〈Review article〉

Glucotoxicity and lipotoxicity induced beta-cell apoptosis in type 2 diabetes mellitus

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Summary   Type 2 Diabetes mellitus (T2DM) is an aggressive metabolic disorder of progressive decline in pancreatic beta-cell mass and function causing impaired insulin secretion and insulin resistance. Insulin resistance heralds the development of hyperglycemia in subjects that ultimately develop T2DM. Dyslipidemia is also a characteristic feature of T2DM with abnormal lipid profiles characterizing high plasma triglyceride concentration, low HDL-cholesterol concentration and increased LDL-cholesterol concentration. Chronic hyperglycemia and hyperlipidemia are toxic to beta-cells and progressively undergoes accelerated apoptosis triggered by metabolic alterations in proposed mechanisms like glucotoxicity, lipotoxicity and glucolipotoxicity which include endoplasmic reticulum stress, mitochondrial dysfunction and oxidative stress. Our review describes the apoptosis of beta-cells induced by hyperglycemia, hyperlipidemia and both.

Key words: Apoptosis, Endoplasmic reticulum stress, Oxidative stress, Glucotoxicity, Lipotoxicity

1. Introduction

Type 2 Diabetes mellitus (T2DM) is a chronic disorder of impaired insulin secretion and insulin resistance leading to hyperglycemia. In addition to hyperglycemia, dyslipidemia is a characteristic feature of T2DM. Progressive deterioration of beta-cell mass and function are the core pathogenic mechanisms underlying diabetes. Persistent hyperglycemia and hyperlipidemia are the major factors for loss of pancreatic beta-cell mass through apoptosis. Glucose is a key physiological regulator of insulin secretion and small changes in glucose concentration exceeding the physiological range induces the pro-apoptotic signals including endoplasmic reticulum stress, mitochondrial dysfunction and oxidative stress. In T2DM, 25-

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50% of beta-cell death occurs by both intrinsic and extrinsic apoptotic pathways. Hyperglycemia and elevated circulating free fatty acids (FFAs) contribute to beta-cell failure due to the cellular stresses and undergo apoptosis contributing to the pathogenesis of the disease.

1.1. Glucotoxicity induced beta-cell apoptosis

Glucotoxicity refers to the structural and functional damage in the pancreatic beta-cells caused by chronic hyperglycemia. Prolonged hyperglycemia induces apoptosis of pancreatic beta-cells through translation of pro-apoptotic signals by the cell glucose-sensing pathways[1]. Pro-apoptotic signals include endoplasmic reticulum stress, mitochondrial dysfunction and oxidative stress[2].

i) Endoplasmic reticulum stress (ER stress)

Beta-cells that are exposed to an increased insulin secretory request place a high demand on endoplasmic reticulum (ER) for the synthesis of proinsulin, progresses to cellular stress. The increase in proinsulin biosynthesis causes an increased flux of protein through the ER of the beta-cell, which is quite high compared with other cell types even under physiological conditions and any further increase, is expected to be conducive to ER stress. In addition to glucose, islet amyloid polypeptide (IAPP), elevated FFAs and chronic over-nutrition in the beta-cell triggers beta-cell ER stress[3]. Synthesis, modification and delivery of proteins into their target sites are impaired during ER stress. This triggers the unfolded protein response (UPR) to restore the ER homeostasis[4]. In case of severe, chronic ER stress and strong UPR, beta-cell apoptosis is mediated by stress kinases and transcription factors like CHOP, MAPK JNK and caspase-12 are activated leading to apoptosis.

ii) Mitochondrial dysfunction

In normal conditions, glucose induces closure of ATP-sensitive potassium channels (K^+ATP) that leads to short-term release of cytosolic Ca^{2+} whereas chronic hyperglycemic condition leads to increase in long term cytosolic Ca^{2+} that turns to be pro-apoptotic. Chronic hyperglycemia leads to decreased number of mitochondria and changes in their morphology like increased volume and outer surface area, reduction of proteins in the inner membrane, increased variability in mitochondrial size in the beta-cells. These changes in morphology and function are associated with impaired glucose stimulated insulin secretion through impaired oxidative phosphorylation, decreased mitochondrial Ca^{2+} capacity and decline in ATP generation[5]. In addition, disruption in Ca^{2+} homeostasis negatively impacts on beta-cell function and on the insulin secretion pathway leading to activation of apoptotic pathways[6].

iii) Oxidative stress

Oxidative stress is resulted due to overproduction and excessive accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cellular environments during cellular metabolic processes or may be introduced via toxic extracellular mediators. These ROS and RNS include superoxides, peroxides and hydroxyl radicals. When cellular antioxidants including glutathione peroxidase (GPxs), catalase (CAT), thioredoxin and superoxide dismutase (SOD) could not neutralize these radicals due to their lower expression in beta-cells compared to other tissues, makes them vulnerable to oxidative stress. Hence, oxidative stress markers such as nitrotyrosine and 8-hydroxy-2-deoxyguanosine concentrations are higher in T2DM patients compared to controls[7]. Mitochondria are an important intracellular source of ROS and in turn, also a target of ROS-mediated injury. Superoxide anion is a very reactive molecule which can be converted to less reactive H_2O_2 by SOD isoenzymes, and then to oxygen and water mainly by CAT, GPxs, and peroxiredoxin. The levels of the H_2O_2-inactivating enzymes GPxs and CAT are extremely low in pancreatic beta-cells. Therefore, their defense against ROS toxicity is very limited, hence subjected to oxidative stress[8]. These ROS and RNS, ROS, particularly hydroxyl radicals, interfere with normal processing PDX-1 (pancreas duodenum homeobox-1) mRNA, a necessary transcription factor for insulin gene expression and glucose-induced insulin secretion besides being a critical regulator of cell survival. The generation of ROS and RNS will

ultimately activate stress-induced pathways - Nuclear factor-kB (NF-kB), c-Jun N-terminal kinase (JNK), stress kinases and hexosamines to induce apoptosis.

1.2. Lipotoxicity induced beta-cell apoptosis

In addition to hyperglycemia, dyslipidemia is a major feature of T2DM with abnormal lipid profiles[9]. Excessive nutrient intake without lack of physical activity leads to metabolic disorders like obesity, characterized by abnormal lipoprotein values, increased circulating free fatty acids (FFAs), leptin and cytokines, key risk factors for diabetes. Lipotoxicity refers to the damage caused by persistently high FFA levels. Persistently elevated FFAs potentiate glucose stimulated insulin secretion which is lipotoxic to beta-cells and contribute to progressive beta-cell failure in diabetic patients whereas increased insulin resistance is compensated by enhanced insulin secretion in non-diabetic subjects. Elevation of FFAs in healthy individuals has stimulatory effects on insulin secretion, but may contribute to progressive beta-cell failure in individuals with a genetic predisposition to diabetes. Increased concentration of saturated fatty acids is toxic to islet cells[10]. Palmitate is the most abundant saturated fatty acid in human plasma and its deleterious effects are mediated via ceramide-mitochondrial apoptotic pathways[11]. Very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) reduces insulin mRNA levels and beta-cell proliferation and were pro-apoptotic whereas high-density lipoprotein (HDL) protects beta-cells against these pro-apoptotic effects[12, 13]. Oxidized LDL reduces preproinsulin expression levels in isolated beta-cells. The protective effects of high-density lipoprotein were mediated by inhibition of caspase-3 cleavage and activation of Akt/protein kinase B, whereas pro-apoptotic lipoproteins seem to act via c-Jun N-terminal kinase[12, 14]. Hence the changes in lipoprotein profile in T2DM contribute to the pathogenesis and progression of beta-cell failure.

1.3. Glucolipotoxicity-Hyperglycemia induced lipotoxicity and beta-cell apoptosis

The deleterious effects of lipids occur on beta-cells only in the presence of high glucose concentrations; this is termed as glucolipotoxicity[15, 16]. The elevated fatty acids (FAs) do not undergo oxidation in mitochondria leading to the esterification of FAs and accumulation of long-chain acyl-CoA esters in the cytoplasm causing glucose induced lipotoxicity[17, 18]. Glucose concentration plays a crucial role in the effect of FAs. The long-chain fatty acyl-CoAs are accumulated in the cytosol of mitochondria due to the inhibition of carnitine-palmitoyl-transferase-1 and accumulation of cytosolic citrate the precursor of malonyl-CoA due to the presence of elevated glucose levels and FAs resulting from glucose as an oxidative fuel. When these long-chain fatty acyl-CoAs are prolonged for longer duration, beta-cell functions are affected but it was not clearly characterized that whether the long-chain acyl-CoAs accumulation directly affects the beta-cell function or it serves as a precursor for other molecules like diacylglycerols or phospholipids directly activating protein kinase C isoforms that synergizes with glucose to enhance insulin secretion. Elevated FAs should readily undergo oxidation in mitochondria in the presence of physiological glucose concentrations in order not to harm beta-cells. When both the FAs and glucose concentrations are elevated, FAs esterification occurs and the metabolites are accumulated that inhibits the glucose induced insulin secretion[19]. Hence glucotoxicity and lipotoxicity are interrelated and combined glucolipotoxicity also induces the apoptosis of beta-cells.

2. Mechanism of pancreatic beta- cell apoptosis

In T2DM, 25-50% of beta-cell death occurs by both intrinsic and extrinsic apoptotic pathways that differ by their involvement of Bcl-2 family proteins and in the identity of the caspases that initiates apoptosis.

2.1. Intrinsic apoptotic pathway or Bcl-2 regulated pathway

Hyperglycemia and hyperlipidemia affects the different stages in apoptotic signaling by increasing oxidative and nitrosative stress and induces intrinsic apoptotic pathway by activating the pro-apoptotic

Bcl-2 family proteins and caspase cascade leading to the release of cytochrome-c from mitochondria and activation of caspase-9. The balance between the pro-apoptotic and the anti-apoptotic members of the Bcl-2 family regulates this pathway. The Bcl-2 family consists of anti-apoptotic proteins (Bcl-2, Bcl-xL) having four Bcl-2 homology domains (BH1, BH2, BH3 and BH4). Pro-apoptotic multidomain proteins (Bax and Bak) having three homology domains. Pro-apoptotic BH-3 only proteins (Bid, Bad, Bim, Puma, Noxa, DP5) having only one BH3 homology domain. This pathway is regulated by the balance between the pro-apoptotic and the anti-apoptotic members of the Bcl-2. Pro-survival factors include Bcl-2, Bcl-xl, Bcl-w and Mcl-1. Cellular stress activates the pro-apoptotic Bcl-2 family members and down-regulates the pro-survival factors, allowing downstream translo-cation of Bax and Bak to the outer mitochondrial membrane resulting in formation of pores. This causes cytochrome-c release into the cytoplasm and activates caspase-9. This in turn activates downstream caspases-3, 6 and 7 eventually causing apoptosis. The two extrinsic and intrinsic pathways can cross-talk through caspase-8 dependent cleavage of Bid to its truncated form (t-Bid). t-Bid can inhibit pro-survival Bcl-2 proteins and activate Bax and Bak[20-22].

2.2. Extrinsic apoptotic pathway or death receptor pathway

Prolonged exposure of pancreatic beta-cells to hyperglycemia, increased FFAs and increased ROS generation triggers the production of inflammatory cytokines such as nuclear transcription factor kB (NF-KB), interleukin-1ß (IL-1ß), interferon-γ (IFN-γ), and TNF-α (tumor necrosis factor-α) which causes endoplasmic reticulum stress and the unfolded protein response activation in beta-cells leading to apoptosis of beta-cells[23, 24]. Persistent activation of NF-kB leads to the reduction of beta-cell protein expression including insulin, GLUT-2, PDX-1 and increased iNOS expression. NF-kB mediates through IL-1ß, the activation of iNOS in pancreatic beta-cells leading to apoptosis. IL-1ß, IFN-γ and TNF-α bind to their receptors on beta-cells and results in the activation of a variety of signal-transduction pathways to induce apoptosis. IL-1ß activates mitogen activated protein kinase (MAPK) and nuclear factor-kB (NF-kB) pathways leading to the activation of iNOS (inducible nitric oxide) and NO (nitric oxide) pathway. IFN-γ signals through Janus-kinase-signal transducer and activator of transcription mediated (JAK-STAT) signaling pathway which ultimately induces beta-cell death. FasL (fas ligand) belonging to the TNF super-family binds to the cell-surface death receptors such as Fas (CD-95) or tumor necrosis factor alpha receptor-1 (TNFαR1) cell surface death receptor in pancreatic beta-cells which results in the recruitment of FAS-associated death domain (FADD) or Tumor necrosis factor receptor type 1-associated death domain (TRADD), subsequent recruitment of caspase-8 and the downstream activation of effector caspases such as caspase-3, caspase-6, and caspase-7. This ultimately results in the activation of proteases, DNA fragmen-tation and cell death[25-27].

3. Genetic variants of apoptotic genes

Genetic variants in several apoptotic genes may contribute to the pathogenesis and severity of the disease. Single nucleotide gene polymorphisms in apoptotic genes like Fas, FasL, Akt and caspase 3, 7, 8 and 9 may contribute to the pathogenesis of the disease. The two promoter polymorphisms of Fas, Fas-670 G > A and FasL-844C > T are associated with beta-cell function and insulin resistance. Fas- 670GG and the FasL-844CC genotypes lead to higher promoter activities[27]. There are several polymorphisms reported in caspase 3, 7, 8 and 9 and several other genes that are to be studied to find out the role of these SNPs in the apoptotic machinery. Linked with the pharmacogenetics, individualized treatment can be tailored for type 2 diabetics for the better management of the disease.

4. Conclusion

Strategies targeting the conservation of beta-cell mass and function are necessary for the optimal treatment of diabetes. The pathogenesis of the disease is under the control of genetic predisposition and

environmental factors. The studies of genetic variants in apoptotic signaling machinery aid in managing the beta-cell conservation and further improve the targets for beta-cell survival. The pharmacogenetic studies relating to the susceptible apoptotic machinery targeting the beta-cell conservation help in aiding the personalized treatment for type 2 diabetics.

Conflicts of Interest: The Author declares that there is no conflict of interest

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