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TITLE: The Apoptotic Role of Cpeb1 in INS-1 Cells

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ABSTRACT

The Apoptotic Role of Cpeb1 in INS-1 Cells

The dedifferentiation of pancreatic β-cells commonly occurs in the pathogenesis of both type 1 and type 2 diabetes. The β-cell identity undergoes unique changes depending on the diabetogenic stress that is encountered. One such change is the lower levels of Cpeb1 in β-cells affected by both types of diabetes relative to healthy mature β-cells. However, the cellular function(s) of Cpeb1 in β-cells has not been explored. We hypothesized that the downregulation of Cpeb1 in INS-1 cells (from a rat insulinoma cell line) will result in higher levels of apoptosis when faced with diabetogenic stress from proinflammatory cytokines (characteristic of type 1 diabetes) or high concentrations of glucose and palmitate (characteristic of type 2 diabetes). We found that the *in vitro* knockdown of Cpeb1 in INS-1 cells results in higher levels of apoptosis when under stress of proinflammatory cytokines but not of high glucose and palmitate concentrations.

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The Apoptotic Role of Cpeb1 in INS-1 Cells

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Senior Honors Thesis

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Abstract

The dedifferentiation of pancreatic β-cells commonly occurs in the pathogenesis of both type 1 and type 2 diabetes. The β-cell identity undergoes unique changes depending on the diabetogenic stress that is encountered. One such change is the lower levels of Cpeb1 in β-cells affected by both types of diabetes relative to healthy mature β-cells. However, the cellular function(s) of Cpeb1 in β-cells has not been explored. We hypothesized that the downregulation of Cpeb1 in INS-1 cells (from a rat insulinoma cell line) will result in higher levels of apoptosis when faced with diabetogenic stress from proinflammatory cytokines (characteristic of type 1 diabetes) or high concentrations of glucose and palmitate (characteristic of type 2 diabetes). We found that the *in vitro* knockdown of Cpeb1 in INS-1 cells results in higher levels of apoptosis when under stress of proinflammatory cytokines but not of high glucose and palmitate concentrations.

Introduction

Type 1 and type 2 diabetes are significantly prevalent diseases among the human population currently. In the United States, diabetes is the seventh leading cause of death and about 37.3 million U.S. adults are affected by some form of this disease as of 2021 (Centers for Disease Control and Prevention, 2021). Broadly speaking, both types of diabetes are characterized by the body's inability to decrease elevated blood glucose levels. However, the mechanisms by which both types achieve such an effect are different.

The islets of Langerhans are clusters of endocrine cells that are found in the pancreas; these islets consist of glucagon-secreting α-cells, insulin-secreting β-cells, and several other cell types. In the case of type 1 diabetes, mature β-cells are lost due to being targeted by an autoimmune attack (Eizirik et al., 2009; Wilcox et al., 2016). In particular, the interactions between β-cells and the pro-inflammatory cytokines of interleukin-1 β (IL-1β), tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ), is one way these insulin-producing cells are destined for dysfunction and apoptotic cell death (Eizirik et al., 2009). In type 2 diabetes, β-cells fail as a result of insulin resistance resulting in chronic hyperglycemia, which then induces β-cell endoplasmic reticulum (ER) stress and overworking due to increased insulin synthesis demands (Back and Kaufman, 2012; Tomita, 2016). It has also been suggested that elevated levels of free fatty acids, such as palmitate, produce a lipotoxic effect to β-cells, which can then contribute to its ER stress and potential apoptosis (Back and Kaufman, 2012; Kharroubi et al., 2004). There has been debate over how relevant lipotoxicity is to pathophysiological circumstances of β-cell dysfunction with regards to insulin secretory functionality (Weir et al., 2013).

A common event occurring in both type 1 and type 2 diabetes is the loss of functional β-cell mass, and this occurs via β-cell apoptosis (Butler et al., 2003; Tomita, 2016; Wilcox et al.,

2016) and the loss of mature β-cell identity (Bensellam et al., 2018; Moin and Butler, 2019; Nimkulrat et al., 2021; Talchai et al., 2012). A recent study has shown that each type of diabetes results in β-cells that differentiate into a unique identity rather than dedifferentiating in the reverse order of the normal β-cell ontogeny (Nimkulrat et al., 2021). It is worth noting that there still exists some similarities when comparing the identities of immature β-cells and those affected by both forms of the disease (Nimkulrat et al., 2021).

The *Modular regulatory network learning with per gene information* (MERLIN) algorithm is one that can essentially infer regulatory networks of individual genes and reveal potential upstream signaling proteins that may regulate them (Roy et al., 2013). In more recent unpublished work, using the MERLIN algorithm, cytoplasmic polyadenylation element-binding protein 1 (Cpeb1) was identified as a candidate regulatory protein potentially involved in directing β-cell maturation and dedifferentiation in both type 1 and type 2 diabetes (Nimkulrat, unpublished). Cpeb1 and its potential regulatory network were found to be present at higher levels in healthy adult β-cells and at lower levels in developing β-cells and both types of diabetes (Dex Nimkulrat, unpublished). Although MERLIN has indicated that Cpeb1 may regulate a key network found within β-cells' development and dedifferentiation processes, it is not exactly known what cellular functions are under the control of this regulator. Elucidating the functions that are controlled by this protein is essential to support the identification of this potential regulator by the MERLIN algorithm.

Cpeb1 is a translation regulator that has been found to be capable of either activating or repressing the translation of mRNAs containing a cytoplasmic polyadenylation element (CPE) (Richter, 2007). This regulator has also been found to repress the translation of signal transducer and activator of transcription 3 (*Stat3*) and phosphatase and tensin homolog (*Pten*) mRNAs in

HepG2 cells (from a human liver cancer cell line) (Alexandrov et al., 2012). Recently, it was shown that the activation of Stat3 protein (via phosphorylation) in β-cells is reduced when they are under stress of hyperglycemic conditions and streptozocin (STZ), which induces toxic stress on the β-cells (Weng et al., 2020). Furthermore, β-cell-specific Stat3 KO mice affected by STZ-induced hyperglycemia exhibited higher levels of hyperglycemia and β-cell apoptosis, and this occurs through an upregulation of Pten, which when inhibited can result in β-cell rescue (Weng et al., 2020). It has also been shown that deletion of the *Pten* gene in type 2 diabetic mice provided protection against loss of β-cell mass and function (Wang et al., 2010). Interestingly, while *Pten* has been known to be a tumor suppressor gene that is capable of promoting cell survival (Chen et al., 2018), its deletion in type 2 diabetic mice showed little sign of deregulated growth or impaired DNA damage repair (Wang et al., 2010).

Here, it is hypothesized that the downregulation of Cpeb1 in pancreatic β-cells induces increased levels of apoptosis when these cells face conditions that model type 1 and type 2 diabetic β-cell stress. To evaluate this hypothesis, the knockdown of *Cpeb1* using siRNA was first optimized in INS-1 cells (a rat insulinoma cell line modeling β-cells). Subsequently, after determining the most efficient knockdown achieved from optimizations, INS-1 cells with and without *Cpeb1* knockdown were prescribed treatments of cytokines (characteristic of type 1 diabetes pathogenesis) or high glucose and free fatty acid levels (representative of type 2 diabetes pathogenesis). The levels of apoptotic death were then measured and compared among the different treatments of these cells.

Materials and Methods

Cell culture. Insulin-secreting rat insulinoma cells of the INS-1 cell line were used for all cell culturing purposes. They were cultured in AddexBio RPMI-1640 medium (optimized 1x, with glucose, HEPES, L-glutamine, and sodium pyruvate) supplemented with 10% v/v HyClone FBS, 1% v/v Corning 100x penicillin-streptomycin solution, and 0.09% v/v Gibco, Life Technologies 1000x 2-mercaptoethanol. Cells were washed with Corning 1x PBS (without calcium and magnesium) prior to treatment with Gibco 0.05% trypsin-EDTA (1x) for splitting purposes. Confluency in between 80% to 90% was reached before cells were passaged. Cells of passage numbers in between 15 and 20 were utilized for all further procedures.

siRNA cell transfection. INS-1 cells were initially plated at a density of either $3.2 * 10^4$ cells/cm^2 (for 48 hour knockdown duration), $2.4 * 10^4$ cells/cm^2 (for 72 hour knockdown duration), or $1.6 * 10^4$ cells/cm^2 (for 96 hour knockdown duration). After 24 hours of incubation at 37°C following initial plating, the siRNA treatments (of either *Cpeb1* siRNA, scramble control siRNA, or no siRNA) were prepared and applied to the wells, replacing their original growth media. The cells were subject to the siRNA treatment for another 24 hours at 37°C, followed by the replacement of the transfection media with regular, complete media. The cells were kept in the complete media for the remaining time of their knockdown durations.

RNA extraction and cDNA preparation. Following siRNA transfection, the treated INS-1 cells were washed, trypsinized, pelleted (spun down at 200 x g to 900 x g for 2 minutes), and stored at -80°C until ready for RNA extraction. The QIAGEN RNeasy Plus Mini Kit and corresponding protocol were used for all RNA extraction purposes. The Invitrogen Qubit 3.0 Fluorometer and

corresponding protocol were used for RNA concentration measurements. cDNA library preparation was performed by first combining 250 ng RNA with a dNTP mix (containing 10 mM of dATP, dTTP, dCTP, and dGTP each) and random primers. The first mix was run in a thermocycler at 65°C for 5 minutes, followed by 4°C for another 5 minutes. Subsequently, 5x 1st strand buffer, 0.1 M DTT, RNaseOUT, and SuperScript III Reverse Transcriptase were all added to the cDNA preparation mixture. This was then run in the thermocycler at 25°C for 5 minutes, 50°C for 45 minutes, 70°C for 15 minutes, and then kept at 4°C.

qPCR analysis of knockdown efficiency. In preparation for qPCR, cDNA was mixed with 2x Brilliant III Ultra-Fast SYBR Green qPCR Master Mix, 50 µM forward primer, 50 µM reverse primer, and ultra-pure water. *Actb* (gene for β-actin), was used as the housekeeping gene for normalization purposes. The forward and reverse primers used for *Cpeb1* amplification are 5'-CCATTGGGTCTGGTCGTGTGA-3' and 5'- CCTTCCATGCTGTGTCGCCA-3', respectively. The forward and reverse primers used for *Actb* amplification are 5'-TGCTCAGCCTGTACGCAACA-3' and 5'-CCCGCAATGTCTACCCGCAT-3', respectively. The qPCR mixtures were then run in a Bio-Rad CFX96 Touch Real-Time PCR Detection System. The $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001) was then utilized to analyze the normalized expression ratio of *Cpeb1* in these INS-1 cells.

Cytokine treatment. Following the siRNA transfection protocol, the cells were introduced to a cytokine cocktail media containing 10 ng/mL IL-1β, 10 ng/mL TNF-α, and 10 ng/mL IFN-γ. This was adapted from a protocol that treated mouse islets with cytokines (Blum et al., 2014).

The cells were treated with media containing this cocktail (or normal, complete media) for 24 hours at 37°C.

High glucose/fatty acid treatment. Following the siRNA transfection protocol, the cells were introduced to high glucose and fatty acid media containing 0.5 mM palmitate (which was originally conjugated in a 20% BSA solution) dissolved in complete high glucose media. This was adapted from a protocol that treated mouse islets with high glucose and fatty acids (Chu et al., 2010). The cells were treated with media containing high levels of glucose and fatty acids (or normal, complete media) for 24 hours at 37°C.

Adherent cell fixation. Following siRNA transfection and either cytokine or high glucose/fatty acid treatment, adherent INS-1 cells were fixed using 4% paraformaldehyde in 1x PBS, followed by 3 1x PBS washes for 5 minutes each. Fixed cells were kept in 1x PBS at 4°C until ready for staining.

TUNEL staining of adherent cells. The positive control wells were first treated with 1000 Kunitz/mL DNase for 5 minutes. All wells were then treated with 3 1x PBST (0.2% triton X-100) washes for 10 minutes each. Each well was then treated with the TUNEL mixture (1:10 dilution of TUNEL enzyme solution with TUNEL labeling solution) for 1 hour at 37°C. All wells were then treated again with 3 1x PBST (0.2% triton X-100) washes for 10 minutes each. A DAPI stain solution was then applied to each well for 25-30 minutes at room temperature. All wells were then treated once more with 3 1x PBST (0.2% triton X-100) washes for 10 minutes each. Cells were then kept in 1x PBS and at 4°C until ready for imaging.

Fluorescence Microscopy. The Keyence BZ-X810 All-in-One Fluorescence Microscope was used for all imaging purposes. The fluorescence of DAPI and TUNEL were captured at exposure times of 1/6 s and 1.2 s, respectively.

Image analysis and apoptotic index calculation. Fluorescence images were analyzed using ImageJ 1.x software (Schneider et al., 2012) along with the StarDist plugin (Schmidt et al., 2018). StarDist was utilized to count the total number of DAPI-positive cells in each image, with a subsequent manual check and removal of cell clusters (containing 2 or more DAPI-positive cells) that were identified as a single cell by the plugin. TUNEL-positive cell bodies were manually counted, with the pixel display values range set to 54-200 for each image. Five randomly-selected images per treatment replicate were analyzed, and the apoptotic index for each replicate was calculated by dividing the total number of TUNEL-positive cells by the total number of DAPI-positive cells of all five images.

Statistical analyses. The Shapiro-Wilk test of normality was utilized to assess whether the apoptotic index samples of all treatment and control groups came from a normally-distributed population at an α-level of 0.05. The one-way ANOVA was used to test if there was a significantly-different mean apoptotic index among all of the treatment groups, and this was followed up with the Tukey's Test for post hoc analysis if a p-value less than the α-level of 0.05 was obtained from the ANOVA; the α-level for the post hoc tests was also 0.05. A similar ANOVA and Tukey's Test for post hoc analysis was run for knockdown qPCR analysis when

comparing ΔC_t values for untransfected, scramble siRNA-transfected, and *Cpeb1* siRNA-transfected INS-1 cells.

Results

Knockdown of *Cpeb1* in INS-1 cell line

A qPCR assay was run to determine the optimal siRNA concentration and knockdown duration to utilize for the purpose of knocking down *Cpeb1* in INS-1 cells. Treatment of INS-1 cells with siRNA concentrations of 10 nM, 20 nM, and 40 nM was initially performed for the knockdown durations of 48 hours, 72 hours, and 96 hours. Both the 20 nM and 40 nM siRNA treatments of the INS-1 cells resulted in *Cpeb1* expression level decreases between 40% and 60% for all time points (Figures 1.C. & 2.C.). The ΔC_t values of INS-1 cells treated with *Cpeb1* siRNA (n = 3) were found to be statistically significant compared to the relative expression levels of the gene in INS-1 cells treated with either scramble control (n = 3) or no siRNA and transfection media at all (n = 2) for all siRNA concentrations and knockdown durations (data not shown). Interestingly, the *Cpeb1* expression fold changes did not decrease for either siRNA concentration as the knockdown duration increased; however, no statistical analysis was performed to compare whether the *Cpeb1* expression fold changes differed significantly between distinct siRNA concentrations or treatment durations. The siRNA concentration of 40 nM and the knockdown duration of 48 hours were then chosen for the subsequent investigations of the effects of *Cpeb1* knockdown on β-cell apoptosis.

TUNEL assay of *Cpeb1* knockdown INS-1 cells treated with cytokines

Following optimization of the knockdown of *Cpeb1* in INS-1 cells, a TUNEL assay was performed to assess if there is any change in the apoptotic index of INS-1 cells following knockdown of *Cpeb1* and treatment of cytokines. The apoptotic index of *Cpeb1*-knocked down INS-1 cells treated with cytokines afterward was found to be significantly higher than that of the

scramble siRNA-transfected and untransfected INS-1 cells that were also treated with cytokines (Figure 2.B.). Furthermore, *Cpeb1*-knocked down INS-1 cells treated with cytokines were found to have a significantly greater apoptotic index relative to similarly-treated cells not prescribed the cytokine treatment. Additionally, in the absence of a post-knockdown cytokine treatment, the *Cpeb1*-knocked down INS-1 cells were observed to have a higher apoptotic index relative to normal, untransfected INS-1 cells; however, whether or not this increase in apoptosis is attributed primarily to transfection stress could not be determined as the scramble control-treated INS-1 cells exhibited an apoptotic index similar to the untransfected and *Cpeb1*-KD cells. Generally, there appeared to be fewer cells per image for wells containing cytokine-treated cells (Figure 2.A.); however, the proper quantification and statistical analysis of this claim is needed to confirm this observation.

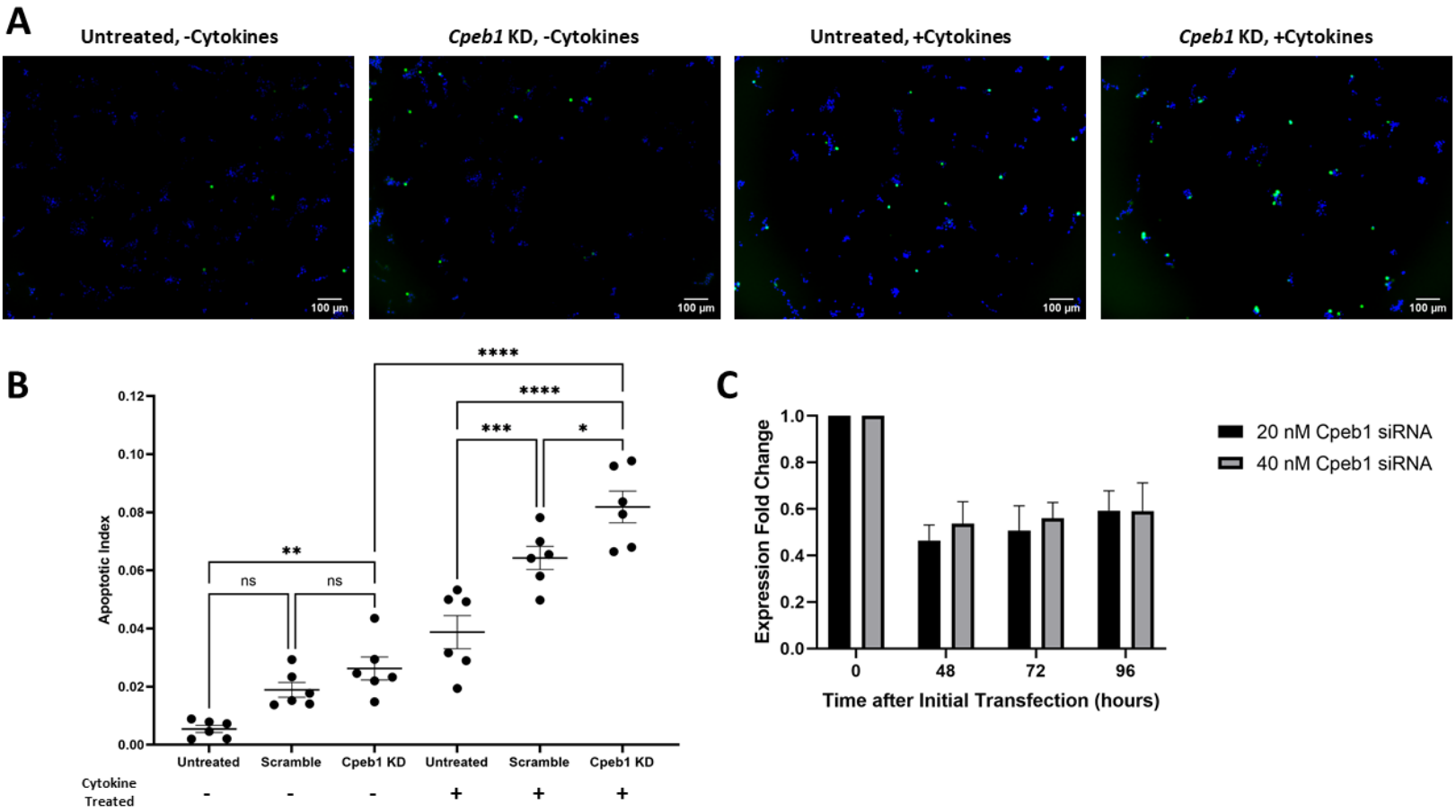

Figure 1. Knockdown of *Cpeb1* results in an increase in the apoptotic index of cytokine-stressed INS-1 cells. (**A**) DAPI labeling (blue) and TUNEL (green) of INS-1 cells with either *Cpeb1*-knockdown or no knockdown (as indicated by "Untreated" label). INS-1 cells were also either treated with cytokines or not following knockdown (as indicated by "+" and "-", respectively). (**B**) Image analysis-derived apoptotic index measurements of INS-1 cells either with or without *Cpeb1* knockdown (or with a scramble siRNA control) and either treated with cytokines or not afterwards ($n = 6$ for each treatment and control group). Error bars represent ±SEM. $^{ns}p > 0.05$, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.0001$, $****p \leq 0.0001$. (**C**) qPCR analysis of *Cpeb1* expression in INS-1 cells following knockdown at differing concentrations of siRNA and durations of knockdown, normalized to β-actin expression. Expression fold changes were determined using $2^{-\Delta\Delta C_t}$ from the Livak method. Error bars represent propagated ±SD originally obtained from ΔC_t measurements.

TUNEL assay of *Cpeb1* knockdown INS-1 cells treated with high glucose/fatty acids

A similar TUNEL assay was also performed to assess any changes in the apoptotic index of INS-1 cells following *Cpeb1* knockdown and treatment of high glucose and palmitate. Interestingly, when faced with a post-transfection HGFA treatment, the apoptotic index of INS-1 cells was found to not change significantly when *Cpeb1* was knocked down in both (Figure 2.B.). A similar observation was made when comparing the apoptotic indices of INS-1 cells with and without *Cpeb1* knockdown and with no post-transfection HGFA treatment.

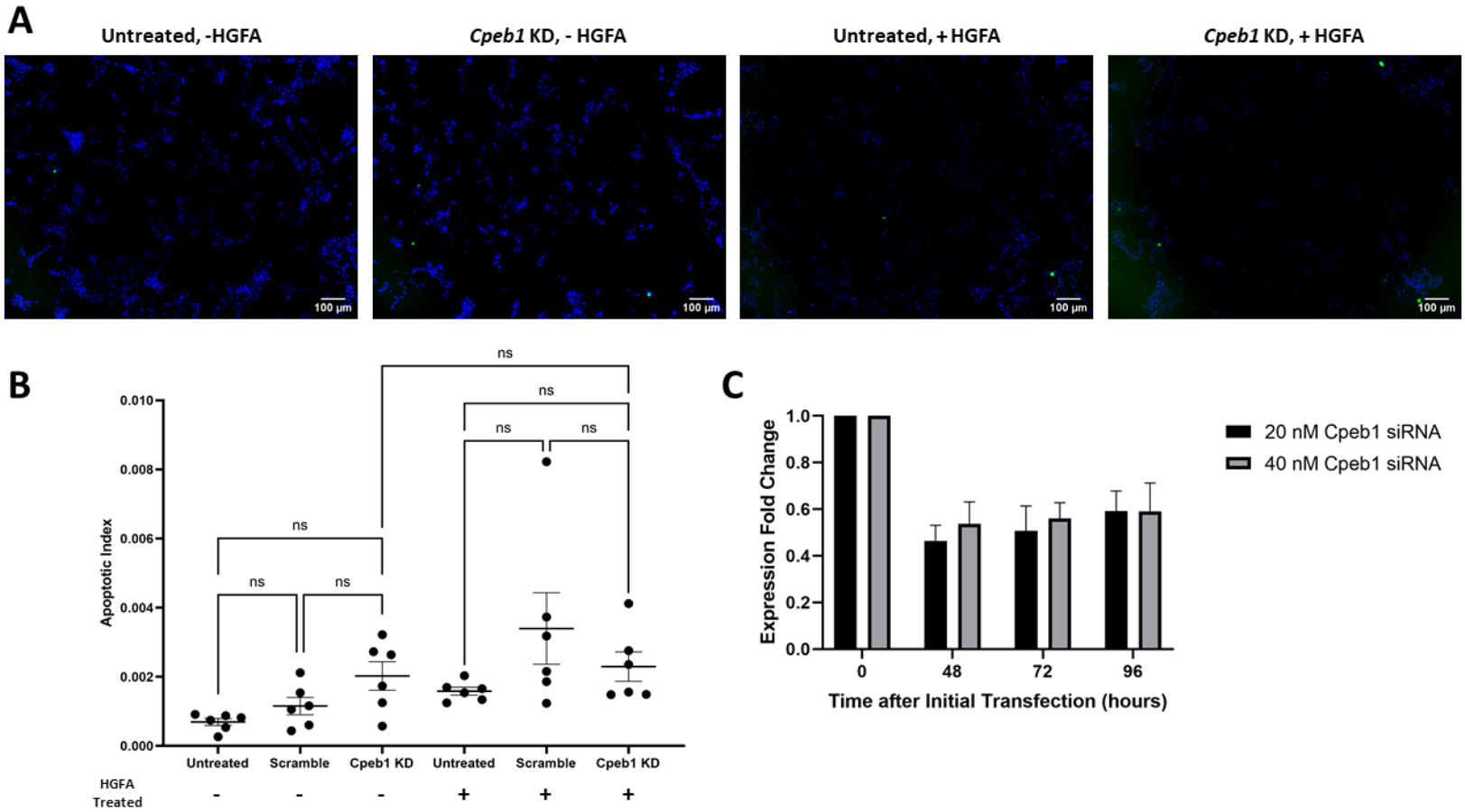

Figure 2. Knockdown of *Cpeb1* results in no change in the apoptotic index of high glucose/fatty acid (HGFA)-stressed INS-1 cells. (**A**) DAPI labeling (blue) and TUNEL (green) of INS-1 cells with either *Cpeb1*-knockdown or no knockdown (as indicated by "Untreated" label). INS-1 cells were also either treated with HGFA or not following knockdown (as indicated by "+" and "-", respectively). (**B**) Image analysis-derived apoptotic index measurements of INS-1 cells either with or without *Cpeb1* knockdown (or with a scramble siRNA control) and either treated with HGFA or not afterwards (n = 6 for each treatment and control group). Error bars represent ±SEM. $^{ns}p > 0.05$. (**C**) qPCR analysis of *Cpeb1* expression in INS-1 cells following knockdown at differing concentrations of siRNA and durations of knockdown, normalized to β-actin expression. Expression fold changes were determined using $2^{-\Delta\Delta C_t}$ from the Livak method. Error bars represent propagated ±SD originally obtained from ΔC_t measurement distributions.

Discussion and Conclusions

It was originally hypothesized that the downregulation of *Cpeb1* in β-cells leads to greater levels of apoptosis when they are situated in conditions of type 1 and type 2 diabetic stress. Firstly, the siRNA knockdown conditions of *Cpeb1* in INS-1 cells (the rat insulinoma β-cell line) were optimized for further investigation of the gene's cellular functionality. Next, cytokine and high glucose and fatty acid treatments were imposed on cells with and without *Cpeb1* knockdown, followed by normalized measures of apoptosis among the cell treatment groups using TUNEL.

The siRNA-mediated knockdown of *Cpeb1* in INS-1 cells was observed to induce around a 40-50% decrease in its expression 48 hours after initial transfection with both 20 nM and 40 nM concentrations of siRNA; this value steadily rose towards around 60% for both siRNA concentrations as the knockdown duration increased to 96 hours. While far from efficient, phenotypic differences regarding apoptosis were still observed as will be discussed next. Further optimization should be done in the future to induce a larger decrease in the expression of *Cpeb1*, and this could potentially be achieved by increasing the concentration of siRNA utilized and/or the amount of transfection reagent applied.

Nonetheless, an increase in the apoptotic index of *Cpeb1*-knocked down INS-1 cells treated with cytokines was observed relative to normal INS-1 cells that were also administered cytokines. This finding supports the initial hypothesis regarding β-cells under type 1 diabetogenic stress. Furthermore, this suggests that *Cpeb1* plays a role in preventing the apoptotic death of β-cells, especially under cytokine stress that occurs in the pathogenesis of type 1 diabetes. This result confers a novel biological role to *Cpeb1* in the context of INS-1 cells and the maintenance of a healthy, mature state. Such a proposed function of the gene should next be

tested *in vivo* using a model organism such as mice. Moreover, assessing this role in human islets could suggest *Cpeb1* is involved in the maintenance of mature human β-cells and that it could be targeted in the context of potentially rescuing cytokine-stressed β-cells (which are observed in type 1 diabetes). Further work needs to be done in order to identify the target mRNAs of this translational regulator in the context of INS-1 and β-cells. Also, it is yet to be determined whether this difference in the levels of apoptosis can be attributed to CPEB1 interacting with *Stat3* and *Pten* as mentioned earlier. Concerning the actual experiment, the visual inspection of TUNEL-positive cell bodies, combined with the StarDist counting of DAPI-stained nuclei, does possess a degree of error that could potentially be reduced to yield even stronger findings. Fluorescence-activated cell sorting (FACS) or an assay for particular apoptotic regulators, like caspases, could help accomplish such a task. It should be noted that this specific experiment was repeated with similar results being obtained, albeit a smaller sample size per treatment group was utilized (data not shown).

Contrastingly, no change in the apoptotic index of INS-1 cells was observed when *Cpeb1* was knocked down and when a high glucose and palmitate treatment was administered afterward. Interestingly, the measured apoptotic index for untransfected INS-1 cells treated with high glucose and palmitate appears to be lower relative to the same measure obtained from comparable experiments in the literature (El-Assaad et al., 2003; Kharroubi et al., 2004). However, the experiments from the literature employed different techniques for identifying apoptotic cell bodies. Furthermore, they also utilized a high glucose and palmitate media containing very little to no FBS, unlike the experiment run here. An explanation of such a choice may be to attain a specific concentration of palmitate unbound to bovine serum albumin (BSA), a protein utilized to dissolve free fatty acids in aqueous solutions and one that is present in FBS

(Lytrivi et al., 2020); thus, the addition of FBS could potentially decrease the actual unbound concentration of palmitate than expected. It should be noted that the high glucose and palmitate treatment durations and cell passage numbers varied in those studies compared to the experiment performed in this paper as well. A repeat of the experiment with a reduced concentration of FBS could be telling of whether *Cpeb1* knockdown regulates apoptosis in INS-1 cells under stress from the synergy of glucotoxicity and lipotoxicity. If this were to be performed, changes to cell survival need to be addressed in the presence of reducing FBS concentration since serum starvation may potentially induce autophagic death of the cells (Fujimoto et al., 2009). Also, repeating the experiment with a greater knockdown of *Cpeb1* could be informative of the gene's apoptotic regulatory role in the face of high glucose and fatty acid stress characteristic of type 2 diabetes.

Identifying the mRNA targets of *Cpeb1* in β-cells for both translation promotion and repression is another step that can be taken in further elucidating the gene's functional role in β-cells and their maturation. Especially, given the identification of a possible apoptotic regulatory role in cytokine-stressed INS-1 cells, the next step would be to determine apoptotic regulators that may potentially be targeted by Cpeb1. As mentioned earlier, the repression of *Stat3* and *Pten* mRNA translation by Cpeb1 in HepG2 cells (Alexandrov et al., 2012) motivates further research into whether such protein-mRNA interactions take place in β-cells, especially since Pten deletion in β-cells has been shown to reduce apoptotic death (Wang et al., 2010). Interestingly, Cpeb1 has been found to stimulate the translation of β-catenin in astrocytes in the context of cell migration to a wound site (Jones et al., 2008). This, combined with the involvement of β-catenin in regulating insulin secretion in β-cells (Sorrenson et al., 2016), presents another avenue to explore with regard to the whole library of β-cell functions involving Cpeb1. More work would also need

to be performed in identifying the regulators of *Cpeb1* in β-cells, given that its expression has been found to be lowered in type 1 and type 2 diabetic mice relative to healthy ones (Nimkulrat, unpublished).

In conclusion, the knockdown of *Cpeb1* in INS-1 cells was found to increase the cell line's apoptotic index when treated with a cocktail of pro-inflammatory cytokines characteristic of type 1 diabetes pathogenesis. No such change in the apoptotic index of INS-1 cells treated with high glucose and palmitate was observed when *Cpeb1* was knocked down. This suggests that the gene may play a role in regulating β-cell apoptosis when under cytokine stress and that such a role may not be present in the face of hyperglycemic and lipotoxic stress. Still, further studies will need to be done to assess whether such a role of *Cpeb1* is observed *in vivo* and to elucidate the mechanisms underlying the potential regulatory role of this gene.

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