Executioners of apoptosis in pancreatic β-cells: not just for cell death

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Choi D, Woo M. Executioners of apoptosis in pancreatic β-cells: not just for cell death. Am J Physiol Endocrinol Metab 298: E735–E741, 2010. First published December 22, 2009; doi:10.1152/ajpendo.00696.2009.—Pancreatic β-cell mass is genetically regulated cell suicide process that is evolutionarily conserved. Upon receiving an apoptotic signal, either at the mitochondria or on the cell surface, the cell undergoes a commitment to die and uses the machinery within it to undergo a highly orchestrated and characteristic process. In multicellular organisms, this process plays a critical role in all stages of life, from development, where apoptosis is important for tissue sculpting and remodeling, to maintenance of homeostasis during physiological turnover and the elimination of unwanted or harmful cells throughout the life of the organism (40, 89). Histological evidence of apoptosis was discovered in the early 1970s, and the genetics of developmental apoptosis in the nematode Caenorhabditis elegans (46, 62) provided the working genetic frame onto which mammalian genetic discoveries were made. Knockout mouse models for many of the components of the apoptotic signaling pathways have provided valuable insights into the mechanisms and the essential roles of each of the apoptotic genes, which are highly tissue and context specific. In this review, we explore the essential role of each of the apoptotic genes in pancreatic β-cells in both homeostatic and diabetes models.

Pancreatic β-Cell Homeostasis

The pancreatic β-cell plays an essential role in regulating glucose homeostasis. β-Cell mass is dynamic and is tightly matched to meet the body’s demand for insulin (28). During the neonatal period, the β-cell population increases rapidly, and this burst in expansion of β-cell mass is followed by a transient increase in β-cell death (6, 45). With increasing age, the rates of β-cell apoptosis and proliferation and/or neogenesis equilibrate at a frequency of 0.5% under steady-state conditions (7).

β-Cell Apoptosis in Type 1 and Type 2 Diabetes

Type 1 diabetes is a chronic autoimmune disease that affects 0.5% of the population in the developed world and continues to increase in incidence (61). This disease is hallmark by immune-mediated destruction of the pancreatic β-cells. Typically, at the time of diagnosis, patients with type 1 diabetes have an estimated 60–80% reduction in β-cell mass (67). The current working model is that in genetically predisposed individuals T lymphocytes are aberrantly activated by the antigen-presenting cells (APCs) in the pancreatic draining lymph nodes. The activated T lymphocytes then circulate, target, and invade the islets, known as insulinitis. These activated T cells can then proceed to destroy the islets. These two phases, insulinitis and islet destruction, are controlled by two distinct genetic processes (61).

Type 2 diabetes, hallmark by underlying insulin resistance, is also characterized by defects in glucose-responsive insulin secretion in addition to an eventual decline in β-cell mass (76). As long as β-cells are able to compensate for the insulin resistance by enhancing insulin secretion and increasing β-cell mass, euglycemia can be maintained. However, in susceptible individuals, perhaps with genetic defects and/or exogenous insults, their β-cells may be unable to meet the body’s demand for insulin, ultimately resulting in diabetes mellitus (29, 30, 60, 73). As such, autopsy studies in which β-cell mass was quantified morphometrically in pancreata from patients with...
type 2 diabetes have shown β-cell apoptosis to account for the dramatic reduction of β-cell mass (8, 54, 77, 96).

Pathways of Apoptosis

The genetics of the apoptotic pathway was first described in *Caenorhabditis elegans*. CED-3 is the executioner of apoptosis, which is activated by CED-4. CED-9 negatively regulates CED-4, which in turn is inhibited by EGL-1. Homologs for each of the components are characterized in mammals. In fact, Bcl-2, which was first discovered as a proto-oncogene as a result of a translocation mutation in chronic lymphocytic leukemia (20), was later discovered to be a CED-9 homolog (32). The demonstration that Bcl-2 was able to inhibit apoptosis in the nematode (90) was strong evidence that indeed the apoptosis pathway was highly evolutionarily conserved. The Bcl-2 family of proteins comprises a large number of proteins of which some are proapoptotic and some are antiapoptotic. The BH3-only proapoptotic Bcl-2 proteins were later found to be homologous to EGL-1, whereas the antiapoptotic Bcl-2 proteins are CED-9 homologs (34). Apoptotic protease-activating factor 1 (APAF-1) is a mammalian CED-4 homolog, which in turn activates CED-3 (26) (Fig. 1).

In mammals, there are 14 CED-3 homologs identified to date, of which some function in inflammation and convert proinflammatory cytokines to their active forms (16). The term “caspase” was later coined for the CED-3 homologs to describe their cysteine aspartate-specific protease function (2). Interleukin 1β-converting enzyme, the first CED-3 homolog discovered and later called caspase-1, does not have a predominant role in apoptosis but is responsible for converting IL-1β and IL-18 to their active forms (11, 98). Caspase-3 was later found to be one of the main executioners of apoptosis (26, 66, 88). The apoptotic caspases are classified as initiators or executioners. Initiator caspases (caspase-2, -8, -9, and -10) have characteristic domains that facilitate recruitment and interaction with other proteins, which allows for proximal activation initiated either at the mitochondria or at the cytoplasmic membrane upon ligand binding, which in turn activates the executioner caspases (caspase-3, -6, and -7) (15, 49).

Caspases are synthesized as inactive precursors, called pro-caspases, which undergo proteolytic cleavage at specific aspartate residues, resulting in conformational change and activation (15). However, emerging evidence shows that noncleaved caspases may also have biological functions, although the mechanisms are not clearly understood.

Extrinsic apoptotic pathway. The extrinsic pathway is initiated at the cytoplasm by activation of death receptors that belong to the tumor necrosis factor (TNF) superfamily of receptors, which are characterized by their death domain in their cytoplasmic tail. Their ligands, which include TNF receptor 1 (TNFR1), Fas (CD95/APO-1), and the TNF-related apoptosis-inducing ligand receptor 1 (TRAIL-R1) bind to the respective receptor. Upon receptor ligation, procaspase-8 is recruited by interaction of its death domain to the death-inducing signal complex (DISC), where it becomes activated (65), which in turn activates caspase-3, -6, and/or -7 (42, 83). c-FLIP, a negative regulator of the extrinsic apoptotic pathway, can directly block caspase-8 activation by competitive binding at the DISC (80). Caspases-2 and -10 have also been shown to be recruited to and be activated at the DISC under certain settings (48, 52, 92).

Intrinsic apoptotic pathway. The intrinsic, or mitochondria-mediated, apoptotic pathway is activated in response to cellular stresses such as hypoxia, survival factor deprivation, heat shock, or DNA damage (1). The principal regulatory step in the intrinsic pathway is the interaction between pro- and antiapoptotic Bcl-2 family members on the mitochondrial outer membrane. Upon activation, proapoptotic Bcl-2 family members, including Bax, Bak, Bad, and Bid, are recruited from the cytoplasm to the mitochondrial outer membrane (47, 56, 87). However, antiapoptotic Bcl-2 proteins, including Bcl-2 and Bcl-xL, block the activity of Bax and Bak to inhibit the apoptotic pathway. Activated caspase-8 also cleaves Bid, which in turn can initiate the mitochondria-mediated apoptotic pathway (56), representing a link between the extrinsic and intrinsic apoptotic pathways. Oligomerization of these proteins leads to pore formation in the mitochondrial membrane and mitochondrial outer membrane permeabilization, resulting in the release of cytochrome c into the cytosol. Cytochrome c will in turn bind to Apaf-1 and procaspase-9 to form the apoptosome, and in the presence of ADP will activate caspase-9 (3, 50, 70). Caspase-9 then cleaves executioner caspases caspase-3, -6, and/or -7, leading to the terminal phase of apoptosis (51). The executioner caspases can become activated in response to either the extrinsic or the intrinsic apoptotic pathway and represent another point of convergence in the apoptotic pathway (Fig. 2). Caspase activation can be directly inhibited by inhibitors of apoptosis (IAPs), which bind to them, thereby preventing apoptosis (82). Other important mechanisms of β-cell apoptosis include endoplasmic reticulum (ER) stress (31, 53) and caspase-independent pathways (37), which will not be described in this review.

Diverse Roles of Apoptotic Proteins in β-Cells

Much evidence has shown that β-cell apoptosis is an essential process in type 1 and type 2 diabetes pathogenesises. Despite this notion, there have been only a few studies that have
investigated the precise role of specific caspases in the mechanism of β-cell apoptosis in vivo. The specific caspase knock-out mice have been invaluable tools for studying the role of each of the caspases under specific context. Emerging evidence from knockout studies suggests that certain apoptotic proteins, which are known largely for their roles in cell death, may also participate in other vital nonapoptotic cellular processes.

Caspase-3. Caspase-3 has been extensively studied in various tissues due to its role as the principal executioner of apoptosis. As such, caspase-3 is an attractive target to inhibit apoptosis in disease settings, including diabetes. Deletion of caspase-3 in the β-cells was shown to be protective against the multiple low doses of streptozotocin (STZ; MLDS) model of type 1 diabetes in mice (57, 59). Not only were these mice protected against type 1 diabetes development by inhibition of β-cell apoptosis, but the caspase-3-deficient mice did not show any evidence of T cell activation or infiltration (57). These results show that the initial T cell activation that is required to initiate autoimmunity is caspase-3 dependent. Therefore, inhibiting β-cell apoptosis not only protects against β-cell destruction, but the initial autoimmunity may also be prevented, which is an attractive strategy, particularly for islet transplantation.

Although caspase-3 was essential in β-cell apoptosis in MLDS-induced diabetes, caspase-3 does not appear to be required for maintenance of β-cell homeostasis (Liadis N, Woo M, unpublished data). These data show that the essential role of caspase-3 is context specific, where it is necessary in the context of diabetes, in the presence of apoptotic stimuli, whereas under basal conditions, caspase-3 appears to have a redundant role.

Although inhibiting apoptosis is a desired effect in the setting of diabetes, a theoretical risk of promoting tumorigenesis always prevails. However, direct evidence of islet tumor promotion as a consequence of specific caspase inhibition is still lacking. A study to directly examine the role of caspase-3 in islet tumorigenesis shows that caspase-3 deletion, with a concomitant activation of the proto-oncogene c-Myc, does not lead to tumor formation (75). Interestingly, in addition to completely protecting against islet apoptosis, caspase-3 deletion also leads to inhibition of cell cycle progression. Indeed, activated caspase-3 has been shown not only to cleave proteins leading to cell death, but also to cleave other vital proteins, including cell cycle inhibitors p21 and p27 (55, 93). As such, caspase-3 deficiency in islets upon c-Myc activation leads to persistent p27 expression (75), thereby inhibiting cell cycle progression. Thus, one can argue, at least in the context of c-Myc activation, that inhibition of caspase-3 has not only antia apoptotic effects but perhaps even paradoxical tumor-suppressive effects.

Caspase-3 has also been reported to have significant non-apoptotic roles in other tissues, including cellular differentiation of keratinocytes, erythroblasts, osteoclasts, and skeletal muscle (9, 27, 55, 68, 85). Furthermore, caspase-3 also cleaves the p50 and p65 subunits of NF-κB, which hinders its transcriptional activity and role in cell survival (43). Thus, by the virtue of caspase-3’s ability to cleave a number of vital proteins, caspase-3 has the potential to regulate many facets of different cellular processes.

Caspase-8. Similar to caspase-3, caspase-8 is best known for its role in mediating apoptotic cell death. In pancreatic β-cells, loss of caspase-8 leads to protection against apoptosis against FasL-induced cell death in vitro, in addition to ceramide, which has been shown to increase susceptibility to Fas-mediated cell death (36, 58). Furthermore, in vivo studies of mice with β-cell caspase-8-deficiency show protection against MLDS- and high-fat diet (HFD)-induced β-cell death and diabetes development (58). These results demonstrate that caspase-8 is indeed essential in mediating the extrinsic apoptotic pathway in pancreatic β-cells and that this pathway is required in the development of diabetes in these experimental models.

Interestingly, in contrast to the essential role of caspase-8 in β-cell apoptosis diabetes models, caspase-8 appears to provide a paradoxical prostimulatory role in the absence of apoptotic stimuli, under basal conditions, such that loss of caspase-8 leads to an age-dependent decrease in β-cell mass and glucose intolerance. The decline in β-cell mass is attributed to a paradoxical increase in β-cell death that is associated with a decrease in phosphorylated IRS-2 and Akt, which are important components of the insulin signaling pathway that play a critical role in maintaining β-cell mass (58). The precise mechanism for this novel role of caspase-8 in regulating growth factor signaling is not yet clear. Despite the decrease in β-cell mass with aging, caspase-8 deletion leads to an increase in insulin secretion in response to glucose with increased exocytosis of insulin granules at the level of individual β-cells and an associated increase in the expression of key proteins involved in the exocytosis machinery (58). Whether this is a primary effect of caspase-8 deletion or a compensatory effect still remains to be elucidated. Caspase-8 has been reported to also play a major role in T and B cell proliferation and activation (5, 44, 78) and differentiation of hematopoietic progenitors and monocytes (44) as well as tumor metastasis and autophagic cell death (84, 97), illustrating the highly context-specific diverse roles of caspase-8 in different tissues.
Fas. The biological role of Fas signaling is also becoming increasingly complex. Although well known for its role in cell death, recent evidence has highlighted nonapoptotic roles for Fas. Similar to caspase-8, Fas deletion in the pancreatic β-cells has been shown to be protective against FasL- and ceramide-induced death ex vivo, which again illustrates its proapoptotic role in the β-cells (14).

Elucidation of the role of Fas/FasL in pancreatic β-cells has been complicated by virtue of the presence of both of these molecules not only on the β-cells but also on lymphocytes. Furthermore, until recently, lpr (lymphoproliferative) mice with a generalized mutation leading to a lack of functional Fas precluded assessment of the definitive tissue-specific role for Fas (13, 38). Studies using mouse models with β-cell-specific Fas deletion have shown Fas to play a redundant role in β-cell apoptosis in type 1 diabetes models (4, 14). In addition, Fas deletion was ineffective in protecting mice against HFD-induced type 2 diabetes (14). These studies indicate that Fas is not essential in β-cell death in the context of diabetes.

Furthermore, Fas does not play an essential role in the homeostatic regulation of pancreatic β-cell mass; however, there appears to be a role for Fas in β-cell function and insulin secretion. Similar to the findings from mice with β-cell-specific caspase-8 deletion, Fas deletion also appears to lead to enhanced insulin secretion and glucose tolerance (14). In contrast, another report showed a defect in insulin secretion (81). The reasons for these discrepant findings are not entirely clear.

Fas has also been shown to have roles in nonapoptotic cellular processes in other tissues. For example, Fas activation has been shown to lead to hepatocyte proliferation and liver regeneration after partial hepatectomy (18, 72, 86). Similarly, mice subjected to experimental sciatic nerve crush injury has been shown to lead to hepatocyte proliferation and liver cellular processes in other tissues. For example, Fas activation has been shown to lead to hepatocyte proliferation and liver regeneration after partial hepatectomy (18, 72, 86). Similarly, mice subjected to experimental sciatic nerve crush injury has been shown to lead to hepatocyte proliferation and liver cellular processes in other tissues.

RIPcre cKO (57, 75) Protection against MLDS-induced diabetes and insulitis No effect on β-cell homeostasis (Liadis, N., Woo, M. Unpublished data)
RIPcre Caspase-8fl/fl (58) Protection against MLDS- and HFD-induced diabetes Cell cycle and apoptosis inhibition in c-Myc tumor model Decline in β-cell mass with aging
RIPcre Fasfl/fl (14, 81) No effect on MLDS- and HFD-induced diabetes Enhanced insulin secretion in individual β-cells
RIP-Bcl-XL transgenic (71, 99) Protection against thapsigargin-induced β-cell death in vitro No effect on β-cell homeostasis Enhanced insulin secretion and glucose tolerance
RIP-XIAP transgenic (74) Promotes long-term islet graft survival in mice by inhibiting β-cell apoptosis and insulin Impaired in insulin secretion and glucose intolerance
PDX-1cre Survivinfl/fl, RIPcre Survivinfl/fl and PAX-6cre Survivinfl/fl (41, 94) No effect on MLDS-induced diabetes Decrease in β-cell mass due to cell cycle defect Glucose intolerance with aging

MLDS, multiple low doses of streptozotocin; HFD, high-fat diet.

**Table 1. Summary of the in vivo role of apoptotic proteins and their regulators in pancreatic β-cells**

<table>
<thead>
<tr>
<th>Genetic Model</th>
<th>Apoptotic Function in Diabetes Models</th>
<th>β-Cell Homeostasis and Nonapoptotic Function</th>
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</thead>
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<tr>
<td>Whole body caspase-3 KO (57, 75)</td>
<td>Protection against MLDS-induced diabetes and insulitis</td>
<td>No effect on β-cell homeostasis (Liadis, N., Woo, M. Unpublished data)</td>
</tr>
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<td>RIPcre Caspase-8fl/fl (58)</td>
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**Role of Endogenous Apoptosis Inhibitors in β-Cell Apoptosis**

Bcl-2 proteins. Strategies of increasing antiapoptotic protein expression in β-cells to protect against cell death have also been shown to be effective. Bcl-XL is an antiapoptotic member of the Bcl-2 family of proteins, and it competes with Bax at the mitochondrial outer membrane to inhibit mitochondrial membrane permeability that leads to apoptosis (47, 87). Overexpression of the antiapoptotic protein Bcl-xL in pancreatic β-cells has been shown to be protective against β-cell apoptosis, although this was associated with impaired β-cell function (99). Furthermore, Bcl-xL overexpression did not have any effect on pancreatic insulin content (99). However, in a tumor model, a concomitant activation of c-Myc and Bcl-xL overexpression led to a rapid dedifferentiation and fulminant tumor progression (71).

**IAPs.** The IAPs are another class of endogenous inhibitors of cell death. Members in this family include cIAP1/hIAP-2/MIHB, cIAP-2/hIAP-1/MIHC, XIAP/hILP-1/MIHA, survivin/TIAP, NAIP, ILP-2/Ts-IAP, ML-IAP/Livin/KIAP, and Bruce/Apollon (79, 91). In contrast to what the name suggests, the majority of these members are involved in regulation of a variety of cellular processes and not restricted to apoptosis modulation (69). XIAP, on the other hand, has been shown to directly bind to and inhibit caspase-3 (82). Overexpression of this inhibitor in transplanted mouse (74) and human (22, 23) islets has been shown to protect against allograft rejection. Similar to the caspase-3-deficient mice, these mice were protected from islet infiltration by immune cells or by activated splenocytes (74), which again supports the importance of caspase-3-mediated β-cell death in the initiation of autoimmune diabetes.

Survivin, another IAP member, has a unique expression pattern that is restricted in embryonic and malignant tissues but generally not in tissues of healthy adults (63). Recent studies have shown that the role of survivin is not limited to apoptosis inhibition but is also involved in the regulation of the mitotic...
spindle checkpoint and cell cycle progression (63). Interestingly, survivin is expressed in the islets during the late embryonic and neonatal periods and gradually declines to a complete absence by early adulthood. Despite the transient expression of survivin, its deletion in the pancreas leads to a persistent decline in β-cell mass and glucose intolerance later in adulthood (41, 94). These results suggest that perinatal gene expression may play a critical role in the establishment of the β-cell mass such that inability to reach the appropriate β-cell mass during this early period in life may lead to defects in glucose homeostasis later in life. Transgenic expression of survivin in transplanted islets has been shown to afford long-term engraftment and correction of hyperglycemia in host mice treated with MLDS (19). These results show that neonatally expressed genes may also be potential candidates for enhancement of β-cell mass.

Pharmacological caspase Inhibitors

Generation of small molecule inhibitors that inhibit specific caspases has been valuable in an experimental setting and more recently in the clinical arena, particularly in acute tissue injury models (12, 36, 39). These inhibitors have also been shown to be potentially ideal in the setting of islet transplantation.

In vitro studies on β-cell lines and caspase inhibitors have shown that the caspase-2 inhibitor is effective in protecting the HIT-T15 β-cell line against an experimental model of cell death (35). Furthermore, murine βTC1 cell lines transfected with human Fas were shown to be protected against Fas-induced β-cell apoptosis by the caspase-3 inhibitor Z-Asp-Glu-Val-Asp-fluoromethyl ketone (95). In the case of palmitate-induced cell death, executioner caspase-6 inhibitors were found to be protective against β-cell apoptosis (33). EP1013, a potent and selective caspase inhibitor, has been used to pretreat isolated human islets for transplantation in vitro in combination with treatment in the murine recipient posttransplant, which significantly improved islet graft survival (21). A pan-caspase inhibitor, zVAD, which has been shown to inhibit caspases 1–10, and -12, has also been shown to be beneficial for human islets in the context of islet transplantation (24, 64) but were suggested to be not as effective as the selective caspase inhibitor EP1013 (21).

Conclusions

Insufficient insulin supply resulting from deficiencies in both β-cell mass and function is the central defect in both type 1 and type 2 diabetes, and apoptosis is inappropriately increased in both diseases. Although the instigating stimuli are significantly different in the two diseases, they both activate the common apoptotic machinery. Therefore, it is of great interest to unravel the specific components of the suicide machinery that culminates in β-cell death. However, emerging evidence shows that caspases are not only executioners of apoptosis but can also modulate other cellular functions in a highly context- and tissue-specific manner. Thus far, caspases do not appear to play a predominant role during β-cell development or homeostasis. Importantly, caspases also do not appear to play a predominant role in tumor formation in β-cells. While some caspases, particularly caspases-3 and -8, appear to play a critical role in β-cell apoptosis in diabetes models, they appear to also have nonapoptotic roles. IAPs also appear to be promising targets for modulating β-cell fate during diabetes development. Together, a comprehensive understanding of these molecules that regulate cell fate must be considered carefully in our efforts to achieve novel strategies for enhancement of β-cell mass for prevention or treatment of both type 1 and type 2 diabetes.

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DISCLOSURES

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