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Type 2 diabetes as a protein misfolding disease

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Type 2 diabetes (T2D) is a highly prevalent and chronic metabolic disorder. Recent evidence suggests that formation of toxic aggregates of the islet amyloid polypeptide (IAPP) might contribute to β -cell dysfunction and disease. However, the mechanism of protein aggregation and associated toxicity remains unclear. Misfolding, aggregation, and accumulation of diverse proteins in various organs is the hallmark of the group of protein misfolding disorders (PMDs), including highly prevalent illnesses affecting the central nervous system (CNS) such as Alzheimer's disease (AD) and Parkinson's disease (PD). In this review we discuss the current understanding of the mechanisms implicated in the formation of protein aggregates in the endocrine pancreas and associated toxicity in the light of the long-standing knowledge from neurodegenerative disorders associated with protein misfolding.

Protein misfolding and disease

PMDs are diseases where at least one protein or peptide has been shown to misfold, aggregate, and accumulate in tissues where the disease-specific damage occurs. There are at least 30 different PMDs, including several neurodegenerative disorders such as AD, PD, Huntington disease (HD), transmissible spongiform encephalopathies (TSEs), and amyotrophic lateral sclerosis (ALS), as well as diverse systemic disorders such as familial amyloid polyneuropathy, T2D, secondary amyloidosis, and dialysis-related amyloidosis [1]. The first line of evidence that linked protein misfolding and aggregation with disease came from post-mortem histopathological studies showing that a typical feature of each disease is the accumulation of protein deposits comprising a different protein, such as amyloid- β (A β) and tau in AD, α -synuclein in PD, poly-Q extended huntingtin in HD, IAPP in T2D and prion protein (PrP) in TSEs [1]. Perhaps the most compelling evidence for the key role of misfolded proteins came from genetic studies. Mutations in the genes encoding the proteins that predominantly comprise the aggregates have been genetically associated with inherited transmission of many PMDs

[1,2]. Inheritance of these mutations resulted in earlier onset and increased severity of the disease than in sporadic cases and were associated with a more extensive burden of protein aggregates [2]. Furthermore, transgenic expression of disease-specific human genes harboring the associated mutations in animal models reproduced several phenotypic and pathological characteristics of PMDs, supporting the key contribution of protein aggregates in these diseases [3]. In the case of TSEs, the prominent role for misfolded protein aggregates is well supported by the fact that these diseases can be transmitted from individual to individual by the sole administration of misfolded prion protein aggregates (Box 1).

Although T2D is often considered a PMD, few studies have investigated the involvement of protein misfolding in the disease pathogenesis. The subject has been mostly neglected in the diabetes field despite the fact that the evidence implicating the accumulation of misfolded protein aggregates in T2D pathogenesis are comparable with other diseases such as AD or PD. Here we review the literature and discuss the role of protein misfolding and aggregation in T2D.

The role of protein misfolding and aggregation in T2D

T2D is a complex metabolic disease characterized by chronic insulin resistance and progressive loss of β -cell function and β -cell mass [4], which leads to impaired insulin release and hyperglycemia. In a prediabetic context, compensatory increases in insulin secretion from β cells protects against hyperglycemia. However, genetic and environmental factors are believed to predispose some individuals (~20% of the population) to β -cell failure under conditions of chronic insulin resistance [5]. This transition to dysfunction and loss of β cells is frequently ascribed to the consequences of glucolipotoxicity [6,7], islet cholesterol accumulation [8], and islet inflammation [9].

Accumulating evidence suggests that toxic aggregates of IAPP may contribute to β -cell dysfunction and disease [10,11]. IAPP is a 37-amino-acid neuroendocrine polypeptide hormone [12] also known as amylin [13]. The phenomenon of IAPP accumulation associated with T2D, particularly in elderly individuals, was first described in 1901 as 'islet hyalinization' [14]. However, the clinical significance of this observation was not appreciated as islet amyloid was not found in many diabetic patients and some nondiabetic individuals also exhibited islet

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Box 1. When misfolded protein aggregates behave as infectious agents: the prion story

Prion diseases represent some of the most intriguing PMDs, which can be transmitted by infection where the infectious agent is thought to solely comprise the misfolded protein [140]. The disease is transmitted by seeding the aggregation of the normal prion protein, resulting in the accumulation of large quantities of these toxic aggregates in the brain [140,141]. Prion infectivity is dependent on the ability of misfolded prion protein aggregates to nucleate the misfolding and aggregation of the host prion protein [141]. Interestingly, the formation of misfolded aggregates in T2D, as well as in all other PMDs, follows a seeding–nucleation mechanism similar to the process of prion replication in which preformed polymers seed the aggregation of the monomeric protein [141]. The similarities between the molecular mechanism of prion propagation and the process of protein misfolding and aggregation in PMDs suggest that misfolded aggregates have an inherent ability to be transmissible [36,141]. Indeed, a series of recent studies has shown that the pathological hallmarks of various PMDs, including AD, PD, HD, and some forms of systemic amyloidosis, can be induced under experimental conditions by administration of tissue homogenates carrying the respective misfolded protein aggregates (for reviews see [141–143]). The transmission of protein misfolding from molecule to molecule and cell to cell may play a major role in the spreading of protein aggregates during the progression of these diseases [144]. Whether IAPP misfolding and aggregation can also be transmitted using the prion principle remains to be explored.

amyloid, albeit in much lower amounts [15–17]. More recently, it has been established that islet amyloid deposits are present in over 90% of T2D patients [16,18–21]. The fact that IAPP aggregates can be observed in nondiabetic individuals is unsurprising considering that in studies of other PMDs (such as AD or PD) it is clear that aged individuals free of disease symptoms who are in the process of developing the disease may show substantial amounts of these aggregates [22,23]. Several studies have linked IAPP aggregation with β -cell loss and progression of T2D. Post-mortem studies suggested that IAPP aggregates are associated with loss of β -cell mass [4,18,21]. IAPP aggregation was suggested to be an important cause of declining β -cell function in clinically transplanted islets [24]. A mutation in the IAPP gene that elevates its aggregation propensity [25,26] is associated with early onset of T2D [27,28]. Longitudinal studies in animal models that spontaneously develop T2D (nonhuman primates and domestic cats) showed that formation of IAPP aggregates precedes β -cell dysfunction and clinical signs of the disease [29–32]. Finally, transgenic mice and rats overexpressing human IAPP (hIAPP) spontaneously developed clinical and pathological hallmarks of T2D [33]. Although the link between IAPP aggregation and β -cell loss is convincing, the cause and origin of IAPP aggregation and the mechanism of toxicity is incompletely understood.

The biological, biochemical, and aggregation properties of IAPP

To conceptualize the process of IAPP aggregation, we will describe the biological and biochemical features of IAPP, its sequence propensity to aggregate, and the pathways responsible for IAPP misfolding and aggregation. The IAPP amino acid sequence, especially at the amino and carboxyl termini, is highly conserved, suggesting an important physiological role [34]. The functions that have been proposed for IAPP

include inhibition of insulin secretion, delay in gastric emptying, diminished appetite, suppression of glucagon release (reviewed in [34]), and suppression of tumorigenesis [35]. However, the exact role of IAPP function and dysfunction in diabetes is not fully understood.

Amyloidogenic proteins/peptides form multimeric aggregates following a seeding–nucleation mechanism (Box 1). This model can be divided in two different kinetic phases [36,37]. The first phase is the lag phase, where the monomeric protein acquires a misfolded structure able to self-associate, forming small oligomers known as seeds or nuclei. Formation of the initial seeds is thermodynamically unfavorable and is a kinetically slow, rate-limiting step. Seeds formed during the lag phase act as a template for the recruitment of other monomeric proteins in the second phase, resulting in exponential growth of the oligomers, forming a continuum of larger aggregates [36,37]. The propensity of a given protein to form amyloid aggregates depends on its sequence, the stability of the folded conformation, the concentration of the protein, and the interaction with various factors including other proteins, membranes, extracellular matrix elements, and other cofactors. In the case of IAPP there is some evidence that interaction with intracellular membranes may play a role in the formation of toxic oligomers [38,39]. Protein sequences highly prone to forming amyloid exhibit a short lag phase and a large amount of the monomer incorporates into the aggregate. In our experience studying the kinetics of amyloid formation of many different amyloid proteins, hIAPP is one of the most amyloidogenic proteins. Compared with other PMD-associated proteins such as A β or α -synuclein, IAPP aggregates much faster at similar concentrations, exhibiting a significantly shorter lag phase (Figure 1A).

Although the IAPP sequence is generally conserved, there are a few critical interspecies differences, especially in residues 20–29, which is considered the domain responsible for amyloidogenicity (Figure 1B) [40]. Interestingly, species that are known to express an amyloid-prone sequence of IAPP (including humans, nonhuman primates, and cats) are also the species known to spontaneously develop T2D [41]. Islet amyloid has been found present in 80% of diabetic cats [31,42] and 100% of diabetic macaca mulatta [30]. The three proline substitutions (proline is a well-known beta-sheet-breaking amino acid [43]) within the 20–29 amyloidogenic region in rodent IAPP substantially reduces the propensity of IAPP to misfold and aggregate compared with hIAPP [43,44]. Spontaneous T2D is not reported in rodents and the rodent models that are often used to recapitulate T2D, such as high-fat diet-induced diabetic mice or ob/ob mice, do not produce islet amyloid [45]. Several groups have developed mice and rats expressing hIAPP (hereafter called tg-hIAPP mice or rats) [46]. Although some of these models require a prediabetic condition or a high-fat diet, overexpressed hIAPP has been reported to form toxic aggregates resulting in β -cell loss leading to T2D pathology [33,47].

The process of amyloid formation produces a dynamic equilibrium of a continuum of aggregates of different sizes and properties, ranging from small oligomers (dimers, trimers, tetramers, hexamers, etc.), larger but still soluble oligomers (containing 12–50 units) to protofibrils and

Figure 1. Islet amyloid polypeptide (IAPP) propensity to form amyloid aggregates. **(A)** The figure shows a schematic representation of the typical kinetics of human IAPP aggregation compared with other protein misfolding disorder (PMD)-associated proteins. In our experience, at low micromolar concentrations, physiological temperature, and pH, IAPP forms aggregates in minutes, whereas A β needs hours and α -synuclein needs days to bypass the lag phase before aggregating exponentially. **(B)** The amyloidogenicity of IAPP from human, monkey (*Macaca mulatta*), cat, and mouse was studied *in silico* using a structure-based algorithm originally described by Eisenberg and colleagues [145]. The amino acid sequence is plotted along the x-axis. Each histogram bar represents one hexapeptide starting at the indicated position in the sequence and is colored according to its Rosetta energy. Orange-red segments with energy below the indicated energetic threshold of -23 kcal/mol (gray line) are predicted to form fibrils.

large amyloid fibrils. The nature of the most toxic structure of IAPP aggregates, as well as the aggregates implicated in other PMDs, is not completely clear. Here we refer to protein aggregates as the heterogeneous mixture of polymers of different sizes that likely comprise the structures present in the patients' tissue. Initially it was thought that large aggregates were the most pathologically relevant structures, but recent reports have provided compelling evidence for smaller and soluble oligomers as the triggering agents in disease [10,48,49]. Some investigators have proposed that the formation and deposition of large amyloid fibrils could be a protective mechanism to sequester and isolate toxic oligomers [50]. Although this is an attractive hypothesis, it is likely that both soluble misfolded oligomers and larger aggregates, even those deposited in tissues, might be toxic, perhaps by different mechanisms

[51,52]. In addition, since protein aggregates appear to be dynamic structures in equilibrium with each other, it is possible that large amyloid deposits may serve as a reservoir for more toxic smaller oligomers [53,54]. Finally, at this time we cannot exclude that the most relevant structures might be off the pathway of the amyloid formation process, such as the recently identified antiparallel β -sheet oligomers known as cylindrins [55].

IAPP synthesis, processing, and clearance

IAPP is predominantly expressed by β cells as the 89-amino-acid pre-pro-IAPP. The 22-amino-acid signaling peptide is cleaved off in the endoplasmic reticulum (ER), producing pro-IAPP, which is further processed by the endoproteases prohormone convertase (PC) 2, PC1/3, and carboxypeptidase E in the late Golgi and secretory

granules in a pH-dependent manner [56–58]. These enzymes are also responsible for processing proinsulin. Post-translational modifications, including amidation of the COOH terminal and disulfide bridge formation between residues 2 and 7, are prerequisites for full biological activity [34]. Processed IAPP remains stored with insulin in secretory granules in a 1–2:50 molar ratio (IAPP:insulin). Insulin and proinsulin inhibit IAPP aggregation, with insulin being more potent [59–62]. These inhibitory effects, together with the low pH in the secretory granules, are believed to maintain IAPP in a soluble state. IAPP is coexpressed and cosecreted with insulin by β cells in response to glucose stimuli [63,64]. However, in a state of high insulin demand such as insulin resistance, IAPP expression increases compared with insulin, enhancing the probability of aggregate formation. Supporting this view, transgenic mice expressing relatively low levels of hIAPP require pharmacological induction of insulin resistance to develop diabetes and islet amyloid [65]. The evidence suggests that IAPP oligomerization begins intracellularly [39], but large amyloid deposits accumulate extracellularly, as in several other amyloid diseases.

In addition to elevated expression, impaired degradation may also contribute to elevated IAPP levels, promoting aggregation (Table 1). Insulin-degrading enzyme (IDE), a Zn^{2+} metalloprotease involved in the clearance of insulin [66], can also degrade IAPP [67]. IDE has been identified as a T2D susceptibility gene [68–70]. Pharmacological inhibition of IDE reduced IAPP degradation in RIN-m5F cells, leading to enhanced formation of toxic IAPP aggregates and cytotoxicity [70]. A recent report suggested that pharmacological inhibition of IDE may restore glucose tolerance and increase

plasma insulin and IAPP levels in obese mice [71]. However, this mouse model does not express amyloidogenic IAPP. The effect of genetic or pharmacological reduction of IDE on IAPP aggregation in a tg-hIAPP model has not been studied. Neprilysin (NEP) is a type 2 zinc-containing metalloprotease present on the surface of various cell types, including β cells [72]. It degrades IAPP [73] and can prevent amyloid formation [74]. Elevated NEP levels are reported in mice overexpressing hIAPP compared with age-matched wild type controls [72]. The interpretation of these results is that sustained elevation of NEP is a compensatory mechanism aimed at degradation of extracellular IAPP and reducing its accumulation in amyloid deposits.

Removing misfolded proteins

In long-lived cells such as neurons and β cells, it is crucial to have an efficient protein degradation machinery to avoid toxicity arising from sustained accumulation of damaged proteins. Cells mainly deploy three mechanisms to remove misfolded proteins: the ubiquitin proteasome system (UPS), autophagy, and aggresome formation. Defects in these mechanisms may result in the accumulation of misfolded and aggregated proteins. Table 1 summarizes current evidence implicating dysfunction of cellular degradation machinery in T2D and neurodegenerative PMDs.

The UPS

The UPS is the predominant member of the cellular clearance machinery for the degradation of short-lived, damaged, abnormal proteins. Compelling evidence has shown impaired UPS function leading to accumulation of polyubiquitinated proteins in neurodegenerative disorders associated

Table 1. Pathways involved in aggregate formation and clearance and cell death in T2D and PMDs of the CNS

Protein (pathway)	Mouse models of CNS PMDs	Mouse models of T2D	Relation to human disease
Insulin degrading enzyme (IDE)	CNS-specific IDE overexpression leads to marked reduction of A β aggregates and associated pathology in AD mouse model [146]		Linkage of IDE to T2D and AD by genome-wide association studies [68,69,147]
NEP	CNS-specific NEP overexpression prevents A β plaque formation [148]	Elevated mRNA level in tg-hIAPP model harboring islet amyloid [72]	NEP activity in cerebrospinal fluid is associated with dementia and A β 42 levels in Lewy body disease [149]
UCH-L1 (UPS)	Age-dependent loss of UCH-L1 activity in the hippocampus of AD mouse model; administered UCH-L1 restores memory deficit in AD mouse model [83]	Hemizygous deficiency leads to significant β -cell loss and severe hyperglycemia [86]	Reduced level of UCH-L1 observed in T2D islets; mutation in UCH-L1 linked to early-onset PD and downregulation of UCHL1 associated with idiopathic PD and AD [82,84]
Atg7 (autophagy)	CNS-specific deletion of Atg7 leads to age-dependent accumulation of inclusion bodies and neuronal loss [150]	β -cell-specific Atg7 deletion induces hIAPP aggregation, β -cell dysfunction, and glucose impairment [93,95]	
XPB1 (ER stress)	sXBP1 is upregulated in the brains of ALS, HD, and TSE patients and genetic reduction of XPB1 was neuroprotective in mouse models of ALS, PD, and HD [124]	Elevated level of sXBP1 observed in mice overexpressing hIAPP [122]	XPB1 polymorphism associated with prediabetic traits in a Chinese population [125]
NLRP3 and caspase-1 (inflammasome)	Deficiency of NLRP3 or caspase-1 prevented pathological and behavioral alterations in AD mouse models; caspase-1 deficiency decreased neuronal loss and increased survival in ALS mouse model [151,152]	IL-1 β production by islet macrophages of mice harboring islet amyloids [134]	Enhanced expression of active caspase-1 in the brains of patients with mild cognitive impairment, AD, and ALS; combined elevation of IL-1 β and IL-6 increases the risk of T2D [153,154]

with protein misfolding [75–78]. Proteins tagged with ubiquitin chains are targeted to the 26S proteasome. Next, deubiquinating enzymes hydrolyze ubiquitin chains, which is required for substrate entry into the proteolytic barrel of the proteasome [79]. Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), a member of the deubiquinating enzyme family, is abundantly expressed in β cells [80]. Mutations in UCH-L1 have been linked to early-onset PD [81] and downregulation of UCH-L1 has been associated with idiopathic PD and AD [82]. Moreover, exogenous administration of UCH-L1 restored memory deficit in an AD model [83]. Reduced levels of UCH-L1 were also observed in islets from T2D patients compared with body mass index-matched controls [84]. This deficiency resulted in the accumulation of ubiquitinated proteins in β cells, leading to apoptosis. Further studies in human islets revealed that elevated levels of hIAPP downregulate the expression of UCH-L1 by an unknown mechanism [84]. UCH-L1-null mice displayed mild glucose intolerance when challenged with a high-fat diet [85]. However, even hemizygous deficiency of UCH-L1 in mice expressing hIAPP, at an otherwise tolerable level, manifested as a significant increase in β -cell apoptosis, loss of β -cell mass, and severe hyperglycemia on a standard diet [86]. Thus, it seems that elevated levels of IAPP, caused by conditions such as insulin resistance, may reduce the efficiency of the UPS system. Inefficient UPS function could eventually promote aggregate formation leading to toxicity, which has been shown to occur in PMDs of the CNS [87,88].

Autophagy dysregulation

Autophagy is a major clearance mechanism for lysosomal degradation of damaged proteins and organelles. Macroautophagy (hereafter referred to as autophagy) involves the sequestration of damaged organelles or large protein aggregates into membrane-bound cargo vesicles known as autophagosomes that transport the contents to the lysosome for proteolysis. Although autophagy is considered an adaptive process, current studies suggest that a basal level of autophagy is always active and is involved in protein quality control [89]. Autophagy abnormalities in PMDs of the CNS lead to accumulation of protein aggregates, which results in cytotoxicity [90]. This can be reversed by pharmacological activation of autophagy [91]. A significantly high volume of autophagic vacuoles and autophagosomes has been detected in islet β cells from T2D patients compared with healthy controls, indicating alterations of the autophagy pathway [92]. Studies in β cells from tg-hIAPP mice suggested that hIAPP aggregates impair lysosomal degradation of the autophagosome's cargo, resulting in accumulation of autophagic vacuoles [93]. However, the mechanism of impairment remains to be determined. Moreover, genetic or pharmacological upregulation of autophagy protected the cells from IAPP-mediated toxicity [93–95]. By contrast, pharmacological downregulation of autophagy increased the vulnerability of cells to IAPP aggregates, suggesting a vicious cycle whereby IAPP aggregates reduce autophagic flux, which further promotes IAPP aggregation, leading to toxicity. β -Cell-specific knockout of Atg7 (a crucial initiator of autophagy) induced toxic IAPP aggregate formation, leading to β -cell

dysfunction and early induction of T2D in tg-hIAPP mice (Table 1) [93,95]. It is important to note that autophagy is important for the maintenance of β -cell homeostasis. Islet β -cell-specific genetic reduction of autophagy (by Atg7 deficiency), even in the absence of hIAPP expression, led to mild glucose intolerance and elevated blood glucose levels [96]. However, in the presence of IAPP aggregates this reduction of autophagy resulted in severe diabetes even on a standard diet [93,95]. It was further shown that obesity-associated insulin resistance led to an enhanced autophagic flux in β cells to meet the increasing demand for insulin. However, in the presence of IAPP aggregates the reduction of lysosomal degradation prevented this compensatory increase in autophagic flux [95].

The aggresome

Proteins that escape canonical degradation machineries can be temporarily sequestered to diminish cytotoxicity via the formation of cytosolic inclusions at the microtubule organizing center known as aggresomes [97]. Recently, p62/SQSTM1 was reported to function in the formation of cytosolic protein inclusions and their clearance by a process of protein aggregate-specific autophagy termed aggrephagy [98]. Evidence indicates that p62-positive aggresomes can be eventually digested by the UPS and lysosomal machinery [99–101]. Accumulation of p62-positive protein aggregates was noted in β cells expressing hIAPP and enhanced formation of p62-positive inclusion bodies were associated with reduced IAPP-induced β -cell apoptosis [93,102]. Furthermore, it has been demonstrated that p62 can specifically bind to aggregation-prone hIAPP and temporarily sequester it in a less toxic form [93]. However, these p62 inclusions must be degraded by the autophagy/lysosome pathway for long-term protection of β cells, as described for α -synuclein- or huntingtin-containing p62 inclusions in neurons [103,104].

Mechanisms of IAPP-induced toxicity

Several mechanisms have been proposed for IAPP aggregate-mediated β -cell dysfunction and death in T2D. Membrane permeabilization, calpain hyperactivation, induction of ER stress, dysregulation of the clearance pathways, and induction of inflammation have all been reported (Figure 2). Interestingly, the same pattern of cellular defects has also been described in other PMDs, mostly those affecting the CNS [49]. Current evidence implicating protein aggregate-mediated cellular dysfunction in T2D and brain PMDs is summarized in Table 1. Neuronal dysfunction and death by disease-specific protein aggregates have been studied in detail. In the following sections we discuss our current understanding of the putative mechanisms by which IAPP aggregates may induce β -cell dysfunction and provide a comparison with other misfolded oligomeric proteins responsible for neuronal death in PMDs affecting the CNS.

Membrane permeabilization

It has been suggested that IAPP aggregates might produce cytotoxicity by forming pores that permeabilize cell membranes, notably those that comprise the protein synthesis and secretory pathways (i.e., the ER, secretory vesicles, and plasma membranes) [39]. It has been reported that

Figure 2. Molecular pathways implicated in β -cell damage mediated by the formation and accumulation of islet amyloid polypeptide (IAPP) aggregates. Illustration of major cellular pathways implicated in the toxicity of IAPP aggregates against β cells. Figure elements are not drawn to scale. Abbreviations: ER, endoplasmic reticulum; ROS, reactive oxygen species; UPS, ubiquitin proteasome system.

IAPP oligomers form pore-like structures in the membrane resulting in leakage followed by Ca^{2+} dysregulation leading to cytotoxicity [105–107]. Similar mechanisms have been reported for $\text{A}\beta$, α -synuclein, and PrP oligomers [108,109]. Accumulating data suggest that lipid bilayers may promote IAPP aggregation by direct interaction with negatively charged phospholipids, such as phosphatidyl serine, promoting interaction of the positively charged IAPP monomer with the membrane [110]. Binding of negatively charged heparan sulfate proteoglycan, present on the cell surface, to the positively charged N terminal of IAPP and pro-IAPP has also been shown to promote the aggregation of the peptide [111,112]. Interestingly, a high concentration of non-amyloidogenic rat IAPP has been shown to induce leakage in artificial lipid bilayers [113]. However, similar concentrations of rat IAPP did not produce cellular toxicity [113], indicating that the relationship between membrane leakage and toxicity may not be direct. It should be noted that studies using lipid bilayer mimetics that are likely to differ in composition to the relevant intracellular and plasma membranes, while potentially providing mechanistic insights, do not mimic the complex intracellular

environment (e.g., ion concentrations, other proteins, cofactors like glycosaminoglycans, pH, chaperones, ubiquitination). Moreover, the itinerary of how toxic oligomers arrive at (or are formed in) various intracellular sites is poorly understood. Perhaps most important, it remains unclear why, in the setting of insulin resistance, some individuals are prone to the formation of β -cell oligomers, β -cell failure, and diabetes while others successfully express, fold, and secrete (or degrade) IAPP.

Mitochondrial damage

Mitochondrial dysfunction is a crucial pathogenic event both in T2D and in PMDs of the CNS (reviewed in [114,115]). $\text{A}\beta$ and α -synuclein aggregates have previously been shown to disrupt mitochondrial function by forming pores in the mitochondrial membrane [116]. Recently, toxic IAPP oligomers have been observed within disrupted mitochondrial membranes in tg-hIAPP mice [39]. Furthermore, studies in cells exposed to IAPP aggregates indicated loss of mitochondrial membrane potential, ATP depletion, and other mitochondrial-associated damage [117]. Unstable mitochondrial membrane potential leads to overproduction

of reactive oxygen species, which has recently been shown to be an initiator of toxicity by IAPP aggregates [118,119].

ER stress

In general the ER is well equipped to meet the high secretory demand of β cells (approximately 10 000 proinsulin molecules per β cell per minute [120]). However, genetic or environmental alterations, as well as the accumulation of protein aggregates, are known to produce chronic stress beyond the capacity of the ER, leading to deleterious consequences. Several groups have reported morphological changes related to ER stress, such as distention of the ER [121], and induction of ER stress-associated death response markers, such as C/EBP homologous protein (CHOP) and activation of caspases in islet β cells from T2D patients [122]. Neither controls nor islets from type 1 diabetic individuals manifested these changes, suggesting that sustained ER stress could be a very specific pathological feature of T2D [122]. Using primary cell lines, islets, and rodent models (tg-hIAPP), it was shown that induction of ER stress is specifically associated with the expression of the amyloidogenic hIAPP sequence [122]. Moreover, IAPP-mediated cytotoxicity was prevented, at least partially, by either reduction of CHOP or increase of the endogenous ER chaperone BIP [123]. X-box-binding protein-1 (XBP1) is a crucial member of the ER stress signaling cascade [124] and a polymorphism in XBP-1 has been associated with prediabetic traits in a Chinese population [125]. Activation of XBP1, evident by increased levels of spliced XBP1 (sXBP1), was observed in the brains of ALS, HD, and TSE patients and genetic reduction of XBP1 provided neuroprotection in mouse models of ALS, PD, and HD (reviewed in [124]). Elevated sXBP-1 was observed only in mice that overexpress hIAPP [122] (Table 1). Similar results were obtained from primary culture of human pancreatic cells on exposure to IAPP aggregates. Although XBP1 deficiency in mice led to reduced insulin secretion followed by mild glucose intolerance [126], the effect of IAPP aggregate formation was not tested in this model.

Inflammation

IAPP aggregates might also induce inflammation in islets of Langerhans. IAPP aggregates were observed in macrophages present in pancreatic tissues of biopsy samples from T2D patients as well as in diabetic monkeys, and tg-hIAPP mice [127]. Although these macrophages could not efficiently remove the aggregates, internalization of IAPP aggregates led to secretion of multiple inflammatory cytokines including IL-1 β in cell culture studies [128–131]. Interestingly, a threefold increased risk of developing T2D in individuals with elevated levels of both IL-1 β and IL-6, but not IL-6 alone, has been reported [132]. Using bone marrow-derived dendritic cells as a model, it has been shown that phagocytized hIAPP oligomers activate the NLRP3 inflammasome, leading to IL-1 β secretion [133]. Moreover, a recent study indicated that hIAPP oligomers induce a proinflammatory response in islet-resident macrophages, including IL-1 β secretion leading to islet dysfunction in a tg-hIAPP mouse model [134]. Interestingly, studies in various PMDs have shown that activation of inflammasome and consequent IL-1 β secretion seems to be a common mechanism by which protein aggregates produce tissue damage [135].

IAPP aggregates in non-pancreatic tissues

Severe and uncontrolled diabetes damages numerous non-pancreatic tissues, including kidney (diabetic nephropathy), sensory neurons (diabetic neuropathy), and heart (diabetic cardiomyopathy). IAPP deposits have been observed in several of these tissues in T2D patients. In a study conducted on biopsy-proven T2D nephropathy patients, 48.3% exhibited IAPP deposits in the kidneys [136]. The incidence of renal lesions such as glomerular nodular lesions and glomerulosclerosis was greater and tubular interstitial lesions were more severe in patients with IAPP deposition than in patients without IAPP deposition, suggesting a pathological role of IAPP deposits [136]. This study also suggested that elevated plasma IAPP may induce this deposition, but is not the only determining factor. IAPP deposition is also observed in temporal-lobe gray matter of brains from T2D patients

Box 2. Crosstalk between IAPP and other protein aggregates

Multiple proteins may aggregate simultaneously in PMDs. Even multiple PMDs may coexist in a single individual [155]. Interestingly, the fact that the process, as well as the end products and intermediates, of the seeding–nucleation mechanism are similar in all PMDs raises the possibility that seeds comprising one protein may catalyze the polymerization of other proteins [155]. This process of heterologous seeding, also known as ‘cross-seeding’, has been extensively described using pure preparations of proteins *in vitro*, especially among proteins with some degree of sequence homology [155].

Epidemiological studies have shown that one PMD may be a significant risk factor for a second PMD. It has been shown that a large percentage of AD patients are simultaneously suffering from T2D or impaired fasting glucose [156]. Furthermore, AD patients show a higher incidence of islet amyloidosis than healthy individuals. T2D patients also exhibit an increased risk of developing AD compared with age-matched nondiabetic individuals [157]. Although the mechanism responsible for the risk association between AD and T2D is unclear, several hypotheses have been proposed, including alterations in insulin signaling, hypercholesterolemia, and oxidative stress. Another interesting possibility is that the misfolded proteins

implicated in AD and T2D may interact with each other, promoting their heterologous seeding and accelerating the pathological onset [155]. This idea is supported by *in vitro* studies showing that IAPP and A β can cross-seed each other, stimulating amyloid formation [158,159]. In addition, IAPP has been found associated with amyloid plaques in AD patients [159]. Further, A β deposits and hyperphosphorylated tau, hallmark features of AD, have been reported in the islets of T2D patients [160] and most of the A β colocalized with IAPP aggregates in severely affected areas. Moreover, a recent study reported that intravenous injection of preformed A β fibrils can trigger islet amyloid formation in the pancreas of a transgenic mouse model overexpressing hIAPP [161]. However, this group did not observe any A β immune reactivity in the pancreas during an analysis of a relatively small number ($n = 4$) of T2D patients. The interaction may not be restricted to AD and T2D proteins, as oligomeric α -synuclein was recently reported to be present in islets of T2D patients and was speculated to impair glucose-stimulated insulin release from β cells [162]. However, the presence of protein aggregates comprising other proteins in the T2D pancreas remains an important question for future studies.

[137]. Congophilic IAPP deposits were found in both blood vessels and perivascular spaces, suggesting influx from the peripheral circulation. Finally, significantly high levels of oligomeric IAPP were reported in the failing hearts of T2D patients [138]. Interestingly, failing hearts from obese prediabetic individuals also exhibited oligomeric IAPP, suggesting that this process of accumulation may initiate decades before the onset of overt T2D. Normal hearts and failing hearts from patients without diabetes did not show IAPP aggregates [138]. Using a transgenic rat model expressing hIAPP, it was further shown that prediabetic and diabetic rats manifest cardiac hypertrophy associated with IAPP accumulation and that pure synthetic IAPP aggregates induce structural and functional defects in cardiomyocytes [138]. These findings certainly warrant further research to explore an as-yet-undiscovered role for IAPP aggregates in non-pancreatic tissue damage associated with T2D. It is also possible that IAPP aggregates may interact with other disease-causing aggregates through a process of heterologous seeding (Box 2).

Concluding remarks

The presence of misfolded IAPP aggregates of various sizes ranging from small soluble oligomers to large fibrillar aggregates deposited in the islets of Langerhans of patients affected by T2D is well established. The crucial question is whether these aggregates are inert bystanders that result as a consequence of the tissue damage during the disease or whether they play a crucial role in the pathogenesis. The diabetes field has mostly ignored the putative relevance of IAPP aggregates in T2D. This is surprising considering that the evidence for a key role of these aggregates in disease pathogenesis is compelling and similar to that which has elevated protein aggregates as the widely accepted cause of various neurodegenerative diseases. Considering T2D as a PMD will open an entire new area of research and uncover novel targets for therapeutic intervention. The mechanism and factors implicated in the transition from prediabetic conditions to β -cell failure and T2D are unclear. Convincing evidence indicates that the formation of IAPP oligomers and accumulation of islet amyloid might significantly contribute to this process. Factors promoting IAPP misfolding and aggregation in a prediabetic milieu are not well understood. However, genetic susceptibility and physiological and cellular changes during aging, such as progressive loss of proteostasis efficiency [139], may play a crucial role. All of the sporadic cases of PMDs affecting the CNS are largely associated with aging, suggesting a link between protein misfolding and aging. Irrespective of the cause of aggregation, misfolded aggregates, including islet amyloid, seem to share common mechanisms of formation, intermediates, and end products, as well as pathways of cellular toxicity. Thus, inhibition of protein misfolding and aggregation might be a common therapeutic strategy to prevent some of the most prevalent, insidious, and chronic diseases of our time.

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