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PMA-triggered PKCε activity enhances Nrf2-mediated antiviral response on fish rhabdovirus infection

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ABSTRACT

Viral infection is often accompanied with alteration of intracellular redox state, especially an imbalance between reactive oxygen species (ROS) production and antioxidant cellular defenses. The previous studies showed that an antioxidant cellular defense system, the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), played an important role against spring viraemia of carp virus (SVCV) infection in fish. To further reveal the mediated mechanism that Nrf2 active state was affected by protein kinase C (PKC), here we evaluated SVCV replication in host cells by treated with a strong activator of PKC phorbol-12-myristate-13-acetate (PMA) and an inhibitor staurosporine. Our results showed that PMA significantly repressed SVCV replication and viral-induced apoptosis in Epithelioma papulosum cyprini (EPC) cell, suggesting that PKC may exhibit an anti-SVCV effect. Likewise, PMA resulted in a higher phosphorylation levels of PKCε rather than PKCα/β to participate in the activation of Nrf2, mainly involved in the activation of Nrf2 phosphorylation of Ser40 to favor Nrf2 translocation to nucleus. Furthermore, the data revealed that PMA up-regulated an antiviral response heme oxygenase-1 (HO1) gene expression that was confirmed as the key player against SVCV infection by HO1 specific siRNA. Overall, this study provided a new therapeutic target for the treatment of SVCV infection, and modulating PKC activity could be used for the prevention and treatment of SVCV.

1. Introduction

Spring viraemia of carp (SVC) is a severe viral disease of cyprinid fish to bring significant economic losses to the aquaculture industry [1,2], and is caused by spring viraemia of carp virus (SVCV), a member of the genus Vesiculovirus in the family Rhabdoviridae. Due to difficult to eradicate the virus from affected ponds, all aquatic life should be destructed to eliminate virus when SVCV infection is established [2]. Even though SVCV has been gotten public attention as a notable animal disease recognized by the International Office of Epizootics [3], the pathogenic mechanism of SVCV remains poorly understood.

Oxidative stress is one of the major pathogenic mechanisms of virus infection associated with the imbalance between reactive oxygen species (ROS) production and antioxidant defense system [4–8]. To counteract oxidative stress, cells have developed a series of endogenous defense systems. Among these systems, the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), a cap'n'collar basic leucine zipper transcription factor, plays a central role on mediating many biological processes, including antioxidative responses and drug metabolisms [9,10]. Nrf2 is mainly activated by oxidative stress or electrophilic compounds to regulate the basal and inducible expression of target genes, such as heme oxygenase-1 (HO1), glutathione-S-transferases (GSTs), superoxide dismutase, glutathione reductase and glutathione peroxidase [11]. Under normal conditions, the transcriptional activity of Nrf2 depends on dissociation from the Kelch-like ECH-associated protein 1 (Keap1), sequestering Nrf2 in the cytoplasm and orchestrating its ubiquitination and degradation. When inactivation of Keap1 is triggered by oxidative stress or chemopreventive agents, Nrf2 is released from the Nrf2-Keap1 complex, rapidly translocates into the nucleus, and binds to antioxidant response elements (AREs) in the upstream promoter area of phase II detoxifying enzymes and antioxidant protein genes [12], which represents a feasible therapeutic approach against SVCV infection [13]. In addition to Keap1, several kinases, such as protein kinase C (PKC), mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase, have been suggested to modulate Nrf2 activity. It should be noted that the PKC family plays a key role in regulating Nrf2/ARE activity through activating phosphorylation of Ser40 of Nrf2 [14–16].

The previous study revealed that PKC appears to be an important mechanism in Nrf2-mediated ARE activation to repress SVCV

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replication [17], however, it was not clearly explained which PKC isoforms acted on the regulation of Nrf2 phosphorylation yet. In this study, we aimed to identify the role of PKC in Nrf2-activated antiviral pathway during SVCV infection and explore how the activated PKC facilitated Nrf2 translocation to nucleus to inhibit SVCV replication in host cells.

2. Materials and methods

2.1. Cells and virus

Epithelioma papulosum cyprini cells were maintained at 25 °C in 5% CO_2 in medium 199 (Hyclone, USA) supplemented with 10% fetal bovine serum (FBS; ZETA LIFE, USA). SVCV multiplication was propagated in EPC cells until cytopathic effect (CPE) observed. The viral medium with cells was cultured and stored at −80 °C for use. Virus titration assay was performed as described previously [18].

2.2. Reagents and antibodies

A PKC activator phorbol-12-myristate-13-acetate (PMA) with a purity of > 99% and an inhibitor staurosporine with a purity of > 98% were purchased from Beyotime Institute of Biotechnology (China). The cytotoxicities of PMA and staurosporine on EPC cells were tested by trypan blue exclusion dye staining. It should be noted that cell viability assay on all doses of drugs used in the present study showed no detectable cell death.

The monoclonal antibodies pNrf2 (phospho S40, ab76026), PKCα (ab323376), pPKCα (phospho T638, ab32502), PKCβ (ab32026), pPKCβ (phospho S660, ab75837) and PKCε (ab124806) were purchased from Abcom (USA). The polyclonal antibodies Nrf2 (ab62352) and pPKCε (Phospho S729, 70-30497-050) were purchased from Abcom and MultiSciences (China), respectively. The α-Tubulin polyclonal antibody (AF0001), histone H3 (AF0009) and α-Tubulin (AT819) monoclonal antibody were purchased from Beyotime Institute of Biotechnology (China).

2.3. Antiviral activity and cell viability

Prior to antiviral assay, cytotoxicities of PMA and staurosporine were tested based on trypan blue exclusion dye staining test, and the initial concentration used here showed no significantly detectable (cell death was less than 10%). EPC cells were cultured in 12-well plates or 96-well plates and grown to a monolayer. Subsequently, the cells were first incubated with SVCV for 2 h and then treated by 1 and 10 μM PMA or 0.1 and 1 nM staurosporine for 24 and 48 h. Subsequently, supernatants were removed and EPC cells were washed three times with 0.1 M phosphate buffer (PBS). Total mRNA from each sample was isolated with Trizol (TaKaRa, Japan) according to the manufacturer's protocols.

For cell viability assay, EPC cells with density of 1×10^4/well were seeded in 96-well plates containing 100 μL 10% FBS M199 and incubated for reaching approximately 80–90% confluence. Then the medium was replaced with 100 μL 5% FBS medium containing SVCV ± PMA or staurosporine for incubation 48 h. The protection efficiency of coumarins on the viability of EPC cells was measured using CCK-8 Kit (Beyotime, China) following the manufacturer's protocol.

2.4. Quantitative PCR

The cells with a total of 1×10^7 were taken from in 12/24-well plates and immediately frozen in liquid nitrogen for subsequent RNA isolation. Total RNA was extracted by the Trizol reagent (Takala, Japan). Under the NanoDrop spectrophotometer (ND-1000, Nano-Drop Technologies Inc., Wilmington, DE), nucleic acid concentrations were measured at 260 nm. Purity of the extracted total RNA was determined by A_{260}/A_{280} ratio, in which ratios of the absorbance at 260 and 280 nm ranged from 1.8 to 2.0. To further ensuring RNA purity, DNA contamination was removed by treating with DNase I (Takara, Japan) following manufacturer's instruction. The purified RNA was reverse transcribed using HiScript Q Select RT SuperMix for qPCR (+gDNA wiper) (Vazyme, China), and 500 ng/μL of RNA was used per reaction in cDNA generation. The qPCR was performed using CFX96 Real-Time PCR Detection System (Bio-Rad, USA) and AceQ® qPCR SYBR® Green Master Mix (Vazyme, China). The PCR was amplified at cycling conditions: 95 °C for 5 min and then 40 cycles at 95 °C denaturation for 15 s, followed by at 60 °C annealing for 30 s and 30 s at 72 °C. Furthermore, expression of Nrf2 was also examined, which was amplified at cycling conditions: 95 °C for 5 min and then 40 cycles at 95 °C denaturation for 15 s, followed by at 55 °C annealing for 30 s and 30 s at 72 °C. To assess the specificity of each amplicon, a melt curve analysis of 5 s per step from 65 to 95 °C was performed at the end of each PCR thermal profile. The sequences of primer pairs are listed in Table 1. Each individual sample was run in triplicate wells.

Table 1
Sequences of primer pairs used for the analysis of gene expression by real-time PCR.

Genes		Primer sequences (from 5′to 3′)	References
Actin	Forward	GCTATGTGGCTCTTGACTTCGA	[41]
	Reverse	CCGTCAGGCAGCTCATAGCT	
SVCV glycoprotein (G)	Forward	GCTACATCGCATTCCTTTTGC	[42]
	Reverse	GCTGAATTACAGGTTGCCATGAT	
SVCV nucleoprotein (N)	Forward	AACAGCGCGTCTTACATGC	[43]
	Reverse	CTAAGGCGTAAGCCATCAGC	
Nrf2	Forward	GGACGAGGAGACCGGAGAGTT	[41]
	Reverse	CTGTTCTGCGAAAGCCTCATTC	
HO1	Forward	AGCTGTACAGGAGTCGCATGAA	[41]
	Reverse	ACCTGGACGTTGAGCTCGAA	
siHO1	Sense	GCUGAUCAAAGCUGCGACUTT	[40]
	Antisense	AGUCGCAGCUUUGAUCAGCTT	

2.5. Apoptosis assay

Apoptosis was enumerated based on cell morphology and by flow cytometry-based Annexin V/propidium iodide (PI) staining (Vazyme, China). Each assay was repeated a minimum of three times. Fluorescence intensity was measured by flow cytometry using a FACSCalibur (Becton Dickinson, USA), and a minimum of 20,000 was counted in each treatment.

2.6. Western blot

EPC cells were treated with 1 and 10 μM PMA or 0.1 and 1 nM staurosporine for 24 and 48 h. The total proteins were extracted by RIPA Lysis Buffer (Beyotime, China) under ice-bath cooling. Subsequently, the homogenates were centrifuged at 8000 g for 15 min at 4 °C to obtain the supernatant. The total or nuclei proteins were isolated by Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, China), and separated in 12% SDS-PAGE and then transferred to a PVDF membrane for western blot assay. The blots were blocked for 2 h at room temperature with 5% skim milk in tris-buffered saline (TBS) containing 0.1% Tween 20, and incubated different time with the primary antibodies that were shown in section 2.2 (1:1000). After washing with TBS containing 0.1% Tween 20, the blots were incubated 1 h with the corresponding secondary antibody (1:1000), namely goat anti-mouse (A0216) or anti-rabbit (A0208) IgG from Beyotime Institute of Biotechnology (China).

2.7. Immunocytochemistry

Immunocytochemistry on Nrf2 and pNrf2 in nucleus was performed

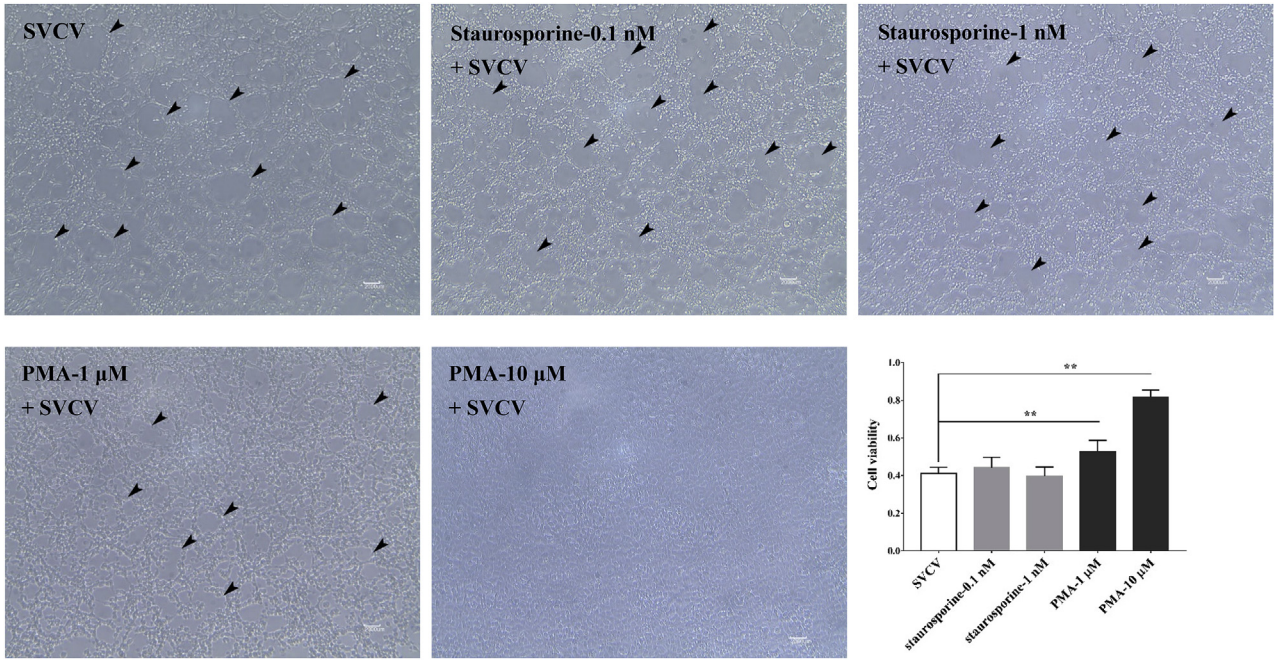

Fig. 1. Morphological effect and cell viability of PMA and staurosporine on SVCV in EPC cells. EPC cells cultured in 12-well plates were exposed to 1×10^3 $TCID_{50}$ SVCV for 2 h and then the medium with SVCV was removed and cells were incubated in fresh medium containing PMA or staurosporine for 48 h. Arrows in Figure showed cytopathic effects of SVCV-infected and drug-treated in EPC cells. Cell viability was tested by CCK-8. The *P* value for each study was determined by Student's t tests (two-tailed assuming equal variances). $^{**}P < 0.01$; $^{*}P < 0.05$.

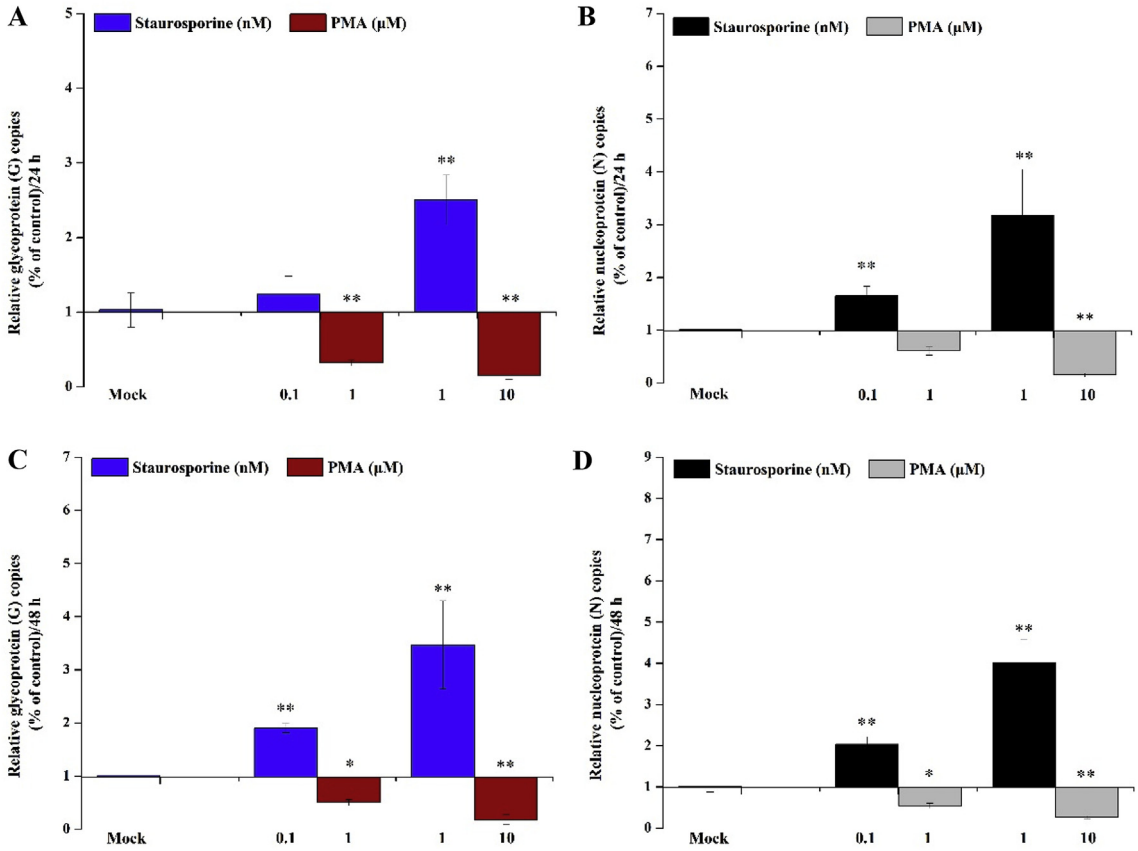

Fig. 2. After SVCV-infected cells exposure to PMA or staurosporine for 48 h, the total RNA from each sample was extracted. The glycoprotein (G) (A and C) and nucleoprotein (N) (B and D) cDNA copies of SVCV in the presence of 1 and 10 μM PMA and 0.1 and 1 nM staurosporine were assayed by qPCR at 24 and 48 h. Error bars indicate the SD. Results were from a minimum of three replicates. The *P* value for each study was determined by Student's t tests (two-tailed assuming equal variances). $^{**}P < 0.01$; $^{*}P < 0.05$.

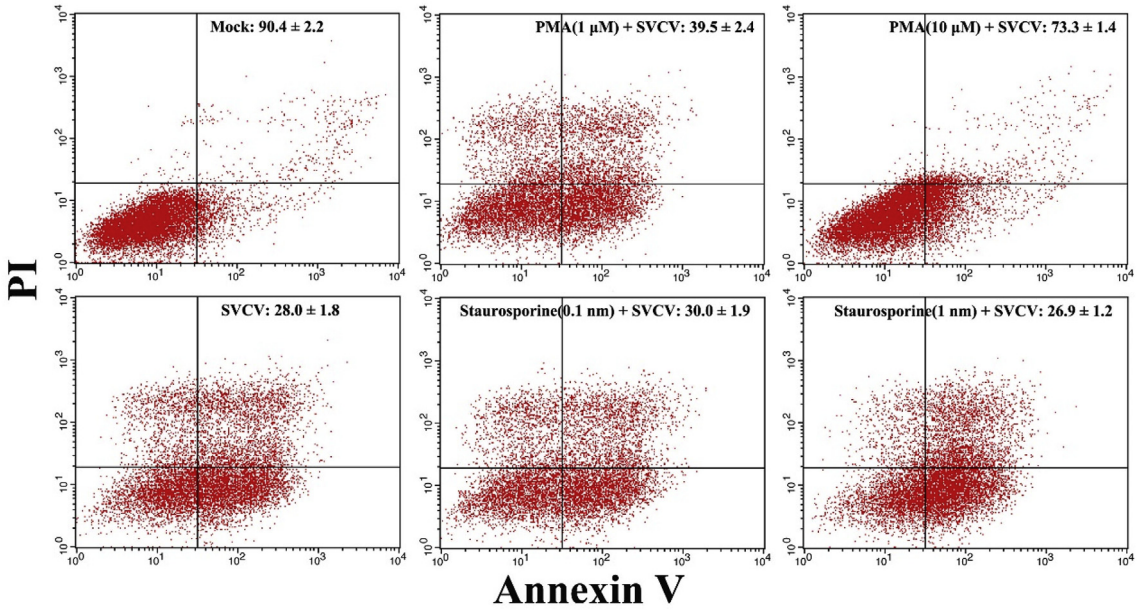

Fig. 3. SVCV triggered apoptosis in EPC cells by treated with PMA and staurosporine. The percentage of apoptotic cells was assessed by Annexin-V/PI staining at 48 h post-infection, with the highest percentage of live cells 90.4 2.2% for Mock, 28.0 ± 1.8% for SVCV, 73.3 ± 1.4% for 10 μM PMA-SVCV and 30.0 ± 1.9% for 0.1 nM Staurosporine-SVCV. Data were the means ± SD from independent experiments performed in triplicate. X and Y exes represented PI and Annexin-V, respectively.

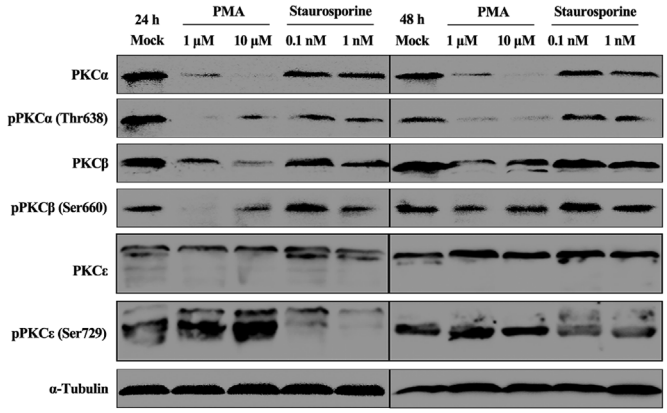

Fig. 4. The expressions of PKCα/β/ε and phosphor-PKCα/β/ε was analyzed by Western blot. A rabbit polyclonal antibodies against PKCα/β/ε with dilution of 1:1000 and pPKCα/β/ε with dilution of 1:1000 were used to monitor the expression profiles of PKCα/β/ε and pPKCα/β/ε. Antibody against α-Tubulin (1:1000) was used as the internal control.

as described previously [19]. Briefly, EPC cells, grown on cover glass, were infected with SVCV ($TCID_{50} \times 10^3$) or treated with PMA and staurosporine for 24 and 48 h. The sampled cells were fixed with 4% paraformaldehyde and permeabilized by the enhanced immunostaining permeabilization buffer (Beyotime, China). After that, cell monolayers were washed and incubated with the specific primary antibody (1:500) at 4 °C overnight. Subsequently, the cells were labeled by Alexa Fluor 488/594 secondary antibody and performed by employing an upright fluorescence microscopy (Leica-DM5000, Germany) to observe monitorization of fluorescence signals.

2.8. Knockdown of HO1 gene expression

The HO1 specific and scrambled siRNAs were synthesized by GenePharma (China) according to the previous study [40], and the siRNA sequences were shown in Table 1. According to the manufacturer's protocol of Lipofectamine 2000 (Invitrogen, USA), EPC cells were transfected with HO1 specific or scrambled siRNAs when the cells were 60–70% confluent in 24-well plates. Briefly, 100 nM final concentration of siRNAs and 2 μL of Lipofectamine 2000 were diluted and mixed gently in 100 μL of serum-free OPTI-MEM culture (Invitrogen, USA) in two separate sterile RNase-free EP tubes after incubation for 5 min at room temperature, respectively. The mixture of siRNAs and Lipofectamine 2000 was further incubated for 20 min at room temperature before being added drop wise to the cells. After the exposed cells incubated for another 6 h, the mixture was replaced with the fresh complete medium, and the cells were cultured for 24 and 48 h.

2.9. Statistical analysis

Relative mRNA expression was calculated using $2^{-\triangle\triangle Ct}$ method with the formula, $F = 2^{-\triangle\triangle Ct}$, $\triangle\triangle C_t = (C_{t,\ \text{target gene}} - C_{t,\ \text{reference gene}}) - (C_{t,\ \text{target gene}} - C_{t,\ \text{reference gene}})_{\text{control}}$ [18]. All data were presented as mean ± standard deviation (SD). Differences between experimental group and control were generated with SPSS (version 18.0) statistical software (SPSS Inc., Chicago, IL, USA) using Student's t tests (two-tailed assuming equal variances). A *p*-value of less than 0.05 or 0.01 was statistically considered significantly.

3. Results

3.1. Antiviral activity of PMA on SVCV infection

As shown in Fig. 1, the result revealed that 10 μM PMA significantly reduced the cytopathic effect (CPE) of EPC cells. By using CCK-8 examination, the viral-infected cell proliferation was increased in 1 and 10 μM PMA treatments with 52.8% and 81.8%, respectively. By contrast, the cell proliferation in 1 nM staurosporine (39.6%) was decreased compared with SVCV infection (41.0%). Besides, the result in Fig. 2 also indicated that SVCV replication was inhibited by PMA after 24 and 48 h drug exposures. In addition, the relative expression of G protein gene in PMA-treated cells decreased approximately 67.9–85.3% in 24 h and 49.4–81.9% in 48 h in comparison with control (Fig. 2A). Meanwhile, 0.1 and 1 nM staurosporine improved G protein expression with 124–252% in 24 h and 191–347% in 48 h, respectively (Fig. 2C).

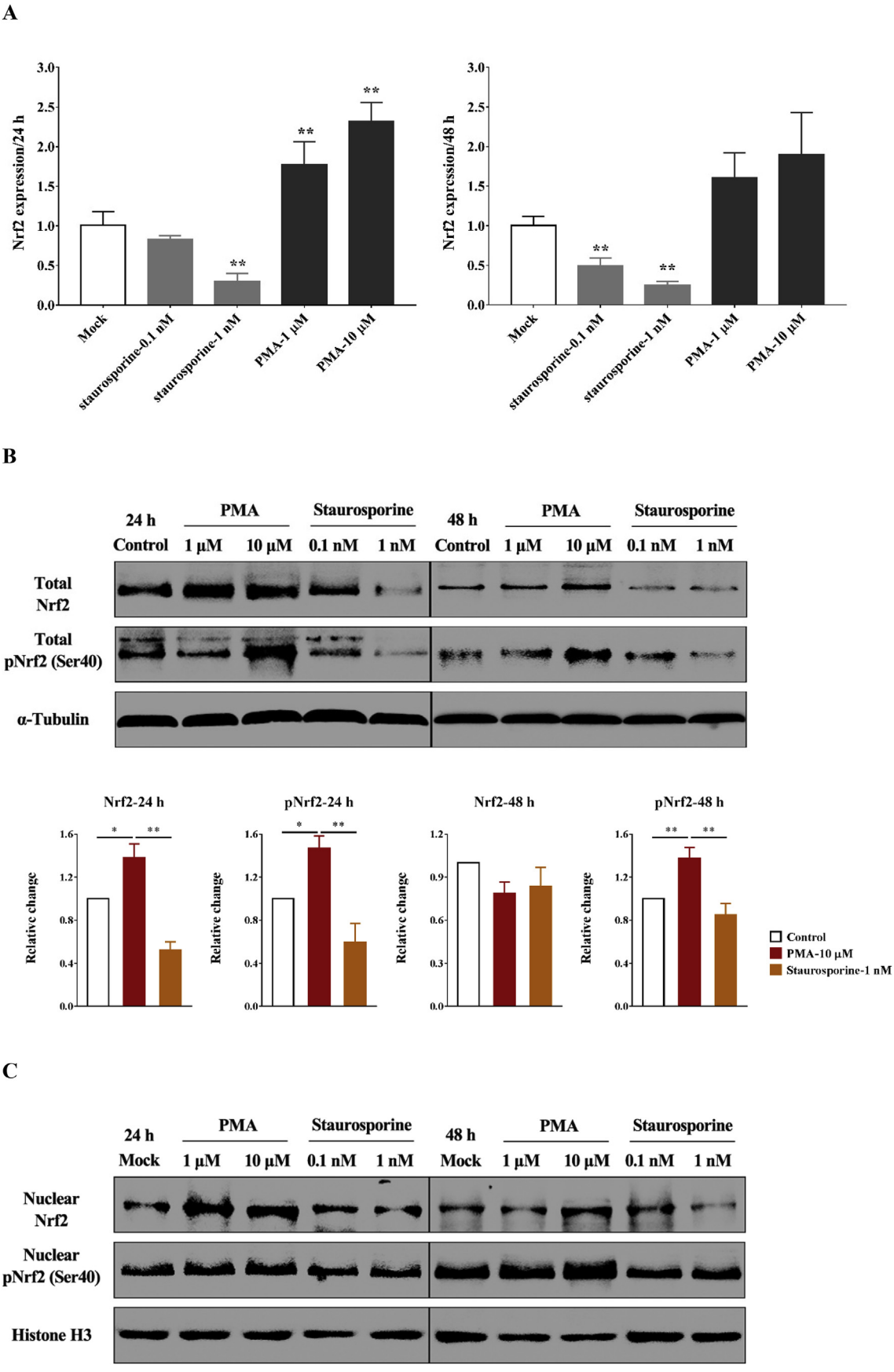

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Fig. 5. Nrf2 protein expressions in cells and nuclei after PMA or staurosporine treatment were analyzed by western blot, and their statistical data (n = 3) were shown. (A) The expression of Nrf2 in PMA or staurosporine exposed cells at 24 and 48 h. (B and C) The total amount of cellular and nuclear Nrf2 protein were analyzed by Western blotting. A rabbit polyclonal antibodies against Nrf2 with dilution of 1:1000 and pNrf2 with dilution of 1:1000 was used to monitor the expression profiles of Nrf2 and pNrf2. Antibodies against α-Tubulin (1:1000) and Histone H3 (1:1000) were used as the internal control. Error bars indicate the SD. The *P* value for each study was determined by Student's t tests (two-tailed assuming equal variances). $^{**}P < 0.01$; $^{*}P < 0.05$.

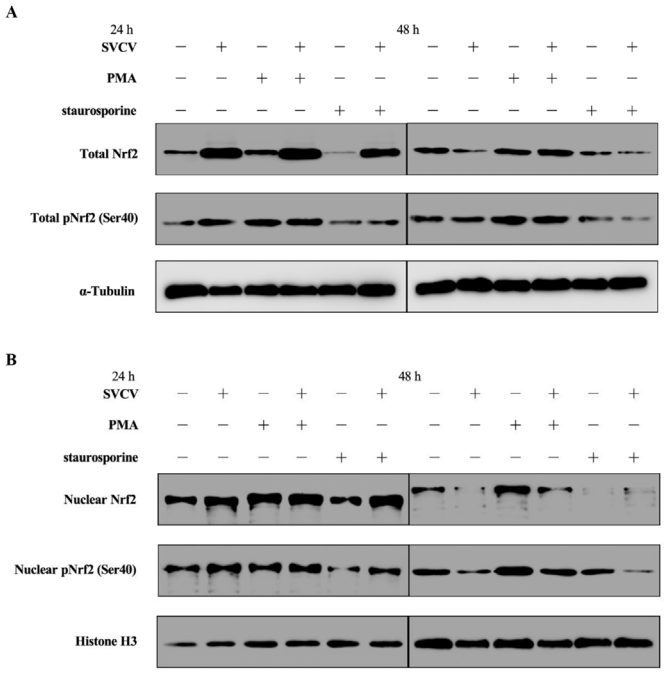

Fig. 6. Translocation of Nrf2 and phosphorylated Nrf2 were regulated in the presence of PMA or staurosporine. (A and B) Translocation of Nrf2 protein to nucleus was analyzed by Western blot.

As a check index for the antiviral effect of drugs, the additional data of N protein expression of SVCV showed a similar trend with G protein in each treatments (Fig. 2B and D).

3.2. Anti-apoptotic effect of PMA on SVCV-infected cells

As shown in Fig. 3, apoptosis in SVCV-infected cells affected by PMA and staurosporine was measured as the percentage of live cells and apoptotic cells by Annexin V/PI. The data reflected that percentage of normal/live cells in the SVCV-infected group was significantly lower than that in PMA-SVCV group, with 45.3% enhancements (10 μM PMA treatment). In contrast, the normal/live cells in response to staurosporine-SVCV treatment versus Mock were reduced. These results suggested that PMA inhibit SVCV-induced apoptosis in EPC cells.

3.3. PMA triggers PKC activity

Consistent with our hypothesis that PMA triggered phosphorylation of PKC isoforms in fish cells, phosphorylation of PKCα, β and ε were detected in the presence of PMA or staurosporine. Indeed, PKCα/β expression and pPKCα/β (Thr638/660) were inhibited by PMA (Fig. 4). On the other hand, pPKCε (Ser729) was activated by PMA-induced phosphorylation, while staurosporine dropped the phosphorylation levels in some extent. It was possible that pPKCε (Ser729) played an important role on promoting antiviral response of PMA against SVCV infection in fish cells, and PKCε isoform was an important target of PMA in EPC cells.

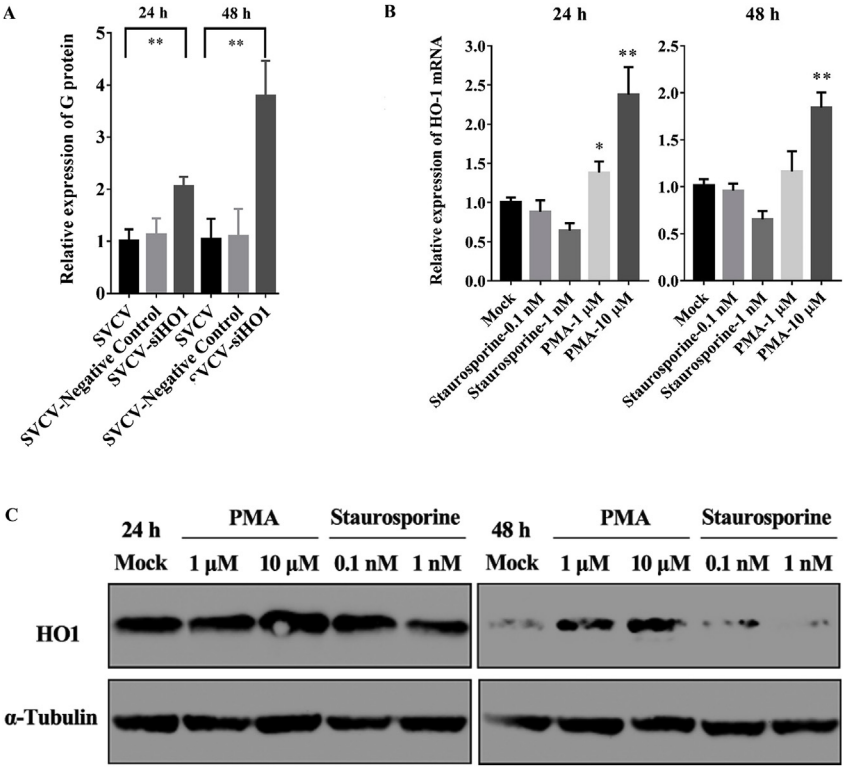

Fig. 7. The inhibition of HO1 with specific siRNAs targeting HO1 reduced SVCV replication. (A) HO1 knockdown increased the mRNA expression of SVCV G protein. (B and C) HO1 gene and protein expressions were analyzed by qPCR and Western blot respectively. The experiments were performed in triplicate, each value represents mean ± SD. $^{**}P < 0.01$; $^{*}P < 0.05$ versus Mock.

3.4. PMA enhances Nrf2 nuclear translocation

Nrf2 expression in EPC cells with PMA or staurosporine treatments was shown an opposite in Fig. 5. Although a nonsignificant change of Nrf2 in the response to PMA was recorded at 48 h, an increase of Nrf2 expression was found in PMA treatment at 24 h. In addition, Nrf2 expression in 0.1 and 1 nM staurosporine was minimized mostly 83.0% and 29.6% versus control at 24 h, respectively. With the exposure time increase, more reduction was examined in staurosporine treatments (Fig. 5A). The further detection on western blot analysis was presented in Fig. 5B and C. As expected, the total of Nrf2 in EPC cells was significantly increased by PMA treatments, confirming that PMA increased Nrf2 expression while staurosporine suppress this expression in EPC cells. Besides, we found that PMA promoted Nrf2 translocation to nucleus through western blot detections, with the maximum rise occurring in 10 μM treatment. Notably, PMA also enhanced Nrf2 phosphorylation at the Ser40 in nucleus and total of normal or viral infected cells at 48 h (Fig. 6A). A similar effect was also observed by performed immunocytochemical tests, for employing a specific antibody against Nrf2 (Fig. S1). These results indicated that PMA facilitated pNrf2 (Ser40) phosphorylation to enhance Nrf2 activity in the protein translocation process.

3.5. PMA promotes HO1 expression

In order to investigate if HO1 is the key player against for SVCV replication in EPC cells, HO1 were knocked down with knockdown rate 68.3% (24 h) and 55.6% (48 h) by gene-specific siRNAs, followed by SVCV infection in EPC cells. As shown in Fig. 7A, siHO1 significantly enhanced the expression of SVCV G protein gene, indicating that HO1 is negative effect for SVCV infection. Additionally, the previous studies replied that Nrf2 as an important nuclear factor mediated HO1 against SVCV infection [13,17]. Obviously, PMA and staurosporine exhibited an opposite effect on HO1 expression, showing that PMA up-regulated HO1 gene expression and protein level in both 24 and 48 h (Fig. 7B and C). Similar with the result of PMA on the activation of PKC-Nrf2, we hypothesized that PMA promoted activation and accumulation of Nrf2 in the nucleus to enhance HO1 expression against SVCV.

4. Discussion

As the result of a dynamic equilibrium between oxidant and antioxidant molecules, the intracellular redox state was considered to affect viral replication [19,20]. As described in the previous study, SVCV infection inducing oxidative stress resulted in a series of physiological changes in EPC cells [21]. Meanwhile, SVCV induced ROS generation to target Nrf2 antioxidant defense pathway which was potential for combating SVCV infection [13]. In this sense, Nrf2 expression of PMA stimulation was responsible for inhibition on SVCV replication in EPC cells. In parallel with this, it was that PMA can induce Nrf2 translocation to nucleus in viral present or absent, confirming that activated PKC was conducive to suppressing SVCV infection. Although virus-induced oxidant stress had exceeded the restriction of Nrf2 that exhibiting Nrf2 expression reduction in 48 h [17], PMA still significantly maintained a plateau of Nrf2 levels into nucleus.

The mechanism of Nrf2 translocation into the nucleus is complicated. In addition to chemopreventive agents and ROS inducing the dissociation of Nrf2-Keap1 to cause Nrf2 nuclear accumulation ultimately [22], Nrf2 phosphorylation also results in the release of Nrf2 from Keap1. In particular, PKC has been recognized as the key regulator and upstream signal to regulate phosphorylation of Ser40 on Nrf2 resulting in the dissociation of Nrf2 from Keap1 and activates its nuclear translocation [23]. In this regard, the results from our previous study showed that an antiviral drug had an intense response on SVCV replication through the activation of PKCα/β phosphorylation triggering in correlation with augmented Nrf2 nuclear accumulation [17]. Thus, here we assumed that the PKC family increased Nrf2 activity in nucleus displaying an anti-SVCV role. Prior to the verified experiments, the result of active analyses indicated that PMA suppressed SVCV replication and weakened virus-induced CPE and apoptosis. As well, staurosporine exhibited an opposite effect on improving viral replication. These results replied that PKC active state regulated SVCV infection in fish cells. Interestingly, not PKCα/β but PKCε (PMA stimulating) appeared to enhance phosphor-Nrf2 expression in this study. Thereby, we considered that the sites of Nrf2 phosphorylation could be mediated by multiple PKC isoforms against SVCV. As a family of phospholipid-dependent serine/threonine kinases, PKC is divided on the basis of structure and response to phosphatidylserine, calcium, and diacylglycerol, into classical (α, β, and γ), novel (δ, ε, η, and θ), and atypical (ζ, ι, and λ) isoforms [24]. Numerous studies have shown that PKCε is deemed to play a crucial role in regulating the mitochondrial membrane potential and H_2O_2 production on Nrf2 translocation [25–28], involving in PKCε mainly being related to mitochondrial protection that prevents the opening of the mitochondrial permeability transition pore [29]. In some mammal cell models, PMA seems to evoke a significant increase in the phosphorylation of PKCα or PKCβII potentially stimulated the Nrf2 or MAPK phosphorylation [30,31]. Unlikely, PMA was determined to activate PKCε phosphorylation (Ser729) for Nrf2 translocation against SVCV in EPC cells. In addition to PKCα/β, it was considered that PKCε also had an intense response on Nrf2 signaling activation during SVCV infection. Furthermore, PMA-stimulated PKC can also enhance NF-κB signaling directly as well as through MAPK-mediated pathway [32]. It is known that NF-κB activation can be evocated both by immune insults and external and internal danger signals associated with senescence and ageing process, such as oxidative and genotoxic stress and tissue injuries [33]. According to the previous study [21], the activity of NF-κB in EPC cells may be altered by SVCV infection through observation on the expression of NF-κB inhibitor alpha-like protein A and B obviously enhanced, and NF-κB could be activated by ROS production to inhibit SVCV replication.

As a highly effective therapeutic target against virus infection controlled by the transcription Nrf2 [34–36], HO1 is one of the cytoprotective enzymes and plays a crucial role in the maintenance of cellular homeostasis and increasing the survival of cells under oxidative stress [37]. Given the known function of HO1 in the innate antiviral immune responses, HO1 inhibition on viral replication generally works with type I IFN responses. Some studies revealed that up-regulation of HO1 activated IFN-related expression for negatively regulating virus replication [38,39], which is contributed to eliminating virus in host cells. Concerning the HO-1 antiviral mechanism, our results suggested that PMA up-regulated HO1 expression via triggering PKCε-Nrf2 signaling pathway against SVCV in EPC cells.

In summary, our study provides the evidence that PKC plays a positive role in antiviral effect of Nrf2 pathway following PMA and staurosporine treatments. As an underlying mechanism, we found that SVCV infection showed a reduced response to PMA through the activation of PKC-Nrf2 signaling. These data support the role of PKC in EPC cells for the host defense to repress SVCV infection.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.02.033.

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