Unravelling the story of protein misfolding in diabetes mellitus

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Abstract

Both environmental and genetic factors contribute to the development of diabetes mellitus and although monogenic disorders are rare, they offer unique insights into the fundamental biology underlying the disease. Mutations of the insulin gene or genes involved in the response to protein misfolding cause early onset diabetes. These have revealed an important role for endoplasmic reticulum stress in β-cell survival. This form of cellular stress occurs when secretory proteins fail to fold efficiently. Of all the professional secretory cells we possess, β-cells are the most sensitive to endoplasmic reticulum stress because of the large fluctuations in protein synthesis they face daily. Studies of endoplasmic reticulum stress signaling therefore offer the potential to identify new drug targets to treat diabetes.

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Key words: Endoplasmic reticulum stress; Diabetes; Unfolded protein response; PKR-like ER kinase

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INTRODUCTION

We place a heavy burden on our pancreatic β-cells. They are expected to deliver a life-long supply of insulin at the precise levels necessary to maintain glucose homeostasis while avoiding hypoglycaemia. This requires that they synthesize and secrete insulin at times of plenty, but rapidly attenuate protein synthesis when the hormone is no longer needed. Consequently, the secretory pathway of a β-cell experiences dramatic changes in client protein flux. As a consequence, it is exquisitely sensitive to defects of protein folding because large increases in the rate at which new proteins enter the endoplasmic reticulum (ER) can overwhelm the resident chaperones. This can allow incorrectly folded proteins to accumulate inside the organelle, a situation termed “endoplasmic reticulum stress”. Evidence accrued over the last decade has shown that ER stress plays an important role in the pathogenesis of diabetes, both through direct toxicity to the β-cell causing loss of β-cell mass and in peripheral tissues where it contributes to insulin resistance. We propose that current views of diabetes should be revised, so that it is seen as another member of the growing list of protein misfolding diseases.

GENETIC STUDIES IDENTIFYING ER PROTEINS IN THE DEVELOPMENT OF DIABETES MELLITUS

A critical observation was made almost forty years ago when three siblings, two brothers and a sister, were reported who had developed permanent neonatal diabetes mel-
litus in association with developmental bone defects[1]. The parents of those first cases were unrelated, but many subsequent case reports were of consanguineous families[2,3]. The condition now eponymously named Wolcott-Rallison syndrome (OMIM #226980)[4] is also known as multiple epiphyseal dysplasia with early onset diabetes mellitus (ME-D-IDDM) to highlight its extra-pancreatic manifestations. Indeed, these are diverse and include osteoporosis, growth retardation, hepatic and renal dysfunction and cognitive impairment. It is now known to be inherited as a classical autosomal recessive trait and has been mapped in two consanguineous families to the region 2p12[5]. Each family harbored a distinct mutation, but both proved to be in the Perk gene.

PERK is a ubiquitously expressed kinase localized to the membrane of the ER[6]. Protein sequence homology showed it to be a member of the eIF2α kinase family that links cellular stresses to the inhibition of protein translation. For example, the prototypical family member GCN2 reduces protein synthesis during periods of amino acid starvation by phosphorylating and thus inhibiting the translation initiation factor eIF2α[7]. Other members of this family inhibit protein synthesis during viral infection (PKR[8]) or iron deficiency in red blood cell progenitors (HRI[9]). In each instance, reduced protein synthesis promotes cellular health, by reducing the consumption of amino acids during starvation or preventing viral replication or matching haemoglobin synthesis to available haeme. In the case of PERK, reduced protein synthesis prevents new proteins entering the ER when they cannot be correctly folded. It transpires that PERK is essential for β-cells to withstand fluctuations in proinsulin synthesis that occur daily. Mice in which the Perk gene is deleted faithfully recapitulate many of the phenotypic features of Wolcott-Rallison syndrome and this has been very useful in understanding this condition[10]. Remarkably, these mice are born with normal islets of Langerhans, but rapidly lose β-cells in the neonatal period. Prior to death, these cells exhibit ER dilatation, due to distention with aggregates of misfolded proinsulin.

When circulating glucose levels are low, so is the demand for insulin and PERK is inactive. When proinsulin enters the ER it is bound reversibly by molecular chaperones, which enable higher levels of client proteins to be folded. This mechanism has been called the Unfolded Protein Response (UPR)[11,12]. The major client protein of the β-cell ER is proinsulin[13]. Recently, defects in insulin folding have been shown to underlie rare cases of familial permanent neonatal diabetes mellitus (OMIM #606176)[11]. Initially discovered in mice by Dr. Akio Koizumi in Akita, Japan, a spontaneous INS2 gene mutation causes β-cell death[14]. In contrast to man, mice possess two insulin genes that are functionally redundant[15] and yet the Akita mutation (C96Y) behaves as a semi-dominant trait[16,17]. This toxic-gain-of-function is caused by a substitution of a conserved cysteine residue required for the formation of an intra-molecular disulphide bond[18,19]. The mutant insulin fails to be secreted and is instead retained in the ER where, crucially, wildtype proinsulin also becomes trapped in complexes with Akita mutant proinsulin impairing secretion of the normal protein[20].

When a three-generation family with permanent neonatal diabetes mellitus was found to have a heterozygous mutation of the insulin gene, this led to 83 similar families being screened, nine of which harbored insulin mutations including one analogous to the Akita mutation of mice[11]. Several subsequent studies have also confirmed that sense mutations of the INS2 gene in man are a rare but important cause of neonatal diabetes[21-24]. When such mutant insulins were expressed in cultured cells, they caused ER stress and impaired cell viability[22,25]. This contrasts with mutations that impair proinsulin synthesis through impaired transcription, which are inherited recessive traits and fail to cause death of the β-cell[26].

EMERGING MODEL FOR ENDOPLASMIC RETICULUM STRESS INVOLVEMENT IN DIABETES

The precise details of UPR signaling and its links to cell death have been reviewed elsewhere[11,12,26]. However, it is worth examining some aspects of ER stress and cell death again, as these may provide a novel target for therapeutic intervention in the future. For example, Dr Seiichi Oyadomari observed that deletion of the Chop gene could delay the onset of diabetes in Akita mice[27] and prevent β-cell death following other toxic stresses[28]. CHOP is a transcription factor that is up-regulated by a number of cellular insults, notably ER stress. It has been linked to the induction of cell death and some have suggested its function is to kill cells when the degree of ER stress is insurmountable[29-31]. However, our work has suggested that the link to cell death is more complex and most likely stems from CHOP acting to boost the secretory capacity of the cell[32]. We showed that the Gadd34 gene is transcriptionally induced by CHOP during ER stress and that deleting Gadd34 was at least as protective as deleting the Chop gene in an animal model of ER stress. GADD34 functions to dephosphorylate eIF2α following ER stress so that protein translation can recover and UPR target genes can be transla-
In this manner, it behaves as a functional antagonist of PERK. Whilst cells lacking PERK are vulnerable to death during ER stress, it appears that the antagonism of PERK by GADD34 during ER stress can also result in cell death. Excessive activity of GADD34 in some circumstances creates a situation similar to that of PERK deficiency. Could GADD34 inhibitors therefore prove useful to treat diabetes? A molecule called salubrinal has been identified that promotes eIF2α phosphorylation during ER stress.[39] Its precise mechanism of action requires further elucidation, but has been suggested to involve inhibition of eIF2α dephosphorylation, which can promote cell survival in some models of ER stress.

When mice were generated with β-cells that were partially resistant to PERK by mutating the phosphorylation site of eIF2α (eIF2αS51A), the animals developed diabetes due to uncontrolled proinsulin synthesis and increased oxidative stress.[37] Feeding them antioxidants in their diet ameliorated this. Interestingly, CHOP induces the transcription of ERαα1a, which increases protein oxidation in the ER to promote disulphide bond formation.[33] While this may improve protein folding, it also imposes an oxidative stress burden on the β-cell. Consequently, preventing the induction of ERαα1a may explain why Chop knockout reduces oxidative damage and improves β-cell survival in models of diabetes.[40] Chop deletion also increases β-cell mass and prevents glucose intolerance both in high-fat fed eIF2αS51A mice and in leptin receptor deficient mice. This appears to be mediated by increased β-cell proliferation and by reduced apoptosis suggesting that CHOP antagonism might help maintain β-cell mass in patients if this could be achieved pharmacologically.

β-cells, therefore, can be subject to ER stress either from poorly regulated proinsulin synthesis in Wolcott-Rallison syndrome or directly from mutant proinsulins. It is less clear, however, why ER stress should be relevant in peripheral tissues in diabetic patients. Nevertheless, ER stress in peripheral tissues plays at least as important a role in diabetes as it does in the β-cell. Some peripheral tissues, for example adipocytes, respond to raised circulating glucose by increasing ER protein synthesis thus increasing ER client load.[39] In addition, obesity increases peripheral tissue inflammation, which can also cause ER stress.[40,41] A consequence of this appears to be impaired insulin signaling and consequently insulin resistance.

While PERK regulates protein translation during ER stress and has further effects on UPR gene transcription, a second ER stress sensor called IRE1 regulates other UPR genes. IRE1 is far older than PERK in evolutionary terms, being found even in yeast. Not only can it trigger gene transcription but it can, at least in mammals, also impair insulin receptor signaling. Activated insulin receptors signal to the cell’s interior via the phosphorylation of target molecules including insulin receptor substrate 1 (IRS1) on tyrosine residues. This can be blocked if IRS1 is phosphorylated on serine residues by the Jun N-terminal kinase (JNK),[42] and is triggered in peripheral tissues of obese subjects through activation of JNK by IRE1.[43] The notion that peripheral ER stress can impair glucose homeostasis is supported by a number of other lines of evidence. For example, if ER function is impaired in the liver by deleting the chaperone Oxygen-Regulated Protein 150 (ORP150), mice display impaired IRS1-dependent insulin signaling and develop glucose intolerance.[51] In contrast, raising the levels of ORP150 protects obese mice from diabetes.[34,45] We may yet be able to use these observations in therapies, since two small molecular “chemical chaperones”, 4-phenyl butyric acid and taurine-conjugated ursodeoxycholic acid, relieve ER stress in animal models in vivo and improve peripheral insulin sensitivity in obese diabetic mice.[46]

CONCLUSION

Substantial clinical and experimental evidence clearly shows that ER stress is important in diabetes both affecting β-cell survival and contributing to peripheral insulin resistance. This novel paradigm has already shed light on poorly understood aspects of diabetes and is providing exciting new targets for therapeutic intervention. Novel molecules, for example salubrinal and guanabenz, are poorly understood aspects of diabetes and is providing clues to the development of new drugs that will enable us to tackle ER stress in diabetes and will eventually help to treat this important cause of human suffering.

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