Abstract

Diabetes mellitus is one of the most common non-communicable diseases, and if uncontrolled, targets multi-organ systems with serious debilitating and life-threatening sequelae. Most diabetic cases fall under the Type 2 category, characterized by relatively late onset, development of insulin resistance and/or deficiency, and amyloidosis. Type 1 diabetes, on the other hand, manifests early during childhood and has an autoimmune component to it that causes a severe deficiency in the circulating levels of insulin. Despite the heterogeneity in etiology and clinical presentation, hyperglycemia is the most common metabolic abnormality in diabetic patients. At the molecular level, pancreatic β-cell loss by apoptosis appears to play an important role in the development of insulin deficiency and the onset and/or progression of the disease. Here, we provide a short review on the apoptotic death circuitry in the pathogenesis of diabetes.

Keywords: Pancreatic β-cells; Apoptosis; Mitosis; Genetic control; Diabetes

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Abbreviations: Akt, protein kinase B; Bad, Bcl-2 antagonist of cell death; Bax, Bcl-2 antagonist X; Bcl, B-cell leukaemia/lymphoma; FADD, Fas-associated death domain containing protein; FFA, free fatty acid; IAPP, islet amyloid polypeptide; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein. kinase; MDM2, mouse double minute 2; NOD, non-obese diabetic mice; PI3K, phosphatidylinositol 3-kinase

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doi:10.1016/j.biocel.2006.09.007
1. Introduction

Apoptosis is a coordinated series of events for the programmed execution of cell death, and plays an important role in the maintenance of tissue homeostasis. Defects in apoptosis regulatory machinery are implicated in a variety of pathological states; inadequate apoptosis may contribute to oncogenesis, while excess apoptosis is the underlying cause for cell loss during HIV/AIDS, neurodegeneration, and diabetes mellitus. A host of physical, chemical and biological factors can trigger apoptotic death by activating complex yet tightly controlled intracellular signal transduction pathways (Fig. 1). The extrinsic pathway is activated upon ligation of the cell surface death receptor(s), which in turn activates downstream effector mechanisms orchestrated by the caspase family of cysteine proteases (Green, 2005). The prototype example of death signaling via the extrinsic pathway is the Fas(CD95) death receptor, which brings about the assembly of the death inducing signaling complex (DISC), a multi-protein complex comprising of the cytoplasmic aspect of the Fas(CD95) receptor, the adaptor protein FADD (Fas-associated death domain containing protein), and pro-caspase 8. Assembly of this complex is the initiating signal for the processing of pro-caspase 8 to its active form. Depending upon the level of caspase 8 activity, the ensuing signal can either directly activate downstream caspase cascade or involve the intermediacy of the mitochondrial death pathway for efficient execution (Scaffidi et al., 1998). The intrinsic or mitochondria-dependent pathway characteristically involves cross-talk between caspases, pro-apoptotic members of the Bcl-2 family (e.g. Bax and Bad) and death amplification factors, such as cytochrome c and apoptosis-inducing factor, released from the mitochondria (Green, 2005). In contrast, anti-apoptotic members of the Bcl-2 family, such as Bcl-2 and Bcl-xL, blunt intrinsic death signaling by blocking the recruitment of pro-apoptotic members to the mitochondria (Green, 2005). Thus, there exists a tight intracellular balance between these two function-
ally contrasting Bcl-2 family members, and the fate of the cell is determined by the tilt in the ratio towards one or the other. Additionally, the phosphorylation status of some of the Bcl-2 members could also determine their distinct role(s) during cell death or survival signaling; phosphorylation status of Bad is critical in maintaining cell survival, as in this conformation Bad is anchored to 14-3-3 and unable to translocate to the mitochondria. Upon initiation of an apoptotic signal or inactivation of the kinase, unphosphorylated Bad is no longer able to bind 14-3-3, is free to translocate to the mitochondria and trigger mitochondrial outer membrane permeabilization (Xiao & Singh, 2006; Zhou et al., 2002).

Interestingly, the kinase involved in Bad phosphorylation is a critical cell survival serine threonine kinase, Akt/PKB, activated downstream of PI3K and regulated by the protein phosphatase PTEN (Sun et al., 1999). In addition to its role in promoting tumorigenesis, Akt/PKB also is a critical regulator of pancreatic β-cell mass and function and carbohydrate metabolism via insulin-dependent uptake of glucose. Upregulation of Akt activity strongly reinforces survival pathways and blunts death signaling in a variety of cell types, and its downregulation renders the intracellular milieu permissive for death execution (Datta et al., 1997). The latter has been documented in the dysfunction and loss of insulin secreting β-cells associated with diabetes mellitus. Indeed, increased rate of apoptosis has been linked to the pathophysiology of diabetes mellitus, and the PI3K/Akt pathway is critically involved in regulating cell fate decision.

2. Apoptosis and insulin deficiency in Type 2 diabetes mellitus

2.1. Apoptosis and β-cell loss in Type 2 diabetes

Insulin resistance and deficiency are characteristic hallmarks of Type 2 diabetes mellitus. However, in addition, deposition of pancreatic amyloid consisting of islet amyloid polypeptide (IAPP) is also a common finding. The relative contribution of insulin insensitivity and deficiency in the pathogenesis of Type 2 diabetes has long been a subject of debate. Insulin resistance precedes insulin deficiency and is thus suspected to play a causative role (Reaven, 1988). However, defects in insulin sensitivity alone are inadequate for diabetic hyperglycemia; in separate cases (i.e., obesity and genetic insulin receptor defects), compensatory insulin production by pancreatic β-cells is capable of maintaining normoglycemia. It is believed that the disease can arise due to failure of β-cell insulin production to counteract the progressive insensitivity to insulin.

Insulin secretion in response to a nutrient load presents a biphasic feature. The first phase of secretion, due to the release of preformed insulin, is rapid and relatively intensive and is followed by a longer and less intense second phase resulting from the synthesis of new insulin. Insulin deficiency can be a consequence of compromised insulin secretion per β-cell and/or reduction in total β-cell mass. Although defects in both phases of insulin secretion are noted in glucose intolerance/diabetes (definitions available on www.diabetes.org), there is no solid evidence that they play a causative role in diabetes (Masiello, 2006). On the other hand, cross-sectional studies of sample size with appropriate control subjects have presented compelling data supporting a role for β-cell loss in the pathogenesis of Type 2 diabetes (Butler et al., 2003, 2004). The important findings include: (i) β-cell mass can increase during insulin resistance, which appears to represent a compensatory state; (ii) β-cell loss is present in diabetes; (iii) β-cell deficit correlates with the level of impairment in glucose tolerance, from around 40% in glucose intolerant subjects to approximately 60% in Type 2 diabetes patients; (iv) β-cell death can directly lead to insulin deficiency. It appears that loss of 60% or more β-cells elicits manifestations of diabetes, particularly in the presence of insulin resistance (Butler et al., 2003, 2004).

There is experimental evidence that β-cell volume is reduced in patients with Type 2 diabetes, and recent reports comparing pancreatic tissues from Type 2 diabetic patients and non-diabetic subjects showed significantly higher rate of apoptosis in diabetic islets as opposed to the non-diabetic counterparts (Butler et al., 2003, 2004). In transgenic diabetic animal models that express IAPP, typical features of β-cell apoptosis were observed concomitantly with a reduction in β-cell mass. Similarly, elevated activities of caspases-3 and -8 in β-cells from islets of Type 2 diabetic patients, which could be inhibited by the anti-diabetic agent metformin, were reported in a separate study, thus reinforcing the link between apoptosis and β-cell loss during Type 2 diabetes (Marchetti et al., 2004).

2.2. Diverse stimuli trigger β-cell apoptosis via complex signaling pathways (Fig. 1)

Apoptotic death of β-cells has been observed in response to diverse stimuli, such as glucose, cytokines, IAPP, and free fatty acids (FFA). In primary cultures of murine β-cells, glucose induces apoptosis in a dose-dependent manner, starting at 11 mM (Efanova et al.,
The importance of functional Akt as somewhat of a rheostat in controlling β-cell death and the consequential development of diabetes. Indeed, the involvement of Akt in β-cell physiology might go beyond apoptosis and include the regulation of insulin secretion, β-cell volume, neogenesis and replication (reviewed in Elghazi et al., 2006).

### 3. Apoptosis and autoimmunity in Type 1 diabetes mellitus

Type 1 diabetes remains a common disease, although it accounts for only a relative small proportion of the overall diabetes population. Its onset occurs early in life due to rapid loss of pancreatic β-cells by cell-mediated autoimmunity; pathological infiltration of islets with lymphocytes occurs prior to clinical manifestations. Both helper (CD4+) and cytotoxic (CD8+) T cells are actively involved, and are under the influence of MHC loci as well as non-MHC determinants. Indeed, the link between hyperactive immune response and Type 1 diabetes is so striking that T cell depleted NOD (non-obese diabetic) mice fail to develop diabetes. In contrast, adoptive transfer of T cells from a diseased donor to a disease-free recipient concomitantly transfers the disease.

Infection-associated molecular mimicry has been a popular hypothesis for the development of autoimmunity in Type 1 diabetes. However, there is evidence that early developmental remodeling and/or homeostasis of β-cell mass involves β-cell apoptosis (Trudeau et al., 2000), which might trigger autoimmunity. In NOD mice, CD8+ cells are primed in the pancreatic lymph nodes, remain undetectable for up to 3–4 weeks after birth and progressively increase to maximal levels by 8 weeks of age. T cell priming is achieved by antigen-presenting cells that transport β-cell antigens specifically to pancreatic lymph nodes (Hoglund et al., 1999). Although the relationship between β-cell apoptosis and auto-immunity remains to be fully established, there is emerging evidence that T cell-induced apoptosis is a dominant effector mechanism in Type 1 diabetes. In this regard, pancreatic β-cells derived from newly diagnosed patients with Type 1 diabetes were found to have increased cell surface expression of Fas(CD95) as compared to β-cells from healthy subjects that did not constitutively express detectable Fas(CD95). The apoptotic signal is then delivered via the Fas(CD95)L (Fas-ligand) expressed on infiltrating T lymphocytes (Stassi et al., 1997). Furthermore, expression of dominant-negative Fas(CD95) or neutralizing antibodies to Fas(CD95)L significantly blocks apoptosis, maintains adequate β-cell function, blocks adoptive
transfer of diabetes by primed T cells, and retards the course of diabetes development (Allison et al., 2005). In contrast, transgenic expression of Fas(CD95) in β-cells accelerates the course of the disease (Petrovsky et al., 2002). A similar mechanism has been demonstrated for cytokine-induced β-cell death (Riachy et al., 2006), thus highlighting the role of the extrinsic apoptotic pathway. Other studies provide evidence to implicate mitochondria as well in β-cell loss leading to Type 1 diabetes (Chang et al., 2004) (Fig. 1).

Similar to Type 2 diabetes, inhibition of Akt is implicated in Type 1 diabetes (Storling et al., 2005) and activation of Akt (i.e., by insulin-like growth factor) delays the onset of the diabetes (Chen et al., 2004). Recent findings have also implicated the transcription factor NF-κB in β-cell apoptosis more so in Type 1 than Type 2 diabetes (Cnop et al., 2005). However, there still is a fair amount of controversy on whether NF-κB is upregulated or downregulated in β-cell apoptosis in diabetes.

4. Mitosis and β-cells apoptosis sensitivity

Apoptosis itself is a rapid event. If β-cell apoptosis plays an important role, why diabetes should take years to develop? Cell cycle-associated apoptosis sensitivity appears to provide an answer. In non-β-cells, constitutive expression of c-myc, which promotes cell proliferation, is essential for apoptosis commitment upon growth hormone withdrawal. Using IAPP toxicity as a model for β-cell death in Type 2 diabetes, Ritzel et al. reported that: (i) β-cells that just had previously undergone mitosis (i.e., 3 h post mitosis) were more vulnerable to IAPP-evoked apoptosis. In particular in the first 3 h post mitosis, cells are highly sensitive to apoptosis triggering by IAPP; (ii) in human islets incubated with IAPP, a high percentage (approximately, 44%) of apoptotic β-cells was in mitotic phase. Similarly, in human autopsy pancreatic tissues from Type 2 diabetes patients, which characteristically present IAPP deposition, more than a quarter of apoptotic cells were those at the mitotic phase. Thus, “replication increases β-cell vulnerability to human IAPP-induced apoptosis” (Ritzel & Butler, 2003). In keeping with the IAPP-elicted β-cell apoptosis, cytokines can also induce apoptosis of β-cells (cell line and β-cells isolated human islets) predominantly in those committed to mitosis. This model seems to be more relevant to Type 1 diabetes. In the light that replication of somatic cells including β-cells occurs at a limited rate in adulthood, β-cell apoptotic death is likely to occur at a slow rate, which is compatible with the slow development of diabetes.

5. Genetic susceptibility and apoptosis machinery

The development of diabetes and possibly its microvascular complications is under genetic control and involves interplays between genetic and non-genetic factors (Lee et al., 2000, 2001). If apoptosis plays a part, then there could be a link between genetic alterations in apoptosis machinery and the pathogenesis of the disease. Emerging evidence has begun to unify the genetic susceptibility and genes in the apoptosis signaling machinery, as increasing numbers of apoptosis regulatory genes have recently been linked to the pathogenesis of diabetes. Some of them are listed in Table 1. Fas(CD95)L, caspase-7, Akt and Pten gene products are important for the induction of apoptosis in Type 1 and/or Type 2 diabetes, and, as shown in Fig. 1, they are well recognized players in the regulation of apoptosis. SUMO (small ubiquitin-like modifier) can regulate apoptosis through evoking posttranslational modification of apoptosis regulatory proteins and/or modifying their transcription (Babic et al., 2006). Calpain 10 is one of the calpain proteases that can cleave various substrates during apoptosis. It is known that calpain 10 is required for β-cell apoptosis triggered by fatty acid palmitate, low glucose and inhibition of the type 2 ryanodine receptor (Johnson et al., 2004). NOS2 (nitric oxide synthase-2) is involved in nitric oxide production, which has been shown to cause apoptosis in various types of cells including β-cells (Bosca & Hortelano, 1999). The plasma concentration of apolipoprotein CIII (ApoCIII) is found to be elevated in Type 1 diabetic patients, and is suspected to cause β-cell apoptosis through increasing free intracellular Ca2+ concentration (Juntti-Berggren et al., 2004). AMP-activated protein kinase (AMPK) maintains cell energy balance; it inhibits ATP-consuming pathways and enhances ATP-synthesis when intracellular energy level is low. AMPKs including the isoform AMPKα2 are often found to promote cell survival, possibly by reg-

### Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disease</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Fas(CD95)L</td>
<td>Type 2 diabetes</td>
<td>Nolsoe et al. (2006)</td>
</tr>
<tr>
<td>Caspase 7</td>
<td>Type 1 diabetes</td>
<td>Babu et al. (2003)</td>
</tr>
<tr>
<td>Akt</td>
<td>Type 2 diabetes</td>
<td>George et al. (2004)</td>
</tr>
<tr>
<td>Pten</td>
<td>Types 1 and 2 diabetes</td>
<td>Ishihara et al. (2003)</td>
</tr>
<tr>
<td>SUMO</td>
<td>Type 1 diabetes</td>
<td>Qu et al. (2005)</td>
</tr>
<tr>
<td>Calpain 10</td>
<td>Type 2 diabetes</td>
<td>Kang et al. (2006)</td>
</tr>
<tr>
<td>NOS2</td>
<td>Type 1 diabetes</td>
<td>Johanssen et al. (2001)</td>
</tr>
<tr>
<td>ApoCIII</td>
<td>Type 1 diabetes</td>
<td>Hokanson et al. (2006)</td>
</tr>
<tr>
<td>AMPKα2</td>
<td>Type 2 diabetes</td>
<td>Horikoshi et al. (2006)</td>
</tr>
</tbody>
</table>
ulating p38 MAPK (Li et al., 2005), which is one of the important players in apoptosis. However, the correlations between genotypes and phenotypes suggest only an association between gene mutations/polymorphisms and disease, and further studies are required to confirm that these genes play pathogenic roles, and if so, are they specific to β-cell death? Do they require non-genetic factors for eliciting the disease phenotype? Can mutations in the genes alter the bioactivity of their products that in turn amplify apoptotic or diminish anti-apoptotic signals? Furthermore, genetic association or positional cloning studies in search for diabetes susceptibility genes may unveil novel apoptosis signal transduction pathways in pancreatic β-cells, if the apoptosis-associated genes confer an altered genetic susceptibility towards diabetes pathogenesis.

6. Remarks

Due to the relative dearth of good models in vitro, delineating specific apoptotic pathways to Type 1 or Type 2 diabetes remains a challenging and almost impossible task; β-cell culture is commonly employed as an experimental model. However, there appear to be a few differences between the two types of diabetes, such as: (i) apoptotic β-cell death in Type 1 diabetes could be an autoimmune response predominantly mediated by the extrinsic apoptotic pathway; (ii) Type 2 diabetes is triggered by metabolic factors, and involvement of various death signaling cascades (i.e., extrinsic, intrinsic and others) are evidenced; (iii) NF-κB pathway is important in apoptosis in Type 1 but not so much in Type 2 diabetes, as it does not respond to elevations in glucose and FFA (Cnop et al., 2005). Cytokine-elicited apoptosis is implicated in both types of diabetes (Fig. 1).

The Akt survival kinase (Fig. 1) appears to play a critical role in this metabolic disease. Inhibition of Akt signaling has been observed in apoptosis of β-cells in both forms of the disease as well as in its microvascular complications. Still there remains an obvious gap in our understanding of the role of apoptotic signaling pathways in the development of diabetes in vivo, particularly in terms of differentiating the two forms of the disease. However, lessons learnt from work on in vitro culture systems and animal models of the disease should provide novel targets for the design and development of effective therapeutic strategies targeting to control β-cell loss and its sequela.

In the light of the existing data, it is plausible that development of diabetes is a function of β-cell apoptotic death; earlier initiation and higher intensity of β-cell apoptosis (i.e., by autoimmunity) leads to earlier disease onset possibly with more severe clinical presentations (Type 1), while triggering of relative mild apoptosis

![Age of onset of diabetes and apoptosis.](image)

Fig. 2. Age of onset of diabetes and apoptosis. It is hypothesized that intensive autoimmune-evoked β-cell apoptosis leads to rapid deterioration of β-cell function and early onset of Type 1 diabetes. Metabolic factor-elicited and possibly mitosis-associated apoptosis occurs at comparatively mild level, which determines the slow loss of β-cell function and late onset of Type 2 diabetes. Moderate apoptosis may be implicated in atypical diabetes outside the classical Types 1 and 2. Genetic determinants might influence the initiation and intensity of β-cell apoptosis. Double arrow bars indicate a stronger influence.
later in life (i.e., by metabolic factors) elicits late onset of diabetes with a slower progression pattern (Type 2). Late onset Type 1 diabetes and early onset Type 2 diabetes cases may involve moderate β-cell apoptosis by the combination of moderate pathophysiological triggers (Fig. 2).

Acknowledgements

SP and SCL are supported by grants from the National Medical Research Council and the Biomedical Research Council of Singapore as well as the Academic Research Fund, National University of Singapore.

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