Cadmium inhibits signal transducer and activator of transcription 6 leading to pancreatic β cell apoptosis

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Abstract. The toxic heavy metal cadmium has been proven to cause pancreatic dysfunction and lead to the development of DM. However, the underlying mechanisms have not been completely elucidated. Here, we investigated the effects of cadmium on the pancreatic β cell line MIN6 and explored the underlying mechanisms. The Cell Counting Kit-8 (CCK8) assay and flow cytometry were used to determine cell viability and apoptosis in MIN6 cells. The expression levels of signal transducer and activator of transcription 6 (STAT6) were assessed by western blotting. We further assessed the effects of cadmium on the function of pancreatic β cells under high glucose levels using enzyme-linked immunosorbent assay (ELISA) and western blotting. Insulin secretion and expression were decreased by cadmium in MIN6 cells. In addition, cadmium suppressed cell viability and promoted apoptosis of MIN6 cells, downregulated insulin secretion and genesis of MIN6 cells under high glucose conditions, while inhibiting STAT6. Furthermore, after treatment with IL-4, the activator of STAT6, the MIN6 cell viability suppression and apoptosis promotion effect caused by cadmium were blocked. In conclusion, cadmium inhibits pancreatic β cell MIN6 growth by regulating the activation of STAT6. Our findings reveal a new mechanism of cadmium toxicity in pancreatic β cells.

Key words: Cadmium, Pancreatic β cell, Signal transducer and activator of transcription 6, Insulin

TYPE 2 DIABETES MELLITUS (T2DM) is a chronic metabolic disease, and primarily characterized by initial insulin resistance, followed by a gradual decline in the function of insulin secreting β cells. The factors leading to pancreatic β cell decompensation warrant clarification. In addition to genetic factors, diet and other environmental factors are thought to contribute to the development of T2DM [1, 2]. Exploring environmental factors contributing to the reduced function of pancreatic β cells is therefore highly relevant.

Among diet and environmental factors, toxic metal pollutants can affect human organs, including pancreatic islets. Cadmium (Cd) is a toxic heavy metal that is widely used in batteries, color pigments, coatings and plating, several alloys, and plasticizers. Cd is known to be ranked the 7th hazardous substance in the Substance Priority List by the US Agency for Toxic Substances and Disease Registry (Agency for Toxic Substances and Disease Registry, ATSDR, 2017). In addition to occupational exposure, diet and smoking are the two main routes through which Cd enters the human body and accumulates in human organs [3, 4]. The biological half-life of Cd is as long as 10–30 years. Individuals with nonoccupational exposure to Cd have the highest levels reached at approximately 50 years of age [5]. With long-term exposure, Cd can accumulate in human organs. Currently, most studies focus on the cytotoxic activity of chronic Cd intoxication in the kidneys, liver, thyroid, and bones [5-8]. Recent studies indicate that Cd could result in pancreatic dysfunction, which is positively correlated with higher levels of blood glucose and lower levels of serum insulin, leading to the development of DM [9]. Cd preferentially accumulates in islets with pancreatic tissue samples and induces islet atrophy thereby decreasing the islet area [9, 10]. Additionally, a study on Cd exposure showed that Cd accumulates in the pancreatic β cell line MIN6 in a time-dependent manner [9]. It can cause a degeneration, necrosis, and weak degranulation in the β
cells of the pancreatic islets of rats [11, 12]. However, the possible mechanisms through which Cd elicits its toxicological effects in pancreatic β cells have not yet been clarified.

Signal transducer and activator of transcription (STAT) family monomers are characterized structurally by the presence of the Src homology 2 (SH2) domain and a C-terminal tyrosine phosphorylation site. Upon ligand binding, STATs are recruited to the phosphorylated residues by virtue of the interaction of C-terminal SH2 domains. STAT6 is a member of the Janus kinases (JAK)/STAT signal transduction pathway and is highly expressed in various human cells, and is considered a regulator of multiple biological processes, such as cell apoptosis. Gene expression studies indicate that IL-4 can activate the tyrosine phosphorylation of STAT6 and lead to the accumulation of its homo- and heterodimers in the nucleus. STAT6 acts as a signal transducer in the cytoplasm and upon phosphorylation at Y641, translocates to the nucleus and binds to the DNA consensus site TTCN4GAA. It has been also found that p-STAT6 (Tyr641) was significantly decreased in peripheral blood mononuclear cells (PBMCs) from patients with T1D compared to controls [13]. Studies have demonstrated that IL-4 stimulation inhibits cell growth and induces apoptosis in human cancer cells and normal hepatocytes by STAT6 activation [14, 15] and by augmentation of caspase-3, 8 and 9 activity [15]. Interestingly, a recent study showed that activation of STAT6 plays an important role in maintaining the cell viability of pancreatic β cells by promoting the transcription of a variety of anti-apoptotic target genes [16]. Thus, we deduce that Cd may inhibit STAT6 to promote cell apoptosis in pancreatic β cells.

Despite this evidence, the mechanisms involved in the suppression of the viability of pancreatic β cells in response to Cd toxicity are unclear, and it is not known whether phosphorylation of STAT6 is reduced during pancreatic β cell apoptosis induced by Cd. Therefore, to address these issues, we used IL-4, the activator of STAT6, to co-treat MIN6 cells with Cd, and studied the effects of Cd on STAT6. This prompted us to study whether Cd inactivates STAT6, triggering pancreatic β cell apoptosis.

**Materials and Methods**

**Materials**

Cadmium chloride (Sigma, St. Louis, MO, USA) was dissolved in sterile distilled water to prepare stock solutions (1 and 10 mmol/L), which were filtered through a 0.22 μm pore size membrane, aliquoted, and stored at room temperature. Dulbecco’s modified Eagle’s medium (DMEM), 0.05% trypsin–EDTA, fetal bovine serum (FBS), and penicillin–streptomycin were purchased from Gibco (Rockville, MD, USA). Enhanced chemiluminescence solution from Millipore (Billerica, MA, USA), whereas normal goat serum was obtained from Chemicon International Inc. (Temecula, CA, USA). Cell Counting Kit-8 from Dojindo (Kumamoto, Japan). The following antibodies were used: caspase-3, insulin, phospho-STAT6 (Tyr641) (all from Cell Signaling Technology, Beverly, MA, USA), β-tubulin (Sigma), goat anti-rabbit IgG-horseradish peroxidase (HRP) and goat anti-mouse IgG-HRP (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA). All other chemicals were obtained from local commercial sources and were of analytical grade quality.

**Cell culture and reagents**

MIN6 cells were maintained at 37°C in a 5% CO₂ atmosphere in DMEM containing 15% FBS, 55 μmol/L β-mercaptoethanol, 25 mmol/L glucose, 3.7 g/L sodium bicarbonate, 100 U/mL penicillin, and 100 μg/mL streptomycin. All chemicals were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

**Plasmid transfection**

In brief, the target gene STAT6 was amplified using PCR, and the product was purified with gel extraction. The product of double enzyme digestion was then ligated with T4 DNA ligase, and the purified product and pcDNA3.1 were integrated into the pcDNA-STAT6 recombinant plasmid. The recombinant pcDNA-STAT6 was cloned into E. coli, and positive clones were selected and amplified (Convenience Biology, Changzhou, China). The recombinant plasmids were extracted from positive clones and transfected into MIN6 cells following identification with restriction analysis.

MIN6 cells were seeded at a density of 1 × 10⁴ cells/well in a 6-well plate, a density of 4 × 10⁴ cells/well in a 24-well plate, or a density of 5 × 10³ cells/well in a 96-well plate. The pcDNA3.1 was used to construct the pcDNA-STAT6 expression plasmids, and the vector pcDNA was used as a control, and they were transfected into MIN6 cells using Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA). After overnight culture, transfected cells were directly treated with different concentrations of Cd (1.2 or 2 μmol/L) for 48 h, followed by western blot analysis or quantification of secreted insulin, or for 72 h, followed by cell viability analysis.

**Analysis of cell viability and morphology**

MIN6, seeded in a 96-well plate (5 × 10³ cells/well), were treated with different concentrations of Cd (0–
Cd induces MIN6 apoptosis via STAT6

MIN6 cells were seeded at a density of 1 × 10^4 cells/well in a 6-well plate, treated with 1.2 or 2 μmol/L Cd for 72 h. Apoptosis was detected by flow cytometry using the FITC Annexin V Apoptosis Detection Kit (BD, San Diego, CA, USA) on a flow cytometer (Guava Technologies, CA, USA).

Statistical analysis

Data are expressed as mean ± SEM. One-way analysis of variance (ANOVA) was used to determine the significance of differences between treatment groups. t-test was used to compare the significance of differences between two groups. A p value of 0.05 was considered to indicate statistical significance.

Results

Cd reduced viability and altered morphology of MIN6 cells

Cd, a toxic heavy metal, can accumulate in the pancreatic β cell line MIN6 in a time-dependent manner. Accumulating evidence indicates that it can cause necrosis and degranulation of pancreatic β cells. To determine if a low concentration of Cd exposure has an impact on the viability of β cells, MIN6 cells were exposed to 0–2 μmol/L of Cd for 72 h, or to 1.2 or 2 μmol/L Cd for different time (0–72 h). We found that exposure of MIN6 to low concentrations of Cd resulted in a dramatic decrease in cell viability in a time- and concentration-dependent manner (Fig. 1B and C). At 2 μmol/L, Cd reduced the cell viability by ca. 50% compared to the vehicle control. This is consistent with the notion that MIN6 cells accumulate Cd in a time- and concentration-dependent manner over 72 h. Cd displays cytotoxicity when its concentration exceeds cell tolerance.

Consistently, we observed that more cells became round or shrunken when exposed to increasing concentrations of Cd (0.4–2 μmol/L) by phase-contrast microscopic observation (Fig. 1A). The data suggest that Cd may induce apoptosis of MIN6 cells, especially at concentrations of ≥1.2 μmol/L within 72 h.

Cd suppressed function of MIN6 cells

Since Cd reduced the viability of pancreatic β cells MIN6, we next assessed the effects of Cd on the function of pancreatic β cells. Insulin secretion in MIN6 cells was significantly suppressed by 1.2 μmol/L and 2 μmol/L Cd treatment for 48 h under 16.7 mmol/L glucose (Fig. 2A), suggesting that low concentrations of Cd can also abolish the high level of glucose-stimulated insulin secretion.
We further determined whether Cd reduced insulin genesis in pancreatic β cells under high glucose conditions. Under 16.7 mmol/L glucose conditions, we observed a significant decrease in the expression of insulin in MIN6 cells cultured with Cd in a dose-dependent manner (Fig. 2B).

**Cd induced apoptosis of MIN6 cells**

Earlier studies have consistently shown that cell death resulting from Cd mediates developmental cytotoxicity, which is attributed to its induction of cell apoptosis [17-19]. In our results, reduced cell viability and morphological changes appeared to be consistent with cell apoptosis. To test whether the changes were due to Cd-induced apoptosis, we performed annexin-V-FITC and...
propidium iodide staining followed by flow cytometry. As shown in Fig. 3, Cd significantly induced cell apoptosis of MIN6 cells in a concentration-dependent manner. At 2 μmol/L, Cd promoted MIN6 cell apoptosis by two-fold compared to the vehicle control. It is consistent with our previous findings. We also observed a higher cell death rate in MIN6 treated with 2 μmol/L Cd.

To gain more insights into the event that Cd induces cell apoptosis in MIN6 cells, we determined the proteolytic cleavage of caspase-3 in MIN6 cells treated with Cd (0–2 μmol/L) for 48 h (Fig. 4). Our western blotting results showed that treatment with Cd resulted in activation of caspase-3 in a concentration-dependent manner in MIN6 cells.
Cd-induced MIN6 cell apoptosis was associated with inhibition of STAT6 signaling

Previous studies have confirmed that STAT6 activation is involved in the reduction of pancreatic β cell viability. IL-13 treatment induces robust and early phosphorylation of STAT6 in pancreatic β cells in a JAK-dependent manner, demonstrating that this pathway is operational in these cells. It is not known whether this pathway is affected during the alteration of MIN6 cell viability after Cd treatment. Therefore, we hypothesized that Cd induces MIN6 cell apoptosis by inhibiting STAT6 activation. To this end, MIN6 cells were treated with Cd (0–2 μmol/L) for 48h, or with 1.2 or 2 μmol/L Cd for different time (0–72 h) followed by western blotting analysis. We found that Cd remarkably inhibited phosphorylation of STAT6 in a time- and concentration-dependent manner. Here we found a dramatic decrease in phospho-STAT6 levels after 48 h of exposure to 2 μmol/L Cd (Fig. 4), and after 72 h of exposure to 1.2 μmol/L Cd. Furthermore, Cd-mediated suppression of phospho-STAT6 (p-STAT6) was consistent with decreased cell viability (Fig. 1) and increased cell apoptosis in MIN6 cells, suggesting that Cd-induced MIN6 cell apoptosis might be associated with its reduction of STAT6 phosphorylation.

IL-4 attenuated Cd-induced cell apoptosis via activation of STAT6 in MIN6 cells

IL-4 is considered to recruit STAT6 and induce phosphorylation of STAT6 [20]. IL-4 binds to the IL-4R subunit, and then is known to preferentially induce STAT6 signaling [21]. Based on the expression data obtained from the BioGPS database, we found that IL-4Rα was expressed in MIN6 cells. Therefore, we hypothesized that IL-4 might protect against Cd-induced MIN6 cell apoptosis by upregulating the phosphorylation of STAT6.

To test the hypothesis, first of all, MIN6 cells were cotreated with IL-4 or and with Cd (1.2 and 2 μmol/L) for 72 h following preincubation with/without IL-4 (40 ng/mL) for 2 h. Then we examined whether IL-4 prevents Cd-induced MIN6 cell apoptosis. We found that IL-4 alone did not alter cell viability of MIN6 cells, but partially prevented Cd-induced MIN6 cell apoptosis (Fig. 5). However, treatment with 40 ng/mL IL-4 partially prevented Cd induced MIN6 cell apoptosis (Fig. 5A and B). In line with this, we also found that IL-4 partially increased the STAT6 activation, and suppressed Cd-induced inhibition of STAT6 signaling in MIN6 (Fig. 5B).

Fig. 5  IL-4 reversed Cd-induced cell apoptosis and suppression of insulin genesis in MIN6 via activation of STAT6. (A). The STAT6 activator IL-4 partially attenuated Cd-inhibited MIN6 cell viability. (B) IL-4 partially inhibited Cd-induced MIN6 cell apoptosis via activation of STAT6. Western blotting analysis was performed using indicated antibodies. The blots were probed for β-tubulin as a loading control. (B and C) IL-4 partially rescued Cd-inhibited insulin genesis in MIN6. All data were presented as mean ± SEM of triplicate independent experiments. ** p < 0.01, difference with 0 μmol/L Cd group; * p < 0.05, difference with 0 ng/mL IL-4 group; ** p < 0.01, + p < 0.05, difference with 16.7 mmol/L glucose plus 0 μmol/L Cd group group.
**IL-4 rescued Cd-inhibited insulin genesis in MIN6 cells**

In this study, we have observed that IL-4 alone did not show any toxicity in MIN6 (Fig. 5A and B). Here, we showed that IL-4 alone increased insulin genesis in MIN6 and partially rescued Cd-induced suppression of insulin genesis in MIN6 (Fig. 5B).

We further determined whether IL-4 has a protective effect against Cd-induced suppression of insulin secretion of pancreatic β cells under high glucose. However, Under 16.7 mmol/L glucose conditions, IL-4 has no significant effect on Cd-induced suppression of insulin secretion in MIN6 (Fig. 5C).

**Overexpression of STAT6 suppressed Cd-induced cell apoptosis in MIN6 cells**

Activation of STAT6 plays an important role in maintaining the cell viability of pancreatic β cells. Our results showed that Cd may inhibit STAT6 to promote cell apoptosis in pancreatic β cells. To demonstrate the importance of STAT6 in the pathogenesis of pancreatic β cell apoptosis induced by Cd, we first tested whether overexpression of STAT6 has any protective effect on Cd-induced MIN6 cell apoptosis. MIN6 cells, transfected with pcDNA-STAT6 or pcDNA3.1 (control), were exposed to Cd (1.2 and 2 μmol/L) for 48 h, followed by western blotting. The results showed that overexpression of STAT6 conferred partial resistance to Cd-induced inhibition of STAT6 phosphorylation as well as activation of caspase-3 (Fig. 6B).

We further determined whether overexpression of STAT6 has a protective effect against Cd-inhibited MIN6 cell viability. We observed that overexpression of STAT6 partially attenuated Cd-induced MIN6 cell viability alternation (Fig. 6A). However, overexpression of STAT6 did not change insulin secretion and genesis in MIN6 (Fig. 6B and C), suggesting that overexpression of STAT6 by tranfection with pcDNA-STAT6 has no effect on reversing Cd-suppressed the MIN6 cell function.

**Discussion**

Cd, an environmental toxin, adversely affects biological systems in various ways, including the kidneys, liver,

![Fig. 6](image-url)
thyroid, testis, and central nervous system. Both acute and chronic Cd exposure can result in nephrotoxicity, immunotoxicity, osteotoxicity, genotoxicity, neurotoxicity, and tumors. Although data are divergent, there is a growing body of evidence indicating that Cd exposure can cause hyperglycemic states in animals, and the toxicity of Cd is related to the etiology of T2DM [22-24].

In the present study, Cd exposure was found to be positively associated with higher risk of T2DM among occupational workers and susceptible populations such as pregnant women in the United States, Korea, China and Pakistan. Published literature has reported that Cd exposure significantly induces hyperglycemia and increases glycemic control biomarkers, such as serum carbohydrate metabolizing enzymes (α-amylase and DPP-IV) [25]. Cd-induced hyperglycemia results in enhanced lipid peroxidation, reduced insulin secretion, enhanced activity of gluconeogenic enzymes and impaired insulin receptor signaling [26].

Among adults in the general population, several studies have demonstrated that high urinary Cd concentration contributes to a higher risk of T2DM. However Barregard found no significant associations between blood and urinary Cd concentrations and the risk of T2DM [27]. The association between Cd exposure and T2DM has not been consistent. Possibly due to variations in research design, study population characteristics, individual Cd exposure assessment, previous studies do not fully address why these studies found no clear consensus regarding the association. It is worthwhile to note that in the published literature, Cd exposure was measured in the blood/urine levels. It has been reported that Cd accumulates in the kidney and urinary Cd is commonly recognized as the biomarker of long-term exposure, but blood Cd tends to reflect current exposure to Cd [28, 29]. Long-term exposure to Cd reflects the total body burden of individuals which is more important regarding its role in chronic diseases instead of recent exposure. However several studies have reported that changes in diet, lifestyle, and excretion rates such as urine creatinine can affect the long-term stability of urinary Cd [30]. Whether the body burden of Cd contributes to an increased risk of T2DM remains to be explored.

It is worth noting that Cd exposure induced a slightly but significant increase in α-amylase levels in Cd-exposed participants, similar to cadmium electroplating workers [25]. Serum α-amylase has been used as a biomarker to evaluate the Cd-induced pancreatic toxicity. Studies have confirmed that Cd gives priority to accumulate in islets of the human pancreas. And in model cell lines of pancreatic β cells MIN6, Cd accumulates in a time-dependent manner [9]. For its extremely long biological half-life and low rate of excretion from the body, cause Cd storage in tissues with a diversity of toxic effect. Cd toxicity leads to β cell death in the pancreatic islets of rats [11]. Other results suggest that Cd has a direct effect on the pancreas by inducing β cell damage and subsequent changes in insulin release [31, 32]. In this study, we found that chronic exposure to low concentrations of Cd (0–2 μmol/L) resulted in a significant increase in cell apoptosis (Figs. 2 and 4). However, the underlying mechanisms remain to be determined.

Caspase-3 appears to play an important role in the regulation of cell apoptosis in pancreatic β cells [33-35]. Activation of caspase-3 has been implicated in type 1 diabetes mellitus or hyperglycemic conditions [36, 37], implying that activation of the caspase pathway may be a common mechanism of pancreatic β cell apoptosis. Because Cd may trigger cell death by caspase-dependent and/or independent apoptotic mechanisms, depending on cell types [38, 39], we investigated the role of caspase-3 in Cd-induced β cell apoptosis. Our results showed that Cd significantly increased the proteolytic cleavage of caspase-3 in MIN6 cells in a concentration-dependent manner. It suggested that Cd induced cell apoptosis in the MIN6 cells in a caspase-3-dependent manner (Fig. 4).

In our study, we found that Cd markedly reduced insulin secretion in MIN6 cells after treatment with 3 mmol/L glucose (data not shown). Similar results were observed when the glucose concentration was elevated to 16.7 mmol/L (Fig. 2). Cd could also decrease insulin secretion. However, we also observed that there was no difference in insulin secretion when MIN6 responded to different concentrations of glucose (3 and 16.7 mmol/L). As a widely used pancreatic β cell line, MIN6 is cultured for the in vitro analysis of β cell function. However, after long-term culture, the function of glucose-stimulated insulin secretion (GSIS) may be lost [40]. Next, we determined whether Cd could suppress the expression of insulin in MIN6 cells. Our results showed that Cd reduced insulin expression in MIN6 treated with high level of glucose. These results implied that Cd-induced decline in insulin secretion in MIN6 treated with high glucose might be associated with its effect on insulin expression. As for how Cd suppresses insulin expression and secretion, it has been speculated that Cd may disrupt lipid metabolism and altered oxidative status and lead to pancreatic β cell dysfunction [41, 42].

Here, for the first time, we showed that Cd induces inhibition of STAT6 in the pancreatic β cell MIN6. This is strongly supported by the findings that: (i) Cd exposure decreases cell number, induces apoptosis, and inhibits cell proliferation [43, 44]; (ii) Cd induces cell toxicity, and its connection with different signaling pathways is complicated, including JAK-STAT [45, 46]; and (iii) direct inhibition of JAK-STAT pathway attenuates heavy
metal-induced cell loss and apoptosis [47]. Our results are in agreement with the finding that Cd induces cell apoptosis in various cells by directly inhibiting JAK-STAT.

Signal transducer and activator of transcription (STAT) family monomers are recruited to phosphorylated residues through interaction with their C-terminal SH2 domains. Traditionally, IL-4 or IL-13 can recruit STAT6, and IL-10 usually signals through STAT3. Once bound to the receptor, the STAT protein is phosphorylated on target tyrosine residues by activated JAK, thereby facilitating the disengagement of STATs from the receptor. Subsequently, STAT molecules dimerize with other phosphorylated STAT molecules and are transported to the nucleus, where they bind to consensus sequences present in specific target genes to promote transcription. In the studies, we also noticed that Cd decreased the phosphorylation of STAT6 in MIN6 cells in a time- and dose-dependent manner. In type 1 diabetes, selective β cell loss occurs within the inflamed milieu of insulitic islets. This milieu is generated via the enhanced secretion of proinflammatory cytokines and by the loss of anti-inflammatory molecules, such as IL-4 and IL-13. Upon binding of their cognate cytokines, each receptor promotes the auto-phosphorylation of associated JAKs, leading to a cascade of events culminating in the recruitment and phosphorylation of the transcription factor, STAT6. In response, STAT6 monomers dimerize and translocate to the nucleus where they bind to consensus sequences in genomic DNA to promote the transcription of target genes [48]. Previous studies have confirmed that IL-13 treatment induces robust and early phosphorylation of STAT6 in β cells in a JAK dependent manner [49]. Moreover, the expression of STAT6 was significantly diminished in the β cells of individuals with type 1 diabetes, suggesting that it is likely to enhance their susceptibility to the actions of proinflammatory cytokines during disease progression. And depletion of STAT6 occurs in vitro when islet cells are exposed to proinflammatory cytokines or the saturated fatty acid palmitate [16], demonstrating that STAT6 plays a critical role in promoting β cell viability and is depleted in islets of individuals with type 1 diabetes.

To gain insights into the molecular mechanism by which STAT6 regulates Cd-induced pancreatic β cell apoptosis, we carried out studies using the STAT6 activator IL-4, which is frequently used in the field [21]. Interestingly, in this study we observed that IL-4 inhibited Cd-induced pancreatic β cell apoptosis. Therefore, our findings strongly suggest that IL-4 might suppress Cd-induced cell apoptosis, at least in part, by activating STAT6.

To further verify the role of STAT6 in induction of pancreatic β cell apoptosis induced by Cd, MIN6 cells, transfected with pcDNA-STAT6 or pcDNA3.1 (as control), were exposed to Cd (1.2 and 2 μmol/L) for 48 h. Western blotting revealed that overexpression of STAT6 partially inhibited Cd-induced inhibition of STAT6 phosphorylation. Consistently, Cd-activated caspase-3 was apparently attenuated by overexpression of STAT6, indicating that pancreatic cell apoptosis due to Cd is related to the inhibition of STAT6. However, we observed that overexpression of STAT6 did not rescue Cd-inhibited insulin secretion and genesis. In line with it, we also observed that transfection with pcDNA-STAT6 or pcDNA3.1 affected the viability of MIN6, suggesting that it might be contribute to the failure of overexpression of STAT6 reversing Cd-inhibited MIN6 function.

A new question that arises from this work is how STAT6 mediates Cd-induced pancreatic β cell apoptosis. It has been reported that the suppressors of cytokine signaling (SOCS) proteins are negative regulators of the JAK-STAT pathway, and SOCSs inhibit STATs phosphorylation by binding and inhibiting JAKs [50]. Sodium arsenite can mediate expression of SOCS. However, it is unknown whether SOCSs or JAKs play an important role in Cd-induced pancreatic β cell apoptosis. Clearly, more studies are needed to address this issue.

Collectively, our findings indicate that Cd induces STAT inhibition, leading to pancreatic β cell apoptosis, and IL-4 prevents Cd-induced cell apoptosis in MIN6 in part by activating STAT6 signaling pathway.

**Disclosure**

The authors declare that there are no conflicts of interest that would prejudice the impartiality of this study.

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