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Transduced Tat-CIAPIN1 reduces the inflammatory response on LPS- and TPA-induced damages

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Cytokine-induced apoptosis inhibitor 1 (CIAPIN1), known as an anti-apoptotic and signal-transduction protein, plays a pivotal role in a variety of biological processes. However, the role of CIAPIN1 in inflammation is unclear. We investigated the protective effects of CIAPIN1 in lipopolysaccharide (LPS)-exposed Raw 264.7 cells and against inflammatory damage induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) in a mouse model using cell-permeable Tat-CIAPIN1. Transduced Tat-CIAPIN1 significantly reduced ROS production and DNA fragmentation in LPS-exposed Raw 264.7 cells. Also, Tat-CIAPIN1 inhibited MAPKs and NF-κB activation, reduced the expression of Bax, and cleaved caspase-3, COX-2, iNOS, IL-6, and TNF-α in LPS-exposed cells. In a TPA-induced animal model, transduced Tat-CIAPIN1 drastically decreased inflammation damage and inhibited COX-2, iNOS, IL-6, and TNF-α expression. Therefore, these findings suggest that Tat-CIAPIN1 might lead to a new strategy for the treatment of inflammatory skin disorders. [BMB Reports 2019; 52(12): 695-699]

INTRODUCTION

Inflammation is a natural defense response to infection or injury, and it may lead to various human diseases, including cancer (1). Under the inflammatory responses, macrophages are activated and secrete pro-inflammatory mediator proteins, such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and reactive oxygen species (ROS) as well as pro-inflammatory cytokines, including interleukin (IL)-6, IL-1β, and tumor necrosis factor-α (TNF-α) (2-5). Several studies have demonstrated that the nuclear factor-kappa B (NF-κB) and the mitogen-activated protein kinases (MAPKs) signaling pathways play a pivotal role in inflammatory responses, suggesting that modulation of NF-κB and MAPKs is a key point for therapeutic approaches to inflammatory diseases (6-10).

Cytokine-induced apoptosis inhibitor 1 (CIAPIN1) is known as an anti-apoptotic and signal-transduction protein, and many studies have revealed that CIAPIN1 may suppress apoptosis and regulate tumorigenesis (11-14). Park *et al*. (2011) reported that the CIAPIN1 protein protects neuronal MN9D cells against oxidative stress-induced cell death by increasing the expression of anti-apoptotic proteins (15). Wang *et al*. (2015) have shown that transfected CIAPIN1 genes in human multiple myeloma significantly inhibited the growth and proliferation of tumor cells, suggesting that CIAPIN1 is a potential tumor suppressor (16) and several studies have reported that chronic inflammation can lead to cancer by increasing pro-inflammatory mediators, ROS, intracellular signaling-pathway mediators, and transcription factors (17-19). Although the CIAPIN1 protein may be associated with the suppression of cancer and inflammation, there is no evidence about its exact roles in inflammation until now. Therefore, we investigated the effects of Tat-CIAPIN1 on inflammation with lipopolysaccharide (LPS)-exposed Raw 264.7 cells and a 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced mouse edema model.

RESULTS AND DISCUSSION

Transduction and effects of Tat-CIAPIN1 against LPS-induced cytotoxicity in Raw 264.7 cells

Since it is known that protein transduction domains (PTDs) can deliver proteins into cells, many studies have suggested that PTDs can be used for application of therapeutic proteins to treat various diseases (20-30). Purified Tat-CIAPIN1 protein was identified (Supplementary Fig. S1). We showed that Tat-CIAPIN1 transduced into the Raw 264.7 cells concentration- and time-dependently as well as transduced Tat-CIAPIN1

levels persisted in the cells for 12 h (Supplementary Fig. S2A-S2C).

We also assessed the distribution of Tat-CIAPIN1 in Raw 264.7 cells using immunostaining with Alexa Fluor 488 and DAPI. Tat-CIAPIN1 transduced into both the cytosol and the nuclei of Raw 264.7 cells. However, CIAPIN1 did not transduce into the cells (Fig. 1A). Other studies have reported that LPS induces ROS production and DNA damage in various cells, including Raw 264.7 cells, finally leading to cell damage (2-4, 31). In agreement with these reports, we showed that ROS generation and DNA fragmentation levels were significantly increased in the cells exposed only to LPS, control CIAPIN1, and Tat peptide. However, transduced Tat-CIAPIN1 markedly inhibited ROS generation and DNA fragmentation in LPS-exposed cells (Fig. 1B and 1C).
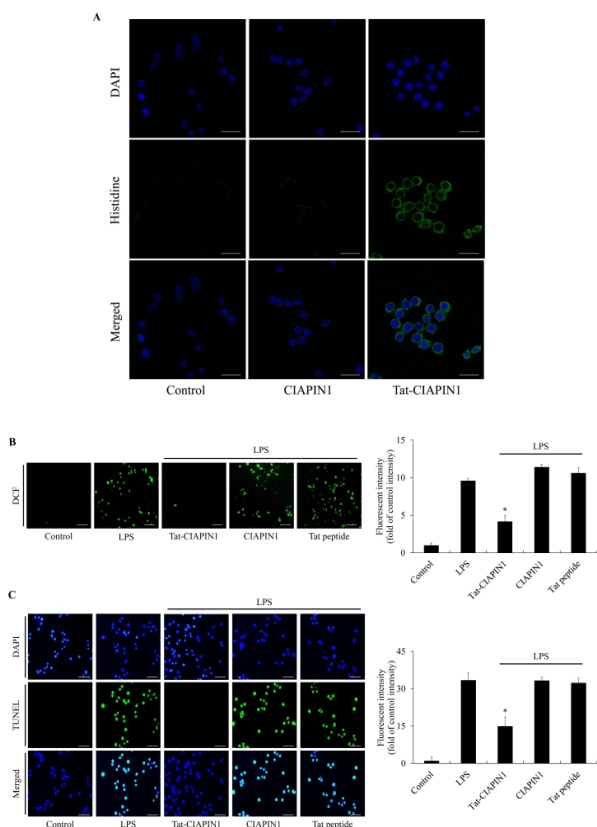
Effects of Tat-CIAPIN1 on LPS-induced inflammatory responses in Raw 264. 7 cells

Other studies have reported that regulation of NF-κB and MAPKs signaling pathways are important to protect against LPS-induced inflammatory responses in Raw 264.7 cells (32, 33). To examine the effects of Tat-CIAPIN1 on LPS-induced signaling pathways (MAPKs and NF-κB), the cells were exposed to LPS (1 μg/ml). In the LPS-exposed cells, phosphorylated MAPKs and p65 expression were higher than in the control cells. CIAPIN1 and Tat peptide-exposed cells showed similar patterns. In contrast, Tat-CIAPIN1 significantly reduced the phosphorylated MAPKs and p65 expression (Fig. 2A). Many studies have described that NF-κB and MAPKs signaling pathways are crucial mediators in various cellular biological processes and play a key role in the process of inflammatory
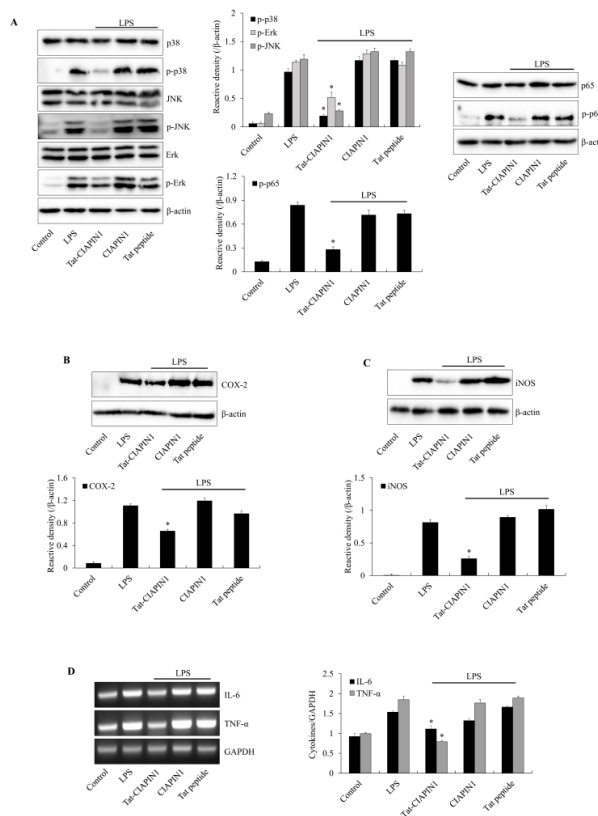

Fig. 1. Effects of transduced Tat-CIAPIN1 protein on LPS-induced ROS production and DNA fragmentation. The localization of transduced Tat-CIAPIN1 protein was examined by confocal fluorescence microscopy (A). Scale bar = 20 μm. Cells were treated with Tat-CIAPIN1 (3 μM) or CIAPIN1 protein for 1 h before treatment with 1 μg/ml of LPS for 3 h or 14 h. Then, intracellular ROS levels (B) and DNA fragmentation (C) were measured by DCF-DA staining and TUNEL staining. Fluorescence intensity was quantified using an ELISA plate reader. Scale bar = 50 μm. *P < 0.05, compared with LPS-treated cells.

Fig. 2. Effects of Tat-CIAPIN1 protein on LPS-induced inflammatory responses in Raw 264.7 cells. The cells were treated with Tat-CIAPIN1 (3 μM) or CIAPIN1 protein for 1 h before being exposed to LPS (1 μg/ml). MAPK and NF-κB activation (A) and the expression levels of COX-2 (B) and iNOS (C) protein were analyzed by Western blotting. Total RNA was extracted from the cells. We analyzed cytokines (IL-6 and TNF-α) and GAPDH mRNA by RT-PCR using specific indicated primers (D). The band intensity was measured by densitometer. *P < 0.05, compared with LPS-treated cells.

response by promoting the release of the pro-inflammatory cytokines (34-36).

We investigated the effect of Tat-CIAPIN1 against LPS-induced Bax, Bcl-2, and Caspase-3 expression levels. Other studies have shown that LPS induced apoptosis via a caspase-dependent mitochondrial death signaling pathway (37, 38). Supplementary Fig. S3A-S3C shows that Bcl-2 and Caspase-3 expression were reduced in the LPS-exposed Raw 264.7 cells. However, Tat-CIAPIN1 significantly increased Bcl-2 and Caspase-3 expression more than did those treated with LPS alone. In contrast, Bax and cleaved Caspase-3 expression showed an effect opposite to that of Bcl-2 and Caspase-3. There was no changes in CIAPIN1 and Tat peptide-treated cells. Consistent with our results, other studies have shown that overexpression of CIAPIN1 reduced cleaved Caspase-3 expression, whereas Caspase-3 expression was increased in CIAPIN1-depleted K562 cells, suggesting that CIAPIN1 has an anti-apoptotic function (39, 40). In addition, CIAPIN1 protected against neuronal cell death caused by increased Bcl-XL under oxidative stress conditions, suggesting that CIAPIN1 plays an important role in protecting neuronal cells against cell death induced by oxidative stress (15).

We also investigated whether Tat-CIAPIN1 suppresses the inflammatory response in LPS-induced Raw 264.7 cells. LPS markedly increased COX-2, iNOS, TNF-α, and IL-6 expression in Raw 264.7 cells. There were no changes in CIAPIN1 and Tat peptide-treated cells. However, Tat-CIAPIN1 drastically reduced COX-2, iNOS, TNF-α, and IL-6 expression in LPS-exposed Raw 264.7 cells (Fig. 2B-2D). Several studies have reported that LPS activated macrophages via the activation of NF-κB, MAPKs, pro-inflammatory proteins, and cytokines and led to cell death (32, 33, 41-43). These data indicate that Tat-CIAPIN plays an important role against LPS-induced Raw 264.7 cell injury. However, the precise mechanism involved in the target site of Tat-CIAPIN1 in inflammatory molecular signal pathways remains to be elucidated.
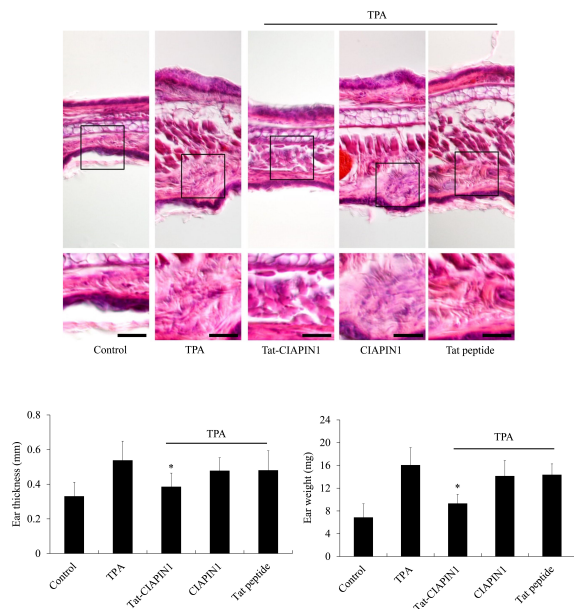

Fig. 3. Effects of Tat-CIAPIN1 protein on TPA-induced mice ear edema. Ears of mice were treated with TPA (1 μg/ear) once a day for 3 days. Tat-CIAPIN1 protein (10 μg) was topically applied to the mouse ears 1 h prior to TPA exposure over 3 days. Protective effects of Tat-CIAPIN1 protein were confirmed by hematoxylin and eosin staining, changes in ear weight, and ear thickness in a TPA-induced mice ear edema model. Scale bar = 50 μm. *P < 0.05, compared with TPA-treated mice.
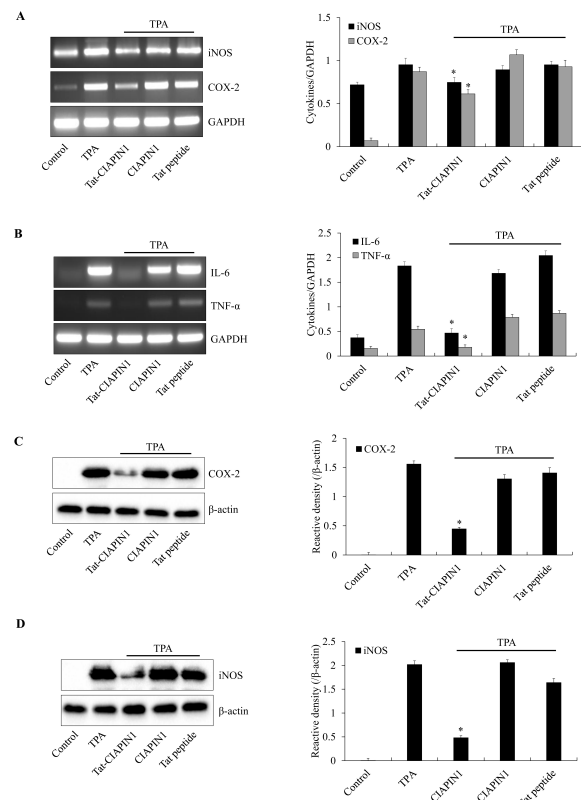

Fig. 4. Effects of Tat-CIAPIN1 protein on TPA-induced pro-inflammatory mediator protein (iNOS and COX-2) and cytokine (IL-6 and TNF-α) expression in mice ears. Mice were stimulated with TPA (1 μg/ear), after which Tat-CIAPIN1 protein (10 μg) was topically applied to the mouse ears for 3 days. Mouse ear extracts were prepared. After total RNA was extracted from ear biopsies, pro-inflammatory mediator proteins (iNOS and COX-2) and cytokine (IL-6 and TNF-α) expression levels were measured by RT-PCR using specific primers (A, B). The expression levels of pro-inflammatory mediator protein (iNOS and COX-2) were confirmed by Western blotting (C, D). The band intensity was measured by densitometer. *P < 0.05, compared with TPA-treated mice.

Effects of Tat-CIAPIN1 on TPA-induced ear edema model

It has been reported that multiple applications of TPA to the ears of mice induce skin inflammation (44). TPA-induced skin tumors are highly related to the inflammatory response, including production of cytokines, iNOS, and COX-2 proteins (45-48). Therefore, we investigated whether transduced Tat-CIAPIN1 protects against inflammation in a TPA-induced ear edema animal model (Supplementary Fig. S4). As shown in Fig. 3, TPA significantly increased ear thickness and weight of mice as compared with the control-, CIAPIN1- and Tat peptide-treated groups. This increase in ear thickness and weight was markedly inhibited by treatment with Tat-CIAPIN1. We also examined whether Tat-CIAPIN1 could inhibit expression of pro-inflammatory cytokines and proteins. TPA markedly increased the COX-2, iNOS, TNF-α, and IL-6 expression in mice ears. CIAPIN1 and Tat peptide did not show the protective effects. However, Tat-CIAPIN1 drastically reduced the COX-2, iNOS, TNF-α, and IL-6 expression in a TPA-induced mice model of edema (Fig. 4). Our results coincide with those of Chung et al. (2007), who reported that pro-inflammatory protein and cytokines expression markedly increased in TPA-induced skin inflammation models (49).

In summary, our study showed that Tat-CIAPIN1 inhibits LPS-induced inflammation damage by suppression of pro-inflammatory proteins and cytokines expression and of the NF-κB and MAPK signaling pathways. These finding imply that Tat-CIAPIN1 exerts a protective role in the inflammatory response. Although further studies are still needed to confirm the precise roles of Tat-CIAPIN1 in inflammation, these finding suggest that Tat-CIAPIN1 may be a potential therapeutic agent for skin inflammatory diseases.

MATERIALS AND METHODS

See supplementary information for this section.

ACKNOWLEDGEMENTS

CONFLICTS OF INTEREST

The authors have no conflicting interests.

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