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Tat-CIAPIN1 protein prevents against cytokine-induced cytotoxicity in pancreatic RINm5F β-cells

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Cytokines activate inflammatory signals and are major mediators in progressive β-cell damage, which leads to type 1 diabetes mellitus. We recently showed that the cell-permeable Tat-CIAPIN1 fusion protein inhibits neuronal cell death induced by oxidative stress. However, how the Tat-CIAPIN1 protein affects cytokine-induced β-cell damage has not been investigated yet. Thus, we assessed whether the Tat-CIAPIN1 protein can protect RINm5F β-cells against cytokine-induced cytotoxicity. In cytokine-exposed RINm5F β-cells, the transduced Tat-CIAPIN1 protein elevated cell survivals and reduced reactive oxygen species (ROS) and DNA fragmentation levels. The Tat-CIAPIN1 protein reduced mitogen-activated protein kinases (MAPKs) and NF-κB activation levels and elevated Bcl-2 protein, whereas Bax and cleaved Caspase-3 proteins were decreased by this fusion protein. Thus, the protection of RINm5F β-cells by the Tat-CIAPIN1 protein against cytokine-induced cytotoxicity can suggest that the Tat-CIAPIN1 protein might be used as a therapeutic inhibitor against RINm5F β-cell damage. [BMB Reports 2021; 54(9): 458-463]

INTRODUCTION

Type 1 diabetes mellitus (T1DM), a chronic autoimmune disease, occurs primarily in childhood and is characterized by hyperglycemia that destroys pancreatic β-cells. T1DM is increasing and affecting millions of people worldwide. It is well known that environmental and genetic factors affect the development of T1DM (1-5). In the early stages of T1DM, the infiltration of inflammatory cells promotes the release of cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interferon-γ (INF-γ), and promotes cytotoxicity in pancreatic β-cells, which may contribute to the impairment of insulin secretion and lead to β-cell death (6-8). Thus, inhibition of β-cell damage may ameliorate T1DM progression.

Cytokine-induced apoptosis inhibitor 1 (CIAPIN1; originally named anamorsin) is an identified apoptosis-associated protein. Several studies have reported that CIAPIN1 is an anti-apoptotic molecule that has no homology with the anti-apoptotic proteins, such as Bcl-2, caspase, the IAP families, or other signal-transduction molecules; CIAPIN1 is known as a regulator of the RAS signaling pathway (9-12). CIAPIN1 is expressed in various tissues, including metabolic tissues, and has a critical role in various cancers, including gastric cancer, hepatocellular carcinoma, and renal cancer (12, 13). Several previous studies showed that the CIAPIN1 protein inhibits the proliferation of various cancer cells; so this protein could be a new anticancer agent (13-16). In the brain, overexpression of the CIAPIN1 protein reduced dopaminergic neuronal cell death in rat brains. Other studies have reported that this protein is involved in inflammation, ROS, and intracellular signaling-pathway mediators (17-20).

Protein transduction peptides (PTDs) are generally composed of 4-30 basic amino-acid-rich sequences and are promising tools for the delivery of proteins into cells (21, 22). In general, the large molecule of protein prevents them from being delivered to cell membranes and the blood-brain barrier (BBB). However, PTD technology permits intracellular delivery of therapeutic molecules without increasing cytotoxicity (23, 24). The Tat PTD is a natural peptide from human immunodeficiency virus type 1 and is commonly used to deliver proteins into cells (25-27). We also have reported that the Tat-CIAPIN1 protein reduced cell deaths in oxidative-stress-induced hippocampal neuronal cells and reduced inflammatory responses in LPS-induced Raw 264.7 cells (28, 29). In addition, many reports have shown that transduced PTD fusion proteins inhibit cell deaths in oxidative stress- or cytokine-induced cells (28-35). In this study, we investigated whether the Tat-CIAPIN1 protein can protect RINm5F β-cells against cytokines-induced cytotoxicity.

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RESULTS AND DISCUSSION

Transduction of the Tat-CIAPIN1 protein into RINm5F β-cells

Much evidence has demonstrated that a transduced fusion protein has been an effective tool for application of therapeutic proteins (28-35). Although the precise mechanism of transduction of Tat fusion proteins has not been well established, this fusion protein seems to be transduced into cells by either direct translocation or endocytosis (36, 37). This protein delivery has been getting the spotlight as an alternative means for gene delivery (38, 39). To investigate the transductive efficiency of the Tat-CIAPIN1 protein in RINm5F β-cells, we prepared the cell-permeable Tat-CIAPIN1 protein as described previously (28).

Fig. 1A shows purified Tat-CIAPIN1 and CIAPIN1 proteins. To assess the ability for Tat-CIAPIN1 protein transduction, we treated RINm5F β-cells with Tat-CIAPIN1 proteins (0.5-3 μM) for 1 h or with Tat-CIAPIN1 proteins at 3 μM for 15-60 min. As shown in Fig. 1B and 1C, the Tat-CIAPIN1 protein was transduced into RINm5F β-cells in a concentration- and time-dependent manner. In addition, the Tat-CIAPIN1 protein levels remained stable for 12 h after transduction and subsequently disappeared over time (Fig. 1D). These results indicate that the Tat-CIAPIN1 protein was transduced into RINm5F β-cells and was maintained in the cells for at least 12 h. In a previous study, we showed that the Tat-CIAPIN1 protein was transduced into HT-22 and Raw 264.7 cells (28, 29).

The Tat-CIAPIN1 protein inhibits cytokine-induced RINm5F β-cell damage

It is well known that cytokines promote cytotoxicity in pancreatic β-cells and lead to β-cell death (6-8). Therefore, we first investigated whether the Tat-CIAPIN1 protein inhibits cytokine-induced RINm5F β-cell deaths. As shown in Fig. 2A, transduced Tat-CIAPIN1 protein increased cell survival in a concentration-dependent manner against cytokine-induced cell death. Also, we assessed the toxicity of the Tat-CIAPIN1 protein by measuring the cell viability. As shown in Fig. 2B, the viability of the cells treated with the Tat-CIAPIN1 protein was consistently maintained.

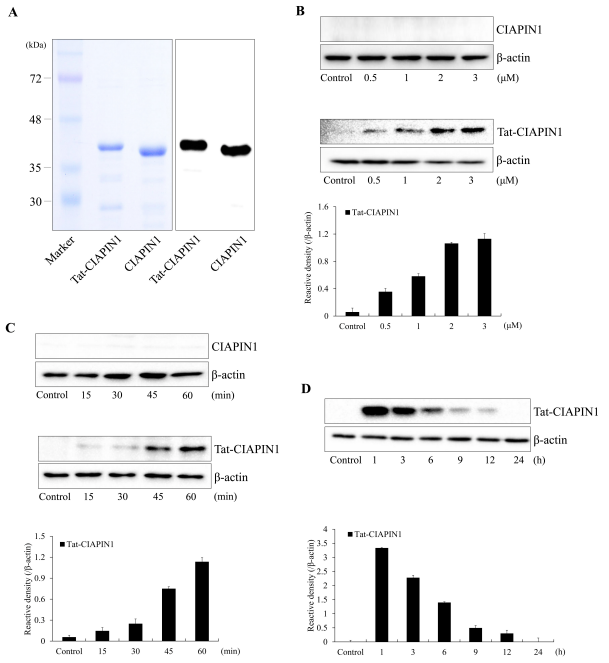

Fig. 1. Purification and transduction of the Tat-CIAPIN1 protein into RINm5F β-cells. We confirmed purified Tat-CIAPIN1 and CIAPIN1 proteins by 12% SDS-PAGE and Western blot analysis (A). Transduction of the Tat-CIAPIN1 protein into RINm5F β-cells. We added the Tat-CIAPIN1 protein (0.5-3 μM) to the culture media for 1 h (B). We then added the Tat-CIAPIN1 protein (3 μM) to the culture media for 15-60 min (C). We assessed the stability of the transduced Tat-CIAPIN1 protein after various time periods. The cells were treated with the Tat-CIAPIN1 protein (3 μM), incubated for 1-24 h, and analyzed by Western blot analysis (D). We repeated all experiments at least three times, and present data as mean ± SEM.

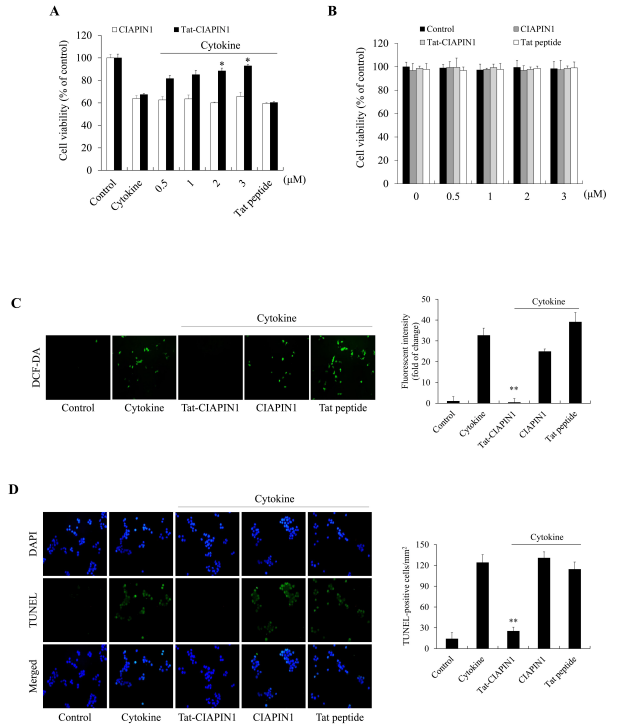

Fig. 2. Effects of the transduced Tat-CIAPIN1 protein on cytokine-induced RINm5F β-cell damage. We pretreated the Tat-CIAPIN1 protein (0.5-3 μM or 3 μM) with RINm5F β-cells for 1 h, and then treated it with cytokines (5 ng/ml IL-1β, 10 ng/ml TNF-α, and 10 ng/ml IFN-γ). Then we assessed cell viability (A and B), ROS production (C), and DNA fragmentation (D) as described in Materials and Methods. *P < 0.01 and **P < 0.001 compared with cytokine-treated cells. We repeated all experiments at least three times, and present data as mean ± SEM.

Next, we investigated whether the Tat-CIAPIN1 protein inhibits ROS production and DNA fragmentation (Fig. 2C, D). In cytokine-exposed RINm5F β-cells, ROS production and DNA fragmentation levels were decreased by the Tat-CIAPIN1 protein. These results indicate that the Tat-CIAPIN1 protein can protect against cytokine-induced cytotoxicity. However, the CIAPIN1 protein or Tat peptide alone did not show the protective effects in cytokine-induced RINm5F β-cells.

Since pancreatic β-cells contain significantly fewer antioxidant proteins, including superoxide dismutase, catalase, and glutathione peroxide, than do other tissues in the rat model, several studies have reported that cytokines and oxidative stress are major factors for the destruction of pancreatic β-cells. The antioxidant proteins play a beneficial role in pancreatic β-cell viability (40-43).

Effects of Tat-CIAPIN1 against cytokine-induced NF-κB, MAPK, and the apoptosis signaling pathway

Some studies have shown that NF-κB is a key factor in cytokine-induced pancreatic β-cell damage and have suggested that inhibition of NF-κB may be a novel strategy for delaying the progression of T1DM (44, 45). Sakai *et al*. (46) have reported that NF-κB and MAPKs are involved in diabetes complications and diabetic nephropathy. Other studies have shown that MAPK and NF-κB activation were increased in streptozotocin-induced rat or INS-1 cells (46-48). In this study, we showed that the levels of NF-κB and MAPK activation were markedly increased in cytokine-exposed RINm5F β-cells by the Tat-CIAPIN1 protein and that this fusion protein reduced NF-κB and MAPK activation levels (Fig. 3).

Since it is known that excessive cytokine leads to cell death via the apoptosis signaling pathway and that Bcl-2 and Bax protein expression is associated with apoptosis (47-50), we examined whether the Tat-CIAPIN1 protein regulates apoptotic signaling (Fig. 4). In cytokine-induced RINm5F β-cells, the Tat-CIAPIN1 protein significantly reduced cleaved Caspase-3 and Bax protein expression, whereas Bcl-2 protein expression was significantly increased by this fusion protein. These results indicate that Tat-CIAPIN1 plays a crucial role for cell survival by regulating signaling pathways in cytokine-exposed RINm5F β-cell (Fig. 4). Zhang *et al*. (51) showed that overexpression of the CIAPIN1 protein significantly increased Bcl-2 protein expression, whereas Bax protein expression was significantly decreased in hypoxia/reoxygenation-damaged H9c2 cells, suggesting that overexpression of the CIAPIN1 protein reduced apoptosis by the changes of expression of apoptosis-associated proteins in hypoxia/reoxygenation injury (51).

In summary, we showed that the Tat-CIAPIN1 protein inhibited pancreatic β-cell death by reducing ROS generation, activating NF-κB and MAPK, and regulating the apoptosis-associated proteins in cytokine-induced RINm5F β-cells. Although the precise function of the CIAPIN1 protein in T1DM remains to be verified, the Tat-CIAPIN1 protein plays a beneficial role in pancreatic β-cells. Therefore, we suggest that Tat-CIAPIN1 may provide a potential therapeutic protein agent for T1DM.

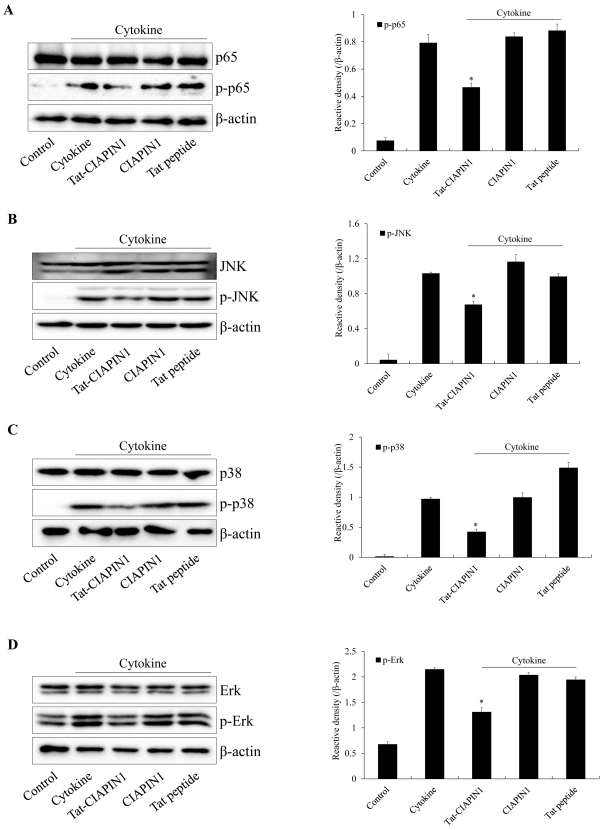

Fig. 3. Effects of the Tat-CIAPIN1 protein on cytokine-induced NF-κB and MAPK activation in RINm5F β-cells. We pretreated the cells with the Tat-CIAPIN1 protein (3 μM) for 1 h and then treated them with cytokines (5 ng/ml IL-1β, 10 ng/ml TNF-α, and 10 ng/ml IFN-γ). Then, we assessed the levels of NF-κB (A) and MAPK (B-D) by Western blotting and measured the band intensity by densitometer. *P < 0.01 compared with cytokine-treated cells. We repeated all experiments at least three times, and present data as mean ± SEM.

MATERIALS AND METHODS

Materials

Tat-CIAPIN1 protein was prepared as described previously study (28). The used antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and Cell Signaling Technology (Beverly, MA, USA). 2′,7′-Dichlorofluorescein diacetate (DCF-DA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Cytokines (IL-1, TNF-α, and IFN-γ) were obtained from R&D system (Minneapolis, MN, USA). Fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin) were purchased from Gibco (Carlsbad, CA, USA). All other agents were of the highest grade available unless otherwise stated.

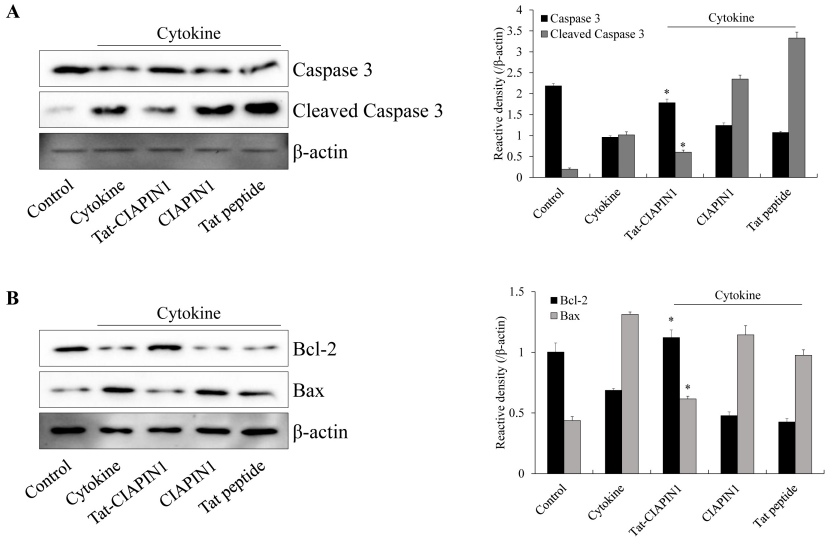

Fig. 4. Effects of the Tat-CIAPIN1 protein on cytokine-induced apoptotic signaling in RINm5F β-cells. We pretreated the cells with the Tat-CIAPIN1 protein (3 μM) for 1 h and then treated them with cytokines (5 ng/ml IL-1β, 10 ng/ml TNF-α, and 10 ng/ml IFN-γ). Then, we assessed the indicated protein expression levels (A, B) by Western blotting and measured the band intensity by densitometer. *P < 0.01 compared with cytokine-treated cells. We repeated all experiments at least three times, and present data as mean ± SEM.

Cell culture and Tat-CIAPIN1 protein transduction

Pancreatic β-cells (RINm5F β-cells) were obtained from the ATCC (Manassas, VA, USA) and maintained in RPMI1640 medium containing 10% FBS and 1% antibiotics.

To assess the ability of Tat-CIAPIN1 protein transduction, RINm5F β-cells were treated with 0.5-3 μM or 15-60 min of Tat-CIAPIN1 protein in culture medium. After washing, transduced levels were determined by Western blotting.

Western blot analysis

Protein concentrations were determined with Bradford assay (52). Equal amount of proteins were loaded onto 12% SDS-PAGE, and transferred to nitrocellulose membrane. The membranes were incubated in 5% milk followed by incubation overnight at 4°C with primary antibodies. After washing, the membranes were incubated with Horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h. Then, protein bands were visualized using ECL reagents (Amersham, Franklin Lakes, NJ, USA) (28, 33).

Effect of Tat-CIAPIN1 protein on RINm5F β-cell viability

Cell viability was analyzed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. After transduced Tat-CIAPIN1 protein (0.5-3 μM) into RINm5F β-cells for 1 h, cytokines (5 ng/ml IL-1β, 10 ng/ml TNF-α, and 10 ng/ml IFN-γ) were exposed the cells for 12 h. Cell viability was determined as described in previous (28).

Measurement of DNA fragmentation levels

DNA fragmentation was determined using a Cell Death Detection Kit (Roche Applied Science, Basel, Switzerland). After transduced Tat-CIAPIN1 protein (3 μM) into RINm5F β-cells for 1 h, cytokines (5 ng/ml IL-1β, 10 ng/ml TNF-α, and 10 ng/ml IFN-γ) were exposed the cells for 24 h. Then, DNA fragmentation levels were confirmed as described in previous (28, 33).

Measurement of ROS level

Intracellular ROS levels were determined using a DCF-DA staining. After transduced Tat-CIAPIN1 protein (3 μM) into RINm5F β-cells for 1 h, cytokines (5 ng/ml IL-1β, 10 ng/ml TNF-α, and 10 ng/ml IFN-γ) were exposed the cells for 22 h and the cells incubated for 30 min with DCF-DA (20 μM). Then, ROS production levels were confirmed as described in previous (28, 33).

Statistical analysis

Data represent the mean of three experiments ± SEM. Differences between groups were analyzed by ANOVA followed by a Bonferroni's post-hoc test using GraphPad Prism software (version 5.01; GraphPad Software Inc., San Diego, CA, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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