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ORIGINAL PAPER

ID2 promotes the expansion and survival of growth-arrested pancreatic beta cells

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Abstract Inhibitors of DNA binding proteins (Ids) are implicated in the control of proliferation and differentiation. Herein, we tested the hypothesis that Id2 could stimulate proliferation and survival in differentiated pancreatic beta cells. We showed that Id2-enhanced proliferation of a growth-arrested pancreatic beta cell line (BTC-tet). This was mediated by the Rb pathway, as shown by an E2F1-driven reporter assay and Western immunoblot of phosphorylated Rb protein. Id2 also induced expression of Bcl-2, accompanied by a significant reduction of critical mediators of cytokine stimulation, including p38 MAPK and NFκB, as well as apoptosis markers, caspase-3 and Annexin-V. Overall, our data suggest that Id2 enhances proliferation and survival of growth-arrested BTC-tet cells.

Keywords Id2 · E2F · Bcl2 · BTC

Introduction

Uncovering signaling pathways of beta cell proliferation and survival is important for the development of effective therapies for diabetes. Inhibitors of DNA binding proteins (Ids), are a family of proteins that are implicated in a number of cellular processes, including control of proliferation and differentiation [1, 2]. Specifically, Iavarone et al. [3] and Lasorella et al. [4] showed that Id2 can inhibit the growth-arresting function of Rb, an atypical Id target. We have demonstrated that Id2 is important in the expansion of pancreas progenitors using in vitro and in vivo models [5]. It is not known if Id2 can affect differentiated beta cell expansion.

The pathogenesis of autoimmune diabetes involves the production of proinflammatory cytokines causing destruction of beta cells by apoptosis. In vitro and in vivo disease models have demonstrated that increased expression of IFNγ, TNFα, and IL-1β precedes the development of diabetes [6–9]. These cytokines activate cellular mediators, including MAPK and NFκB, which promote apoptosis of pancreatic islets [10], as well as inhibit the actions of prosurvival proteins such as Bcl-2. Furthermore, NFκB is a key transcription factor that is activated by cytokines during the progressive loss of beta cells [11], and rat beta cells that were transfected with an NFκB repressor gene prevented expression of Fas and enhanced cell survival [12]. Similarly, the overexpression of Bcl-2 protected a pancreatic beta cell line [13] and human islets [14] from cytokine-induced apoptosis. Here, we asked whether Id2 could affect pancreatic cell survival through modulation of cytokine signaling pathways.

In this study, we investigated mechanisms through which Id2 could enhance proliferation and survival of pancreatic beta cells. Mature pancreatic beta cells show relatively low mitotic activity. Therefore, to investigate the influence of Id2 on pancreatic cell growth and survival, we used a cell line that was amenable to experimental growth-arrest. We found that stable transfection of Id2 significantly enhanced proliferation of growth-arrested Beta Tumor Cells (BTC-tet), measured by an E2F1 reporter assay and ^{3}H-thymidine uptake. Id2 also enhanced basal Bcl-2 expression, concomitant with a decrease of apoptotic markers, including Annexin-V and cleaved caspase-3, following cytokine treatment. Moreover, Id2 abrogated cytokine-induced NFκB luciferase reporter gene expression. Taken together, our data provide evidence that Id2

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stimulated proliferation of differentiated beta cells and can possibly act as a survival factor.

Materials and methods

Reagents and antibodies

Phosphorylated p38 MAPK, cleaved caspase-3, and phosphorylated Rb were from Cell Signaling; Id2 and Bcl-2 were from Santa Cruz; Insulin was from Dako; and actin was from Sigma. BMP4, TNFα, IFNγ, and IL-1β were from R&D Systems, Inc.; Annexin V-FITC and 7AAD were from Pharmingen. The Id2 construct was a generous gift from Dr. Jayshree Sarnater and Dr. John Kessler (Northwestern University Feinberg Medical School), and the E2F1-luciferase reporter was from Dr. Joseph Nevins (Duke University Medical Center).

Cell culture

BTC-tet cells were a gift from Shimon Efrat [15, 16]. These cells express the SV40 T antigen oncoprotein under control of bacterial tetracycline-resistance operon regulatory system. Therefore, in the presence of tetracycline, production of the SV40 T antigen oncogene is repressed and growth is arrested. BTC-tet cells are maintained in DMEM media at 37°C and 5% CO_2.

Transfection

For establishment of BTC-tet clones that express Id2, we transfected the cells with Id2 using FuGene (Roche) and geneticin-resistant clones were evaluated for Id2 protein expression by Western immunoblot analysis. Transient transfections were performed using either FuGene or TransIT-LT1 (Mirus) transfection reagent.

E2F1-luciferase reporter assay

Our lab established a stable BTC-tet cell line expressing the E2F1-driven luciferase reporter construct. We have found that tet-treated cells show at least a ten times decrease in E2F1-driven luciferase reporter reading compared to non-tet treated cells. In this report, we also stably transfected Id2 into these cells. To assess E2F1-dependent proliferation, we growth-arrested the cells by treating with tetracycline (10 μg/ml) for 7 days and cells were harvested using Reporter Lysis buffer (Promega). Luciferase activity was read in a luminometor and expressed as relative light unit (RLU)/μg of protein. In some cases, BMP4 (50 ng/ml) was added to BTC-tet cells with and without Id2 for 24 h before lysis.

^{3}H-thymidine uptake

BTC cells with or without the Id2 construct were growth-arrested and stimulated with BMP4 (50 ng/ml) for 24 h with the addition of ^{3}H-thymidine during the last 4 h. The growth index of the cultured BTC-tet cells was determined by quantitatively measuring ^{3}H-thymidine uptake using a beta scintillation counter and expressed as counts per minute (cpm).

Western immunoblotting

BTC-tet cells were lysed and protein extracts were prepared and separated by SDS-PAGE as described previously [5]. Briefly, cells were lysed in 2× sample buffer (0.13 mol/l Tris-base, pH 6.8, 20% glycerol, and 4% SDS) and aliquots were taken for protein determination using the BCA kit (Pierce, Rockford, IL). The remaining cell extracts were denatured in 4× sample buffer (0.13 mol/l Tris, 40% glycerol, 8% SDS, 4% β-mercaptoethanol, and 0.02% bromophenol blue). Equal amounts of protein were separated by SDS-PAGE and transferred to Immobilon polyvinylidine fluoride membranes (PDVF) (BioRad, Hercules, CA) overnight in transfer buffer (25 mmol/l tris-base, 192 mmol/l glycine, pH 8.3, and 20% methanol). The membranes were blocked in 5% skim milk powder in Tris buffer containing 0.05% Tween-20, then probed with the indicated antibody and visualized with enhanced chemiluminescence. The membranes were reprobed for actin to normalize for protein loading. Densitometry analysis was carried out using Image J software. The intensity of images of scanned Western blots was determined and the ratio of each band to its actin control was calculated.

Annexin-V detection

BTC-tet cells were growth-arrested with tetracycline and then treated with TNFα/IL-1β (100 U/ml each) or IFNγ/IL-1β (100 U/ml), followed by staining for Annexin-V-FITC conjugated and 7AAD, as instructed by the manufacturer, and analysis by flow cytometry.

Confocal microscopy

BTC-tet cells with and without Id2 were fixed in 4% paraformaldehyde, blocked with casein and incubated with

anti-insulin antibody (Dako, Denmark) followed by anti-guinea pig FITC. Images were acquired using a Zeiss 2100 confocal microscope with a 60× objective. RGB micrographs were compiled with Image J software.

RT-PCR

Cells were growth-arrested and RNA was isolated using TRIZol (Invitrogen). Reverse transcription was performed using the First Strand Synthesis kit (Invitrogen) and PCR was performed using primers for insulin and β-actin. The sequences for the primers are as follows:

Insulin: 5′-TgCCCAggCTTTTgTCAAACA-3′; 5′-CTCCAgTgCCAAggTCTgAA-3′,
Actin: 5′-CATgTTTgAgACCTTCAACACCC-3′; 5′-gCCATCTCCTgCTCgAAgTCTAg-3′.

Statistics

Data were expressed as mean ± SEM and analyzed using Instat (GraphPad Software Inc.). Comparisons were made by Student's t-test. A value of $P < 0.05$ was considered statistically significant.

Results

Transfection of Id2 into a pancreatic beta cell line (BTC-tet)

To elucidate the role of Id2 on pancreatic beta cell proliferation, BTC-tet cells were transfected with Id2 and evaluated for protein expression by Western immunoblot analysis. We showed that non-transfected BTC-tet cells did not express Id2, whereas lysates from Id2 transfected clones (clones 11 and 12) demonstrated the expression of Id2 (Fig. 1a). One clone did not express Id2 (clone 17). Confocal imaging and RT-PCR of BTC-tet and Id2 cells showed that both express insulin (Fig. 1b, c). Previous reports also showed that tet treated BTC cells provide sufficient insulin to normalize glucose levels in vivo [17]. We used clone 12 for all subsequent experiments, unless otherwise indicated.

Id2 stimulates proliferation in BTC-tet cells through the Rb/E2F pathway

We first investigated the influence of Id2 on BTC-tet cell proliferation by measuring ^{3}H-thymidine incorporation.

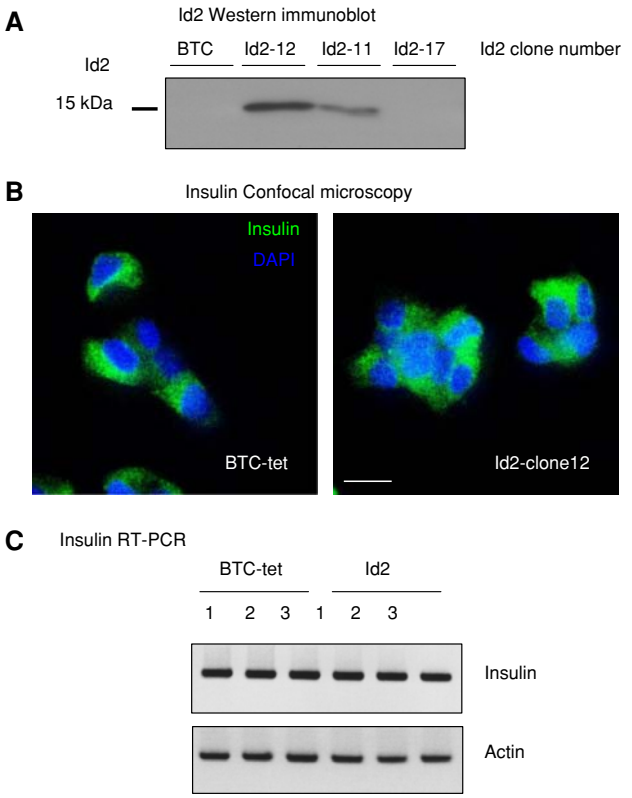
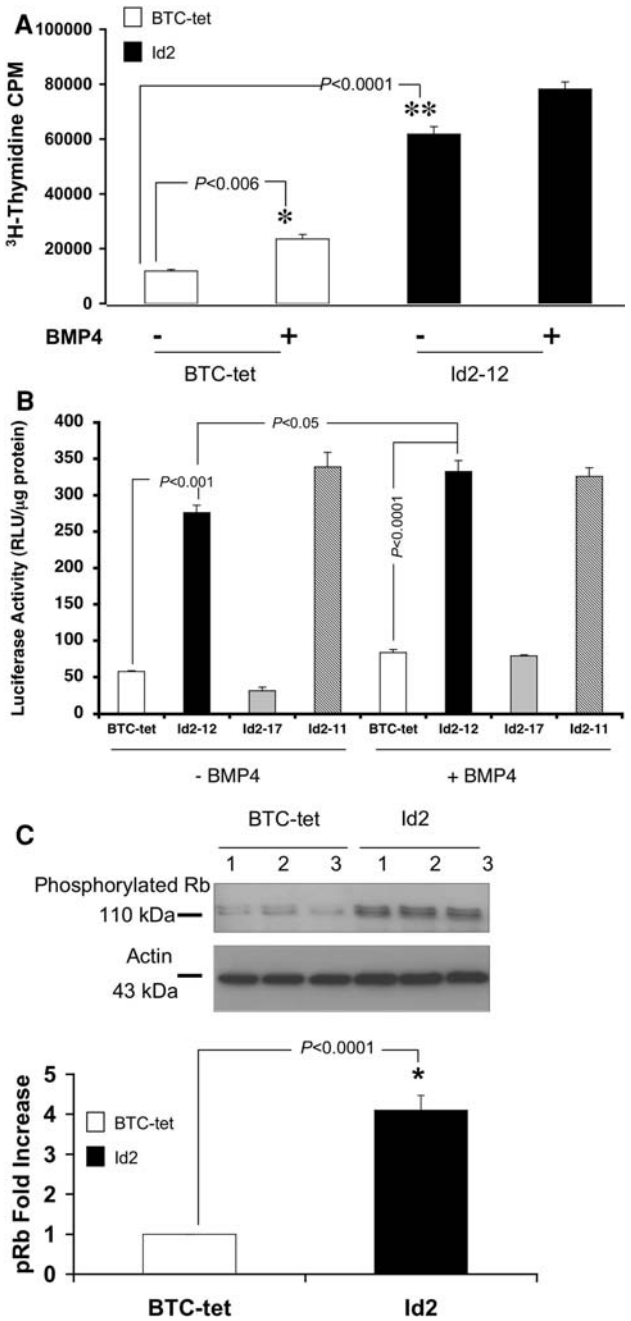


Fig. 1 Id2 expression in BTC-tet cells. (**a**) Western immunoblot demonstrating the expression of Id2 in transfected BTC-tet clones but not in control BTC-tet cells in non-growth-arrested condition. (**b**) Confocal micrographs of cultured cells showing expression of insulin (green) in BTC-tet and Id2-expressing cells. Nuclei are depicted by DAPI staining (blue). Bar represents 50 μm. (**c**) RT-PCR results showing unaltered expression of insulin Id2-expressing cells compared to BTC-tet controls. The numbers 1–3 represent different, independent samples

We growth-arrested the cells by tetracycline treatment for 7 days, which has been shown to inhibit cell proliferation by inhibiting the expression of T antigen [16], so that the effect observed would be independent of the cellular transformation. As we have previously shown that growth of pancreas progenitor cells is stimulated by bone morphogenetic protein 4 (BMP4), we also tested to see if this growth factor could also enhance proliferation in BTC-tet cells. The growth index of the cultured BTC-tet cells was determined by quantitatively measuring ^{3}H-thymidine uptake (Fig. 2a). In these experiments, BMP4 alone stimulated ^{3}H-thymidine uptake to twofold ($P < 0.006$ compared to BTC-tet control). BTC cells that overexpressed Id2 showed enhanced ^{3}H-thymidine uptake (fivefold, $P < 0.0001$ compared to BTC-tet control) and the proliferation effect was further stimulated with BMP4 (6.6-fold, $P < 0.0001$ compared to BTC-tet control). Therefore, we found that Id2 stimulates proliferation of growth-arrested pancreatic beta cells.

We next sought to determine if Id2 modulates the activity of key regulators of the cell cycle. The E2F1 transcription factor is essential for normal cell cycle progression. To investigate the influence of Id2 on E2F1 activity, we established stable transfectants of an E2F1-driven luciferase reporter construct to provide us with a measure of E2F1 activity in BTC-tet cells. These cells served as our control cells (unless otherwise indicated) for all subsequent studies. To ask whether Id2 increases E2F1 activity, BTC-tet cells carrying the E2F1-luciferase reporter with and without stably transfected Id2 were growth-arrested by tetracycline treatment for 7 days, after which they were tested for luciferase activity (Fig. 2b). BTC-tet cells that express both E2F1-luciferase and Id2 (clone 12) showed significantly enhanced basal luciferase activity to 4.7-fold ($P < 0.001$ compared to BTC-tet alone). Similarly, a different clone expressing Id2 (clone 11) also increased luciferase activity by 3.4-fold. Next, we tested the effect of BMP4 (50 ng/ml) in growth-arrested BTC-tet cells and found it increased luciferase activity 1.5-fold. In the growth-arrested BTC-tet Id2-expressing cells, BMP4 had an additive effect by increasing luciferase activity to 5.7-fold. As a control, we also determined the effects of cells transfected with empty vector (in the absence of Id2) and found E2F1-luciferase activity was comparable to BTC-tet control cells. Furthermore, clones that did not express Id2 (clone 17) also showed comparable luciferase activity as control BTC-tet cells (Fig. 2b). Therefore, the effects observed are directly related to the expression of Id2 and not artifact effects of transfection selection.

Phosphorylation of Rb is required for disengagement of E2F1 during the transition into S phase of the cell cycle. Since Id2 is known to bind to the Rb protein, we sought to determine if the Id2-enhanced proliferation of growth-arrested BTC-tet cells was mediated through the Rb pathway. We found by Western immunoblotting that expression of Id2 in growth-arrested cells increased the phosphorylation of Rb (by fourfold, Fig. 2c). Therefore, our data demonstrate that Id2 expression stimulates proliferation of pancreatic beta cells by affecting the activity of key molecules involved in cell cycle progression.



◀**Fig. 2** Overexpression of Id2 enhances proliferation in BTC-tet cells. (**a**) Id2 increased ^{3}H-thymidine uptake in BTC-tet cells. BTC-tet or Id2 clones (clone 12) were growth-arrested by tetracycline for 7 days (it should be noted that wherever the term "growth-arrested" is indicated, it alludes to treatment of tetracycline for 7 days), with the addition of BMP4 (50 ng/ml) in the last 24 h, after which ^{3}H-thymidine incorporation was measured. BMP4 and Id2-enhanced proliferation of BTC-tet cells. $^*P < 0.006$ (BTC-tet vs. BMP4), $^{**}P < 0.0001$ (BTC-tet vs. Id2), $N = 4$. (**b**) Id2 increased E2F1-luciferase reporter activity and enhanced the growth promoting effect of BMP4. BTC-tet or Id2 clones (clones 11 and 12 express Id2, whereas clone 17 does not), expressing the E2F1-luciferase reporter, were growth-arrested by tetracycline for 7 days, with the addition of BMP4 (50 ng/ml) in the last 24 h and luciferase activity measured as reported in Materials and Methods. Id2 and BMP4 enhanced basal luciferase activity in Id2 expressing cells but not in cells without Id2 (clone 17). $^*P < 0.001$ (BTC-tet vs. Id2), $^{**}P < 0.05$ (Id2 vs. Id2 + BMP4), $^{***}P < 0.001$, $N = 3$. (**c**) Id2-enhanced expression of phosphorylated Rb protein. BTC-tet and Id2 expressing cells were growth-arrested using tetracycline and phosphorylated Rb protein was detected by Western immunoblot. Membranes were stripped and reprobed for actin to confirm equal protein loading. Shown is a representative blot with the corresponding graph of analyzed densitometry. The data demonstrate that the Id2-mediated increase in proliferation of growth-arrested BTC-tet cells correlated with an increase in Rb phosphorylation. 1,2,3 refers to samples from independent experiments. $^*P < 0.0001$ (BTC-tet vs. Id2), $N = 3$

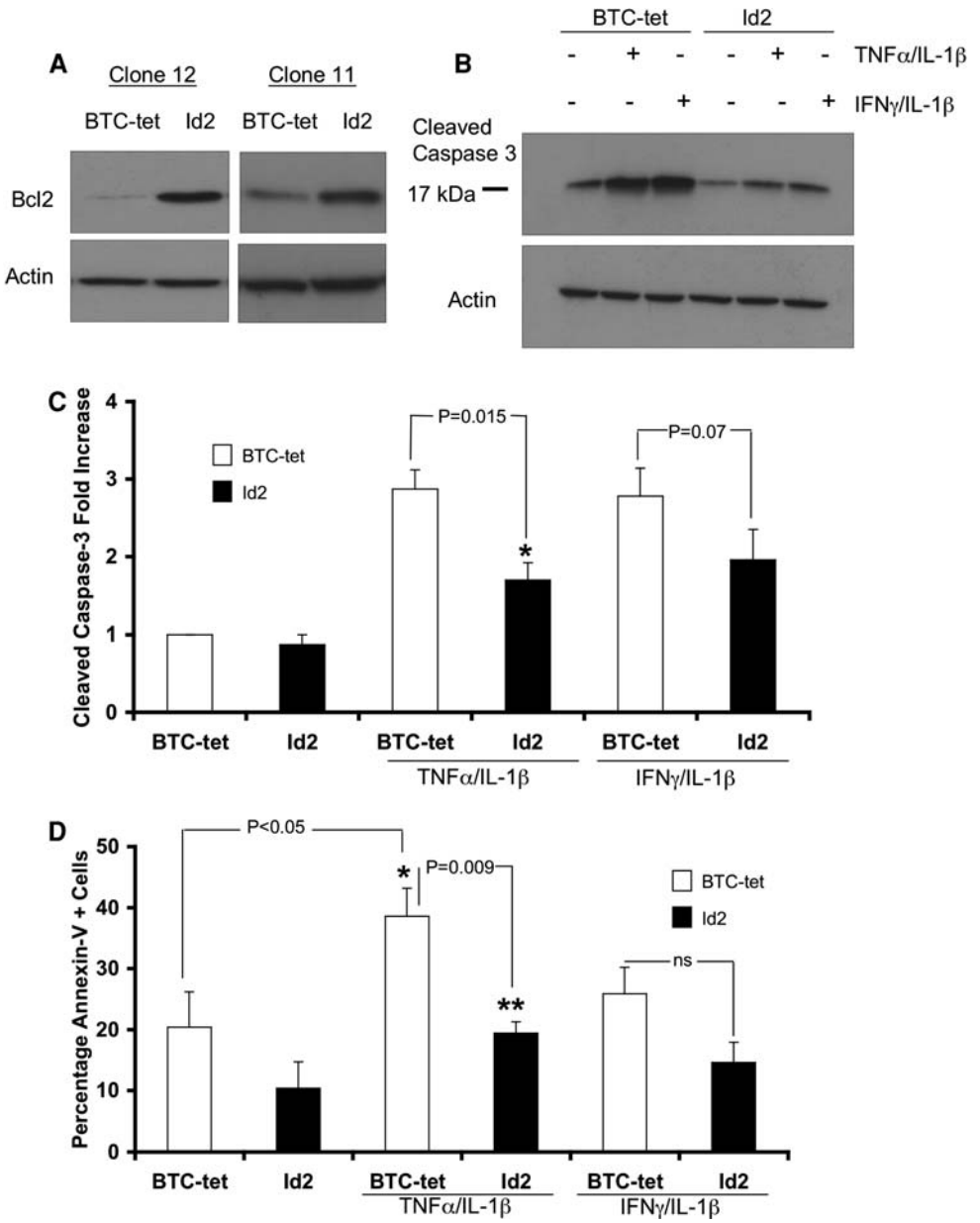


Fig. 3 Id2 augments Bcl-2 expression and reduced cytokine activation of cleaved caspase-3 and Annexin-V. (**a**) Representative Western immunoblot showing enhanced basal expression of Bcl-2 in growth-arrested Id2-expressing cells (clones 11 and 12) compared to control BTC-tet cells. Membranes were stripped and reprobed for actin to confirm equal protein loading, $N = 5$. (**b**) Growth-arrested BTC-tet or Id2-expressing cells were treated with for TNFα/Il-1β or IFNγ/Il-1β for 24 h and lysed. Western blot demonstrating expression of cleaved caspase-3, and its corresponding actin. BTC-tet cells showed a significant increase of cleaved caspase-3 expression with cytokine stimulation compared to Id2-expressing cells. (**c**) Densitometry of results is represented graphically. Both TNFα/Il-1β and IFNγ/Il-1β increased expression of cleaved caspase-3. In cells expressing Id2, the increase of caspase-3 is significantly reduced in the TNFα/Il-1β treatment group. $^*P < 0.05$, Id2 vs. Id2 + TNFα/Il-1β, $P = 0.065$ and for Id2 vs. Id2 + IFNγ/Il-1β, $P = 0.07$, $N = 6$. (**d**) BTC-tet or Id2-expressing cells were growth-arrested with tetracycline for 7 days with the addition of TNFα/Il-1β or IFNγ/Il-1β for 24 h and Annexin-V (apoptosis marker) along with 7AAD (to distinguish from necrotic cells) were analyzed by flow cytometry. The percentage of the cell population that was Annexin-V+/7AAD−, demonstrating apoptotic cells, is represented graphically. TNFα/Il-1β increased Annexin-V expression in BTC-tet cells compared to Id2 expressing-cells. $^*P < 0.05$ (BTC-tet vs. BTC-tet + TNFα/Il-1β), $P = 0.5$ (BTC-tet vs. BTC-tet + IFNγ/Il-1β, $^{**}P = 0.009$ (BTC-tet + TNFα/Il-1β vs. Id2 + TNFα/Il-1β), $P = 0.08$ (BTC-tet + IFNγ/Il-1β vs. Id2 + BTC-tet + IFNγ/Il-1β), $P = 0.1$ (Id2 vs. Id2 + TNFα/Il-1β), $P = 0.5$ (Id2 vs. Id2 + IFNγ/Il-1β), ns: not significant, $N = 4$ experiments

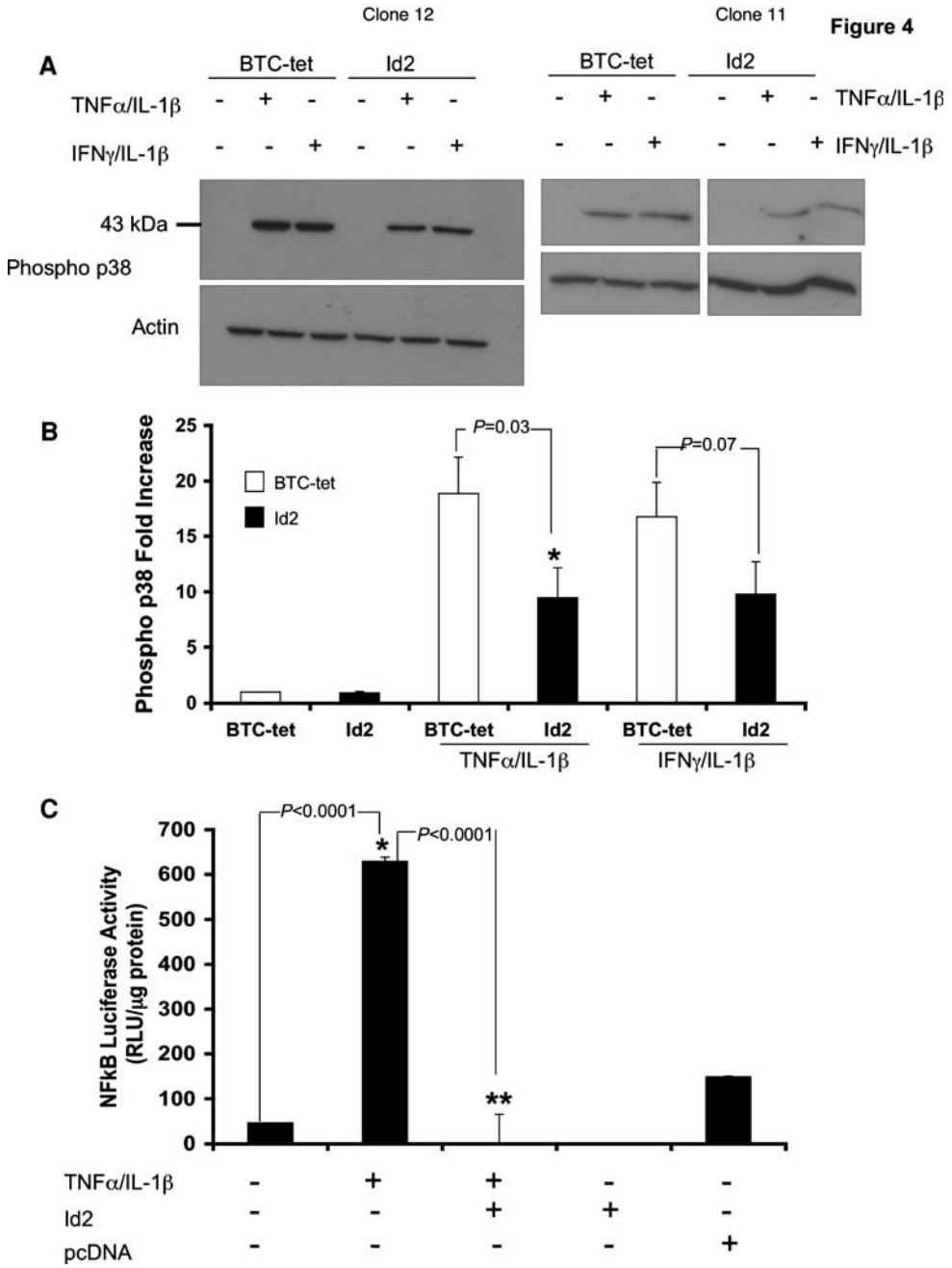


Fig. 4 Id2 reduced cytokine-mediated phosphorylated p38 MAPK expression and NFκB activity. (**a**) To determine if Id2 affects cytokine-induced p38 MAPK activation, BTC-tet or Id2-expressing cells (clone 11 and 12) were growth-arrested and treated with for TNFα/Il-1β or IFNγ/Il-1β for 30 min and cell lysates were immunoblotted using a phosphorylated antibody to p38 MAPK and reprobed for actin. TNFα/Il-1β or IFNγ/Il-1β activated p38 MAPK in BTC-tet cells but to a lesser extent in Id2-expressing cells. (**b**) Densitometry of results (from clone 12) is represented graphically. Id2 cells showed significantly decreased expression of phosphorylated p38 MAPK compared to BTC-tet cells with TNFα/Il-1β stimulation and a decreased trend with IFNγ/Il-1β stimulation, although not quite significant. $^{*}P = 0.03$ (BTC-tet + TNFα/Il-1β vs. Id2 + TNFα/Il-1β), $P = 0.07$ (BTC-tet + IFNγ/Il-1β vs. Id2 + IFNγ/Il-1β) $N = 6$ experiments. (**c**) To determine whether Id2 alters cytokine-induced activation of NFκB, BTC-tet cells (without the E2F-luciferase reporter and not growth-arrested) were transiently transfected with NFκB-dependent luciferase reporter, stimulated with TNFα/Il-1β (24 h) and luciferase activity was measured. TNFα/Il-1β enhanced the NFκB transcriptional activity. However, transient transfection with Id2 significantly reduced TNFα/Il-1β-dependent luciferase activity. BTC-tet cells were also transfected with the NFκB reporter and empty pcDNA3 vector (in place of Id2) as a control. $^{*}P < 0.0001$ (NFκB reporter vs. TNFα/Il-1β), $^{**}P < 0.0001$ (NFκB reporter + TNFα/Il-1β vs. Id2 + NFκB reporter + TNFα/Il-1β), $N = 4$

Reduced cytokine-induced apoptosis in Id2-expressing BTC-tet cells

Next, we asked whether Id2 altered cell survival in BTC-tet cells. Therefore, we determined whether there was a change in the expression level of Bcl-2 when Id2 is present. We found by Western immunoblotting that the basal level of Bcl-2 expression in growth-arrested Id2 cells was increased by fourfold over control BTC-tet cells (assessed by comparing the ratio of Bcl-2 over actin) (Fig. 3a). Similar results were also observed for clone 11, which also expresses Id2.

Since we observed increased levels of Bcl-2 in Id2-transfected BTC-tet cells, we tested whether Id2 could prevent cytokine-induced apoptosis. Growth-arrested BTC-tet cells were treated with a combination of apoptosis-inducing cytokines (TNFα/IL-1β or IFNγ/IL-1β), and caspase-3 activation was measured by Western immunoblot using an antibody that specifically recognizes the active cleaved form of caspase-3 (Fig. 3b). We found that TNFα/IL-1β increased expression of active caspase-3 to 2.9-fold over the control, and IFNγ/IL-1β enhanced it 2.8-fold. In cells expressing Id2, the effect was diminished to 1.7- and 2-fold, respectively (Fig. 3c). We also observed that cytokine treatment decreased Bcl2 levels in Id2-expressing cells; however, the amount of Bcl2 in these instances was still above that observed in BTC-tet control cells (data not shown). These results demonstrated that Id2 is able to counteract the influence of pro-inflammatory cytokines on BTC-tet cell survival.

In addition to the above-described analyses, we also monitored apoptosis of growth-arrested BTC-tet cells through flow cytometry using Annexin-V (a marker for apoptosis) and 7AAD (to distinguish cells undergoing necrosis) (Fig. 3d). We found that control cells showed 20% Annexin-V+/7AAD− (representing the population of apoptotic cells). The combination of TNFα/IL-1β increased the percentage to 39% and IFNγ/IL-1β increased it to 26% (Fig. 3d). In contrast, the basal level of Annexin-V+/7AAD− cells that expressed Id2 was 10%. Likewise, Id2 significantly reduced the percentage of Annexin-V expressing cells to 19% ($P = 0.009$) and 14% ($P = 0.08$) for TNFα/IL-1β and IFNγ/IL-1β treatments, respectively (Fig. 3d). These results demonstrate that Id2 significantly reduced cytokine-induced apoptosis in the BTC-tet cell line.

Id2 reduces cytokine-induced phosphorylation and activation of p38 MAPK

Activation of p38 MAPK is an early event during cytokine-induced apoptosis. We treated growth-arrested BTC-tet cells with a combination of TNFα/IL-1β, IFNγ/IL-1β and measured phosphorylated p38 MAPK activation by Western immunoblotting. We found that TNFα/IL-1β and IFNγ/IL-1β increased phosphorylation of p38 MAPK to 19- and 17-fold, respectively (Fig. 4a, b). However, in BTC-tet cells that overexpress Id2, there was reduced activation of p38 MAPK compared to non-transfected cells. Likewise, a separate clone containing Id2 expression also showed decreased activation of phosphorylated p38 MAPK. Therefore, in BTC-tet cells, Id2 is able to partially suppress cytokine-induced p38 MAPK activation.

Id2 inhibits NFκB activation

In pancreatic beta cells, NFκB activation is critical for cytokine-induced apoptosis. We asked whether cytokine-induced activation of NFκB is diminished in Id2-expressing BTC-tet cells. For this purpose, we transiently transfected an NFκB-dependent luciferase reporter construct into BTC-tet cells (the cells used in this experiment did not contain the E2F1-luciferase reporter) with and without Id2 and stimulated them with TNFα/IL-1β (Fig. 4c). The cells in these particular experiments were not growth-arrested due to the brief nature of transient transfection. We found that the basal NFκB-dependent luciferase activity was increased by 13-fold following TNFα/IL-1β treatment ($P < 0.0001$ TNFα/IL-1β vs. NFκB plasmid alone). However, co-transfection with Id2 significantly inhibited basal and cytokine-induced NFκB-dependent luciferase activity (Fig. 4c). Therefore, Id2 can prevent cytokine-induced NFκB activation.

Discussion

BTC-tet cells can be used as a sensitive assay for measuring factors that promote beta cell proliferation [18], as well as to study the effects of overexpressing specific genes of interest [19–23]. In this report, we have demonstrated that expression of Id2 promoted proliferation and survival of growth-arrested pancreatic beta cells, using the BTC-tet system. Id2 increased E2F1-luciferase reporter activity concomitant with phosphorylation of Rb, a regulator of E2F1. Overexpression of Id2 also increased expression of Bcl-2. This was accompanied by a significant reduction in cytokine-mediated apoptosis, reflected by a decrease in activated caspase-3 and Annexin-V levels. Id2 also suppressed the TNFα/IL-1β mediated increase of phosphorylated p38 MAPK levels and NFκB activity.

Id proteins function in the control of proliferation and differentiation [1, 2]. The major role of Ids is to bind to and inhibit the function of bHLH transcription factors; however,

non-bHLH targets of Id have been described [3]. Id1 overexpression has been related to tumor angiogenesis in human pancreatic cancer [24], but very little is known regarding the expression and function of Id proteins in the pancreas. Our data, using immunohistochemical staining, suggest that Id2 is not expressed in beta cells of normal adult mouse pancreas. In our previous study, we showed that Id2 immunoreactivity in E16 mouse pancreas does not co-localize with insulin-positive cells. However, Id2 is expressed in duct cells of the IFNgNOD mouse model of pancreas regeneration. It should be noted that Id expression (including Id2) has been documented in gene array and transcription factor analysis studies of the fetal pancreas [25, 26]. Nonetheless, the precise function of Id proteins in normal pancreas development remains to be determined.

We found that overexpression of Id2 can stimulate cell cycle progression in growth-arrested pancreatic BTC-tet cells, as well as enhance the effect of BMP4, a pleiotropic growth factor previously shown to stimulate proliferation in pancreatic progenitor cells [5] and embryonic stem cells [27]. Since Rb is an atypical target of Id, we postulated that Id2-enhanced proliferation in BTC-tet cells is mediated by Rb. It has been shown that ectopic Id1 expression increased proliferation and induced phosphorylation of Rb in nasopharyngeal carcinoma [28]. However, Id1 also downregulated p21Waf1/Cip1 (cyclin kinase inhibitor) with no effect on Rb [29]. Id2 also inhibited p57Kip2 in neuroblastoma cells, an effect that is counteracted by Rb [30]. We showed that expression of Id2 resulted in hyperphosphorylation of Rb in BTC-tet cells and augmented E2F1-dependent proliferation.

Studies have shown that cytokines are critical instigators in beta cell apoptosis [6, 8]. IL-1 and TNFα activate p38 MAPK, inducing cell death through the caspase pathway [31]. Moreover, cytokine-mediated activation of NFκB is a key downstream event in the progressive loss of beta cells in diabetes. Beta cell specific expression of the degradation-resistant NFκB protein inhibitor prevented IL-1β and IFNγ-induced apoptosis in vitro and in vivo [11]. Prosurvival proteins like Bcl2 prevent the pathogenic effects of cytokines. For example, INS-1 cells overexpressing Bcl2 are resistant to the cytotoxic effects of ROS [32]. Likewise, in human islets, Bcl2 protected against ER stress-induced apoptosis [33]. Although Florio et al. demonstrated that Id2 induces apoptosis in myeloid progenitors and osteosarcoma cells [34], here, we show that Id2 is able to prevent cytokine-induced apoptosis in conjunction with a significant reduction in p38 MAPK activation, NFκB reporter stimulation, and enhanced Bcl2 expression. In an esophageal cancer model cell line, HKESC-3, Id1 also protected cells from TNFα-induced apoptosis by upregulation of Bcl2 [29]. Therefore, Id proteins can potentially function in prosurvival signaling, and the function may be dictated by cell specificity.

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