 6.

Results

The expression levels of PRDX6 protein and *Prdx6* mRNA

The expressions of PRDX6 protein and *Prdx6* mRNA were both significantly decreased in the *Prdx6*-RNAi (P6-RNAi) HEECs compared to the control cells (Figure 1A - C). This confirmed that our shRNA sequence targeting *Prdx6* was effective.

Sequencing Quality

The distribution patterns of total and distinct tags were similar between HEECs transfected with P6-RNAi and non-targeting shRNA construct. This suggested the absence of bias in the construction of the two libraries (Figure 2). Tags with copy numbers > 100 were dominant for both P6-RNAi and control HEECs, while the majority of distinct tags had copy numbers < 5 (Figure 2). This indicated that low mRNA levels were more significant.

Differential Gene Expression

There were 903 differentially expressed genes between the control and P6-RNAi transfected HEECs. Compared to the control cell, 589 genes were up-regulated and 314 were down-regulated (including *Prdx6*) in P6-RNAi transfected cells (Figure 1D).

Real-time PCR

Ten up-regulated and ten down-regulated genes were randomly selected for real-time PCR validation. Consistency was observed between real-time PCR and DGE results (Figure 3).

Functional annotation of DEGs

DEGs were categorized into biological processes, molecular function, and cellular components (Figure 4). The biological processes contained cellular protein metabolic, metabolic, multi-organism, response to other organism, and response to biotic stimulus. The molecular function was cytokine activity. The cellular components involved in ribosomal subunit, ribosome, cytoplasmic part, cytoplasm, organelle part, intracellular organelle part, membrane-bounded organelle, intracellular membrane-bounded organelle, organelle, intracellular organelle, intracellular part, and intracellular.

Pathway analysis of DEGs

There were 13 significantly different pathways between P6-RNAi and control HEECs (Table 1). The total number of related genes in the significantly different pathways were 80, of which, 44 genes belonged to the CCL, CXCL, IL, and IFIT family. The four gene families are reported to be associated with immunity.

The mRNA expression levels of *JAKs*, *STATs*, and *SOCs*

Compared to control cells, the expression levels of *JAK1* and *STAT1* were significantly increased, while the expression level of *SOCs3* was significantly reduced in P6-RNAi HEECs (Table 2).

The expressions of proteins in JAKs / STAT signaling pathway

We measured the expression levels of JAKs, STATs, and SOCs in HEECs. Compared to control HEECs, expression levels of JAK1 and STAT1 were significantly increased in P6-RNAi HEECs. In addition, the expression level of SOCs3, which is an inhibitor of the JAK / STAT signaling pathway, was significantly reduced in P6-RNAi HEECs (Figure 5A-B).

Activation of the JAK1 / STAT1 signaling pathway by the IL-6 receptor family and IFN γ in P6-RNAi HEECs

Compared to control cells, the expression levels of *IL-6*, *IL6ST*, and downstream genes (i.e., *PIM-3*, and cytokines) were significantly increased in P6-RNAi HEECs (Table 3).

Although differences of expression levels in IFN γ and IFN γ receptor were not observed between the control and P6-RNAi HEECs, the majority of IFN γ related genes (*IFRD1*, *IRF9*, *IFI6*, *IFI27*, *MX1*, *IFI44*, *IFI44L*, *IFIT1*, *IFIT3*, *IFITM1*, *IFIT2*, *IFIH1*, *IFIT5*, *IFI30*, *IFITM3*, *GBP1*, *IFI16*, *IRF7*) showed significantly higher expression (Table 4). Furthermore, the expression levels of downstream genes of IFN γ signaling (*OAS1*, *OAS2*, *OAS3*, *OASL*, *HLA-A*) were significantly higher in P6-RNAi HEECs (Table 4).

The Malondialdehyde (MDA) levels and total antioxidant capacity of HEECs

The MDA level in Prdx6-RNAi HEECs was significantly increased compared to that of control. The total antioxidant capacity, meanwhile, showed the opposite trend (Figure 6).

Discussion

Our previous study demonstrated that Prdx6 was expressed in human epididymis, seminal fluid, and spermatozoa[29], and the protective role of Prdx6 in human spermatozoa was also reported[28]. Here, we try to investigate the potential role of Prdx6 in HEECs.

The DEG results showed that the differentially expressed genes between P6-RNAi and control HEECs mostly belonged to the CCL, CXCL, IL, and IFIT families that are associated with immunity. On the other hand, it is well known that multiple developmental and immunological processes were regulated by the JAK / STAT signaling pathway[39]. This prompted us to investigate whether down-regulation of Prdx6 could activate the JAK/STAT signaling pathway. Interestingly, as we had speculated, we were able to detect the up-regulated expressions of JAK1 and STAT1, and the significantly decreased expression of SOCS3 in P6-RNAi HEECs. The same expression trend of *JAK1*, *STAT1*, and *SOCS3* also be found in the DEG analysis.

To further verified the activation of JAK1/STAT1 signaling pathway, we analyzed the upstream and downstream genes of the pathway. The JAK-STAT pathway has been widely reported to be activated by IL-6 and IFN γ . IL-6 is required for the activation of the JAK-STAT pathway during myocardial infarction[40], in myelomas[41], head and neck tumors[42], and primary breast cancers[43]. IFN γ is one of the most important cytokines and plays an important role in defense against microbial infections by producing various cytokines and inducing autophagy[44]. IFN γ activation of the JAK2 / STAT1 signaling pathway has been reported in EBV (+) gastric cancers[45], though the activation of STAT1 in primary mammary carcinomas was found to be IFN- γ -independent[46]. Our DEG results showed that expression levels of *IL-6*, *IL6ST*, and their downstream targets, i.e., *PIM3* and various cytokines, were significantly increased in P6-RNAi HEECs. Although we did not observe significant differences in expression levels in IFN γ and IFN γ receptor between P6-RNAi and control HEECs, the expression levels of eighteen IFN related genes were significantly increased, as well as the expression levels of downstream targets (*OAS1*, *OAS2*, *OAS3*, *OASL*, *HLA-A*) of IFN γ signaling in P6-RNAi HEECs. We speculate that IFN γ and IFN γ receptor expression levels might be transiently increased after *Prdx6* knockdown but returned to normal levels by the time when we performed our measurements. Additional time course studies need to be performed to determine if IFN γ and IFN γ receptor expression levels are transiently increased after Prdx6 knockdown. Nevertheless, our results clearly demonstrated a relationship between *Prdx6* and JAK1 / STAT1 signaling pathway in HEECs.

In addition, we tried to reveal how *Prdx6* regulate JAK1 / STAT1 signaling pathway activation. Prdxs play an important role in reducing several cellular peroxide substrates, such as ROS[47]. Although high levels of ROS may result in oxidative stress to promote apoptosis in lymphocytes[48], certain amount of ROS is required to maintain cellular homeostasis[49]. Leong et al. (2000) in their paper described the immunomodulatory effects of ROS, in which ROS acts as a second messenger to participate in lymphocyte activation and as a third signal in T cell activation[50]. Our results showed that the MDA level and [total antioxidant capacity](#) in Prdx6-RNAi HEECs was significantly increased and decreased, respectively. Therefore, it can be speculated that knockdown of *Prdx6* resulted in higher levels of ROS in

HEECs, which in turn, activated the *IL-6* receptor and *IFN* γ to induce the JAK1 / STAT1 signaling pathway. However, the specific underlying molecular mechanisms need to be further investigated.

Conclusion

We speculated that knockdown of *Prdx6* resulted in higher levels of ROS in HEECs, which in turn, activated the JAK1 / STAT1 signaling pathway induced by *IL-6* receptor and *IFN*.

Declarations

Funding

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Conflicts of interest/Competing interests

The authors declare that they have no competing interests.

Availability of data and material

Not applicable.

Code availability

Not applicable.

Authors' contributions

Hui Shi performed transfections, Western blotting experiments, DGE analysis, and drafted the manuscript. Xiaoyu Liu performed RT-PCR experiments and participated in DGE analysis. Yanwei Wang performed the cell culture experiments. Haiyan Wang performed statistical analysis. Bochen Pan and Jianyuan Li were involved in the design, coordination and helped to draft the manuscript. All authors have read and approved the final version of the manuscript, and agreed with the order of the author ships.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Tables

Table 1
The significantly different pathways

	Pathway	Qvalue	Pathway ID
1	Hepatitis C	0.001	ko05160
2	Rheumatoid arthritis	0.009	ko05323
3	Toll-like receptor signaling pathway	0.021	ko04620
4	Ribosome	0.021	ko03010
5	Cytosolic DNA-sensing pathway	0.026	ko04623
6	Cell cycle	0.026	ko04110
7	NOD-like receptor signaling pathway	0.026	ko04621
8	Cytokine-cytokine receptor interaction	0.026	ko04060
9	Chagas disease (American trypanosomiasis)	0.026	ko05142
10	Osteoclast differentiation	0.026	ko04380
11	RIG-I-like receptor signaling pathway	0.026	ko04622
12	Ubiquitin mediated proteolysis	0.026	ko04120
13	Other glycan degradation	0.028	ko00511

Table 2
The expressions of JAKs and STATs

Gene symbol	log ₂ Ratio (P6-RNAi / control cell)	P-Value	FDR	significant difference
JAK1	1.34	8.28226E-14	2.6014E-12	*
JAK2	1.35	0.01359128	0.045822224	-
JAK3	-1.94	0.001423868	0.006742904	-
JAKMIP3	-5.906890596	0.2498	0.419522358	-
STAT1	1.02	2.1736E-12	4.39471E-11	*
STAT2	1.12	0.1156098	0.245160301	-
STAT3	-0.09	0.728464	0.827680221	-
STAT4	-0.42	0.725936	0.828291933	-
STAT5A	1.19	0.0641106	0.154290407	-
STAT5B	-0.45	0.0708682	0.166866442	-
STAT6	0.83	0.00974278	0.03466438	-
SOCS1	0.485426827	0.58175	0.720043576	-
SOCS2	-0.599324599	0.00657522	0.0251786	-
SOCS3	-1.605721061	7.16074E-07	6.9378E-06	*
SOCS4	0.426804556	0.0877092	0.197829067	-
SOCS5	-0.237136417	0.419994	0.576760444	-
SOCS6	0.358265781	0.207484	0.370426821	-
SOCS7	0.502753645	0.0282318	0.08299928	-
<p>P-Value: indicates the probability that a gene is expressed equally between two samples.</p> <p>FDR: false discovery rate.</p> <p>*: represents a significant difference between P6-RNAi and control cell groups.</p> <p>-: represents no significant difference between P6-RNAi and control cell groups.</p>				

Table 3
The significantly different gene expressions related to IL-6

Gene symbol	log ₂ Ratio (P6-RNAi / cell)	P-Value	FDR	Description
IL-6	3.08	8.81672E-07	8.43333E-06	interleukin-6 precursor
IL6ST	1.21	1.86073E-13	5.11834E-12	interleukin-6 receptor subunit beta isoform 3 precursor
PIM-3	1.61	1.94098E-05	0.000148023	threonine-protein kinase pim-3
CXCL10	8.71	6.12788E-05	0.000417574	C-X-C motif chemokine 10 precursor
CXCL11	9.91	2.34882E-10	3.66318E-09	C-X-C motif chemokine 11 precursor
CCL20	9.23	9.5901E-07	9.10857E-06	C-C motif chemokine 20 isoform 1
CCL2	3.17	6.55254E-13	1.49744E-11	C-C motif chemokine 2 precursor
CXCL3	2.93	3.16648E-09	4.2176E-08	C-X-C motif chemokine 3
CCL4	9.43	1.19972E-07	1.30567E-06	C-C motif chemokine 4 isoform 1 precursor
CXCL1	2.99	1.27232E-13	3.75194E-12	growth-regulated alpha protein precursor
CXCL2	2.92	1.03249E-11	1.87682E-10	C-X-C motif chemokine 2
CCL5	4.01	1.67203E-09	2.33772E-08	beta-chemokine RANTES precursor
IL8	2.48	1.25233E-13	3.70921E-12	interleukin 8
IL1A	2.81	5.68782E-05	0.000390146	interleukin-1 alpha proprotein
IL17RA	1.32	2.43992E-09	3.31192E-08	interleukin-17 receptor A precursor
IL15	1.24	1.26275E-06	1.177E-05	interleukin-15 isoform 1 preproprotein

Gene symbol	log ₂ Ratio (P6-RNAi / cell)	P-Value	FDR	Description
IL4I1	2.43	9.41942E- 05	0.00061488	L-amino-acid oxidase isoform 2

Table 4
The significantly different gene expressions related to IFN γ

Gene symbol	log ₂ Ratio (P6-RNAi / cell)	P-Value	FDR	Description
IFRD1	1.04	4.20214E-07	4.21025E-06	interferon-related developmental regulator 1 isoform 1
IRF9	1.03	3.04816E-11	5.25108E-10	interferon regulatory factor 9
IFI6	5.39	0	0	interferon alpha-inducible protein 6 isoform a
IFI27	5.07	1.03249E-11	1.87935E-10	interferon alpha-inducible protein 27, mitochondrial isoform 2
MX1	4.46	4.58758E-05	0.000319052	interferon-induced GTP-binding protein Mx1
IFI44	4.31	4.6491E-10	6.97675E-09	interferon-induced protein 44
IFI44L	4.29	8.81672E-07	8.41545E-06	interferon-induced protein 44-like
IFIT1	3.93	2.77556E-13	7.12675E-12	interferon-induced protein with tetratricopeptide repeats 1 isoform 2
IFIT3	3.92	4.996E-14	1.68269E-12	interferon-induced protein with tetratricopeptide repeats 3
IFITM1	3.29	2.19824E-14	7.7721E-13	interferon-induced transmembrane protein 1
IFIT2	2.81	0	0	Interferon-induced protein with tetratricopeptide repeats 2
IFIH1	2.81	7.4848E-08	8.41713E-07	interferon-induced helicase C domain-containing protein 1
IFIT5	1.64	2.5313E-14	8.87993E-13	interferon-induced protein with tetratricopeptide repeats 5
IFI30	1.56	1.4997E-05	0.00011708	gamma-interferon-inducible protein precursor
IFITM3	1.46	8.88178E-14	2.75131E-12	interferon-induced transmembrane protein 3
GBP1	1.4	6.01984E-07	5.90443E-06	interferon-induced guanylate-binding protein 1
IFI16	1.22	8.68612E-10	1.26281E-08	Interferon, gamma-inducible protein 16 variant
IRF7	1.17	2.98494E-12	5.89395E-11	interferon regulatory factor 7 isoform d

Gene symbol	log ₂ Ratio (P6-RNAi / cell)	P-Value	FDR	Description
OAS1	3.85	0.000027594	0.000203764	2'-5'-oligoadenylate synthase 1 isoform 3
OAS2	11.17	4.4409E-16	1.80117E-14	2'-5'-oligoadenylate synthase 2 isoform 3
OAS3	3.71	7.4848E-08	8.42414E-07	2'-5'-oligoadenylate synthase 3
OASL	1.71	0	0	59 kDa 2'-5'-oligoadenylate synthase-like protein isoform b
HLA-A	1.23	2.51284E-11	4.39617E-10	HLA class I histocompatibility antigen, A-1 alpha chain precursor

Table 5
Target sequence of interference for human Prdx 6

shRNA name	Position on CDS	Target sequence	GC content (%)
si Prdx6	667	agctggcaccagaatttgccaagag	52
irrelevant (control)		agctagcactagaatctgccgagag	52

Table 6
The sequences and melting temperature of primers

Gene name	Primer(5'-3')	T _m (°C)
GAPDH	F: AACGGATTTGGTCGTATTG; R: GGAAGATGGTGATGGGATT	51.5
ICAM1	F: TAGCAGCCGCAGTCATAAT; R: AGAAAGTTGGGCAGGGAG	53
IL6	F: GTCCAGTTGCCTTCTCCC; R:GCCTCTTTGCTGCTTTCA	53.7
BIRC3	F:TGGTGGTATGTGCCTGTA; R:TGGAAAGAGTCTGGGAGT	48.1
TSNAX	F: TTAATATCGTGCCAAGCC; R: CCTCGTGATCTGCCTACC	49
CCL5	F: CCCTCGCTGTCATCCTCA; R: CCCTCGCTGTCATCCTCA	56.1
IDO1	F: CTGGAACTGCCTCCTATT; R: ATGCGAAGAACACTGAAA	49.5
TLR4	F:GACCTGTCCCTGAACCCTA; R: AAATGTTGCCATCCGAAA	49
EDN2	F:ACTTGGACATCATCTGGGTG; R:GAGGCTCTTGACTGTGGAAA	57
MTOR	F: AAAACCTCGTCACATTACCC; R: CAGCGAGTTCTTGCTATTCC	54.1
CCL2	F: TGCTTCCCTTTCCTACTT; R: TGGATGTTTCTGGGTTAGT	51.1
ASNS	F: CTTCTGAGGGAACTCTATT; R: AGCTGACTTGTAGTGGGT	49.1
MMP14	F: CATCATTGAGGTGGACGAG; R: CATCATTGAGGTGGACGAG	56.9
FOSL	F: ATTCAAATCCGCCCTGTC; R: ATGCGTCGTTTCTCCTCC	53.1
PPIA	F: TTTGCAGACAAGGTCCCA; R: TTGCCATCCAACCACTCA	52.4
BYSL	F: GGGAGCAAATCCTCTACG; R: CACAGCACAGGCAGTTCA	54
GAMT	F:CCTGCCTGACGGTCACTT; R:CCTCCATACGGATGTTCTCC	56.5
UCP2	F: GCTGGAGGTGGTCGGAGAT; R: GGAGGCGATGACAGTGGT	55.4
WNT7B	F: TAGACACCCTCTGTTTCCTTTC; R: TAGACACCCTCTGTTTCCTTTC	56
SHC1	F: GGGGAGGAGTAACCTGAAA; R: TGGCAACATAGGCGACAT	53.8
SPATA20	F: AGTCACCATACCTCCTACAACA; R: TCCTCACGGTCTACCTTCAC	53.7
Prdx6	F: AATTTGCCAAGAGGAATG; R: GTGGTAGCTGGGTAGAGG	50.8
T _m : the melting temperature		

Figures

Figure 1

Expression levels of PRDX6 protein and Prdx6 mRNA (A-C) and differentially expressed genes between control and P6-RNAi HEECs (D). Compared to control HEECs, the expression level of PRDX6 protein significantly decreased in P6-RNAi HEECs (A-B). The expression level of Prdx6 mRNA in P6-RNAi HEECs were reduced by 43.2% compared to control HEECs (C). Compared to control HEECs, there were 589 up-regulated genes and 314 down-regulated genes (including Prdx6) in P6-RNAi HEECs (D).

Figure 2

Evaluation of Sequencing Quality. The distribution patterns of total and distinct tags were similar between P6-RNAi and control HEECs. Tags with copy numbers > 100 were dominant in both control and P6-RNAi HEECs, while the majority of distinct tags had copy numbers < 5.

Figure 3

The differentially expressed genes measured by real-time PCR. Twenty genes were randomly selected to do real-time PCR for validation. Consistency was observed between real-time PCR and DGE results.

Figure 4

GO functional classification of the DEGs. Y-axis represents GO terms. All GO terms were grouped based on biological process, molecular function, and cellular component. The numbers in the bars represent DEGs annotated for biological process, molecular function, and cellular component. The percentage in the round brackets represent DEGs with relative annotation / All genes with relative annotation.

Figure 5

Western blot analysis of JAK1, STAT1, and SOCS3 expression levels (A) and gray degree analysis (B). Compared to control HEECs, the expression levels of JAK1, phosphorylated JAK1, STAT1, and phosphorylated STAT1 were significantly increased, while expression levels of SOCS3 were significantly reduced in P6-RNAi HEECs.

Figure 6

The MDA levels (A) and total antioxidant capacity (B) of HEECs. The MDA level in Prdx6-RNAi HEECs was significantly increased compared to that of control. The total antioxidant capacity, meanwhile, showed the opposite trend (Figure 6).