Lack of TXNIP protects β-cells against glucotoxicity

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Abstract
Glucotoxicity plays a major role in pancreatic β-cell apoptosis and diabetes progression, but the factors involved have remained largely unknown. Our recent studies have identified TXNIP (thioredoxin-interacting protein) as a novel pro-apoptotic β-cell factor that is induced by glucose, suggesting that TXNIP may play a role in β-cell glucotoxicity. Incubation of INS-1 β-cells and isolated primary mouse and human islets at high glucose levels led to a significant increase in TXNIP as well as in apoptosis. Very similar results were obtained in vivo in islets of diabetic mice. To determine whether TXNIP plays a causative role in glucotoxic β-cell death, we used TXNIP-deficient islets of HcB-19 mice harbouring a natural nonsense mutation in the TXNIP gene. We incubated islets of HcB-19 and C3H control mice at low and high glucose levels and assessed them for TXNIP expression and apoptosis. Interestingly, whereas in C3H islets, high glucose levels led again to significant elevation of TXNIP and apoptosis levels as measured by TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling) and cleaved caspase 3, no increase in apoptosis was observed in TXNIP-deficient HcB-19 islets, indicating that TXNIP is required for β-cell death caused by glucotoxicity. Thus inhibition of TXNIP protects against glucotoxic β-cell apoptosis and therefore may represent a novel therapeutic approach to halt diabetes progression.

Introduction
Diabetes and the associated elevated blood glucose levels have deleterious effects on multiple different organ systems including pancreatic β-cells. In the pancreas, this glucotoxicity leads to progressive β-cell dysfunction, impaired insulin gene transcription [1,2] and irreversible β-cell loss due to apoptosis [3–14], resulting in a vicious cycle with worsening of the hyperglycaemia. However, the factors and mechanisms by which glucotoxicity leads to apoptotic β-cell loss have remained largely unknown.

Interestingly, our oligonucleotide microarray study using isolated human islets exposed to high glucose levels revealed that TXNIP (thioredoxin-interacting protein) was the gene induced most dramatically in response to glucose, with a more than 10-fold increase [15]. TXNIP [16–18] was first isolated from a 1,25(OH)2D3 (1,25-dihydroxyvitamin D3)-treated HL-60 human promyelocytic cell line [19] and therefore called VDUP1 (vitamin D3-up-regulated gene 1) [19–21]. It contains 391 amino acid residues and is encoded on human chromosome 1 and mouse chromosome 3. TXNIP binds to and inhibits thioredoxin and thereby can modulate the cellular redox state and/or induce oxidative stress [21–25]. Thioredoxin is a thiol-oxidoreductase and part of a major cellular reducing system protecting cells against oxidative stress [26]. The thioredoxin system reduces oxidized proteins, resulting in oxidation of the two cysteine residues of thioredoxin. In order to return to a reduced and active state, thioredoxin has to be reduced back by the NADPH-dependent thioredoxin reductase [23,27]. Thioredoxin is involved in multiple cellular processes including induction of cell proliferation and inhibition of apoptosis [23,28–30]. As an inhibitor of thioredoxin, TXNIP would be expected to have antiproliferative and pro-apoptotic properties. Indeed, TXNIP overexpression has been shown to render different cell types more susceptible to oxidative stress and apoptosis [22,31]. To investigate whether TXNIP might also induce apoptosis in pancreatic β-cells, we created a stably transfected INS-1 β-cell line with constitutive TXNIP overexpression. In fact, we found that TXNIP-overexpressing INS-1 cells were significantly more susceptible to apoptosis compared with the control cell line overexpressing LacZ [32]. Taken together, the fact that glucose induces β-cell TXNIP expression and that TXNIP induces β-cell apoptosis raised the possibility that this protein may act as a mediator of β-cell glucotoxicity. In our most recent study, we therefore addressed this question by analysing the effects of diabetes on β-cell TXNIP expression in vivo and by studying the susceptibility of TXNIP-deficient islets to glucotoxicity-induced apoptosis [33].

Increased TXNIP expression is associated with increased β-cell apoptosis in vivo
Analysis of islets isolated from obese, insulin-resistant and diabetic ob/ob mice harbouring the leptinab mutation revealed that TXNIP protein levels were elevated almost 10-fold in islets of obese diabetic mice as compared with lean normoglycaemic controls and this increase in TXNIP was accompanied by an equal rise in cleaved caspase 3 levels as a measure of apoptosis [33]. Elevation of TXNIP expression was also observed in islets of non-obese, insulin-resistant
C57BL/6.azip mice as another model of diabetes [32], further supporting the in vivo role of TXNIP in diabetes and β-cell death.

Induction of endogenous TXNIP expression by incubating INS-1 β-cells, isolated primary mouse islets or human islets at high glucose levels (25 mM for 24 h) also demonstrated that the increase in TXNIP protein levels was associated with a ∼10-fold increase in β-cell apoptosis [33]. These findings suggest that TXNIP-mediated β-cell apoptosis does not require constitutive overexpression of TXNIP, but rather that the glucose-induced increase in endogenous TXNIP is sufficient for this effect. In addition, these results are consistent with the pro-apoptotic properties of TXNIP described in extrapancreatic tissues [22,31].

To address further the question of whether increased TXNIP expression is essential for the observed glucose-induced β-cell apoptosis, we took advantage of a unique mouse model of TXNIP deficiency. These HcB-19 mice are an inbred congenic C3H mouse strain [34,35] that harbours a spontaneous inactivating nonsense mutation in exon 2 at codon 97, resulting in dramatically reduced TXNIP mRNA and protein levels [16].

β-Cells lacking TXNIP are protected against glucotoxicity-induced β-cell death

Analysis of control C3H islets with normal TXNIP expression incubated at high glucose levels resulted again in elevated TXNIP protein levels and increased apoptosis, as measured by increased cleaved caspase 3 and a significantly higher percentage of TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling)-positive β-cells compared with C3H islets incubated at low glucose levels [33].

In contrast, parallel experiments using isolated islets of TXNIP-deficient HcB-19 mice revealed no increase in TXNIP expression (as expected), but interestingly also failed to demonstrate any increase in cleaved caspase 3 or TUNEL-positive β-cells in response to glucose [33]. These results provide strong evidence for the protective role of TXNIP deficiency against β-cell glucotoxicity and suggest that TXNIP expression is essential for the increase in β-cell apoptosis observed in response to high glucose exposure.

Conclusion

In summary, our studies have shown that glucose induces TXNIP expression in human pancreatic islets, primary mouse islets and INS-1 β-cells and that this increase in TXNIP is associated with increased β-cell apoptosis. In addition, TXNIP expression was elevated in islets of different mouse models of diabetes. Moreover, the results of the most recent study revealed that TXNIP is required for β-cell death caused by glucotoxicity (Figure 1) and therefore suggest that TXNIP may represent a potential target to interfere with the toxic effects of glucose. Such an intervention could help prevent β-cell apoptosis and the gradual β-cell loss of Type 2 diabetes and delay the need for daily insulin injections. In fact, we found that TXNIP reduction mediates the anti-apoptotic effects of exenatide, an antidiabetic drug recently approved for Type 2 diabetes [36]. Taken together, inhibition of TXNIP may represent a novel therapeutic approach to halt diabetes progression by protecting against glucotoxic β-cell apoptosis and interrupting this vicious cycle.

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