The role of p53 in pancreatic β-cell apoptosis

Clara Ortega Camarillo1, Luis Antonio Flores López2, Alejandro Ávalos Rodríguez3

1Unidad de Investigación Médica en Bioquímica, HE, Centro Médico Nacional Siglo XXI. IMSS. México, D.F. C.P: 06720. México

Correspondence: Clara Ortega Camarillo
E-mail: cocamarillo2014@gmail.com
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β-cells regulate glucose serum levels by means of production and excretion of insulin. Chronic hyperglycemia diminishes the pancreatic β-cell mass due to an increase in the rate of apoptosis. Although the precise mechanism of glucotoxicity on the β-cells is not fully known, several mechanisms have been proposed, the most outstanding being: the increase of Reactive Oxygen Species (ROS), the loss of the mitochondrial membrane potential, and the activation of the intrinsic route of the apoptosis due to p53. This paper will focus on the mobilization of p53 towards the mitochondrion and its phosphorylation, and the activation of the intrinsic route of the apoptosis by hyperglycemia. Since p53 has important functions over the regulation of the cellular cycle, proliferation, and apoptosis, it is subject to strict regulation. The elimination of p53 occurs in the proteasome, and depends on its ubiquitination by Mdm2 (murine double minute 2). Hyperglycemia affects the concentration, ubiquitination and phosphorylation of Mdm2, as well as the phosphorylation of p53, therefore also affecting its average life. Studies made by our group demonstrated that the increase of glucose promotes the interaction between p53 and Mdm2; however, the ubiquitination of p53 diminishes. Thus, it is likely that hyperglycemia interferes with the capacity of Mdm2 to ubiquitinate p53, and leads it to degradation, which allows for p53 to move towards the mitochondria, and for apoptosis activation. Knowing what mechanisms activate the death of the pancreatic β-cells, will allow proposing alternative treatment to prevent dysfunction and decreased of pancreatic β-cell.

Keywords: p53; apoptosis; β-cells; mitochondria; Mdm2; ROS

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homeostasis. Nevertheless, under metabolic stress, the demand for insulin increases, β-cell functions declines, glucose tolerance decreases, and diabetes is then established [1, 2].

The progressive loss of the β-cell functions is characterized by a loss in tolerance to glucose and sensitivity to insulin. It has been demonstrated that previous to clinical manifestations of type 2 diabetes, the β-cell mass diminishes up to 60%, due to increase in the apoptosis rate [3]. Hyperglycemia, which is characteristic of type 2 diabetes, triggers the β-cells apoptosis. There are several proposals regarding the mechanisms emphasizing the β-cells death such as: the increase of Reactive Oxygen Species (ROS), the loss of the mitochondrial membrane potential, the decrease of the interaction of glucokinase with the mitochondria, and the increase in the expression of the c-Myc transcription factor and the transactivator responding to Ca^{2+} (CREST).

The research herein is focused on the participation of p53 in the decrease of pancreatic β-cell mass under hyperglycemic conditions. It was observed that an increase in glucose induces DNA degradation into characteristic fragments of apoptosis, promotes the mobilization of p53 towards the mitochondria, augments the production of ROS, and modifies the mitochondrial membrane potential. While p53 is located in the mitochondria, it also phosphorylates in this organelle [4]. The suppression of p38 MAPK decreased the p53 phosphorylation and the apoptosis of RINm5F cells induced by hyperglycemia [5]. Extending this logic further, it was decided to look further into p53 regulation. Previous studies have demonstrated that the average life of p53 is regulated by ubiquitination, for which Mdm2 protein is responsible. Under the conditions presented here, the p53 ubiquitination decreased, which

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**Figure 1. Regulation of p53 due to high glucose in pancreatic β-cells.** The poly-ADP-ribosylation appears in very early stages of RINm5F cells culture with high glucose, which allows p53 to regulate the DNA damage repair, and the early apoptosis stages. p53 O-GlcNAc is increased in the cytosol, and stabilizes p53, enabling its migration to the mitochondria, where phosphorylation occurs via p38 MAPK. Phosphorylation of p53 probably promotes its interaction with anti-apoptotic proteins, and the release of pro-apoptotic elements such as cytochrome c. These events lead to the activation of caspases. High glucose conditions also inhibit phosphorylation, the activation of Mdm2, and the ubiquitination of p53, as well as its degradation by the proteasome.
probably suggests that the capacity of Mdm2 to lead the p53 degradation is altered by hyperglycemia [6].

**Apoptosis and oxidative stress**

Apoptosis is a mechanism of cellular clearance, which may be triggered by several factors [7]. Thus, hyperglycemia – which is characteristic of diabetes – increases the production of ROS and oxidative stress [8], which contributes to the activation of the mechanisms that emphasizes β-cell death, and the decrease of cell mass. The exposure of β-cells to high glucose concentrations during short periods of time, about 15 min, is characterized by the increase in the production of ROS and by oxidative stress. ROS also alters the mitochondrial membrane potential, and modifies its permeability, which enables cytochrome c and apoptosis-inducing factor release, which triggers the proteolytic cascade, and β-cell death.

Hyperglycemia increases flow of reducing equivalents in the mitochondrial electron transport chain and the production of ROS, mainly superoxide anion (O2•−), generating a state of oxidative stress, which may have several consequences [8]. The interaction of ROS with other molecules may cause an auto-catalytic chain reaction, which damages the cellular components and may cause the cell’s death. Therefore, it is highly important that cells have antioxidant systems that neutralize and/or inhibit ROS production. It is important to point out that pancreatic β-cells are highly sensitive to oxidative stress since the activity of catalase antioxidant enzymes and glutathione peroxidase is smaller in the pancreatic islets than in other organs such as the liver [9].

**Apoptosis in β-cells and p53**

The apoptotic β-cell death due to exposure to high glucose concentrations has been associated with p53 protein mobilization to the mitochondria [4]. Furthermore, the regeneration of the β-cell population and the rescue of the diabetic phenotype have been demonstrated in p53 knockout mice. This emphasizes the importance of this protein in the development of diabetes [10].

P53 protein is a transcription factor, which is responsible for monitoring the damages in the DNA and regulates cell viability. If the DNA repair mechanisms fail, then p53 induces cell death. The activation of p53 occurs as a response to several types of stress, which leads to stabilization and accumulation. It is known that p53 also participates in apoptosis induction acting directly upon the mitochondria, where it forms complexes with proteins Bel-2 and Bcl-XL through its DNA binding domain, and precedes the cytochrome c release and the activation of caspase 3 [11].

Apoptosis of β cells under high glucose was confirmed by the presence of cytochrome c in the cytosol fraction and the decrease in the Bcl-2/Bax ratio in the mitochondrial fraction. These events are associated with the mobilization of p53 to mitochondria, the increase of ROS, and the decrease of mitochondrial membrane potential. It was thus concluded that the glucose increase modifies the intracellular distribution of p53, and favors its mitochondrial location. In addition, it promotes the phosphorylation of p53, prevents its degradation, and increases its biological activity.

**Post-translational modifications of p53 by hyperglycemia**

The permanence and functions of p53 are controlled by different post-translational modifications, which include phosphorylation, poly-ADP-ribosylation, and N-acetylgucosaminylation among others, depending on the intracellular environment [12].

**Phosphorylation**

The phosphorylation has a decisive role upon p53 final functions. Hyperglycemia promotes p53 location and phosphorylation in Serine 392 (equivalent to Ser 389 in mice), in the mitochondria, which correlates with a Bel-2 decrease, and a Bax increase. The chemical suppression of p38 MAPK stopped p53 phosphorylation and shortened the pancreatic β-cells apoptosis by hyperglycemia, which suggests its participation in the reduction of pancreatic β-cell mass [5].

Besides, it knows that p38 MAPK plays an important role in the regulation of insulin secretion [13]. ERK 1/2 (extracellular signal-regulated kinase 1/2) and ATM (ataxia telangiectasia mutated kinases) are other kinases affected by glucose phosphorylation in Serine 392, and probably suppresses the rate of β-cells proliferation. ERK 1/2 phosphorylation has been associated with the activation of genes involved in proliferation, differentiation, cellular growth and apoptosis suppression [14]. ATM activation in cytosol and its participation in p53 Ser15 phosphorylation as a response to the damage in the DNA may be related to the recognition of p53 by Mdm2, its ubiquitination, and nuclear degradation. Ser15 phosphorylation is also necessary so that Tre18 then phosphorylates, and prevents the binding of p53 with Mdm2, and p53 degradation; this has been associated to apoptosis activation by increasing Bax expression [15].

It has been suggested that p53 phosphorylation in Serine 392 is necessary for the translational activation and the apoptotic functions of p53; nevertheless, it is likely that it has independent functions for binding p53 to DNA. Since p53 forms complexes with anti-apoptotic and pro-apoptotic proteins within the mitochondria, and triggers the process of
mitochondrial permeability [11], it is likely that its phosphorylation is a requirement that facilitates its interaction with these proteins, and induces the cell’s death. In spite of existing studies, the importance of phosphorylation in the NH-terminal end for the stability, and translational activation of p53 has proven elusive. This study presents evidence that hyperglycemia increases the percentage of time-dependent apoptosis without changes in p53 expression. In addition, it shows a correlation between the increase of apoptosis, and the location and phosphorylation of p53 within the mitochondria with an increase in glucose, and indicates the importance of p53 phosphorylation as one of the factors contributing to the elimination of pancreatic β-cells under hyperglycemic conditions via the mitochondria.

O-N-acetylglucosaminylation

The O-N-acetylglucosaminylation (O-GlcNAc) is another observed post-translational modification in p53. This modification depends on glucose availability, and constitutes a mechanism of cellular regulation according to the nutritional environment. It is regulated by two enzymes: the OGT (O-linked N-acetyl glucosamine transferase), which adds N-acetyl glucosamine residues to several proteins in the hydroxyl group of serine and threonine, and the O-GlcNAcase (N-acetylglucosaminidase), which eliminates the N-acetyl glucosamine residues from the proteins [18]. O-GlcNAc has been linked to type 2 diabetes since the expression of the RNAm of OGT in the pancreas and the brain is increased [17, 18].

In a high glucose environment, it has been observed the p53 O-N-acetylglucosaminylation is related to its stability and stops its degradation. O-GlcNAc does not interfere with the phosphorylation, but it stimulates p53 apoptotic function. In the studies presented here, it was observed that O-GlcNAc precedes the apoptosis, and increases as the apoptosis signals show up under hyperglycemic conditions. In addition, the protein p53 O-GlcNAc is mobilized to the mitochondria, where it can contribute to the release of pro-apoptotic factors [19]. O-GlcNAc of p53 in Ser149 increases its stability upon interfering with the Thr155 phosphorylation, which is translated into a greater stability for p53. Thus, we suggest that O-GlcNAc stabilizes p53, and may create a signal for its mobilization to the mitochondria.

Poly ADP-ribosylation

Poly (ADP-ribose) polymerase (PARP) is another protein which activates in the presence of damages in the DNA. PARP catalyzes the transference of ADP-ribose units from NAD⁺, an essential co-factor in ATP synthesis, and the balance of the redox potential upon the carboxylic glutamic acid, and aspartic acid residues of several nuclear proteins. Poly ADP ribosylation is important for the repair and replication of the DNA, translation, inflammatory response, and cellular death caused mainly by genotoxic agents, infection and stress [20, 21, 22].

PARP fragmentation matches with the induction of apoptosis in β-cells by hyperglycemia. These results agree with previous reports where it was proven that hyperglycemia induces β-cell apoptosis, and pancreatic cellular lines such as RINm5F. The results showed that the PARP enzyme, which is responsible for adding polymers of ADP-ribose to p53, is active in stages prior to the apoptosis onset in RINm5F cells, and as the treatment time goes by, the PARP activity decreases since it fragments. PARP break by caspase 3 is a marker of caspase-dependent apoptosis. The poly-ADP-ribosylation of p53 is an early response to high glucose conditions that probably contributes to the stability of the protein and to its mobilization to the mitochondria [19]. The fragmentation and inactivation of PARP matched with: the increase of Bax in the mitochondria, release of cytochrome c, and increase in RINm5F cell apoptosis due to high glucose.

Regulation of p53 by Mdm2

The cellular survival depends to a great extent on the balance between the synthesis and degradation of the p53 protein. The expression and activation of Mdm2 (murine double minute 2) is one of the mechanisms of p53 regulation. Mdm2 is an E3 ubiquitin ligase, which links to p53 and transfers ubiquitin residues to it, allowing its recognition by the proteasomes for its degradation. Depending on the amount of ubiquitin added to p53, this protein will be degraded or exported to the cytosol. P53 mono and/or polyubiquitylation is determined by Mdm2 concentration and activation [23].

As previously mentioned, the interaction between p53 and Mdm2 depends on the intracellular environment, which affects the post-translational modifications presented by these proteins. As for Mdm2, the ubiquitylation, sumoylation, and phosphorylation interrupt the formation of the p53-Mdm2 dimer, and stabilize the p53 levels. It has been demonstrated that Mdm2 Ser395 phosphorylation by activation of ATM decreases Mdm2 capacity to direct p53 degradation.

It was previously reported that Mdm2 phosphorylation by Akt participated in p53 regulation, since it reduced transactivation and increased p53 ubiquitination [24, 25]. Akt is a critical regulator of proliferation and cellular survival. Mdm2 phosphorylation in Ser166 and Ser186 by Akt increases its E3 ligase activity; it can protect the cells against the apoptosis induced by p53 under hyperglycemic conditions. It is important to mention that other kinases such...
as ERK 1/2 can phosphorylate Mdm2 into Ser166. Nevertheless, under hyperglycemic conditions, this kinase is decreased, which correlates with an increase in apoptosis rate [5].

The increase of glucose decreases the expression of Mdm2 RNAm, and the concentration of the protein in the nucleus and cytosol. The expression of Mdm2 is regulated by p53. Though we previously demonstrated the permanence of p53 in the presence of high glucose concentrations, this protein does not go to the nucleus, but it is mobilized to other organelles, such as the mitochondria, and thus it cannot stimulate the expression of Mdm2. Additionally, the DNA fragmentation due to hyperglycemia may also affect Mdm2 RNAm expression [5].

The formation of the complex p53-Mdm2 in the cytosol increased with high glucose, but p53 ubiquitination was not observed. This demonstrates that glucose increase induces Mdm2 activation in the cytosol, and promotes its interaction with p53, but inhibits p53 ubiquitination [6]. It is known that the activity of Mdm2 E3 ubiquitin ligase is located within the RING-finger domain, in the carboxyl-terminus region, which in addition contains the lysines acceptor of the substrate, whose main function is to label p53 for its degradation. Mdm2 central acid domain binds to RING-finger domain, and stimulates its catalytic activation, which promotes E2 enzyme release of ubiquitin. The interaction between the acid domain and the RING-finger domain is dependent on phosphorylation by ATM [6]. An increase in ATM phosphorylation by hyperglycemia was also observed in the model herein, so that it is not ruled out that the stress and the cascade of phosphorylations induced by high glucose could phosphorylate some present or near residues to the RING-finger domain, and Mdm2 acid central domain, and suppress p53 polyubiquitination and degradation.

On the other hand, p53 ubiquitylation is also dependent on ATP concentration. Under hyperglycemic conditions, the ATP concentration is reduced due to the increase of ROS and mitochondrial undocking. Therefore, if there is an ATP decrease, the ubiquitins are unable to condense the glycine residues of their carboxyl-terminus region with the p53 lysine residues, and p53 degradation is inhibited.

Conclusions

The mechanisms triggering pancreatic β-cells apoptosis by hyperglycemia are not fully known yet. In this review, we suggest that an ROS increase promotes the activation of phosphorylation cascades, which may interfere with the interaction between p53 and Mdm2, and suppress Mdm2 E3 ubiquitin ligase activity. Thus, it avoids p53 degradation, and promotes its recruitment to the mitochondria, apoptotic mechanisms activation, and β-cells dysfunction. Other alterations caused by hyperglycemia such as Poly (ADP-ribosylation), and O-GlcNAc also contribute to p53 stabilization and activation (Fig. 1). These studies aim to shed light on the understanding of biochemical events causing β-cell death in type 2 diabetes. They provide a reference point for future studies concerning possible therapeutic targets on post-translational modifications of p53.

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Conflict of Interest

The authors declare herein that there is no conflict of interests whatsoever.

Authors' contributions

LAFL: participated in drafting the manuscript and designed the figure. AAR: actively participated in drafting the manuscript. COC: drafted the manuscript and participated in the design of the figure. All authors read and approved the final manuscript.

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