Biochemical and Biophysical Research Communications 448 (2014) 182–188

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

ATF3 attenuates cyclosporin A-induced nephrotoxicity by downregulating CHOP in HK-2 cells

Yong-Min Choi, Hey-Young Cho, Muhammad Ayaz Anwar, Han-Kyul Kim, Ji-Woong Kwon, Sangdun Choi *

Department of Molecular Science and Technology, Ajou University, Suwon 443-749, Republic of Korea

ARTICLE INFO

Article history:
Received 12 April 2014
Available online 24 April 2014

Keywords:
Activating transcription factor 3
C/EBP homologous protein
Cyclosporin A
HK-2 cell
Nephrotoxicity

ABSTRACT

Calcineurin inhibitors such as cyclosporin A (CsA) are widely used to treat organ transplantation-associated complications. However, CsA use is limited due to renal dysfunction. This study attempts to characterize the mechanism of CsA-induced nephrotoxicity using a human embryonic kidney cell line (HK-2). We performed microarray-based whole-genome expression analysis in HK-2 cells. CsA treatment induced the expression of endoplasmic reticulum (ER) stress-related and apoptosis-inducing genes at 6 and 24 h, respectively, indicating that ER-stress predisposed the cells to apoptosis. G1 phase cell-cycle arrest was also observed via ER stress in CsA-treated cells. Furthermore, we found an inverse relationship between activating transcription factor 3 (ATF3), a stress-inducible protein, and C/EBP homologous protein (CHOP), an apoptosis-inducing protein. Moreover, when ATF3 knockdown cells were exposed to CsA, a prompt induction of CHOP was observed, which stimulated ROS production and induced cell death-related genes as compared to wild type. Taken together, our data demonstrate that ATF3 plays a pivotal role in the attenuation of CsA-induced nephrotoxicity by downregulating CHOP and ROS production mediated by ER stress.

1. Introduction

Cyclosporin A (CsA) is widely used as an immunosuppressant in patients, following organ transplantation and various autoimmune diseases [1]. CsA inhibits calcineurin, which in turn impedes the translocation of nuclear factor of activated T cells (NFAT) into the nucleus and the subsequent transcription of regulated genes such as interleukin 2 (IL-2) [2,3]. CsA has been proposed for the treatment of various autoimmune diseases and transplantation-related complications. However, the therapeutic usage of CsA is limited due to the occurrence of chronic renal dysfunction [1,4].

Recently, the involvement of CsA in endoplasmic reticulum (ER) stress has been revealed [5]. This stress is a natural outcome resulting from the disruption of calcium homeostasis, accumulation of oxygen radicals, inhibition of chaperone-related enzymes, and the impediment of post-translational modifications that altogether increase the misfolding of newly synthesized proteins [6]. In response to this stress, cells generally initiate the unfolded protein response (UPR), which alleviates the damaging effects due to the misfolded proteins. In case of failure, apoptosis, a widely appreciated cellular mechanism for cell death, is initiated [7]. C/EBP homologous protein (CHOP) is among the cell death mediators induced in response to ER stress [8], and CHOP expression is known to trigger cell-cycle arrest and apoptosis in several cell types [9].

Activating transcription factor 3 (ATF3) belongs to ATF/cAMP response element binding-protein (CREB) family [10,11]. This transcription factor modulates the transcription of target genes containing a cognate-binding element in a cell context-dependent manner [10,12,13]. Several studies have delineated its role in stress conditions, regulation of the cell cycle, and cell death [14,15]. Adenovirus-mediated expression of ATF3 protects cells against ROS-induced cell death in the human embryonic kidney cell line (HK-2) [12], enhancing its protective role. Furthermore, renal ischemia–reperfusion induces higher mortality, kidney dysfunction, and apoptosis in ATF3-deficient mice [16].

The objective of the current investigation was to evaluate the protective function of ATF3 in CsA-induced nephrotoxicity via ER stress in HK-2 cells. Furthermore, the correlation of ATF3 and CHOP expression in stress conditions was also investigated.

Abbreviations: ATF3, activating transcription factor 3; CHOP, C/EBP homologous protein; CsA, cyclosporin A; HK-2, human renal proximal tubular epithelial.

* Corresponding author. Fax: +82 31 219 1615.
E-mail address: sangdunchoi@ajou.ac.kr (S. Choi).

http://dx.doi.org/10.1016/j.bbrc.2014.04.083
0006-291X/

2. Materials and methods

2.1. Cell culture

HK-2 cells derived from normal kidney were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). HK-2 cells were grown in Keratinocyte-SFM medium kit supplemented with L-glutamine, epithelial growth factor (EGF; Gibco, Carlsbad, CA, USA), and maintained at 37 °C under 5% CO_2.

2.2. Cell proliferation assay (MTS assay)

Cell proliferation was quantified using a colorimetric 3-(dimethylthiazol-2-yl)-5-(carboxymethoxyphenyl)-2-(sulfophenyl)-2H-tetrazolium (MTS) solution (Cell Titer 96H; Promega, Madison, WI, USA). HK-2 cells (1×10^4 cells per well) were cultured in 96-well plates. After CsA (Sigma–Aldrich, St. Louis, MO, USA) treatment for the indicated times, MTS solution (10 μL/well) was added, the cells were incubated at 37 °C for 3 h, and absorbance was measured using a VERSA Microplate reader (Turner Biosystems, CA, USA).

2.3. Cell cycle analysis

Cells were washed once with cold phosphate buffer saline (PBS), and EtOH fixation was performed by treating the cells for 4 h with 70% EtOH. The cells were stained with propidium iodide (40 μg/mL) for 1 h, and then RNase (0.5 mg/mL Sigma–Aldrich) was added. The cell cycle was analyzed by quantifying the DNA content using a flow cytometer (FACSAria™III, BD Biosciences, Franklin Lakes, NJ, USA), and the data were processed using the FACSDiva software (BD Biosciences).

2.4. ROS detection assay

ROS levels were measured using the fluorescent dye dichlorodihydro-fluorescein diacetate (DCFH-DA) (Molecular Probes, NY, USA). Briefly, the cells were washed once and resuspended in PBS, 1 mL of the solution was transferred to a 1.5-mL culture tube, and 1 μL of the DCFH-DA staining solution was added. The cells were then gently mixed and incubated for 15 min at 37 °C in the dark. Next, the cells were washed once and resuspended in 300 μL of cold PBS. Finally, ROS levels were quantified using a flow cytometer (FACSAria™III), and the data were processed using the FACSDiva software.

2.5. Western blot analysis

M-PER Mammalian Protein Extraction Reagent (Thermo Scientific, IL, USA) was employed to isolate protein from treated and control cells with Halt Protease Inhibitor Cocktail (Thermo Scientific). The mixture was incubated for 10 min at 4 °C and centrifuged at 16,000×*g* for 10 min to remove the cell debris. Extracts were loaded onto 10% polyacrylamide gels for electrophoresis, followed by transfer to Hybond-ECL membranes (Amersham, Buckinghamshire, UK). Membranes were blocked using 5% skim milk for 2 h at room temperature, washed, and then incubated with primary antibodies specific for ATF3, CHOP, and β-actin (Santa Cruz Biotechnology, Dallas, TX, USA) that had been diluted with PBS containing 0.05% Tween 20 (PBST) (1:1000) at 4 °C with gentle shaking overnight. After several washes, the membranes were incubated with either Immunopure Goat anti-Mouse or anti-Rabbit IgG peroxidase-conjugated antibodies (Thermo Scientific) diluted in PBST (1:1000) for 2 h. Detection was performed using the Pierce ECL Western Blotting Substrate (Thermo Scientific) and by exposing the blots to film.

2.6. RNA isolation and quantitative reverse transcriptase (qRT)-PCR

Total RNA was isolated using the RNeasy mini kit (Qiagen, Germantown, MD, USA) and converted to cDNA by using the Reverse Transcription Kit (Qiagen). Quantitative PCR was performed using the Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific). Thermal cycling was carried out with an initial denaturation phase of 10 min at 95 °C, followed by 40 cycles of amplification, with denaturation for 30 s at 95 °C, annealing for 30 s at 57 °C, and elongation for 1 min at 72 °C. Amplification was carried out in Roter-Gene Q (Qiagen). The melting curve was evaluated to validate the results and primer specificity, and then relative quantification (ΔΔCt method) was determined using the Rotor-Gene Q Series Software version 2.2.3. Primers used in this study were as follows: ATF3-F; 5′-GAG GAT TTT GCT AAC CTG ACG C-3′ and R; 5′-CTA CCT CGG CTT TTG TGA TGG-3′; CHOP-F; 5′-GGA AAC AGA GTG GTC ATT CCC-3′ and R; 5′-CTG CTT GAG CCG TTC ATT CTC-3′; GAPDH-F; 5′-CCA CCC ATG GCA AAT TCC ATG GCA-3′ and R; 5′-TCT AGA CGG CAG GTC AGG TCC ACC-3′.

2.7. siRNA design and transfection

siRNA was designed and purchased from Bioneer (Daejeon, Korea). The sequence of the siRNA obtained for ATF3 was 5′-GAGA-ACAGCAUUUAGUGAA-3′. Turbofect reagent (Thermo Scientific) was used to transfect HK-2 cells with siRNA. These cells were seeded at 4×10^5 cells in a 6-cm dish containing 2 mL of Keratinocyte-SFM medium and L-glutamine, EGF, and BPE for 12 h. PBS (200 mL) containing 50 nM of siRNA and 4 μL of Turbofect reagent were incubated at room temperature for 15 min and added to each well. After transfection with ATF3 siRNA for 48 h, the transfection medium was swapped with normal medium, and the cells were used in the subsequent experiments.

2.8. Microarray analysis

Total RNA was isolated using the RNeasy mini kit and quantified using a Micro UV–Vis fluorescence spectrophotometer (Malcom, Tokyo, Japan). Afterwards, probe-labeling and hybridization to Affymetrix GeneAtlas Human Genome U219 chip containing 49,386 probe sets were performed. Microarrays were normalized using RMA (Robust Multichip Average) via the Affymetrix Expression Console Ver. 1.1 module. When examining the expression profiles of genes modulated by CsA, only values with an average $\log_2$-(treated/control) (in absolute values) of $\geqslant 0.58$, corresponding to a 1.5-fold change in expression, at a *p*-value of <0.05, were considered differentially expressed.

2.9. Data analysis by DAVID and Cytoscape

Analysis of the 6717-transcriptomic probes was performed using the DAVID (Database for Annotation, Visualization and Integrated Discovery) software, which facilitates the interpretation of microarray data with its online annotation tools [17,18]. The generated data was visualized via the Cytoscape by enabling its enrichment Map plug-in. Enrichment Map is the Cytoscape network software for functional enrichment visualization, which also enables the comparison of two different enrichment results in the same map. Therefore, this plug-in was used to compare the microarray data [19,20].

3. Results

3.1. Cytotoxic evaluation of CsA in HK-2 cells

HK-2 cells were treated with various concentrations of CsA to evaluate its toxicity. The cells were treated with 0.5, 1, or 5 μM CsA for 6, 12, 24, and 48 h and proliferation decreased in a time- and dose-dependent manner (Fig. 1A). There was no significant alteration in the viability at a concentration of 0.5 μM, while 1 and 5 μM profoundly reduced cell viability. Cell viability was decreased to 81.34% and 72.81% at 24 h and 71.86% and 58.53% at 48 h for 1 and 5 μM, respectively. However, the treatment of 5 μM CsA for 48 h severely hampered growth, resulting in an insufficient number of cells for analysis. Therefore, we used 1 μM of CsA for the subsequent experiments.

CsA also negatively affected the morphology of treated cells (Fig. 1B). Cells treated with 1 μM concentration had intact cellular morphology for up to 12 h, but prolonged treatment significantly altered the morphology as evidenced by cellular damage, including enlarged cell size and the cessation of proliferation. Similarly, mild cell-cycle arrest was observed (Fig. 1C) at 6 and 12 h; however, by 24 and 48 h, there was significant cell-cycle arrest. The G1 phase was increased to 11% and 15.9% of the population at 24 and 48 h, respectively. Taken together, 1 μM of CsA plays a critical role in the reduction of HK-2 cell viability and cell-cycle arrest.

3.2. CsA treatment promoted transcriptomic alterations

To evaluate transcriptomic perturbations in HK-2 cells promoted by CsA treatment, mRNA levels were measured using the Affymetrix GeneAtlas system and the U219 array containing 49,386 probe sets. Data were analyzed using the Affymetrix Expression Console software (Ver. 1.1), and expression changes of 1.5-fold were considered significant at p value of <0.05. The expression of 6717 genes was significantly altered, and Gene Ontology (GO) functions were analyzed using the DAVID software. This software is valuable for the analysis of gene expression profiles via its online features, with a focus on omics data, including microarray and high-throughput proteomic data [17,18]. We visualized the expression patterns of differentially expressed genes using Cytoscape (Fig. 2). The number of genes and p-values for each category are summarized in Supplementary Table 1. Functional analysis identified a wide array of disturbances in ER stress-related genes (e.g., UPR, protein folding, and Golgi apparatus) in response to CsA treatment after 6 h. However, change in genes involved in cell death, cell cycle, kidney development, and reactive oxygen species (ROS) were significantly altered after 24 h. Functional analysis indicated that apoptosis might originate from ER stress in this scenario.

3.3. CsA induced ROS production through CHOP expression

We examined the expression of ER stress-related genes in microarray data (Fig. 3A). Careful examination of the microarray data suggested that there was an inverse relationship between the expression patterns of ATF3 and CHOP. Intriguingly, *atf3* mRNA was substantially expressed for up to 6 h and then the expression gradually decreased in the following phase. In contrast, *chop* mRNA expression was initially low, but started to increase at 12 h. The

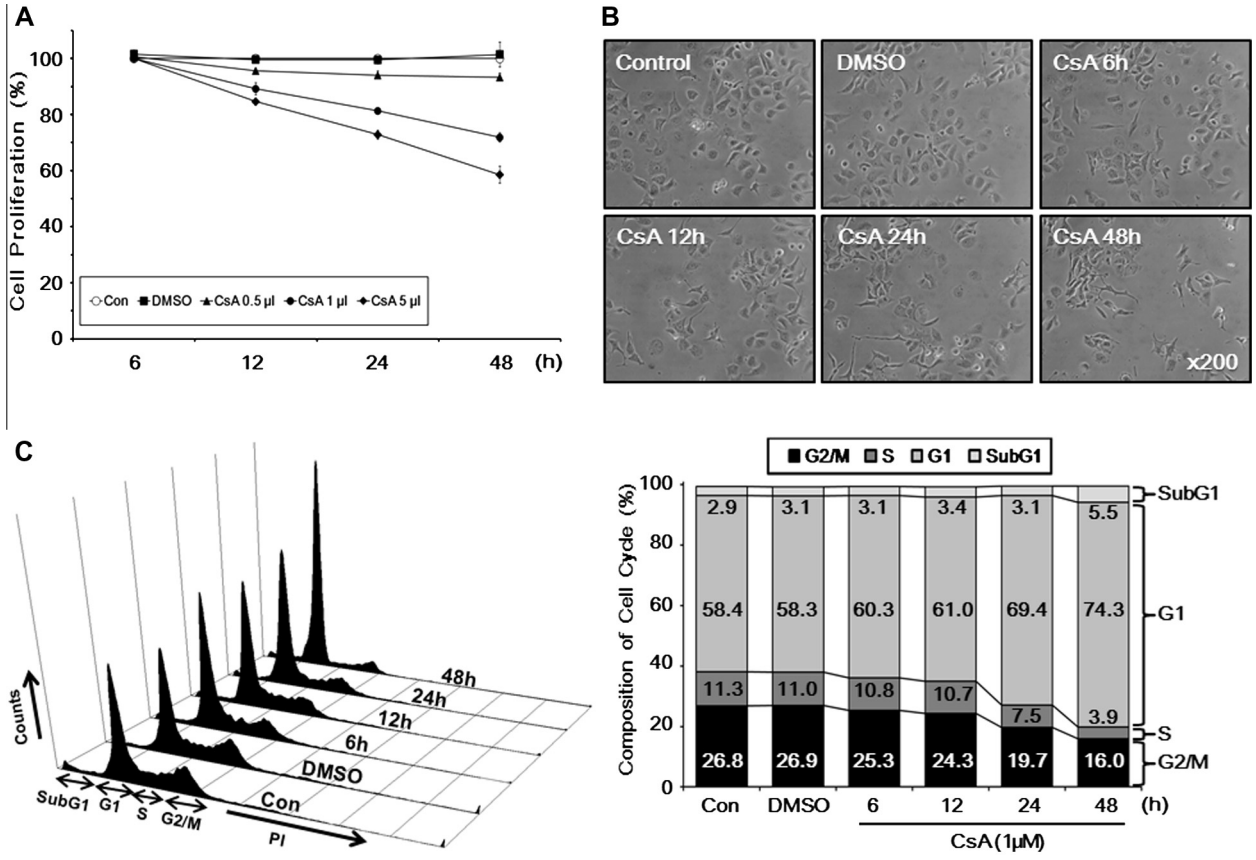

Fig. 1. Time- and dose-dependent nephrotoxicity of CsA in HK-2 cells. (A and B) CsA caused dose- and time-dependent cytotocixity (A) and morphological changes (B) in HK-2 cells. Cell proliferation was quantified using the MTS assay 6, 12, 24, and 48 h after treatment with increasing concentrations of CsA (0.5, 1, and 5 μM). (C) We applied 1 μM of CsA to HK-2 cells for 6, 12, 24, and 48 h. The cells were stained with PI solution, and cell cycle stages were analyzed by FACS. The G1 phase was observed in 69.4% and 74.3% of the cell population at 24 and 48 h, respectively.

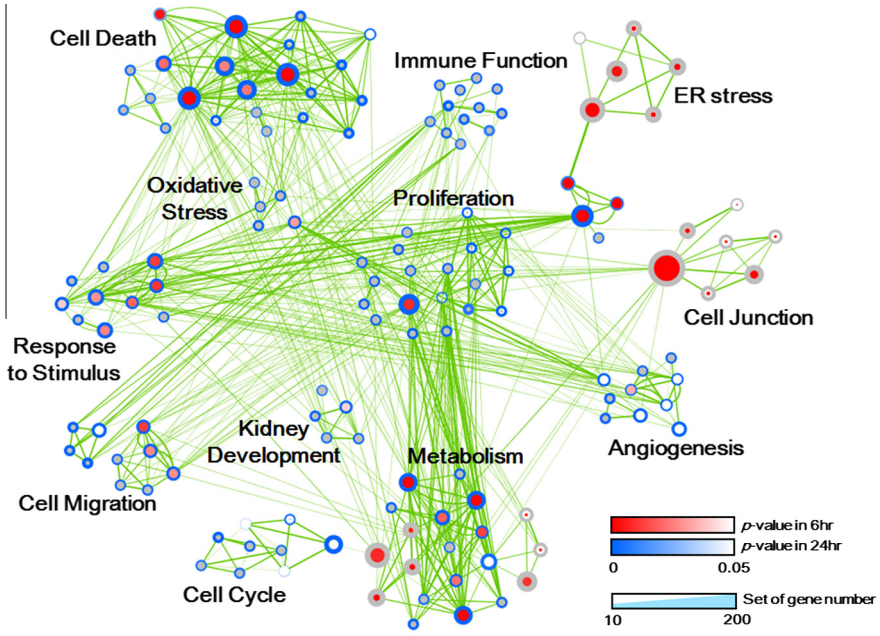

Fig. 2. Visualization of the functions of genes displaying altered expression following treatment with CsA in HK-2 cells. DAVID network derived from the CsA treatment of HK-2 cells provided an overall view of the functional consequences of CsA treatment. Each circular node denotes a gene group with the number of genes proportional to the circular size. The outer node color represents the *p*-value of enrichment in 24-h CsA-treated cells, and the inner node color represents the *p*-value of enrichment in 6-h CsA-treated cells. Each edge of the gene sets represent gene overlap among gene sets, and the similarity of the genes are indicated by the thickness of the edges among the linked nodes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

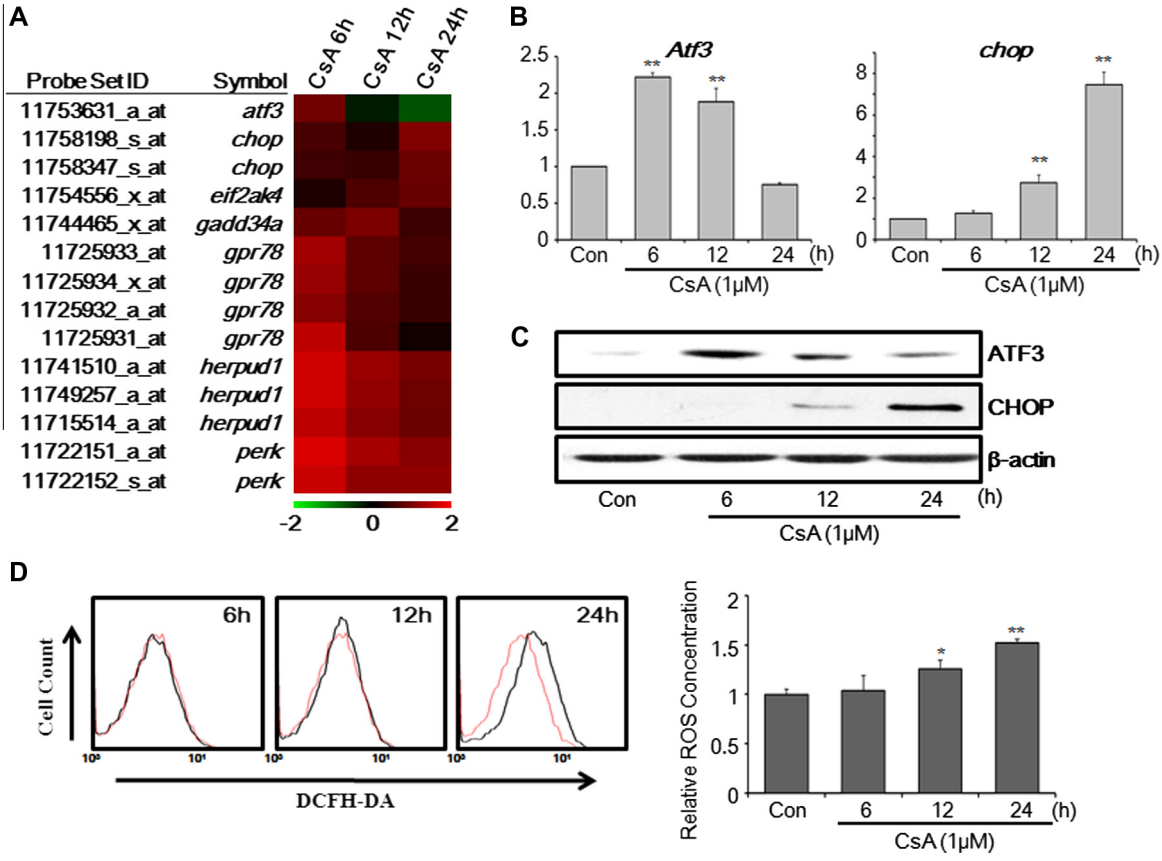

Fig. 3. CsA-induced ROS generation via ER-stress in HK-2 cells. (A) Microarray data indicated that ATF3 gene expression was increased initially (6 h), and then it started decreasing by 12 h, while CHOP gene expression started increasing at 12 h. (B) Using qRT-PCR, we confirmed that the expression levels of selected genes were similar to those observed during the microarray analysis. (C) Western blot replicated the results from the microarray analysis for ATF3 and CHOP proteins. (D) ROS production was analyzed following CsA treatment of HK-2 cells for 6, 12, and 24 h using the oxidant-sensitive probe DCFH-DA. ROS accumulation began at 12 and 24 h, as evaluated by FACS analysis. The mean ± S.D. of three individual experiments are presented; $^{*}p < 0.05$, $^{**}p < 0.01$, by the paired *t* test.

expression pattern of these genes was confirmed using qRT-PCR (Fig. 3B) and Western blot analysis (Fig. 3C).

ROS generation is invariably related to cell-cycle arrest, and we observed CsA-induced ROS generation that might contribute to the nephrotoxicity of the drug. CsA-induced intracellular ROS generation was evaluated using DCFH-DA fluorescent dye. As shown in Fig. 3D, there was no change in the ROS levels at 6 h; however, it increased from 20.7% to 52.2% after 12 and 24 h of treatment, respectively. The increase in the ROS levels corresponded to the expression pattern of CHOP, and therefore, we suspected that CHOP is involved in CsA-induced ROS generation.

3.4. Downregulation of ATF3 induces CsA-induced nephrotoxicity via CHOP expression

As shown in Fig. 3, a reduction in ATF3 expression was observed in contrast to CHOP, which exhibited increased expression after 12 h. Furthermore, CsA-induced ROS generation presented a pattern similar to that of CHOP expression. Previously, it was reported that ATF3 inhibits CHOP expression at the transcriptional level [21,22]. Furthermore, CHOP stimulates ROS generation, which culminates in oxidative stress and cell death [23,24]. Therefore, we knocked down ATF3 expression using siRNA and evaluated the expression of CHOP. Expectedly, early CHOP expression was observed (even at 6 and 12 h), confirming the transcriptional inhibition of CHOP by ATF3 (Fig. 4A).

From these results, it was apparent that CHOP induces ROS generation, causing nephrotoxicity. However, ATF3 suppresses CHOP to protect the cells from CsA-induced nephrotoxicity (Fig. 4B). Cells displayed higher levels of ROS generation after ATF3 knockdown with siRNA, as opposed to scrambled siRNA. Similarly, cell viability was reduced in cells transfected with ATF3 siRNA relative to the wild-type cells (Fig. 4C). Finally, the number of cells that were arrested in the G1 phase after CsA treatment was higher in the case of the cells transfected with ATF siRNA, as compared to the cells transfected with scrambled siRNA (Fig. 4D). Sub G1 phase was also increased in the ATF3 siRNA-treated group, and this phase change was profound at 24 h. The increase in Sub G1 phase revealed DNA damage; therefore, it can be concluded that ATF3 deficiency may influence DNA damage.

4. Discussion

CsA binds to members of the cyclophilin family (Cyp), causing the inhibition of NFAT translocation and calcineurin-dependent IL-2 cytokine expression [2,3]. This group is comprised of chaperone proteins that catalyze the isomerization between the cis- and trans-conformations of amino acids during protein folding in the

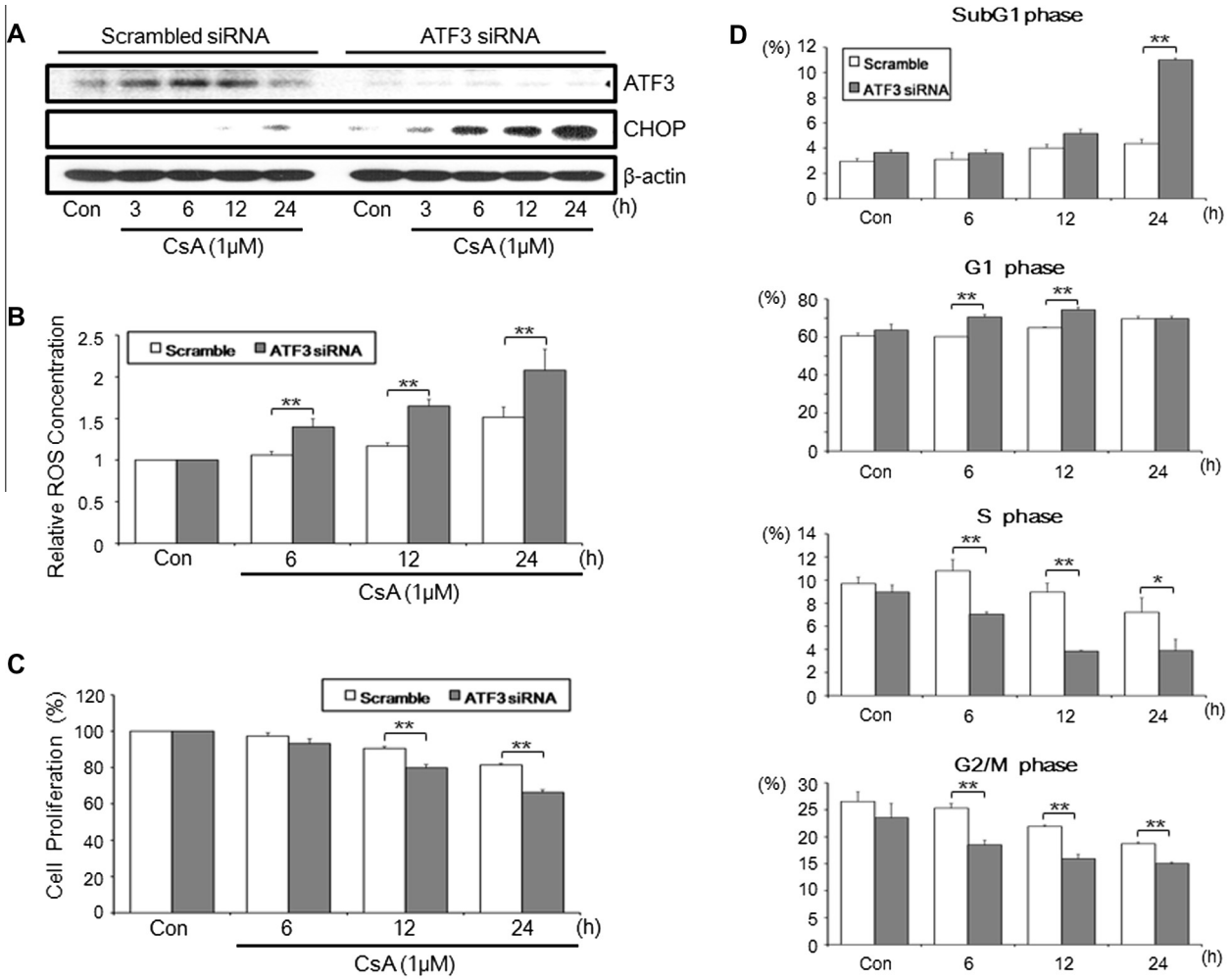

Fig. 4. The effect of ATF3 siRNA on CsA-induced ER stress. (A) CHOP expression increased after ATF3 knockdown, unlike that in cells transfected with scrambled siRNA. (B) The effect of ATF3 siRNA on CsA-induced ROS was measured using the DCFH-DA dye and FACS analysis. Cell viability (C) and cell cycle (D) results also indicate that ATF3 siRNA-treated cells exhibited enhanced nephrotoxicity when compared to CsA-only treated cells. The mean ± S.D. of three individual experiments are presented; $^{*}p < 0.05$, $^{**}p < 0.01$, by the paired t test.

ER. CsA impedes the activity of Cyp, reducing protein-folding activity, culminating in ER stress [25,26]. In this report, we demonstrated that ATF3 inhibits CsA-induced nephrotoxicity (include G1 phase arrest and cell death) by downregulating CHOP transcription in HK-2 cells.

Initially, the application of CsA to HK-2 cells resulted in the reduction of cell viability and stalled the cells at the G1 phase in a dose- and time-dependent manner (Fig. 1). Furthermore, DNA microarray analysis elucidated the perturbations in the gene expression patterns at different time points (6, 12, and 24 h), and that reinforced our earlier observations (Supplementary Table 1). At 6 h, ER stress-related genes were substantially upregulated; however, with time, the expression of the genes involved in cell-cycle regulation, ROS generation, and apoptosis were significantly induced (Figs. 1C and 2D). These results suggest that CsA treatment induces the generation of ROS and cell-cycle arrest after 12 h.

CsA treatment widely influenced the gene expression patterns of cells. Careful inspection revealed an inverse relationship between ATF3 and CHOP expression. Initially, ATF3 was highly expressed, while CHOP was minimally expressed. After 12 h of CsA treatment, ATF3 levels started to decrease, whereas CHOP expression increased (Fig. 4A–C). These results suggest that ATF3 opposed CHOP expression, which is in accordance with the results of other studies [8,21,27]. Moreover, it was reported that ATF3 is known to be upregulated, and it protects renal cells from stress-related conditions [28], while downregulating the transcription of various inflammatory mediators, cell damage-, and survival-related genes [29,30].

When ATF3 was knocked down in cells treated with CsA, a strong and an early expression of CHOP and the robust generation of ROS were observed (Fig. 4A and B). Physiologically, CHOP is involved in the generation of ROS and ER stress that may lead to cell death, whereas ATF3 is a transcription factor that downregulates CHOP transcription. Deficiency in ATF3 may render CHOP unchecked, triggering ER stress and exerting a detrimental physiological outcome [23]. ROS generation coincided with CHOP expression in ATF3-lacking cells, which led to the conclusion that CsA induced ROS generation via CHOP induction. Furthermore, CsA treatment in ATF3 knockdown cells halted the cells at the G1 phase and reduced cell viability, unlike that in the untreated cells (Fig. 4C and D). These observations provide conclusive evidence regarding the protective role of ATF3 in CsA nephrotoxicity by downregulating CHOP and attenuating ROS generation.

Cumulatively, CsA imperils cell survival by inducing ROS generation, cell cycle arrest, and apoptosis. In response to CsA challenge, stress-inducible genes such as ATF3 are expressed, facilitating cell survival. ATF3 downregulates CHOP, which is the principal mediator of CsA-induced nephrotoxicity. The knockdown of ATF3 enhances cellular disintegration and triggers apoptosis. These results strongly indicate that ATF3 acts as a stress-inducible transcription factor, and in particular, as a negative regulator of CHOP transcription, following CsA treatment. Further elucidation of the mechanism of ATF3 regulation and its dependent gene transcription would offer novel approaches for the treatment of organ transplantation-related complications.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by the Mid-Career Researcher Program through the National Research Foundation of Korea, funded by the Ministry of Education, Science and Technology (2012R1A2A2A02016803) and partly supported by a grant from the Priority Research Centers Program (NRF 2012-0006687).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.04.083.

References

[1] A.J. Olyaei, A.M. de Mattos, W.M. Bennett, Nephrotoxicity of immunosuppressive drugs: new insight and preventive strategies, Curr. Opin. Crit. Care 7 (2001) 384–389.
[2] W.M. Flanagan, B. Corthesy, R.J. Bram, G.R. Crabtree, Nuclear association of a T-cell transcription factor blocked by FK-506 and cyclosporin A, Nature 352 (1991) 803–807.
[3] S. O'Connell, N. Tuite, C. Slattery, M.P. Ryan, T. McMorrow, Cyclosporine A-induced oxidative stress in human renal mesangial cells: a role for ERK 1/2 MAPK signaling, Toxicol. Sci. 126 (2012) 101–113.
[4] E. Sarro, O. Tornavaca, M. Plana, A. Meseguer, E. Itarte, Phosphoinositide 3-kinase inhibitors protect mouse kidney cells from cyclosporine-induced cell death, Kidney Int. 73 (2008) 77–85.
[5] N. Pallet, M. Rabant, Y.C. Xu-Dubois, D. Lecorre, M.H. Mucchielli, S. Imbeaud, N. Agier, A. Hertig, E. Thervet, C. Legendre, P. Beaune, D. Anglicheau, Response of human renal tubular cells to cyclosporine and sirolimus: a toxicogenomic study, Toxicol. Appl. Pharmacol. 229 (2008) 184–196.
[6] K. Zhang, R.J. Kaufman, Signaling the unfolded protein response from the endoplasmic reticulum, J. Biol. Chem. 279 (2004) 25935–25938.
[7] D. Acosta-Alvear, Y. Zhou, A. Blais, M. Tsikitis, N.H. Lents, C. Arias, C.J. Lennon, Y. Kluger, B.D. Dynlacht, XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks, Mol. Cell 27 (2007) 53–66.
[8] S. Oyadomari, M. Mori, Roles of CHOP/GADD153 in endoplasmic reticulum stress, Cell Death Differ. 11 (2004) 381–389.
[9] X.Z. Wang, B. Lawson, J.W. Brewer, H. Zinszner, A. Sanjay, L.J. Mi, R. Boorstein, G. Kreibich, L.M. Hendershot, D. Ron, Signals from the stressed endoplasmic reticulum induce C/EBP-homologous protein (CHOP/GADD153), Mol. Cell. Biol. 16 (1996) 4273–4280.
[10] B.P. Chen, G. Liang, J. Whelan, T. Hai, ATF3 and ATF3 delta Zip. Transcriptional repression versus activation by alternatively spliced isoforms, J. Biol. Chem. 269 (1994) 15819–15826.
[11] M. Nilsson, R. Toftgard, S. Bohm, Activated Ha-Ras but not TPA induces transcription through binding sites for activating transcription factor 3/Jun and a novel nuclear factor, J. Biol. Chem. 270 (1995) 12210–12218.
[12] T. Yoshida, H. Sugiura, M. Mitobe, K. Tsuchiya, S. Shirota, S. Nishimura, S. Shiohira, H. Ito, K. Nobori, S.R. Gullans, T. Akiba, K. Nitta, ATF3 protects against renal ischemia–reperfusion injury, J. Am. Soc. Nephrol. 19 (2008) 217–224.
[13] T. Hai, C.D. Wolfgang, D.K. Marsee, A.E. Allen, U. Sivaprasad, ATF3 and stress responses, Gene Expr. 7 (1999) 321–335.
[14] D. Lu, C.D. Wolfgang, T. Hai, Activating transcription factor 3, a stress-inducible gene, suppresses Ras-stimulated tumorigenesis, J. Biol. Chem. 281 (2006) 10473–10481.
[15] B. Hua, M. Tamamori-Adachi, Y. Luo, K. Tamura, M. Morioka, M. Fukuda, Y. Tanaka, S. Kitajima, A splice variant of stress response gene ATF3 counteracts NF-kappaB-dependent anti-apoptosis through inhibiting recruitment of CREB-binding protein/p300 coactivator, J. Biol. Chem. 281 (2006) 1620–1629.
[16] H.F. Li, C.F. Cheng, W.J. Liao, H. Lin, R.B. Yang, ATF3-mediated epigenetic regulation protects against acute kidney injury, J. Am. Soc. Nephrol. 21 (2010) 1003–1013.
[17] G. Dennis Jr., B.T. Sherman, D.A. Hosack, J. Yang, W. Gao, H.C. Lane, R.A. Lempicki, DAVID: database for annotation visualization, and integrated discovery, Genome Biol. 4 (2003) P3.
[18] W. Huang da, B.T. Sherman, R.A. Lempicki, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources, Nat. Protoc. 4 (2009) 44–57.
[19] T. Ideker, V. Thorsson, J.A. Ranish, R. Christmas, J. Buhler, J.K. Eng, R. Bumgarner, D.R. Goodlett, R. Aebersold, L. Hood, Integrated genomic and proteomic analyses of a systematically perturbed metabolic network, Science 292 (2001) 929–934.
[20] A.H. Tong, M. Evangelista, A.B. Parsons, H. Xu, G.D. Bader, N. Page, M. Robinson, S. Raghibizadeh, C.W. Hogue, H. Bussey, B. Andrews, M. Tyers, C. Boone, Systematic genetic analysis with ordered arrays of yeast deletion mutants, Science 294 (2001) 2364–2368.
[21] C.D. Wolfgang, B.P. Chen, J.L. Martindale, N.J. Holbrook, T. Hai, Gadd153/Chop10, a potential target gene of the transcriptional repressor ATF3, Mol. Cell. Biol. 17 (1997) 6700–6707.
[22] Y. Cai, C. Zhang, T. Nawa, T. Aso, M. Tanaka, S. Oshiro, H. Ichijo, S. Kitajima, Homocysteine-responsive ATF3 gene expression in human vascular endothelial cells: activation of c-Jun NH(2)-terminal kinase and promoter response element, Blood 96 (2000) 2140–2148.
[23] K.Y. Tsang, D. Chan, J.F. Bateman, K.S. Cheah, In vivo cellular adaptation to ER stress: survival strategies with double-edged consequences, J. Cell Sci. 123 (2010) 2145–2154.

[24] S.J. Marciniak, C.Y. Yun, S. Oyadomari, I. Novoa, Y. Zhang, R. Jungreis, K. Nagata, H.P. Harding, D. Ron, CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum, Genes Dev. 18 (2004) 3066–3077.
[25] F. Allain, A. Denys, G. Spik, Characterization of surface binding sites for cyclophilin B on a human tumor T-cell line, J. Biol. Chem. 269 (1994) 16537–16540.
[26] V. Mikol, J. Kallen, M.D. Walkinshaw, X-ray structure of a cyclophilin B/cyclosporin complex: comparison with cyclophilin A and delineation of its calcineurin-binding domain, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 5183–5186.
[27] T.W. Fawcett, J.L. Martindale, K.Z. Guyton, T. Hai, N.J. Holbrook, Complexes containing activating transcription factor (ATF)/cAMP-responsive-element-binding protein (CREB) interact with the CCAAT/enhancer-binding protein (C/EBP)-ATF composite site to regulate Gadd153 expression during the stress response, Biochem. J. 339 (Pt 1) (1999) 135–141.
[28] T. Hai, M.G. Hartman, The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor proteins and homeostasis, Gene 273 (2001) 1–11.
[29] M. Gilchrist, V. Thorsson, B. Li, A.G. Rust, M. Korb, J.C. Roach, K. Kennedy, T. Hai, H. Bolouri, A. Aderem, Systems biology approaches identify ATF3 as a negative regulator of Toll-like receptor 4, Nature 441 (2006) 173–178.
[30] M.G. Hartman, D. Lu, M.L. Kim, G.J. Kociba, T. Shukri, J. Buteau, X. Wang, W.L. Frankel, D. Guttridge, M. Prentki, S.T. Grey, D. Ron, T. Hai, Role for activating transcription factor 3 in stress-induced beta-cell apoptosis, Mol. Cell. Biol. 24 (2004) 5721–5732.