

## New Perspectives on the Role of Hyperglycemia, Free Fatty Acid and Oxidative Stress in $\beta$ -Cell Apoptosis

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### Abstract

Apoptosis is a complex network of biochemical and molecular pathway with fine regulatory mechanisms that control the death event during several pathological situations in multi cellular organisms. It is the part of normal development that occurs in a variety of diseases and is known as aberrant apoptosis. Pancreatic  $\beta$  cell apoptosis is also a pathological feature which is common in both type 1 and type 2 diabetes. There are several reasons through which apoptosis can be induced in  $\beta$ -cell. Metabolic abnormalities such as hyperlipidemia and hyperglycemia have been cited as critical mediator of cell death and may either trigger  $\beta$ -cell apoptosis. Persistent hyperglycemia causes increased production of free radicals that can damage initial  $\beta$ -cell in type I diabetes, impaired insulin production, release or function in type II diabetes. Also apoptosis plays an important role in several diabetic complications. The role of metabolic factors and their mechanism in  $\beta$ -cell apoptosis have been surveyed in this paper.

**Keyword:** Apoptosis, Diabetes, Caspase, Free fatty acids

## Introduction

Diabetes mellitus is one of the major metabolic disorders (1). The number of people with diabetes grows faster than expected. In 2007, 246 million people (roughly 6%) were affected by diabetes worldwide and it is estimated that this will increase to 380 million in 2025. It is caused by a combination of insulin resistance and impaired insulin secretion by pancreatic  $\beta$ -cells. Pancreatic  $\beta$ -cell apoptosis is also a pathological feature that is common in both

type 1 and type 2 diabetes. There are several mechanisms through which apoptosis can be induced in cells. Metabolic abnormalities such as hyperglycemia and free fatty acid (FFA) are important factors in apoptosis of  $\beta$ -cell (2,3). In this article, apoptosis, its pathway and caspase is explained in section 2. In section 3,  $\beta$ -cell apoptosis and the metabolic abnormalities is reviewed from various literatures.

## Apoptosis

A complex network of biochemical and molecular pathways with fine regulatory mechanisms that control the death event in cells is called apoptosis. Several pathological situations in multi cellular organisms can cause cell death. This process constitutes a common mechanism for cell replacement, tissue remodeling and removal of damaged cells (4). Apoptosis is known as a natural process that eliminates the unnecessary single cells by an organism (5). The term of apoptosis has been invented to clarify the main process leading to controlled cellular self-demolition (6). In response to certain stimuli, apoptosis is the end point of an energy-dependent cascade of molecular events (6).

### 1- Pathway of apoptosis

There are two pathways that mediate apoptosis in mammalian cells, Intrinsic and Extrinsic pathway.

#### ➤ *The intrinsic dependent pathway*

Various cellular stresses like radiation exposure, high concentration of glucose and growth factor deprivation activate intrinsic pathway (7). Balance 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 regulate intrinsic or mitochondria dependent pathway (8). Family members of pro apoptotic have only one Bcl-2 homology domain. This is called the BH3-only proteins. These groups include factors like Bim, Puma, Noxa, DP5, Bid and others. Different types of cellular stresses activate different BH3-only proteins in tissues and stimulus specific manner. Activating the pro-apoptotic Bcl-2 family members and down-regulating the pro-survival factors (Bcl-2, Bcl-xl, Bcl-w and Mcl-1) are done by cellular stresses. Then translocation of Bax and Bak to outer mitochondrial membrane result to formation of pores. This process releases cytochrome c into cytoplasm, activates caspase-9 and downstream caspase-3, 6 and 7 and eventually causes apoptosis (7). In contrast, blunting

intrinsic death signal can be performed by anti-apoptotic members of Bcl-2 family, such as Bcl-2 and Bcl-xL via blocking the recruitment of pro-apoptotic members to mitochondria.

#### ➤ *The extrinsic dependent pathway*

Apoptosis can be induced by binding of death inducing ligands to cell surface receptors (9) like Fas R or tumor necrosis factor (TNF) receptor or death receptors, this demonstrates a pathway almost individually controlled by caspases. Assembly of a series of proteins "death-inducing signaling complex" can be done by ligand binding to its receptor which then activates an apical caspase, procaspase-8. The ensuing events are the strongest evidence that caspases act in cascade pathway. Caspase-8 causes activation of caspase-3 which can activate other caspases and ultimately cleave a variety of other cellular proteins (7).

#### ➤ *Caspases*

Caspases are Cysteine-dependent aspartate directed proteases. They are important part of apoptosis process. At least 14 caspases have been identified. After cleaving of aspartic acid from this molecule, caspase becomes active and assembles into hetero tetramer (8). Generally, they can be classified into 3 groups. The first group includes caspase-1, caspase-4, caspase-5 that play a main role in inflammatory response. The second group is composed of initial transducers (caspase-2, caspase-8, caspase-9, caspase-10), and the third group (caspase-3, caspase-6, and caspase-7 effector) is a family of cellular proteins regulators. Fas R/CD95/Apo-1 is a cell surface receptor that transduces apoptotic death signals following activation and has been implicated in triggering apoptosis in infected or damaged cells in disease states. Eighteen members of Bcl-2 family of proteins have been detected. Proapoptotic proteins include Bax, Bad, Bak and Bid whereas Bcl-2 and Bcl-xL are anti-apoptotic proteins (8). The sensitivity of cells to apoptotic stimuli is determined by balance between pro and anti-apoptotic signaling pathway. The proapoptotic

bcl-2 proteins act as sensor in cellular damage or stress. It is found in cytosol of cells. In status of cell stress, these compounds move to surface of mitochondria where the anti-apoptotic proteins are located. Interactions between pro and anti-apoptotic proteins result to formation of Permeability Transition pores (PTP) in the mitochondrial membranes. The release of proapoptotic proteins such as Apoptosis Inducing Factor (AIF), Smac/DIABLO, and cytochrome C in mitochondria lead to the formation of the apoptosome and the activation of the caspase cascade. When cytochrome C is released to cytosol, it interacts with apoptotic peptidase activating factor-1 (APAF-1), this interaction leads to the recruitment of procaspase 9 into a multiprotein complex called the apoptosome. Apoptosis is caused by activation of caspase 9 via formation of the apoptosome (9,10).

## 2- $\beta$ -cell apoptosis

The balance between proliferation, differentiation and apoptosis of  $\beta$ -cell, regulates beta cell mass function. Evidence proposed an important role for  $\beta$ -cell apoptosis in the pathogenesis of diabetes. However, pancreatic  $\beta$ -cell apoptosis is also a pathological feature that is common in both type 1 and type 2 diabetes (T1DM and T2DM) respectively. In T1DM,  $\beta$ -cells are selectively destroyed after lymphoid infiltration of the islet. This autoimmune destruction results to insulin deficiency and hyperglycemia. In T2DM, the decreased insulin secretion in association with insulin resistance lead to glucose toxicity effect that in the presence or absence of hyperlipidemia contributes to  $\beta$ -cell death by apoptosis (11). Diverse stimuli can cause apoptotic death of beta cells. Recent studies indicate that  $\beta$ -cells can initiate apoptotic pathways in response to hyperglycemia with different mechanisms. These mechanisms include oxidative stress, increased intracellular  $\text{Ca}^{2+}$ , mitochondrial dysfunction or mitochondria apoptosis pathway, change in intracellular fatty acid metabolism, activation of Mitogen activated

protein kinase (MAPK) signaling pathway and impaired phosphorylation activation of protein kinase Akt (10).

### ➤ *Diabetes, hyperglycemia and $\beta$ -cell apoptosis*

Hyperglycemia is a main feature of type 2 diabetes. High concentration of plasma glucose is toxic to pancreatic  $\beta$ -cells. Studies on mouse and rat islets showed that exposure to high glucose concentrations for 3–6 days resulted in significant  $\beta$ -cell apoptosis. Similarly, treatment of human islets with high concentration of glucose led to significant increase of  $\beta$ -cells apoptosis. These studies confirmed that pancreatic  $\beta$ -cell apoptosis happened in high concentrations of glucose (7). Also high-D glucose level in human and bovine aortic endothelial cells is associated with a significant increase in the Bax/Bcl-2, caspase-3 activity,  $\beta$ -cell death and apoptosis (9). Another study showed that exposing of human pancreatic islets cultures with elevated glucose levels is associated with over expression of proapoptotic genes Bad, Bid, Bik and unchanged expression of anti-apoptotic gene Bcl-2 (11). In response to chronic exposure of hyperglycemia,  $\beta$ -cell produce and release interleukin (IL)-1b. It activates apoptotic pathway (NF- $\kappa$ B (Nuclear Factor Kappa-light-chain- enhancer of activated  $\beta$ -cell) activation, FAS R upregulation, DNA fragmentation). Also insulin-like growth factor with inhibition of AKT delays the onset of diabetes. Recent studies have been shown that transcription factor NF- $\kappa$ B in  $\beta$ -cell apoptosis is more in type 1 than type 2 diabetes (9).

Some studies reported that proapoptotic role for pro-oxidants in diabetes is due to disturbance of mitochondrial function and releases of cytochrome C. Disruption of mitochondrial function via proapoptotic role for prooxidants in direct as well as indirect evidence shows that  $\text{Ca}^{2+}$  is an important determinant of  $\beta$ -cell apoptosis and has a main role in  $\beta$ -cell apoptosis (4). Some studies showed that a presented factor in the serum of many patients with type 1 diabetes activate

voltage-gated L-type  $\text{Ca}^{2+}$  channels in primary  $\beta$ -cell and in a pancreatic  $\beta$ -cell line (4). Also activation of L-type  $\text{Ca}^{2+}$  channels was associated with DNA fragmentation characteristic of apoptosis. The serum factor that acted on the  $\text{Ca}^{2+}$  channels has not been identified; but depleted serum from IgM fraction had no effect on cytoplasmic  $\text{Ca}^{2+}$ . Also high concentrations of glucose in diabetic patients caused an increase in the cytosolic  $\text{Ca}^{2+}$  and oligonucleosomal DNA fragmentation. Some inducers of  $\beta$ -cell apoptosis are directly regulated by  $\text{Ca}^{2+}$ . Also inhibitors of calcineurin can block IL-1B induced apoptosis. Thus,  $\text{Ca}^{2+}$  seems to be a main denominator in  $\beta$ -cell apoptosis. Further detailed analysis of targets and regulators of  $\text{Ca}^{2+}$  signaling in  $\beta$ -cell should reveal novel therapeutic options for the management and treatment of diabetes (4). One example is the effect of  $\text{Ca}^{2+}$  on mitochondrial function. High intracellular  $\text{Ca}^{2+}$  or inositol 1,4,5-trisphosphate (InsP3) can cause depolarization of mitochondria, induction of mitochondrial permeability transition and release of cytochrome c. This initiates apoptosome formation and subsequent caspase activation. Calcineurin,  $\text{Ca}^{2+}$ /calmodulin-dependent protein phosphatase, is another target for  $\text{Ca}^{2+}$  and plays a main role in apoptosis. Calcineurin may mobilize the proapoptotic Bcl-2 family member like Bad by dephosphorylating and allowing it to localize to mitochondria. Theoretically, Bad can be dimerized with other Bcl-2 family members in the mitochondrial membrane and create a conductance pore with ability to release cytochrome c (4).

#### ➤ **Free fatty acid and $\beta$ -cell apoptosis**

Lipid abnormalities such as hyperlipidemia and fatty acid distribution changes could participate in development of vascular lesions in diabetes (12). Also a high fat diet is implicated in development of diabetes by declining of  $\beta$ -cell survival and function (13,14).

Elevated free fatty acid (FFA) level in blood can be due to the absorption of excess fatty acids. They lead to many negative effects via

multiple pathways in pancreatic  $\beta$ -cells and may lead to  $\beta$ -cell apoptosis (14). Overloading lipid pancreatic cells and dysregulating insulin secretion can be caused by elevated levels of FFA (15). Another study reported increased concentration of saturated fatty acids is toxic to islet cells (6) and generation of fatty acid can be reduced by insulin and prostaglandin (14). Palmitate is the most abundant saturated fatty acid in human plasma. Unsaturated fatty acid oleate compared palmitate induced much less apoptosis in cells. Apoptosis has been observed in human islet after treatment with 0.5 mM palmitate conjugated to 1% BSA for 3 days (6). This study showed strong evidence that high palmitate concentrations induce apoptosis in islet cells. Free fatty acid stimulates beta cell apoptosis via caspase function and down-regulation Bcl-2 mRNA. In addition, FFA-induced apoptosis is linked to down regulation of Akt phosphorylation, which is reversed by expression of constitutively active Akt. Down regulation of Akt has also been observed as a central mechanism in  $\beta$ -cell apoptosis induced by dominant negative form the hepatocyte nuclear factor-1 $\alpha$ . In contrast, activation of Akt (i.e., by simvastatin in islet transplantation) inhibits the activation of apoptosis machinery such as Bad, cytochrome c release and caspase-9, which protects  $\beta$ -cell from apoptotic death and helps maintain  $\beta$ -cell mass (7). The protective role of active Akt has been confirmed to block  $\beta$ -cell apoptosis in human islets. Defecting Akt function disposes  $\beta$ -cell apoptosis and develops diabetes (8,11).

#### ➤ **Oxidative stress and $\beta$ -cell apoptosis**

Oxidative stress occurs as a result of an imbalance between productions of reactive oxygen species (ROS) and their neutralizations by antioxidants or both (16-25). Persistent hyperglycemia causes increased production of free radicals (26), especially reactive oxygen species (ROS) (1). It can cause initial  $\beta$ -cell damage in type I diabetes, or impaired insulin production, release or function in type II diabetes (1,17). In diabetic heart, increased ROS production is due to defective



mitochondria, glucose autooxidation, protein glycation and increased activity of cytosolic xanthine oxidase (2). Studies showed  $\beta$ -cell in compared with other tissues have lower expression of antioxidants that make them vulnerable to oxidative stress (6). Therefore, people with diabetes may also have greater antioxidant requirements and antioxidants supplementation can be of interest by preventing or delaying the development of diabetic complications (1). Also high levels of fatty acids can lead to enhanced biosynthesis of ROS that is generated by mitochondria in  $\beta$ -cell. On the other hand, permeability of cell membrane can be increased by elevated levels of ROS via oxidizing lipid components and lead to calcium influx and activation of phospholipase, which degrade cell membrane phospholipids and induce  $\beta$ -cell apoptosis. Also the level of NF- $\kappa$ B protein is up regulated by ROS and finally causes  $\beta$ -cell death (14). The intra cellular elevated levels of

NF- $\kappa$ B are also closely related to the inflammatory response in  $\beta$ -cell. Overproduction of free radicals can destroy biomolecules such as lipids, proteins and DNA (1). ROS formation can propagate lipid and protein oxidation which leads to loss of mitochondrial membrane potential (16). As a result, loss of mitochondrial membrane potential may augment ROS generation; pore formation, caspase9 and 3 activation and finally apoptosis begin (27).

## Conclusions

Apoptosis was greater in type 1 and type 2 diabetes groups than in non diabetes group.  $\beta$ -cell can initiate apoptotic pathways in response to metabolic abnormalities such as free fatty acids (FFAs), hyperglycemia and other factors such as ROS and etc. Also Apoptosis plays an important role in neuronal, kidney and myocardial cells.

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