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Review article

Pancreatic adaptive responses in alcohol abuse: Role of the unfolded protein response

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ABSTRACT

The majority of those who drink excessive amounts of alcohol do not develop pancreatic disease. One overarching hypothesis is that alcohol abuse requires additional risk factors, either environmental or genetic, for disease to occur. However, another reason be a result of alcohol-induced activation of adaptive systems that protect the pancreas from the toxic effects of alcohol. We show that mechanisms within the unfolded protein response (UPR) of the endoplasmic reticulum (ER) that can lead to protection of the pancreas from pancreatic diseases with alcohol abuse. The remarkable ability of the pancreas to adapt its machinery to alcohol abuse using UPR systems and continue functioning is the likely reason that pancreatitis from alcohol abuse does not occur in the majority of heavy drinkers. These findings indicate that methods to enhance the protective responses of the UPR can provide opportunities for prevention and treatment of pancreatic diseases.

Introduction

Alcohol abuse is associated with a spectrum of pancreatic diseases from acute self-limiting episodes of pancreatitis to recurrent acute pancreatitis, chronic pancreatitis and pancreatic cancer [1–3]. However, clinically detected pancreatic disease occurs in only a small minority of heavy drinkers [1]. The reasons why most heavy drinkers do not develop clinically manifest pancreatic diseases are not known. One overarching hypothesis is that alcohol abuse requires additional risk factors, either environmental or genetic, for disease to occur [4–6]. Of note, in animal models we and others also have found that alcohol by itself does not cause pancreatitis but that alcohol feeding sensitizes that pancreas to pancreatitis caused by other pancreatic stressors [7,8].

Another reason why alcohol abuse leads to pancreatic disease in so few individuals could also be a result of alcohol-induced activation of adaptive systems that protect the pancreas from the toxic effects of alcohol. In other words, alcohol could activate both damaging effects and protective effects; and that disease occurs when the damaging effects outweigh the protective effects or when the adaptive mechanisms are impaired. Such a combination could also explain the combined actions of alcohol and another risk factor resulting in pancreatic disease [4–6,9]. That is, with an additional risk factor such as smoking or gene mutations, the alcohol-mediated protective responses are overwhelmed by the combination of its toxic effects and those of the second "hit."

In order to pursue the hypothesis that alcohol intake induces both toxic and protective mechanisms in the pancreas we have turned to investigating the potential role of the unfolded protein response (UPR) of the endoplasmic reticulum (ER) in these dual actions of alcohol using animal models. In our research we have focus mainly on the acinar cell of exocrine pancreas. However, the mechanisms discussed here may also be relevant to the ductal and/or endocrine cells of the pancreas.

Pancreatic acinar cell endoplasmic reticulum (ER)

The acinar cell requires an extensive endoplasmic reticulum network and protein secretory system to sustain its high rate of digestive enzyme production. ER biogenesis, function and turnover are regulated according to the demands of the secretory pathway. The ER recruits translating ribosomes, translocates newly synthesized polypeptides into its lumin, and accommodates post-translational modifications including glycosylation and disulfide bond formation, and chaperone-facilitated protein folding. Correctly folded proteins are tagged, sorted into specific vesicular compartments, and transported to the Golgi, where they are further

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processed, sorted and stored in mature zymogen granules and other organelles. Upon neurohormonal stimulation, zymogen granules undergo exocytosis at the apical pole of the cell, secreting their contents into the acinar lumin and ductal system of the exocrine pancreas. Correct ER processing and sorting are especially critical to prevent inappropriate intracellular activation of digestive proenzymes in the acinar cell [10].

In eukaryotic cells, protein folding is governed by an efficient team of ER molecular chaperones and folding enzymes that include disulfide isomerases, oxidoreductases and enzymes related to glycosylation of newly synthesized proteins. This process is monitored by quality control machinery to ensure that only properly folding proteins progress into the secretory pathway. Aberrant proteins are retro-translocated into the cytosol for proteosomal degradation by a process known as ER-associated degradation (ERAD). Autophagy is also an important mechanism for degradation of superfluous damaged or misfolded proteins and obsolescent organelles. Accumulated evidence underscores the importance of both ERAD and autophagy in preventing the accumulation of toxic proteins within the ER [11,12].

Protein-folding and chaperone functions within the ER are dependent on the presence of sufficient levels of intralimenal Ca^{2+} and ATP, and an oxidizing environment that favors disulfide bond formation in folding polypeptides. Thus, major perturbations in ER luminal and cellular Ca^{2+} fluxes, ATP levels and redox that can occur in the pancreas induce ER "stress" by hindering chaperone and folding activities [13–18]. Ca^{2+}, ATP and a controlled oxidative environment are key factors to maintain ER homeostasis for protein synthesis, processing and transport.

The unfolded protein response (UPR)

In order to adjust to changing demands encountered by the ER protein synthesis and processing machinery, eukaryotic cells have developed the Unfolded Protein Response (UPR) signaling system. The UPR is activated by accumulation of unfolded proteins in the ER lumin, a condition termed "ER stress" [19]. ER stress has several sources, including a physiologic increase in the demand for protein folding, decreased chaperone function, accumulation of permanently misfolded mutant proteins, restricted ER-Golgi protein trafficking, decreases in cellular ATP levels or in $[Ca^{2+}]_{ER}$, and perturbed ER redox status. In particular, repeated cycles of folding-refolding of misfolded proteins via thioredoxin-fold protein disulfide isomerase (PDI) family and ER oxidase-1 (Ero1) activities consumes cellular energy reserves and generates high levels of reactive oxygen species (ROS) and redox imbalance. Thus, protein misfolding shifts the redox status of the lumin to more oxidizing, possibly favoring aberrant disulfide formation.

The UPR has three major outputs that coordinate to maintain ER homeostasis: 1) global reduction in mRNA translation decreases the demand for processing newly synthesized proteins; 2) increased transcription of numerous chaperones and foldases, and phospholipid synthesis to augment the ER folding and export capacity and to expand the ER network, 3) activation of ERAD and autophagic systems to eliminate accumulated unfolded and misfolded proteins, and 4) degradation of ER associated mRNAs to reduce protein folding load [12,19–22]. Three trans-membrane ER stress sensor-transducers are responsible for these UPR outputs [19,20]. Inositol-requiring protein-1 α (IRE1α), activating transcription factor-6 (ATF6), and RNA-activated protein kinase (PKR)-like ER kinase (PERK). Each sensor transmits information from the folding status of proteins in the ER lumin to the nucleus by distinct mechanistic pathways (Fig. 1).

Although these sensors can be simultaneously activated by ER stressors, the UPR signaling outputs vary depending on the nature and duration of the ER stress, and the cell type [23]. For example, the IRE1 branch is critical for adaptation to long term ER stress [24]. Upon its activation, endonuclease activity within the IRE1α polypeptide splices X-box binding protein-1 (XBP1) mRNA resulting in the translation of a multifunctional transcription factor, called sXBP1. sXBP1 regulates a broad spectrum of UPR genes involved in protein folding, including chaperones and oxidoreductases of the PDI family, protein degradation (ERAD), vesicular trafficking and redox metabolism, as well as lipid biosynthesis/metabolism and ER/Golgi biogenesis, in a cell-specific manner [22,25]. The effects of sXBP1 generally adapt to ER stressors and enhance normal function in many cells, but play an especially important supportive role in secretory cells (described in the next section).

Activation of the PERK pathway by ER stressors results in inhibition of general protein translation by phosphorylation and inhibition of eukaryotic Initiation factor 2-α (eIF2α), a factor necessary for most protein translation. In the short term, this inhibition of protein synthesis relieves the cell of the demands of protein processing and can be beneficial. However, in the face of general translational inhibition, eIF2α favors translation of the transcription factor ATF4. ATF4 expression upregulates the proapoptotic transcription factor, C/EBP homologous protein (CHOP), as well as many target genes involved in translation, amino acid import and redox metabolism. The ER sensor ATF6 regulates the expression of XBP1 and several ER chaperones in response to short term ER stress, providing protective adaptation.

Whereas short term perturbations of ER function are normally resolved by the UPR, severe ER stress or defective UPR can lead to inflammation and cell death (10) [27]. Several intermediate responses have been identified. Sustained severe ER stress can trigger cell death downstream of PERK/CHOP and IRE1/JNK activation, and as a result of promiscuous IRE1α-dependent decay (RIDD) of ER associated mRNAs [26,27].

ER stress can favor translocation of proapoptotic Bax/Bak to the ER membrane causing Ca^{2+} release and mitochondrial dysfunction. This process can be positively regulated by Bim-only proteins such as Puma and Noxa that may be transcriptionally induced by ER stress [28]. Notably, it was reported that Bax or Bak exogenous

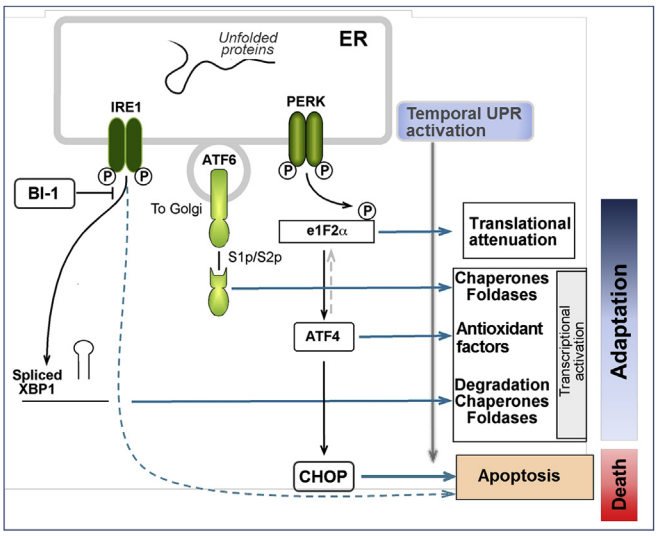

Fig. 1. Activation and outputs of the Unfolded Protein Response (UPR). The scheme summarizes the signaling pathways and outputs of the three branches of the mammalian UPR: IRE1α, ATF-6 and PERK. Upon disturbances of ER function, unfolded proteins accumulate within the ER lumin and the UPR sensors respond by activating adaptive signaling pathways. Cell growth arrest and cell death signaling prevail when ER stress is persistent or too severe.

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