

Original Article

Overexpression of IFIT1 suppresses proliferation and promotes apoptosis of mouse podocytes

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Received March 9, 2016; Accepted June 10, 2016; Epub November 15, 2016; Published November 30, 2016

Abstract: IFN-induced protein with tetratricopeptide repeats 1 (IFIT1) was identified as a candidate gene for systemic lupus erythematosus (SLE). SLE affects multiple organs, including kidney. Podocytes are implicated in the onset and development of kidney disease. In this study, we investigated the effects of IFIT1 on mouse podocytes. IFIT1 expressing lentivirus was packaged, produced and infected mouse podocytes. CCK-8 assay showed that ectopic expression of IFIT1 significantly inhibited proliferation of mouse podocytes. Flow cytometry analysis revealed that IFIT1 overexpression suppressed podocyte apoptosis. Importantly, IFIT1 overexpression reduced the expression of anti-apoptosis protein (Bcl-2), but increased the expression of apoptosis marker protein (cleaved caspase 3), apoptosis promoting protein (Bax) and phosphorylation of p38. Taken together, our results demonstrate for the first time that overexpression of IFIT1 in podocytes prevents cell proliferation and induces cell apoptosis.

Keywords: IFIT1, podocytes, proliferation, apoptosis

Introduction

IFN-induced protein with tetratricopeptide repeats 1 (IFIT1, also known as ISG56) belongs to a family of IFN-induced proteins, which are characterized as regulators of viral replication [1, 2] as well as cellular processes [3-6], including translation, proliferation, apoptosis and signaling. Through microarray analysis on peripheral blood samples, *IFIT1* was identified as a candidate gene for systemic lupus erythematosus (SLE), a chronic, multisystem, inflammatory disorder of autoimmune etiology [7]. SLE affects multiple organs, including kidney [8]. Whether IFIT1 was involved in kidney disease was not clear.

Podocytes, the visceral glomerular epithelial cell, are critical for the formation of glomerular basement membrane (GBM), the maintenance of the glomerular filtration barrier and the regulation of glomerular filtration [9]. Perysinaki et al. reported that slit diaphragm components of podocytes were significantly reduced in lupus nephritis mice [10], suggesting the implication of podocytes in the pathogenesis of lupus nephritis. Podocytes are the target of various

forms of damage. Decrease in the number of podocyte leads to the development of kidney disease [11]. Apoptosis and the lack of proliferative capacity are the possible cause for loss of podocyte number. A recent study suggested that IFIT1 may participate in regulating proliferation of immune cells during stroke-related processes [12]. Expression of IFIT1 elicits apoptotic cell death of Hela cells [6]. However, few investigation has been performed on the functions of IFIT1 on podocytes.

In the current study, we investigated the effects of IFIT1 overexpression on the proliferation and apoptosis of podocytes, thus suggesting a role of IFIT1 in kidney disease.

Materials and methods

Cell culture

Mouse podocytes were obtained from Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). The podocytes were maintained in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA), 100 U/

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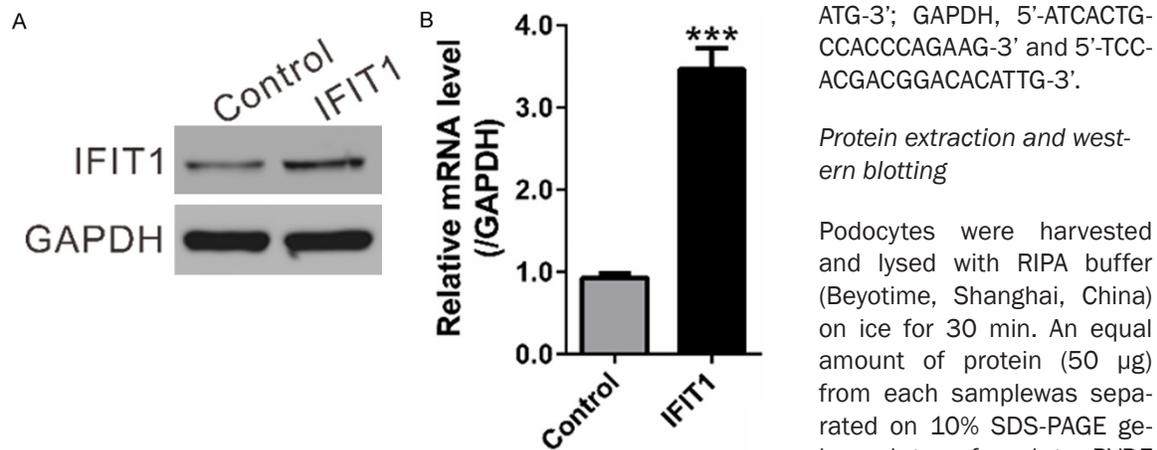


Figure 1. Overexpression of IFIT1 in podocytes by lentiviral transduction. Podocytes were infected with IFIT1 expressing lentivirus (IFIT1) and control lentivirus (control). At 48 h after virus infection, cells were collected. IFIT1 protein (A) and mRNA (B) expression was detected by Western blot and real-time PCR, respectively. *** $P < 0.001$ compared with control.

ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin and 10 U/ml interferon- γ (Sigma, St Louis, MO, USA) in a humidified atmosphere at 33°C with 5% CO_2 . Before the following assays, the podocytes were differentiated by cultured in the above medium without interferon at 37°C for 10-14 days.

Lentiviral production

The full-length mouse IFIT1 were cloned into the expression vector pLVX-AcGFP1-C1 (Clontech, Palo Alto, CA, USA). IFIT1 expressing lentivirus (IFIT1) and control lentivirus (control) were generated by transfecting HEK 293T cells with lentiviral expression and packaging vectors.

Quantitative real-time PCR

Total RNA was extracted from podocytes with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) per the manufacturer's instructions. The first strand cDNA was then synthesized from 2 μg RNA by the two-step RT-PCR system (Thermo Fisher Scientific, Rockford, IL, USA). Quantitative real-time PCR was performed on ABI7300 thermal cycler (Applied Biosystems, Foster City, CA, USA) with a standard SYBR Green PCR kit (Thermo Fisher Scientific) to measure the mRNA levels of IFIT1. GAPDH was served as an internal control. Relative gene expression was calculated using the $2^{-\Delta\Delta\text{CT}}$ method. Primers were list as follows: IFIT1, 5'-TTTGAGATGG-CCTATGTTT-3' and 5'-TTGTCTTCTGACTGCTT-

ATG-3'; GAPDH, 5'-ATCACTGCCACCCAGAAG-3' and 5'-TCC-ACGACGGACACATTG-3'.

Protein extraction and western blotting

Podocytes were harvested and lysed with RIPA buffer (Beyotime, Shanghai, China) on ice for 30 min. An equal amount of protein (50 μg) from each sample was separated on 10% SDS-PAGE gels and transferred to PVDF membranes. After blocking in 5% skim milk for 30 min, the blots were incubated with the primary antibodies for IFIT1 (1:200, Santa Cruz Biotech., Santa Cruz, CA, USA), Bcl-2 (1:200, Santa Cruz Biotech.), Bax (1:200, Santa Cruz Biotech.), p38 (1:1000, Cell signaling Technology, Danvers, MA, USA), p-p38 (1:1000, Cell signaling Technology) and GAPDH (1:1500, Cell signaling Technology) at 4°C overnight. The blots were then incubated with appropriate HRP-conjugated secondary antibody at room temperature for 1 h. Signals were detected with enhanced chemiluminescence (BioRad, Richmond, CA, USA) and quantified using Image J software (National Institutes of Health, Bethesda, MD, USA).

Cell proliferation assay

Cell proliferation was assessed using the CCK-8 assay according to the manufacturer's instruction. Briefly, podocytes were trypsinized and seeded onto 96-well plates at a density of 3×10^3 cells per well. At 24 h after seeding, podocytes were infected with IFIT1 expressing lentivirus and control lentivirus. At 0, 12, 24, 48 and 72 h post virus treatment, CCK-8 solution (10 μl in 100 μl RMPI-160 medium) was added to each well and incubated for 1 h. The absorbance was measured at 450 nm by using a microplate reader (Bio-Rad, Hercules, CA, USA).

Flow cytometric analysis of apoptosis

Cell apoptosis analysis was performed by using Annexin V apoptosis detection kit (eBioscience, San Diego, CA, USA). Briefly, podocytes plated in 6-well plates were infected with lentivirus. At

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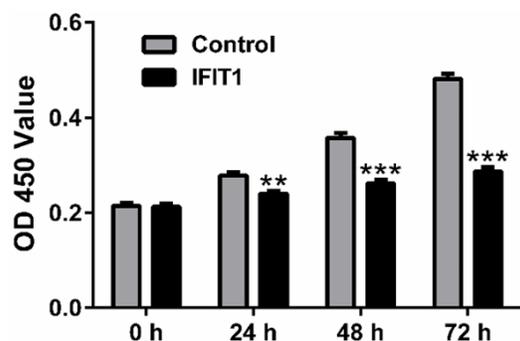


Figure 2. IFIT1 inhibited cell proliferation of podocytes. The chondrocytes viability as tested by CCK-8 at 0 h, 12 h, 24 h, 48 h and 72 h after virus transduction. *** $P < 0.001$ compared with control.

48 h post virus treatment, podocytes were harvested, incubated with Annexin V and Propidium iodide (PI) for 20 min in dark at 4°C and analyzed on a flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA). The early apoptotic cells are represented in the lower right quadrant of the FACS histogram.

Statistical analysis

The data was presented as the mean value \pm SD (standard deviation). The paired, two-tailed Student's t-test was used to analyze the significance of difference between groups. A difference was considered significant when the P value was less than 0.05.

Results

Ectopic expression of IFIT1 in podocytes

In order to study the function of IFIT1 on podocytes, IFIT1 lentivirus was packaged, produced and infected mouse podocytes. As shown in **Figure 1**, IFIT1 lentivirus infection of podocytes resulted in a notable increase of IFIT1 expression at both mRNA and protein levels.

Ectopic expression of IFIT1 inhibited podocyte proliferation

Decreasing podocyte number contributes to the development of kidney disease [11]. The inability or lack of podocytes to proliferate is one of the possible causes for loss of podocyte number. A previous study suggested that IFIT1 may regulate the proliferation of immune cells during stroke-related processes [12]. Therefore, we then evaluated the effects of IFIT1 overex-

pression on cell proliferation by CCK-8 assay. Ectopic expression of IFIT1 significantly suppressed the proliferation of podocytes (**Figure 2**).

Ectopic expression of IFIT1 induced podocyte apoptosis

Apoptosis is another possible cause for loss of podocyte number. Thus, we assessed the effects of IFIT1 overexpression on cell apoptosis by Annexin V-PI staining and flow cytometry analysis. As shown in **Figure 3**, IFIT1 expressing lentiviral treatment for 48 h significantly induced apoptosis of podocytes (from 3.47% \pm 0.39% to 22.70% \pm 0.51%).

Ectopic expression of IFIT1 changed protein expression associated with apoptosis pathway in podocytes

IFIT1 overexpression significantly reduced the expression of anti-apoptosis protein (Bcl-2 [13]), but increased the expression of apoptosis marker protein (cleaved caspase 3 [14]), apoptosis promoting protein (Bax [13]) and phosphorylation of p38 (p-p38) (**Figure 4**). These findings indicated that IFIT1 induced apoptotic death of podocytes via regulating Bcl-2 family proteins and p38 phosphorylation.

Discussion

IFIT1 was identified as a candidate gene for systemic lupus erythematosus (SLE), which affects multiple organs, including kidney. Perysinaki et al. suggested the implication of podocytes in the pathogenesis of lupus nephritis [10]. In the present study, we revealed the effects of IFIT1 on mouse podocytes. Our study indicated a possible role of IFIT1 in kidney disease.

IFIT1 is one of four related human (ISG56/IFIT1, ISG54/IFIT2, ISG60/IFIT3, ISG58/IFIT5) and three related mouse proteins (Ifit1, Ifit2, Ifit3) [15]. These proteins are characterized as inhibitors of viral replication [1, 2], translation [3] and proliferation [4] and promoters of apoptosis [5, 6]. In the present study, we overexpressed IFIT1 in mouse podocytes via lentivirus infection (**Figure 1**). CCK-8 assay (**Figure 2**) showed that IFIT1 can suppress cell proliferation of podocytes, which was consistent with those findings in immune cells [12]. It was reported that overexpression of IFIT1 was able to induce apoptotic cell death of Hela cells [6]. In line with

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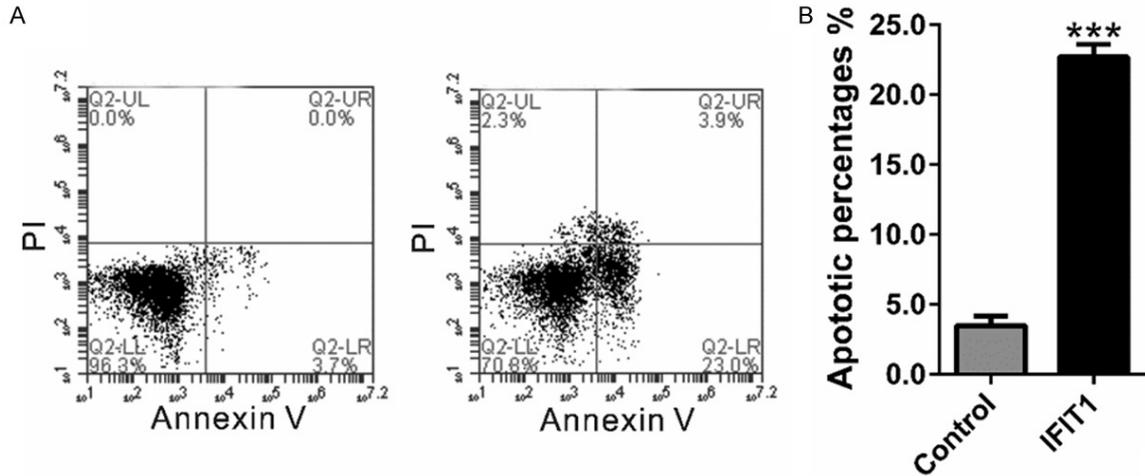


Figure 3. IFIT1 promoted cell apoptosis of podocytes. Cell apoptosis was analyzed at 48 h after virus transduction by Annexin V/PI staining and flow cytometry. *** $P < 0.001$ compared with control.

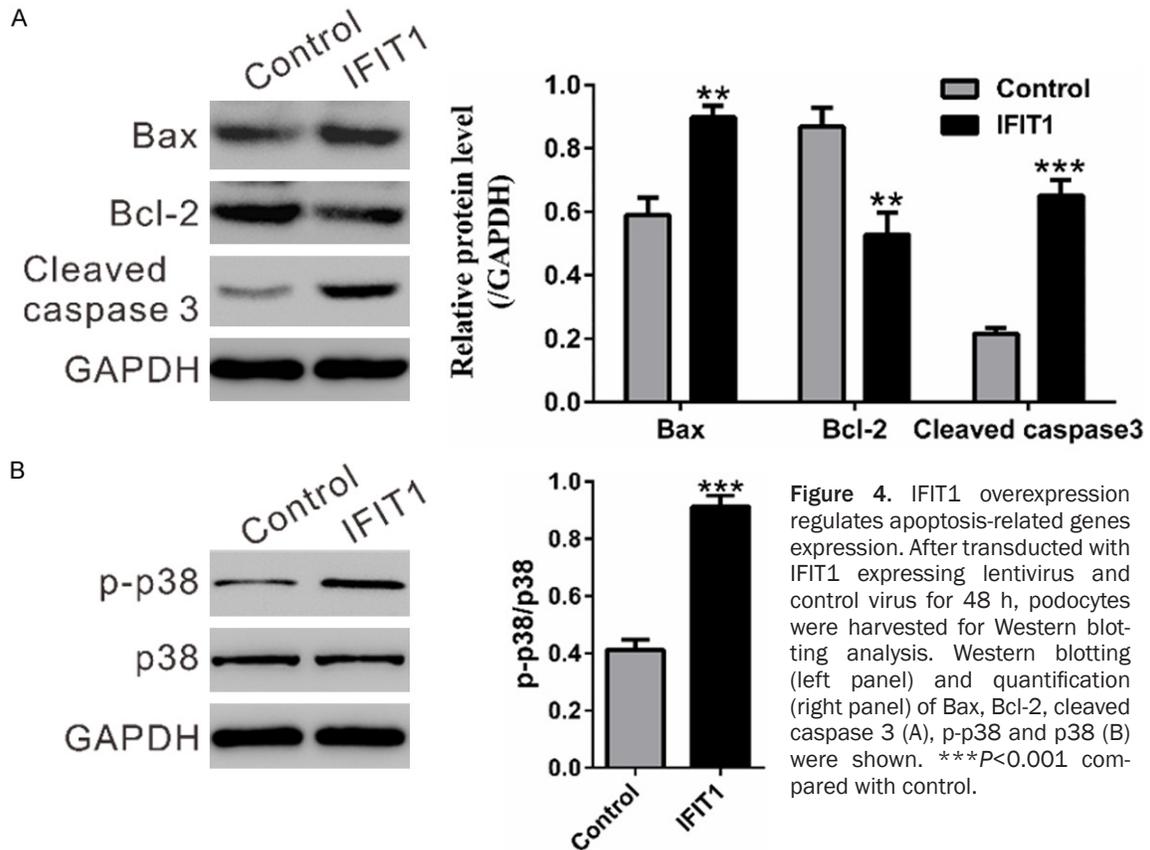


Figure 4. IFIT1 overexpression regulates apoptosis-related genes expression. After transduced with IFIT1 expressing lentivirus and control virus for 48 h, podocytes were harvested for Western blotting analysis. Western blotting (left panel) and quantification (right panel) of Bax, Bcl-2, cleaved caspase 3 (A), p-p38 and p38 (B) were shown. *** $P < 0.001$ compared with control.

these findings, we found the apoptosis-promoting effects of IFIT1 on mouse podocytes (Figure 3). Our data suggested that IFIT1 can decreased podocyte number through promoting cell apoptosis and inhibiting cell survival, thus might participate in the development of kidney disease.

However, further investigations are needed based on clinical samples or animal models.

Mitochondrial pathway plays a critical role in cell apoptosis and cleaved caspase 3 is a marker of mitochondrial pathway of apoptosis [16].

Bcl-2 and Bax are well-known regulators to suppress and promote cell apoptosis, respectively [13]. Phosphorylation of p38 results in cell apoptosis [17]. To further indicate the pathway through which IFIT1 promotes cell apoptosis, we examined the protein levels of Bcl-2, Bax [13] and cleaved caspase 3. We found that IFIT1 overexpression led to an increase in the levels of Bax, cleaved caspase 3 and p-38, and a decrease in Bcl-2 expression (**Figure 4**). These data suggested that IFIT1 exerted an apoptosis-promoting role via regulating phosphorylation of p38 and Bcl-2 family proteins.

In conclusion, we revealed the proliferation-inhibitory and apoptosis-promoting effects of IFIT1 on mouse podocytes. The present study suggest that IFIT1 may decrease podocyte number and be a good candidate target for kidney disease, although further studies are needed.

Acknowledgements

This research was supported by grants from National Natural Science Foundation of China (No. 81302589) and Fujian Provincial Health and Family Planning Commission (No. 2014-ZQNZD19).

Disclosure of conflict of interest

None.

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