Chapter 10

The Role of Human IAPP in Stress and Inflammatory Processes in Type 2 Diabetes

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Additional information is available at the end of the chapter

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Abstract

Understanding the mechanisms regulating whole-body glucose homeostasis is important in order to understand what happens in a disease such as type 2 diabetes (T2D). Insulin resistance, inflammation, dysfunction of islet β-cells, and the presence of amyloid deposits in the pancreas are some of the major causes involved in the process of β-cell deterioration. The unique peptide constituent of amyloid deposits, human islet amyloid polypeptide (hIAPP), is capable of inducing endoplasmic reticulum (ER) stress and the resulting unfolded-protein response activation. Additionally, hIAPP has been shown to induce interleukin-1β expression, the main cytokine involved in inflammation and T2D causing inflammation and eventually, inducing apoptosis. Nevertheless, the mechanisms behind the process of hIAPP aggregation and amyloid formation are still unknown. In this chapter, we describe the different mechanisms by which hIAPP induces ER stress and inflammation. This should open the door for designing therapeutic interventions aimed at modulating the immune system and the ER stress response.

Keywords: Diabetes, pancreatic amyloid, inflammation, ER stress, IAPP, chaperones, diabetes

1. Introduction

Type 2 diabetes (T2D) is a chronic metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia is associated with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels. The genetic and molecular basis of the pathogenesis of T2D is not completely elucidated; however, a growing body of evidence has reported

that the progression from normal to impaired blood glucose level regulation in individuals with T2D is mostly influenced by insulin resistance and β-cell dysfunction. During the last decade, the reduction in β-cell function has been attributed mostly to a decrease in β-cell mass; however, its precise role for the etiology of T2D remains controversial due to the lack of longitudinal studies. In spite of this, several studies in patients with T2D have shown a significant β-cell mass reduction ranging from 20 to 65% [1, 2]. Although, the reasons underlying this deficit are not clearly understood, some factors responsible for this decline have been suggested, for example, metabolic abnormalities (gluco- and lipotoxicity), hormonal changes (inadequate incretin secretion and action), aging, genetic abnormalities, etc. [3]. Additionally, a growing body of evidence has demonstrated the contribution of the hypersecretion of islet amyloid polypeptide (IAPP), together with amyloid deposition for the establishment of T2D.

The process of amyloid deposition is a remarkable physiopathological finding in individuals with T2D (**Figure 1**). The term amyloid emerged from the Latin word *amylum*, which means starch. For a long time, it was thought that these deposits were starch-like, but later it was discovered that they were actually a mass of proteins with a particular β-sheet structure. From that, pathologies with conformational changes in normally soluble proteins or peptides that result in the formation of intermolecular hydrogen bonds, β-sheet conformation, and fibril formation are namely conformational diseases [4]. Besides T2D, these conditions have been also implicated in different human disorders, including Alzheimer's disease (AD), Parkinson's disease, and Huntington's disease.

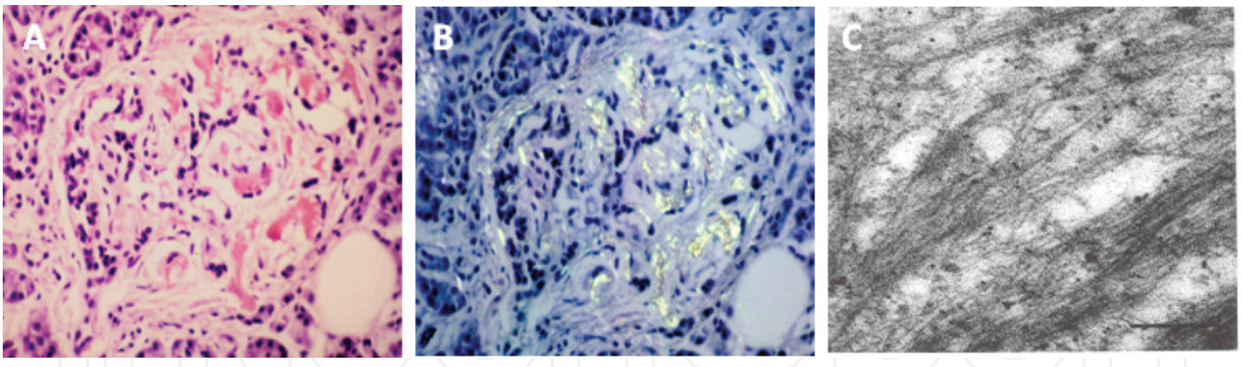

Figure 1. Amyloid deposits from cadaveric human pancreata. Amyloid deposits are observed in human pancreas by (A) hematoxylin/eosin staining, (B) Congo Red staining, or (C) electron microscopy, kindly provided by Anne Clark (unpublished data).

The contribution of IAPP in T2D is still controversial; several studies question whether amyloid deposition is a cause or a consequence of islet decline and whether it occurs intra- or extracellularly [5, 6]. However, numerous evidences correlate the role of IAPP with the severity of the disease. The facts indicate that amyloid deposits are seen in 90% of patients with T2D at autopsy and can possibly correspond to stages of the pathology [7, 8].

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While IAPP and amyloid formation seem to have a substantial relation in T2D, it is unknown whether aggregation of IAPP plays any role in the development of type 1 diabetes (T1D). One can assume that during β-cell destruction, as in T2D, cells are exposed to high levels of IAPP. Indeed, children with new onset of T1D presented with high levels of IAPP [9]. Nevertheless, further studies need to be done in order to determine the importance of IAPP in the development of T1D.

2. IAPP and T2D

2.1. Islet amyloid polypeptide

Islet amyloid polypeptide or IAPP, also known as amylin, is a normal product of pancreatic β-cells. It is stored along with insulin in secretory granules and co-secreted in response to nutrient stimuli. This 37-amino acid peptide was identified in 1987, although the gene was isolated and characterized in 1989 [7]. Nishi et al. located it in chromosome 12, containing three exons and two introns that codified for an 89-amino acid precursor termed preproIAPP with an amino-terminal signal sequence. This signal peptide is then cleaved from the precursor to generate a 67-amino acid propeptide termed proIAPP. This peptide undergoes further translational modifications by the prohormone convertases, which include the formation of disulfide bridges between cysteine residues and an amidation of a C-terminal tyrosine. These prohormone convertases are as well responsible for proteolytic conversion of proinsulin to insulin, supporting the idea that the processing of proIAPP might also be impaired in T2D [10].

The function of IAPP has been suggested to be involved with glucose homeostasis. In general, this hormone inhibits gastric emptying and is important in controlling and delaying the rate of meal-derived glucose. It has also been shown to inhibit secretion of other pancreatic hormones, such as glucagon and somatostatin. Indeed, physiological concentrations of IAPP are responsible for the regulation of food intake and body weight. Several other effects have been described, including the regulation of renal filtration [11], calcium homeostasis [12], and vasodilatation [13]. Nevertheless, a critical role positions IAPP as the main responsible for the pathogenesis of T2D through formation of amyloid deposits and destruction of pancreatic β-cells.

Non-toxic bioactive variants of IAPP have been shown to be clinically important for the treatment of T1D, T2D, and obesity. For example, co-administration of modified non-toxic variants of IAPP and insulin helped normalization of oscillating glucose levels to a greater extent than insulin alone [14, 15]. Furthermore, combinations of IAPP and leptin have also been used for the treatment of obesity [16]. Nevertheless, aggregation, engineering, and solubility problems at physiological pHs have been affecting the different approaches.

2.2. Molecular mechanisms of IAPP aggregation and amyloid formation

Although there has been considerable progress, the exact mechanism of abnormal aggregation of IAPP is still largely unknown; however, several studies pointed the overproduction or

mutations in IAPP as the main causes of amyloid formation. The role of overproduction of IAPP is associated with the increased secretory demand for insulin due to insulin resistance and increasing hyperglycemia [17]. Because IAPP and insulin are co-secreted in β-secretory granules, this increased production and secretion could result in accumulation and aggregation of IAPP. Many studies have reported that transgenic mice overexpressing hIAPP develop islet amyloid deposits [18, 19]. However, other studies contradicted this hypothesis, claiming that IAPP levels are several times higher than normally. To confirm these findings, Kahn et al. demonstrated that non-diabetic obese and/or insulin-resistant individuals with elevated IAPP levels do not develop amyloid deposits per se. However, in both cases, the presence of some factors, such as genetic predisposition, high fat diet, and obesity, might be critical for the development of extensive islet amyloid [18, 19].

Another possible mechanism for amyloid formation concerns the mutations in the IAPP gene or promoter regions, by producing more fibrillogenic forms [20]. For example, based on several studies, the S20G mutation in the IAPP gene appears to be associated with an early onset and more severe form of T2D [21].

In addition, the existence of hydrophobic amino acids in the mid-portion of IAPP could also be responsible for its propensity to aggregate into β-pleated sheets. Several algorithms, such as discrete molecular dynamics simulations [22] or specifically designed software such as TANGO [23–25], have been developed to identify the amyloidogenic regions of hIAPP. In this line, the residues at positions 20–29 of the polypeptide chain have been determined to be the amyloidogenic region of the peptide. Accordingly, the proline substitutions in the 24–29 regions of rodent IAPP are thought to prevent amyloid fibril formation completely [26]. Proline is known to be a β-sheet breaker, and a total inhibition of amyloid formation was seen when substitutions in the 20–29 wild-type area were performed [27]. Proteoglycans, the major components of the extracellular matrix, have been implicated with several pathologies, including AD and T2D, and have been associated with amyloid deposits in the human body [28]. In particular, together with IAPP, heparan sulfate proteoglycan perlecan is the main component of amyloid deposits in pancreatic islets. Several hypotheses indicate that the impaired processing of proIAPP may result in an elevated secretion of the IAPP precursor with a strong affinity for heparan sulfate proteoglycans, which could eventually result in a generation of a nucleus from amyloid formation [17, 18].

2.3. IAPP toxicity

Aggregated hIAPP has cytotoxic properties and is believed to be of critical importance for the progression in patients with T2D. Early studies have shown that the formation of islet amyloid is strongly associated with reduction of insulin secretion and with loss of approximately 50% of the β-cell mass [29, 30]. Human IAPP aggregation has been suggested to occur in a stepwise manner, with soluble monomeric hIAPP forming oligomeric structures, protofibrils, and eventually amyloid fibrils (**Figure 2**).

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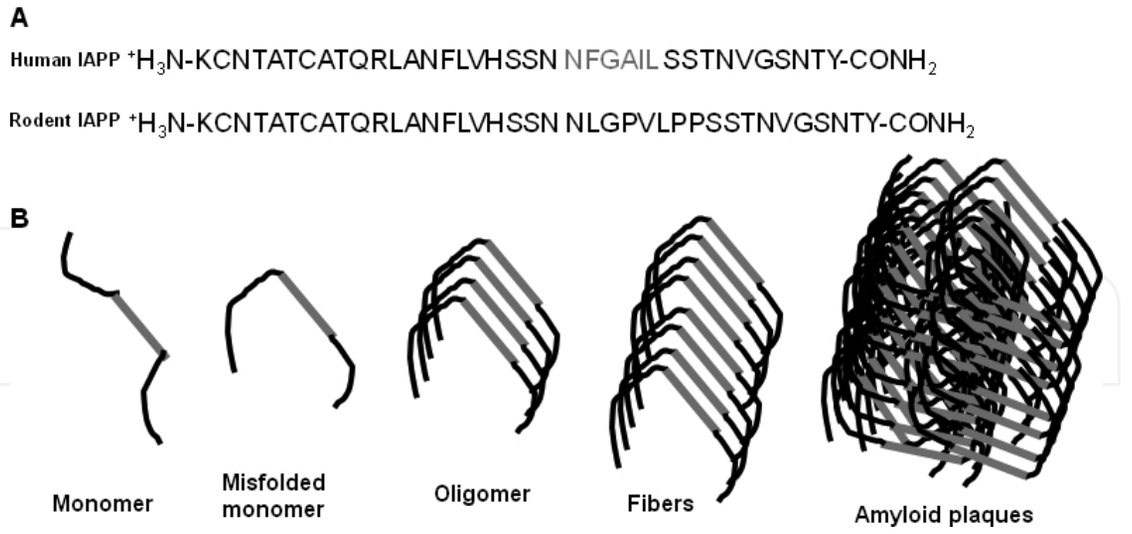

Figure 2. Proposed model of amyloid formation. (A) Primary sequences of human and murine islet amyloid polypeptide. Amyloidogenic region predicted by intrinsic propensities for aggregation is indicated in gray. Note the presence of proline residues in rodent IAPP sequence. (B) Folding or trafficking alterations can induce hIAPP misfolding leading to aggregation. Misfolded monomers aggregate to oligomers that eventually form the characteristic amyloid plaques from T2D.

Initially, there was general acceptance about the concept that the fibrillar forms of hIAPP are the toxic species [31]. Moreover, amyloid fibrils are less toxic than small oligomers formed by aggregates of IAPP. Studies have shown a strong correlation between islet amyloidosis and hIAPP cytotoxicity and eventually β-cell death. Yanker et al. have demonstrated that toxicity is mediated by IAPP fibrils by direct contact of fibrils with the cell surface causing DNA fragmentation, chromatin condensation, and protuberances in the plasma membrane leading to islet cell apoptosis [32]. The common feature for hIAPP fibrils lies on the classical cross β-structure, polymorphic, and typically unbranched [33]. Additionally, in vitro studies have provided evidences that synthetic hIAPP readily forms fibrils and amyloid deposits, which allowed studying the overall morphology and formation process [33, 34].

According to the structural information available for hIAPP fibrils and oligomers, it is clear that hIAPP as an amyloid protein has shown to be toxic through similar mechanisms as other amyloid proteins. One of the most widely accepted mechanisms refers to membrane interaction which leads to cell membrane permeabilization or disruptions [35]. Concerning hIAPP oligomers, the membrane leakage occurs via direct interaction and/or formation of ionic pores and depends on the lipid composition, peptide ratio, pH, and ionic strength. In the case of fibril formation, the damage in the membrane may happen through interaction of fibrils with specific channels located on the cell surface, such as potassium channels [36]. Moreover, oligomers of hIAPP have been shown to increase inflammation in β-cells via the inflammasome, a large group of intracellular multiprotein complexes that induce inflammation and play a central role in immunity [37].

Comparably, hIAPP can form oligomers and fibrils that contribute to islet inflammation. In this line, hIAPP oligomers and fibrils (but not rodent IAPP) have been shown to induce synthesis of interleukins and other inflammatory mediators by pancreatic islets that recruit

and activate macrophages in vivo [38]. Further, endoplasmic reticulum (ER) stress has been proposed to be an important contributor to hIAPP-induced-β-cell death since exogenously added hIAPP has been confirmed to induce ER stress. Some reports showed that ER stress-mediated apoptosis is exacerbated in rodent β-cells expressing amyloidogenic isoforms of hIAPP, leading to a reduction of β-cell mass in hIAPP transgenic mice and rats [6, 39]. In addition, Casas et al. [40] demonstrated that extracellular hIAPP aggregation is associated with ER stress responses in mouse β-cells. Although the mechanism responsible for β-cell cytotoxicity during the process of hIAPP aggregation is still not well defined, a growing body of evidence firmly indicates that IAPP fibrils or oligomers have a crucial role in the progressive β-cell dysfunction in T2D.

Prevention of IAPP amyloid formation may represent a potential treatment for T2D. Thus, the use of several small-molecule inhibitors is being exploited. Several small inhibitory peptides [26, 41], natural polyphenols (such as resveratrol and epigallocatechin gallate; EGCG) [42, 43] or specific antibodies [44, 45] have been successfully used to validate the application of anti-amyloid compounds. For example, the peptide D-ANFLVH inhibited the formation of islet amyloid deposits and contributed to the preservation of β-cell area and improved glucose tolerance in mice [46]. These results validate the application of anti-amyloid compounds as therapeutic strategies to maintain β-cell function in patients with T2D.

3. The endoplasmic reticulum

3.1. Physiological role

Protein folding begins as the nascent polypeptide chain is co-translationally translocated through the ER membrane into the ER lumen. The unique environment of the ER lumen allows for both oxidative protein folding and post-translational modification such as glycosylation and disulfide bond formation, and accounts for approximately one-third of all proteins in eukaryotic cells. Since ER is mainly associated with protein synthesis, if the protein is not properly folded/maturated, it will remain in the ER and will eventually be degraded without reaching its normal cellular site of action [47]. This sophisticated supervision carried by the ER is regulated by sensitive quality control systems that can discriminate between the proper folded proteins from the misfolded ones. For example, folding chaperones consist of a considerable number of proteins that have the capacity to recognize properties common to non-native proteins, such as exposed hydrophobic areas and in most cases through the expenditure of ATP [48]. Thus, chaperones have the role of correctly folding and assembling secreted proteins, aiding oligomerization, and performing post-translational modifications [49]. The other quality control system is the ubiquitin-proteasome system (UPS) in which irreparably damaged proteins are identified and sorted for degradation. This system is responsible for the clearance of intracellular misfolded and aggregated proteins [50]. Many stimuli can disrupt this process, and its failure can give rise to the malfunctioning of living systems leading to the development of an increasing number of disorders, including Parkinson, AD, Huntington, and T2D [51].

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3.2. ER stress and the unfolded protein response

Some situations (oxidative stress, energy deprivation, metabolic challenge, or inflammatory stimuli) can represent a major problem for the cell due to a probable production of unfolded or misfolded proteins. These physiological and pathological conditions may interfere with protein maturation and trafficking processes, leading to the accumulation of unfolded and/or misfolded proteins.

A complex homeostatic mechanism known as the unfolded protein response (UPR) has evolved linking the load of newly synthesized proteins with the capacity of the ER to mature them. The UPR works as a multifaceted strategy to protect the integrity of the ER and the associated functionality of the secretory pathway. In mammalians, the first response consists in attenuating the translation of most peptides, followed by an induction of ER chaperone translation that promotes the correct folding [52] and finally, the activation of the ER-associated degradation (ERAD), in which misfolded proteins are retrotranslocated from the ER lumen to the cytosol and degraded by the ubiquitin-proteasome [52, 53]. Conversely, if this mechanism of adaptation and survival fails to relieve ER stress, a continued accumulation of misfolded proteins takes place within the ER, and consequently UPR will generate pro-apoptotic signals to eliminate the diseased cell [52].

In mammals, the UPR consists of three main classes of proteins that act as sensors of ER stress: inositol-requiring enzyme 1 (IRE1), double-stranded RNA-activated 9 protein kinase RNA (PKR)-like ER kinase (PERK), and activating transcription factor 6 (ATF6) (**Figure 3**). Despite the difference, each of these proteins activates different signaling pathways and activates transcription factors that mediate the induction of several ER stress genes. IRE1 is considered a central regulator of the ER stress signaling and plays an important role in protein biosynthesis. Under non-stressed conditions, IRE1 remains in an inactive monomeric form. Upon accumulation of unfolded proteins, IRE1 is activated and released by the binding immunoglobulin protein or BiP, an endogenous chaperone located in the lumen of the ER that binds newly synthesized proteins and helps them through the process of folding and maturation. Subsequently, IRE1 activates a transcription factor named X-box-binding protein (XPB1), which once translocated into the nucleus initiates several transcriptional programs that upregulate UPR-associated genes. In a similar way, once chaperone BiP releases from its interaction with PERK, PERK is able to dimerize, promoting autophosphorylation and activation [54]. Once activated, PERK phosphorylates eukaryotic initiation factor 2a (elF2α), its only recognized target. Once activated, elF2α leads to a rapid reduction in the number of proteins entering the already overwhelmed ER [55]. However, in some circumstances, other transcription factors such as activating transcription factor 4 (ATF4) are translated and modulate the expression of C/EBP homologous protein (CHOP). CHOP acts by inducing apoptosis [56]. A major mediator of transcriptional induction by ER stress is the basic leucine zipper domain transcription factor ATF6. This protein is also regulated not only by BiP but also by intra- and inter-molecular disulfide bridges which are thought to keep ATF6 inactive [57, 58]. In response to ER stress, BiP is released from ATF6 and disulfide bonds are reduced, which eventually target the expression of transcription chaperones, upregulation of XBP1, and transcription of elements of the ERAD (**Figure 3**).

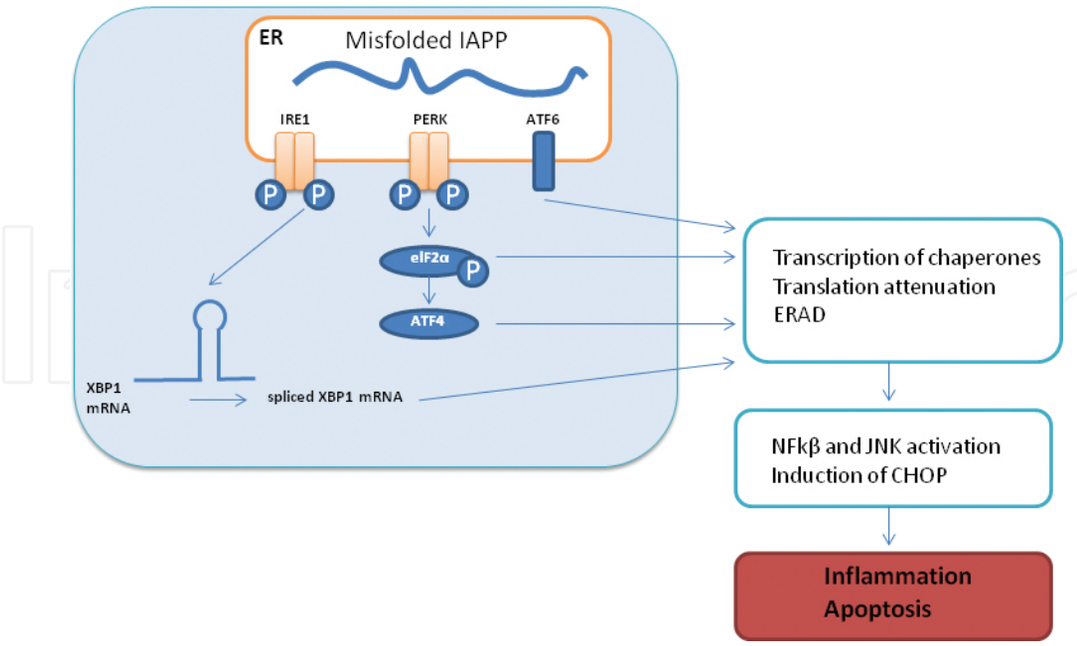

Figure 3. Branches of the UPR and inflammation signaling pathways. The three sensor-transducers of the UPR are inositol-requiring protein-1 (IRE-1), PERK, and ATF6. These three sensor-transducers determine the state of unfolded proteins in the ER lumen. If persistent, NF-κβ and JNK become activated, leading to inflammation and apoptosis.

3.3. Link between ER stress and apoptosis

A growing number of studies implicate ER stress with β-cell death during the evolution of T2D [59, 60]. Several physiological, environmental, and genetic factors can provoke alterations in ER homeostasis leading to a state of stress. Evidences suggest that a continuous increase in insulin biosynthesis might overwhelm the folding capacity of the ER, leading to a chronic state. As a consequence, the UPR signaling pathways are triggered with the objective of maintaining β-cell function and promoting β-cell survival. In general, cells have the capacity to adapt to substantial ER stress, but if the ER stress is too severe and long-standing, the UPR-mediated efforts ultimately fail and the apoptotic pathway is activated in order to protect the organism by eliminating damaged cells (**Figure 3**). At least, three parallel pathways are involved in the stress-mediated apoptosis: activation of CHOP (recognized as a key mediator of apoptosis in ER stress), activation of IRE1–JNK pathway, and activation of caspase 12 [61, 62]. β-Cell apoptosis is also observed in human pancreatic sections and postmortem islet grafts in correlation with amyloid deposition levels [6, 63, 64].

As previously discussed, ER stress-mediated apoptosis is exacerbated in rodent β-cells expressing hIAPP in β-cells, leading to a reduction of β-cell mass [39]. In addition, extracellular hIAPP aggregation is associated with ER stress, contributing to β-cell apoptosis [40, 65]. Nevertheless, in a rat pancreatic β-cell line overexpressing hIAPP, the detection of toxic intracellular oligomers, which lead to defective insulin and IAPP secretion levels in response to glucose, did not change the expression of genes involved in ER stress and apoptosis was not induced [36]. These results agree with other findings with hIAPP transgenic mice, in which

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the authors demonstrated that amyloid formation was not associated with significant increases in the expression of ER stress markers [66]. As discussed elsewhere, the discrepancy in these results may be explained by differences in the ratio of IAPP and insulin produced by the different models used in vitro and in vivo, ranging from low to significantly high levels of hIAPP [67].

4. ER stress and inflammation

The three branches of UPR response can trigger inflammatory signals through different branches that converge in signaling pathways involving c-Jun N-terminal kinases (JNKs) and the nuclear factor κ-light-chain-enhancer of activated B cells (NF-κβ). JNK is considered to play an important role in ER stress in mouse models of diabetes. For instance, an increase in JNK activity promotes insulin resistance in peripheral tissues and in pancreatic β-cells without affecting cell viability [68]. The importance of the JNK pathway in stress has also been observed in knock-out (KO) mice where suppression of the JNK pathway protects β-cells against oxidative stress induction [69].

The pathway of the UPR involving IRE1 can, by different mechanisms, trigger an inflammatory signaling pathway through the activation of JNK (**Figure 3**) [70]. In addition, through multiple mechanisms, both the IRE1 and PERK pathways can also lead to the activation of the NF-κβ pathway, which also plays a critical role in the induction of multiple inflammatory mediators and has been implicated in insulin resistance [60, 71]. ATF6 has also been linked to inflammatory signaling. Genetic and pharmacological inhibition of ATF6 significantly suppresses NF-κβ activation, which can transcriptionally regulate many other inflammatory genes [72]. Activation of either JNK or NF-κB pathways in pancreatic β-cells has been reported to cause increased expression of proinflammatory molecules that can act as mediators, such as inflammatory interleukins 8 (IL8) and 6 (IL6), monocyte chemotactic protein-1 (MCP-1 or CCL2), and the cytotoxin tumor necrosis factor-α (TNF-α), which may have a detrimental effect on cell survival and function [73, 74]. Local chemokine and cytokine release can also contribute to the inflammatory milieu, attracting host macrophages to the pancreatic β-cells, which further propagate local inflammation [38, 75]. In addition, the NF-κβ pathway has been shown to activate the NLRP3 inflammasome, a multiprotein, cytosolic molecular platform that controls the activation of caspase 1, and the secretion of other proinflammatory cytokines such as interleukin-1β (IL-1β) and interleukin 18 (IL-18) in metabolic stress [76]. Inflammation induced by inflammasome-dependent proinflammatory cytokines may produce insulin resistance or cause the death of pancreatic β-cells, leading to the development of diabetes [37, 77].

NLRP3 inflammasome formation is reported to be induced by a variety of compounds, including hIAPP [37]. Oligomeric hIAPP has been shown to induce inflammasome activation and subsequent production of IL-1β. This is supported by observations with a transplantation model, in which hIAPP-expressing islets were transplanted into immunodeficient NOD-SCID mice treated with and without the IL-1β receptor agonist (IL1Ra) [38]. In this study,

IL-1Ra was able to protect transplanted hIAPP-expressing islets from impaired glucose tolerance. Islet grafts expressing hIAPP contained amyloid deposits in close association with macrophages. Moreover, early aggregates of hIAPP induced production of inflammatory cytokines and chemokines, such as CCL2, TNF-α, IL-1α, IL-1β, chemokine (C–C motif) ligand 3 (CCL3), and the chemokine (C–X–C motif) ligand 1, 2, and 10 (CXCL1, CXCL2, and CXCL10, respectively) [38].

5. The role of hIAPP in ER stress, inflammation, and apoptosis

Multiple physiological and pathological conditions, including the accumulation of misfolded proteins, such as insulin or hIAPP, are responsible for the loss of ER homeostasis in β-cells [6, 60].

5.1. Exogenous hIAPP induces ER stress and apoptosis in vitro

Several approaches have been applied to study amyloid toxicity in vitro; synthetic peptides, corresponding to either fragments or the whole protein, have been useful attempt in defining the amyloidogenic pathology. Several studies have reported that amyloid peptide is proficient to induce cytotoxic cell death by external addition of synthetic hIAPP [32, 40, 78–80]. Although the precise mechanism by which IAPP aggregates lead to β-cell death is still unknown, it has been recognized that this aggregation is a concentration dependent on synthetic hIAPP in vitro. Bailey et al. suggested a progressive increase in cell toxicity according to the initial peptide concentration, as well as the time exposed for the process of IAPP fibrillation [34]. In addition, Casas et al. demonstrated that extracellular hIAPP aggregation is associated with ER stress responses in mouse β-cells, by an intracellular signaling that involves downstream inhibition of the ubiquitin-proteasome pathway, contributing to β-cell apoptosis [40]. In line with these studies, evidences have demonstrated that in aqueous solution, synthetic hIAPP spontaneously forms β-sheets and aggregates, whereas synthetic rat IAPP does not [81, 82], and the aggregation process seems to be extremely sensitive to amyloid concentrations [83].

5.2. Intracellular hIAPP

The mechanism by which amyloid oligomers and/or fibrils are formed within the β-cell is not completely understood. For that reason, many attempts to express this protein in various vectors and hosts have been designed. O'Brien et al. [84] were able to transfect fibroblast-like cell line (COS-1) cells with vectors expressing amyloidogenic IAPP; however, those cells containing amyloid fibrils were degenerated or dead when compared to rat IAPP overexpression. Years later, the same group, with the effort to understand the mechanism by which intracellular hIAPP causes cell death, demonstrated that in transfected COS-1 cells, the accumulation of hIAPP initiates a cascade of intracellular signaling events that trigger the apoptotic pathway [85]. However, this cell line was not expected to prevent such event due to

the lack of the cellular machinery needed for the processing and trafficking of immature IAPP, such as secretory granules or the presence of prohormone convertases.

Recent studies have also reported successful cloning and expression of recombinant hIAPP in cultured mammalian cells. Several in vitro approaches allowed the successful expression, purification, and characterization of the amyloidogenicity and cytotoxicity of the human mature IAPP in sufficient amounts using, for example, the Lacl-T7 RNA polymerase-based heterologous expression system for *Escherichia coli*. This *E. coli* expression system has been shown to remove potential toxic proteins and, at the same time, generate high levels of recombinant proteins [86]. Likewise, other studies were capable to clone the hIAPP full-length peptide into not only COS-1 cells but also in rat insulinoma (RIN) and Chinese hamster ovary (CHO) cells [87]. Nevertheless, when studies were performed in INS1E cells, a β-cell line with all the equipment for the processing and regulation of IAPP, the expression of hIAPP by adenovirus has not resulted in cell death unless hIAPP was high enough to cause impaired proIAPP processing [88].

Furthermore, Soty et al. established an in vitro model in which INS1E cells were stably transfected with hIAPP cDNA under the cytomegalovirus promoter (CMV). Under hIAPP overexpression, these cells showed intracellular oligomers and a strong alteration of glucose-stimulated insulin and IAPP secretion. Moreover, inhibition of insulin and secretion of IAPP affected the activity of K_{ATP} channels, leading to an increased mitochondrial metabolism in order to counteract the secretory defects of the β-cells [36]. Nevertheless, hIAPP-expressing INS1E cells were able to completely restore insulin secretion and prevent ER stress upon treatment with molecular (BiP and protein disulfide isomerase; PDI) and chemical (tauroursodeoxycholic acid; TUDCA and 4-phenylbutyrate; PBA) chaperones [89]. Amelioration of insulin secretion upon high glucose stimulation and prevention of β-cell death was further confirmed by the same group using hIAPP transgenic mouse islets and molecular chaperone PDI [90].

5.3. In vivo models of hIAPP overexpression

One of the most active research areas that have contributed substantially to our current understanding of the molecular basis in a multifactorial disease such as T2D is the creation and development of diverse animal models. Nowadays, in vivo studies of human pancreas morphology are not possible by obvious ethical considerations, and the collective human material comes from either autopsy or surgical resection from pancreatic cancer. It is interesting that apart from humans, the only species capable of spontaneously developing T2D are non-human primates and cats; nevertheless, besides the cost of working with such big species, these models not always progress toward T2D, making the use of these models not optimal for research [91]. Studies performed with rodent models of diabetes are then greatly useful and advantageous, especially regarding islet amyloidosis studies. As previously mentioned, unlike the human IAPP, the rodent IAPP is not amyloidogenic due to the proline substitutions in the 20–29 amino acid region. This lack of amyloid development in these models makes impossible to assess the role of IAPP aggregation in islet physiology. Since only a limited

number of species spontaneously form islet amyloid, several groups have developed transgenic mice strains choosing hIAPP as a model for islet amyloidogenesis [91].

Nonetheless, some reports showed that the mere hIAPP overproduction did not lead to amyloid formation or deposition despite elevated plasma concentration of hIAPP. Thus, other factors beyond overexpression had to be involved in the mechanism of islet amyloid formation since these mice were normoglycemic and normoinsulinemic (94–96). Islet amyloid was reported in transgenic mice fed a diet high in fat. Verchere et al. [18] have shown that approximately 80% of male transgenic mice (<13 months old) presented amyloid deposits and were always associated with severe hyperglycemia. In the case of hemizygous transgenic mice for hIAPP, the treatment with growth hormone or dexamethasone induced small intra- and extracellular amyloid deposits [92].

Another strategy used to overexpress hIAPP was to cross-bread hIAPP mice onto a mouse with obese background (ob/ob) [19] or obese Agouti viable yellow (Avy/Agouti) [93]. These mice developed amyloid formation and loss of β-cells, which was associated with progression of diabetes (**Figure 4**).

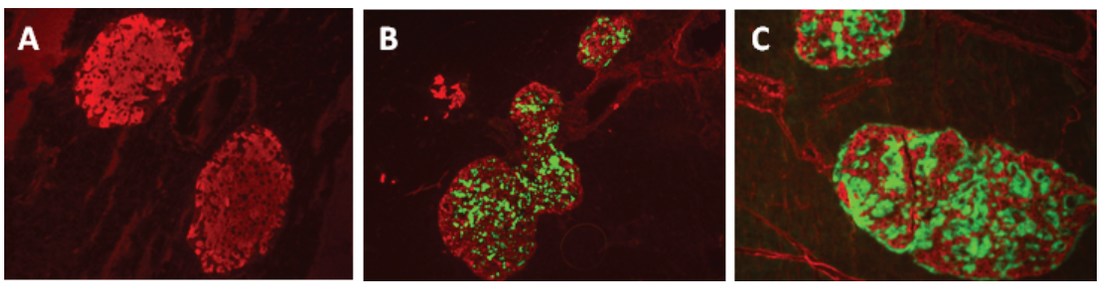

Figure 4. Amyloid formation in hIAPP transgenic mouse islets in obese Agouti yellow mice. Amyloid staining of hIAPP Tg mice in (A) FVB background, Avy/Agouti background at (B) 16 weeks of age and (C) 22 weeks of age. Note the presence of amyloid deposits in Avy/Agouti background as shown by Thioflavin S staining (unpublished data).

Moreover, it was found that female transgenic mice do not increase the occurrence of amyloid when oophorectomized, suggesting a protective role of ovarian hormones in islet amyloidosis. In recent years, Butler et al. showed that transgenic β-cell expression of human proIAPP in rats (HIP rats) that are homozygous for hIAPP develop diabetes within 5-10 months, together with the presence of extracellular amyloid, decreased β-cell mass, and increased β-cell apoptosis [94]. In addition, the loss of approximately 60% of β-cell mass at diabetes onset is comparable to the loss observed in humans with T2D [1].

In conclusion, with a variety of transgenic hIAPP models, it has been possible to clearly highlight that the process of islet amyloid formation is a complex event associated with a great number of factors considered important in the pathogenesis of T2D.

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