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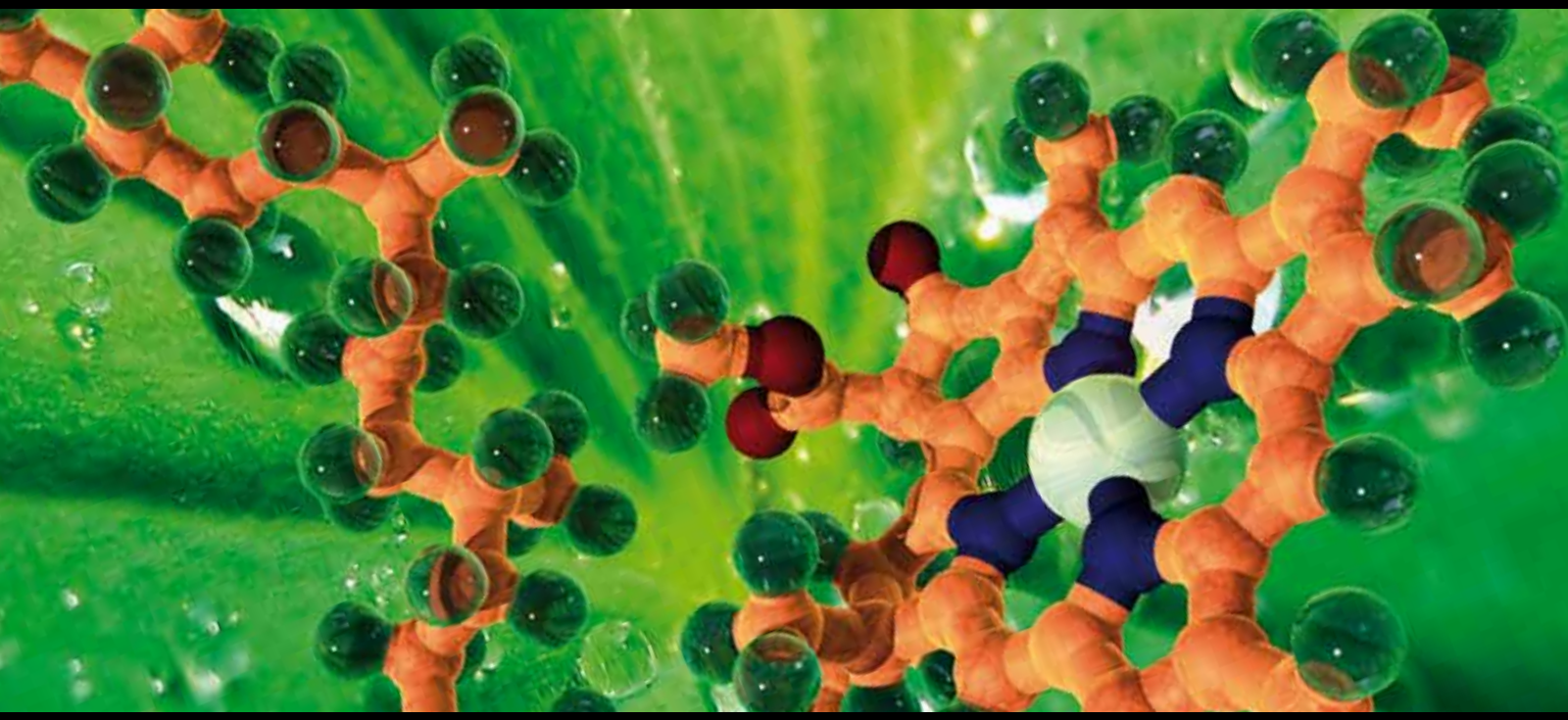
Endoplasmic Reticulum Stress and Lipid Metabolism

Guest Editors: Huiping Zhou, KezHong Zhang, Sabina Janciauskiene, and Xiaokun Li



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Editorial Board

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Editorial

Endoplasmic Reticulum Stress and Lipid Metabolism

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Endoplasmic reticulum (ER) is an elaborate cellular organelle essential for protein folding, calcium homeostasis, and lipid biosynthesis. Disruption of ER homeostasis imposes stress on the ER and subsequently leads to accumulation of unfolded or misfolded proteins in the ER lumen—a condition termed ER stress. In response to ER stress, a group of intracellular signaling pathways originated from the ER, collectively termed ER stress response, are activated to alter transcriptional and translational programs in the stressed cells. ER stress response has been linked to various human diseases associated with dyslipidemia, such as inflammatory diseases, obesity, diabetes, alcoholic and nonalcoholic liver diseases, and cardiovascular diseases. Understanding the impact of ER stress signaling pathways on lipid metabolism will provide important information for the prevention and treatment of these common human diseases in the modern world.

The paper by C. Ji provided an updated overview of the potential mechanisms involved in alcohol-induced ER stress and organ injuries. The alcoholic injuries and roles of ER stress in major organs including liver, pancreas, brain, and heart were discussed. In addition, several important mechanisms underlying alcohol-induced ER stress were described in detail.

The paper by S. Basseri and R. C. Austin provided an overview of recent findings related to ER stress and hepatic lipid metabolism. Liver is the central organ involved in lipid metabolism. Disruption of hepatic lipid metabolism has been linked to various metabolic diseases. Regulation of hepatic lipid metabolism and ER-stress signaling pathways was described. Furthermore, the therapeutic potential of targeting ER stress signaling pathways in dyslipidemia and obesity was discussed.

The paper by E. B. Thorp discussed the Unfolded-Protein-Response-(UPR)-induced apoptosis in ischemic cardiovascular disease. Ischemia is the major cause of heart failure which is secondary to dyslipidemia, atherosclerosis, and myocardial infarction. The ER stress signaling pathways in cardiomyocyte and role of ER stress in ischemia-induced apoptosis were discussed.

The paper by J. W. Brewer and S. Jackowski focused on the physiological UPR in the regulation of lipid synthesis and membrane biogenesis during the differentiation of B lymphocytes into antibody-secreting plasma cells. This paper described the current understanding of the relationship between the UPR, lipid biosynthesis, and organelle biogenesis in activated B cells. In particular, the authors provided up-to-date information regarding the roles and mechanisms of the UPR signaling pathways in regulating phosphatidylcholine synthesis and ER biosynthesis.

The paper by X. Zhang and K. Zhang discussed the links between ER stress, lipid droplet formation, and type II diabetes. The excessive deposition of lipid droplets in adipocytes, hepatocytes, and macrophages has been recognized as a feature of many metabolic diseases, including type II diabetes. Increasing evidence suggests that ER stress response regulates the lipid droplet formation that is associated with the pathogenesis of type II diabetes. This

paper summarized the recent advances in understanding ER stress-associated mechanisms in lipid droplet formation and its involvement in type II diabetes.

The paper by B. S. Zha and H. Zhou provided an updated overview of ER stress response in lipid metabolism in adipocytes. Adipocytes are one of the major cell types involved in the pathogenesis of the metabolic syndrome. Recent advances in identifying the role of ER stress in regulating lipid metabolism in adipocytes and potential links among ER stress, inflammation, and autophagy were discussed.

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Review Article

The Myocardial Unfolded Protein Response during Ischemic Cardiovascular Disease

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Heart failure is a progressive and disabling disease. The incidence of heart failure is also on the rise, particularly in the elderly of industrialized societies. This is in part due to an increased ageing population, whom initially benefits from improved, and life-extending cardiovascular therapy, yet ultimately succumb to myocardial failure. A major cause of heart failure is ischemia secondary to the sequence of events that is dyslipidemia, atherosclerosis, and myocardial infarction. In the case of heart failure postmyocardial infarction, ischemia can lead to myocardial cell death by both necrosis and apoptosis. The extent of myocyte death post-infarction is associated with adverse cardiac remodeling that can contribute to progressive heart chamber dilation, ventricular wall thinning, and the onset of loss of cardiac function. In cardiomyocytes, recent studies indicate that myocardial ischemic injury activates the unfolded protein stress response (UPR) and this is associated with increased apoptosis. This paper focuses on the intersection of ischemia, the UPR, and cell death in cardiomyocytes. Targeting of the myocardial UPR may prove to be a viable target for the prevention of myocyte cell loss and the progression of heart failure due to ischemic injury.

1. Introduction

Heart failure (HF) is a common condition and leading cause of hospitalization in the United States and developed countries. HF can be debilitating and lead to reduced cardiac output, physical disability, and mortality. The numbers of HF cases in the USA are increasing, in line with a rise in the elderly population who are at increased risk [1, 2]. Common causes of HF include ischemic heart disease (including myocardial infarction), hypertension, cardiomyopathy, and valvular heart disease. In the case of ischemic heart disease and myocardial infarction (MI), advances in patient care have reduced the risk of susceptibility to MI and of immediate death. Thus, while there has been an increase in the numbers who initially survive an acute MI, this improvement has been offset by more survivors progressing to HF [3]. This deterioration often leads to left ventricular systolic dysfunction and can be linked to the initial cardiac damage and remodeling early after myocardial ischemia. Thus, new therapeutic targets and treatments are needed to combat the morbidity and mortality caused after MI-induced HF.

1.1. Cardiomyocyte Death in HF. To date, the failure of a heart to deliver blood that is sufficient for the metabolic needs of the body is largely irreversible. Loss of cardiomyocytes by cell death contributes to reduced cardiac output. In the case of myocardial infarction, acute ischemia can lead to significant levels of cardiomyocyte death. Myocardial ischemia after MI is a significant cellular stress that promotes cardiomyocyte death by either necrosis or apoptosis [4]. In patients, increased myocardial apoptosis has been associated with unfavorable ventricular remodeling and early symptoms of post MI heart failure [5]. Adverse cardiac remodeling involves scar and fibrous tissue formation, whereby the chambers of the heart enlarge and contractility become less efficient [6]. At the cellular level, death of the cardiomyocyte depends on the duration of ischemia and also on the capacity of the myocyte to respond to the ischemic stress. Numerous cellular responses have been identified in cardiomyocytes under ischemic stress and HF. For example, autophagy is activated in HF and may suppress hypertrophy through increased protein degradation [7]. Accumulating evidence

indicates that another significant stress response in cardiomyocytes can affect cell survival. During ischemia, the unfolded protein response (UPR) or integrated stress response is activated in myocytes, as described below.

1.2. The UPR. In noncardiac cells, the UPR signals from the endoplasmic reticulum [8], which is responsible for the synthesis and folding of proteins, as well as calcium storage and other signaling pathways. Under conditions that perturb endoplasmic reticulum homeostasis, the ER has the capacity to adapt and activate the UPR to compensate and attempt to restore organelle equilibrium [8]. The function of the UPR is to protect the ER from normal and pathophysiological perturbations in development and disease that include elevated protein synthesis, disruption of ER calcium homeostasis, changes in redox potential, and disturbances in the physical properties of the ER membrane bilayer [9, 10]. The UPR is composed of three main signaling branches. These include inositol-requiring enzyme-1 (IRE-1) [11] activating transcription factor-6 (ATF6) [12] and PKR-like eukaryotic initiation factor 2 kinase (PERK) [13]. Activation of the UPR regulates multiple compensatory gene expression pathways, including induction of protein-folding chaperones, phospholipid biosynthesis, oxidoreductases, and the promotion of terminally misfolded protein degradation, through the ER-associated degradation pathway (ERAD) [8, 14, 15]. The UPR also exerts translational control by phosphorylating the eukaryotic initiating factor eIF2α and selectively reduces protein translation to lessen the load on the ER [16]. These compensatory pathways act first in an attempt to reconstitute cell and ER homeostasis. If homeostasis is restored, this induces a negative feedback of the UPR [17]. If disequilibrium persists, proapoptotic pathways can be induced [18], as discussed below.

1.3. SR/ER and the UPR. Within the cardiomyocyte, the sarcoplasmic reticulum (SR) is a specialized endoplasmic reticulum and extensive network within the cell that regulates calcium (Ca^{2+}) flux and excitation contraction coupling. Under conditions of heart disease, the SR is expanded, consistent with a compensatory response to stress [19]. Through the years, the terms sarcoplasmic reticulum and endoplasmic reticulum have been used interchangeably. Indeed, numerous canonical ER proteins, including protein chaperones, can be found in myocytes after relatively crude biochemical fractionations of the SR. Such ER proteins that have been identified in cardiac tissue include Bip, Grp94, calnexin, PDI, and others [20–22]. Cardiomyocytes, like other cells, require these proteins and chaperones to promote protein folding and other housekeeping functions synonymous with the ER. In addition to encoding canonical ER-resident proteins, cardiomyocytes can also activate the UPR in response to characteristic UPR inducers, such as protein-folding disequilibrium. For example, the Lys-Asp-Glu-Leu (KDEL) receptor, an ER retrieval receptor for protein chaperones, promotes chaperone accumulation in the ER/early secretory pathway. In an experimental model of forced gene activation, transgenic expression of a dysfunctional KDEL receptor induced UPR markers in myocardial tissue [23]. Such protein-folding disorders in the heart have also been linked to cardiomyocyte death, as transgenic overexpression of preamyloid oligomers induces apoptosis in cardiomyocytes [24]. In another example of myocardial protein dysregulation, a R120G mutation in CryAB (crystallin, alpha B), a small heat shock protein, is linked to familial cardiomyopathy. This mutation induces CryAB protein aggregation and in mice, overexpression of R120G mutant CryAB induces cardiomyopathy, whereas overexpression of its wild-type counterpart does not [25]. Furthermore, conditions of increased protein synthesis, such as during hypertrophy, appear to activate the UPR [26]. Some have interestingly suggested that the SR and ER are spatially and functionally distinct [27, 28]. Regardless of this distinction, cardiomyocyte stress induces the UPR, and conditions that can adversely affect protein folding, similar to in noncardiomyocytes, are toxic in the myocardium and linked to activation of UPR pathways.

1.4. Ischemic Stress. In experimental models of myocardial ischemia, activation of UPR chaperones has been shown to occur during development of ischemic heart disease [29]. Ischemia is a major contributor to heart failure, and the reduction in supply of oxygen to the heart is a significant stress on myocardial tissue. Even prior to myocardial infarction, expanding atherosclerotic plaque in coronary arteries reduces blood flow and oxygen in downstream coronary tissue. Loss of perfusion leads to a drop in oxygen and a transition to glycolytic energy production. Ischemic myocardium is characterized by reduced oxidative phosphorylation and increased anaerobic metabolism [30]. Reliance on glycolysis and accumulation of inorganic phosphate also lead to cellular acidification through increases in lactic acid production [31]. These factors in combination can significantly compromise cellular energy production by reducing generation of adenosine triphosphate (ATP). Ischemia also contributes to mitochondria dysfunction. In heart cells, mitochondria swell and release cytochrome C, contributing to contractile dysfunction [32]. When prolonged, ischemia will promote caspase-mediated apoptosis in cardiomyocytes. *In vitro*, ischemia can be simulated through deprivation of serum, glucose, and oxygen (SGO). Ischemia, in other tissues, has been shown to lead to the impairment of protein folding in the ER, leading to activation of the UPR. Hypoxia alone leads to dysfunctional disulfide bond generation by oxygen-dependent protein disulfide isomerase and this in turn leads to protein misfolding and activation of the UPR [33]. Reoxygenation effects on the UPR in cardiomyocytes also are a significant factor [34]. Below, we highlight how ischemia can lead to modulation of the UPR in the heart. Ischemia has been linked to the activation of all three arms of the UPR as described next.

2. The Cardiomyocyte UPR

2.1. The Cardiomyocyte IRE-1α Pathway and Ischemia. The ER transmembrane protein IRE-1α is homodimerized during ER stress to induce autophosphorylation. Homodimerization is induced by sequestration of GRP78/Bip through

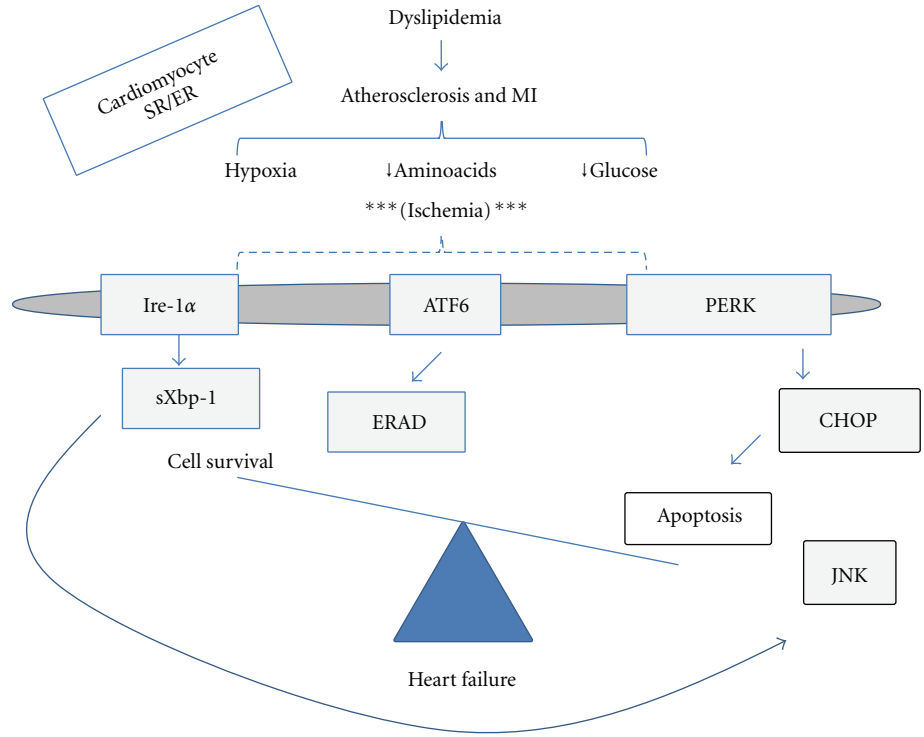

Figure 1: Working model of the cardiomyocyte unfolded protein response during ischemia. Activators of the UPR in cardiomyocytes at the sarcoplasmic reticulum (SR)/endoplasmic reticulu (ER) include ischemia during cardiovascular disease. Proximal effectors of the UPR include IRE-1α, ATF6, and PERK. IRE-1α induces splicing of *Xbp-1* mRNA and can promote prosurvival or proapoptotic pathways. ATF6 proteolysis leads to transcription of ER stress responsive genes and has been implicated in cardiomyocyte cell survival. Initial effects of PERK include translational arrest that reduces the load on the SR/ER folding machinery. Downstream and distal effector responses of PERK include CHOP, which promotes cardiomyocyte apoptosis and may contribute to heart failure.

an accumulation of misfolded protein in the ER [35]. ER stress also activates an IRE-1α endoribonuclease activity that splices X-box-binding protein-1 (*Xbp-1*) mRNA (Figure 1). Spliced *Xbp-1* (*sXbp-1*) encodes a basic leucine-zipper and active form of XBP-1, which induces ER stress response genes [36, 37] that escape PERK-mediated translational arrest (discussed below). The requirement for the UPR in the heart and the IRE-1α pathway begins during embryonic development. GRP78 (guanine-nucleotide-releasing protein 78), an IRE-1α and ATF6 downstream target, is upregulated in the embryonic mouse myocardium. In addition, *Xbp-1* is required for heart formation as *Xbp-1* deficient mice die in utero. *Xbp-1* knockout mice death occurs in association with significant cardiomyocyte death [38]. Consistent with a prosurvival role for XBP-1, inhibition of IRE-1α reduces chemokine-induced autophagic cell death in H9c2 cardiomyocytes [39]. In experimental models of ischemia (in hearts post MI), the IRE-1α downstream target GRP78 is upregulated in myocardial tissue proximal to the infarct [40]. *Ex vivo* (in a Langendorff heart perfusion system), GRP78, and *sXbp-1* are induced during simulated ischemia and reperfusion [41]. *In vitro*, primary neonatal rat cardiomyocytes exposed to serum, glucose, and oxygen deprivation (SGO) can induce spliced *Xbp-1* mRNA, and this occurs within hours [42]. As evidence for a causal role of XBP-1 during ischemia, adenoviral dominant negative XBP-1 expression resulted in increased hypoxia-reoxygenation-induced apoptosis. The IRE-1α pathway has also been implicated in proapoptotic pathways as well. For example, in noncardiomyocytes, IRE-1α can interact with the adaptor protein TNF receptor-associated factor (TRAF2). IRE-1α and TRAF2 subsequently act on ASK1 (mitogen-activated protein kinase kinase kinase), which phosphorylates proapoptotic JNK [43]. Less is known regarding how such a proapoptotic IRE-1α pathway may function in cardiomyocytes. In addition, calcium dysregulation is an important component of ischemic heart failure and upregulation of sarco/endoplasmic reticulum calcium-ATPase isoform 3f (SERCA3f) is associated with heart failure [44]. Experimental overexpression of SERCA3f has been shown to induce *Xbp-1* splicing. Also, cardiomyocyte-specific disruption of the calcium regulator *Serca2* induces the UPR and promotes apoptosis [44]. Overexpression of the downstream target of XBP-1, GRP94 reduced H9c2 cardiomyocyte necrosis induced by both calcium overload and ischemia [45]. Thus, although the aforementioned examples indicate a significant role for the IRE-1α pathway in cardiomyocyte survival and during calcium regulation, much remains to be understood, including how the prosurvival roles of IRE-1α signaling may differentially act during development versus after ischemic injury.

2.2. Activating Transcription Factor 6 (ATF6) in the Heart. On activation of the UPR, ATF6 travels to the Golgi, where its cleavage leads to the translocation of its cytosolic fragment to the nucleus and binding to ER stress response elements (ERSEs). Cleaved ATF6 then promotes transcription of ER-targeted genes, such as the ER chaperone, GRP78. In mice after MI, inhibition of ATF6 activation with 4-(2-aminoethyl) benzenesulfonyl fluoride, an inhibitor of ATF6, impaired cardiac function and increased mortality. In contrast, cardiac function after MI was improved in mice expressing a constitutively active mutant of *Atf6*, compared with wild-type littermates [46] and consistent with a protective role. In primary murine cardiac myocytes exposed to oxygen and nutrient deprivation, membrane-associated ATF6 was reduced with a concomitant increase in nuclear ATF6 [47]. This ischemia-induced event was accompanied by ATF6 binding to the ERSE of GRP78, transcriptional upregulation of GRP78 and was reversible by simulated reperfusion *in vitro*. More importantly, a dominant-negative form of ATF6 prevented inducement of Grp78 and promoted cardiomyocyte cell death, indicating a prosurvival role for ATF6. ATF6 has also been shown to induce ER-associated degradation (ERAD). ERAD has been shown to alleviate ER stress by degrading misfolded protein in the ER [15]. Interestingly, Belmont et al. discovered that Derlin-3, a component of ERAD, is induced by ATF6 in the mouse heart [48]. Furthermore, overexpression of Derlin-3 protected cardiomyocytes *in vitro* from simulated ischemia-induced apoptosis. In another article by Belmont, transcriptional profiling indentified *modulatory calcineurin interacting protein-1* (*MCIP1*), also known as *regulator of calcineurin 1* (*RCAN1*), as a novel ATF6-inducible gene. They found that ATF6 was able to induce RCAN1 in cultured cardiac myocytes and that adenoviral overexpression of activated ATF6 further induced RCAN1 and modulated cell growth [49]. Thus, ATF6 is induced under ischemic conditions and can play a role to help protect cardiomyocyte survival. Interestingly, an ATF6 isoform and other ATF6-related proteins may play a role in regulating the UPR, however, their full roles in cardiomyocytes remain undetermined and should be subject of future investigation [50].

2.3. Cardiomyocyte PERK (dsRNA-Activated Protein Kinase-Like Endoplasmic Reticulum Kinase). Though the IRE-1α and ATF6 branches have for the most part been associated with prosurvival roles in cardiomyocytes, prolonged activation of the PERK/ATF4/CHOP pathway is principally implicated in cardiomyocyte cell death. Downstream of PERK, phosphorylation of eIF2α can be detected as early as one hour after ischemia *in vitro* in cardiomyocytes [42]. Eukaryotic translation initiation factor 2α (eIF2α) phosphorylation leads to a transient downregulation of the majority of protein synthesis through inhibition of cap-dependent protein translation. Only transcripts encoded by ER stress response genes are induced, reducing the demands on the ER. This may have implications in prevention of cardiac hypertrophy and is part of the initial compensatory pathway of the PERK branch towards promoting survival. Under prolonged ER stress, C/EBP homologous protein (CHOP) is induced. Myocardial tissue from patients with heart failure exhibits increased *Chop* mRNA. Okada et al. reported that prolonged ER stress occurs in hypertrophic and failing hearts after aortic constriction [51]. Also, *Chop* deficiency reduces cardiac apoptosis in a pressure overload model of heart disease [52]. CHOP has also been implicated in dilated cardiomyopathy [23]. *In vitro*, in heart cells, prolonged ER stress induced by ischemia promoted the activation of CHOP [42], processing of procaspase-12 and induction of apoptosis. Consistent with activation by ischemia, *Chop* transcription is also regulated by amino acid starvation. For example, an upstream cis amino acid response element in *Chop* has been found to bind activating transcription factor 2 (ATF-2) and expression of ATF-2 is required for the transcriptional activation of *Chop* by leucine starvation *in vitro* [53]. In support of this pathway being activated during ischemia, ATF-2 is stabilized by hypoxia [54]. More recently, prostatic androgen repressed message-1 or PARM was identified to be predominantly expressed in cardiomyocytes and a negative regulator of CHOP-mediated apoptosis [55]. Finally, *Chop* deficiency has been shown to reduce myocardial reperfusion injury in a mouse model of MI [56]. Future studies *in vivo* are warranted to separate the effects of CHOP after ischemia as opposed to after reperfusion.

3. Discussion

Although treatments for heart failure have advanced, the incidence of HF is still rising and new therapies remain an important goal. There is now mounting evidence of a significant role for the UPR in cardiomyocytes during ischemic heart disease. Much remains to be understood with respect to how individual branches of the UPR differentially or synergistically contribute to progression of heart failure and how these pathways differ from requirements of the UPR during development. In addition, the therapeutic and prophylactic potential of modulating the heart is far from complete. Some have suggested that ischemic preconditioning of the heart and activation of the UPR may promote cardiac cell survival. Interesting proofs of principle have been published. For example, *in vitro*, overexpression of ER-stress-induced Grp94 has been shown to inhibit cardiomyocyte necrosis after calcium overload and simulated ischemia [45]. Overexpression of GRP78 has also been shown to have an effect. Forced GRP78 expression inhibited apoptosis in rat ventricular myocytes [57]. Also, preconditioning of H9c2 neonatal cardiomyocytes cells with the ER-stressor tunicamycin has been shown to protect against ATP deletion [58]. These are laudable starts, but much work remains to be done. Future questions remain. For example: What is the effect of chemical chaperones on cardiac stress pathways and cardiac function [59]? Future studies will also be required to dissect the effects of cell-specific deletion of UPR genes in the heart, including cardiomyocytes, myofibroblasts, and inflammatory cells that infiltrate into the myocardium after injury. UPR-targeted therapies may be realized by promoting the cytoprotective function of the UPR in the myocyte. Such an approach may induce UPR-specific ER chaperones and downstream

prosurvival pathways that work to enhance cardiac function and prevent cardiomyocyte death.

Abbreviations

ATF6: Activating transcription factor-6
BIP: Binding immunoglobulin protein
eIF2α: Eukaryotic initiation factor 2
ER: Endoplasmic reticulum
ERAD: ER-associated degradation pathway
Grp94: Glucose-regulated protein
HF: Heart failure
IRE-1: Includes inositol-requiring enzyme-1 (IRE-1)
MI: Myocardial infarction
PERK: PKR-like eukaryotic initiation factor 2 kinase
PDI: Protein disulfide isomerase
SR: Sarcoplasmic reticulum
UPR: Unfolded protein response.

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Review Article

Endoplasmic Reticulum Stress-Associated Lipid Droplet Formation and Type II Diabetes

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Diabetes mellitus (DM), a metabolic disorder characterized by hyperglycemia, is caused by insufficient insulin production due to excessive loss of pancreatic β cells (type I diabetes) or impaired insulin signaling due to peripheral insulin resistance (type II diabetes). Pancreatic β cell is the only insulin-secreting cell type that has highly developed endoplasmic reticulum (ER) to cope with high demands of insulin synthesis and secretion. Therefore, ER homeostasis is crucial to the proper function of insulin signaling. Accumulating evidence suggests that deleterious ER stress and excessive intracellular lipids in nonadipose tissues, such as myocyte, cardiomyocyte, and hepatocyte, cause pancreatic β-cell dysfunction and peripheral insulin resistance, leading to type II diabetes. The excessive deposition of lipid droplets (LDs) in specialized cell types, such as adipocytes, hepatocytes, and macrophages, has been found as a hallmark in ER stress-associated metabolic diseases, including obesity, diabetes, fatty liver disease, and atherosclerosis. However, much work remains to be done in understanding the mechanism by which ER stress response regulates LD formation and the pathophysiologic role of ER stress-associated LD in metabolic disease. This paper briefly summarizes the recent advances in ER stress-associated LD formation and its involvement in type II diabetes.

1. Introduction to ER Stress

ER is an intracellular organelle where dynamic protein folding and assembly, storing cellular calcium, and lipid biosynthesis occur. A variety of biochemical or pathophysiological stimuli can interrupt protein folding process in the ER by disrupting protein glycosylation, disulfide bond formation, or ER calcium pool. These disruptions can cause the accumulation of unfolded or misfolded proteins in the ER lumen, a condition termed as "ER stress" [1, 2]. To protect cells from proteotoxicity caused by ER stress, the unfolded protein response (UPR) is activated through attenuating general protein translation, increasing in protein folding capacity, and expediting degradation of misfolded proteins. Three major ER stress sensors or transducers have been found: inositol-requiring 1α (IRE1α), double-stranded RNA-dependent protein kinase- (PKR-) like ER kinase (PERK), and activating transcription factor 6 (ATF6), which have been comprehensively reviewed [2, 3]. The UPR signaling, mediated through ER stress sensors, modulates transcriptional and translation programs in cells under ER stress. As a double-edged sword, the UPR provides survival signals at the initial phase of stress response, leading to cell adaption to ER stress [1, 2, 4]. When ER stress gets prolonged, the UPR can induce cell death programs to kill the stressed cells. In recent years, the scope and consequence of ER stress and UPR have been significantly expanded. Many pathophysiologic stimuli, such as oxidative stress, proinflammatory stimuli, fatty acids, and energy fluctuations, can directly or indirectly cause ER stress and the UPR activation in specialized cell types, such as macrophages, hepatocytes, and pancreatic β cells [2, 5]. The UPR signaling is fundamental to the initiation and progress of a variety of diseases, including metabolic disease, cancer, cardiovascular disease, and neurodegenerative disease [2, 6, 7].

2. LD Formation

LD, also known as adiposome or fat body, has been found ubiquitously present in lipid-overloaded cells from yeast to mammals [8, 9]. For a long time, LD was thought simply as an inert lipid storage reservoir since its earliest description in 19th century. The discovery of perilipin, an LD-associated protein that coats LD in adipocytes, makes researchers to challenge the understanding of LD as lipid storage [10]. LD is now recognized as a dynamic organelle composed of a monolayer phospholipid, embedded with numerous proteins without transmembrane spanning domains, and a hydrophobic core that contains triacylglycerols (TGs) and sterol esters [11, 12]. TGs are the key neutral lipid required for LDs formation in adipocytes. Deletion of genes encoding enzymes responsible for neutral lipid synthesis eliminated LDs formation [13]. Evidence showed that, without DGAT enzymes, LDs cannot form in adipocytes. Therefore, by segregation of extra TG or hydrophobic molecules into LDs, cells are protected from lipotoxicity. These features make LD a regulatory organelle in lipid homeostasis. The biogenesis and assembly of LD are still largely unknown. It has been suggested that ER is the site where LD is synthesized and assembled. Over ninety percent of LDs were found in close apposition to the ER [14]. ER budding model, Bicelle model, and vesicular budding model have been suggested to explain how LD is formed in ER [15]. Perhaps, the most accepted model is ER budding model in which LD originated between the two leaflets of ER bilayer buds into the cytosol. Newly formed LD can increase its size (0.2 μm–20 μm in diameter) by homotypic fusion that depends on microtubule system, most likely motor protein dynein. Under this mechanism, the growth of LD may proceed without ongoing biosynthesis of TGs and sterol esters [16, 17].

3. ER Stress and LD Formation

LD formation has been proposed as an exit model in the removal of unfolded or misfolded proteins or some ubiquitinated proteins from the ER [18, 19]. LD may serve as a transient depot to sequester unfolded or misfolded as well as excessive proteins to alleviate ER stress (Figure 1). Diverse groups of LD-associated proteins were found in yeast *S. cerevisiae*, *Drosophila* embryos, and human hepatocyte cell line Huh7 [20–22]. Some of the LD-associated proteins, such as Acl-CoA synthetases, lanosterol synthetase, and GAPDH, are conserved from yeast to human. The proteins detected in LD seem to be specific, since the organelle-specific proteins, including lactate dehydrogenase (LDH) (cytosolic marker), integrin (plasma membrane marker), calnexin (ER marker), and GS28 (Golgi marker), were hardly detected in LD fractions [22]. Interestingly, a number of proteins which were thought to be organelle-specific, including histones (nucleus), caveolins (plasma membrane), HSP70 (cytosol), ApoB (ER), and Nir2 (Golgi), were detected in LD fraction [23]. Furthermore, LD dynamically interacts with ER, peroxisomes, mitochondria, and plasma membrane [15]. LD can be transported along microtubules, following the same way that the ER, Golgi, and mitochondria were positioned and delivered [24]. It was proposed that the dynamical interactions between LD and the other compartments facilitate the exchange of proteins and lipids in cells. The LD is functionally and structurally similar to the extracellular counterpart of lipoprotein particles [15, 21]. This notion was supported by the finding that LD provides a platform for degradation of excessive ApoB protein by converging ubiquitin-proteasomal and autophagy-lysosomal pathways, thereby preventing cytotoxicity resulted from aggregation of excessive proteins [25]. Previous studies have shown that disruption of ER functions leads to the accumulation of intracellular lipids [26–28]. Disrupted protein glycosylation or ER-associated protein degradation by ER stress-inducing reagents, such as tunicamycin and brefeldin, has been demonstrated to increase LD accumulation in budding yeast *Saccharomyces* cerevisiae or mammalian cells [28, 29]. Previously, it is known that intracellular LD formation is through the lipogenic program activated by sterol regulatory element-binding proteins (SREBPs). Recent study suggested that more ER-localized, stress-responsive protein factors, such as hepatocyte-specific cAMP responsive element-binding protein (CREBH), can also regulate lipogenic programs to promote LD formation under metabolic stress signals, such as insulin and saturated fatty acids [30]. Moreover, ER stress response may directly facilitate LD synthesis and assembly as a mechanism to defend intracellular stress [29, 31] (Figure 1). This is consistent with the observations that lipids can be recruited to the stressed cells to sequester misfolded proteins in the ER at the early stage of ER stress and that the ER is expanded significantly to alleviated ER stress independent of the UPR [23, 32].

4. LD Formation and Type II Diabetes

Previous studies demonstrated that excessive accumulation of lipids in peripheral tissues is closely associated with insulin resistance in type II diabetes [33, 34]. Although ER stress and UPR pathways in metabolic disease have been extensively reviewed, ER stress-associated LD formation, which is independent of UPR pathway, did not draw much attention. The interaction between LD and mitochondrial might affect the peripheral tissue insulin resistance [35, 36] (Figure 1). Recent studies indicated that insulin resistance is not simply associated with the amount of intracellular lipids. Despite elevated lipids content in skeletal muscle of the trained enduring athletes, the insulin-signal in these individuals is still markedly sensitive [36]. The combination of weight loss and physical activity in obesity improves insulin sensitivity and reduces the size of LD, but not the overall intramyocellular lipid [37]. One possible explanation for these phenomena is that increased mitochondrial oxidative activity for lipid oxidation may decrease insulin resistance. This is supported by the facts that lower oxidative capacity is found in insulin resistant skeletal muscle and that exercise can improve the capacity for lipid oxidation [36]. Several mitochondrial proteins including prohibitin, a subunit of ATP synthase, and pyruvate carboxylase were identified in LD fractions by proteomic analysis [35]. In addition, numerous lipid metabolic enzymes, such as hormone-sensitive

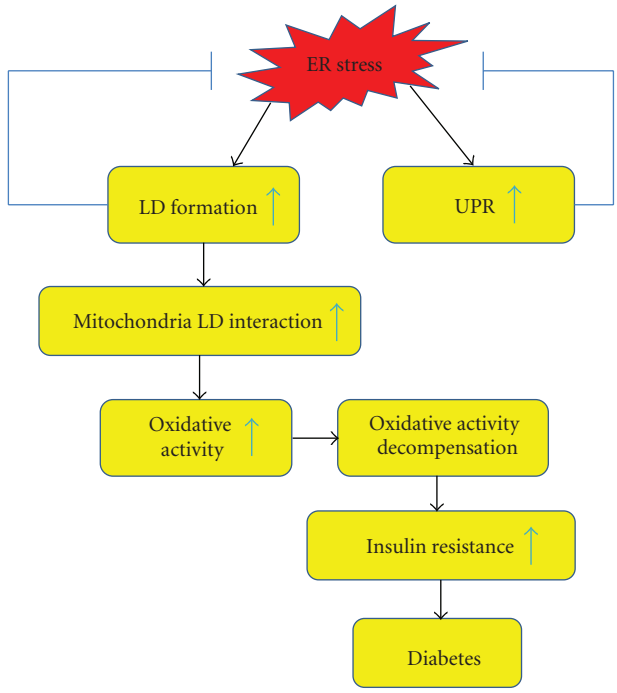

Figure 1: Interactions between ER stress, oxidative stress, and lipid droplets in type II diabetes. LD, lipid droplet; UPR, unfolded protein response.

lipase, lanosterol synthase, and acyl-CoA synthetase, were also found to be associated with LD complex, and the overall LD protein composition can be changed in response to lipolysis stimulation [35, 38]. Despite these observations, further study is required to explore how mitochondria communicate and interact with LD in metabolic processes.

Fat-specific protein 27 (Fsp27) is a member of cell death-inducing DNA fragmentation factor family proteins that is localized to LD. Fsp27 plays an important role in lipid storage and mitochondrial activity in adipocytes [39–41]. Genetic depletion of Fsp27 in mice is characterized by increased glucose uptake, improved insulin sensitivity, and significantly increased mitochondrial metabolism [39, 40]. Small sizes of LDs and increased mitochondrial activity were found in Fsp27-deficient white adipocytes, suggesting that ectopic LD formation represents an imbalance between lipid supply and lipid oxidation in peripheral tissue. Likely, LD-associated proteins and the interactions between LD and the other intracellular organelles may play direct roles in the pathogenesis of diabetes [42]. Type II diabetes is often correlated with increased serum levels of proinflammatory cytokines secreted by ER stress-activated macrophage. Previous research demonstrated that the proinflammatory cytokine TNFα blunts the insulin signaling pathway therefore causing insulin resistance by activating the JNK1/2 signaling pathway which is involved in serine phosphorylation of IRS1 (insulin receptor substrate 1) [43, 44]. However, a new study by Ranjit found that proinflammatory cytokines, such as TNFα, IL1β, and INFγ, act on lipolysis by decreasing the expression of FSP27 and the size of LD in adipocytes [45]. Since decreased FSP27 is evidenced to improve insulin resistance and LDs, it is likely that the proinflammatory cytokines play double-edged roles in type II diabetes.

5. Conclusion

Accumulating evidence demonstrated a strong link between ER stress, LD formation, and type II diabetes. It is important to note that ER stress response is a fundamental stress signaling underlying many life styles, such as air pollution, chronic alcohol consumption, and smoking, which may be associated with the development of metabolic disease [46–48]. Therefore, for the future research, it is important to delineate ER mechanisms in LD formation that is associated with the development of type II diabetes. Key questions include what is the mechanism by which ER stress regulates LD formation? Is there any ER chaperones or UPR targets present in the LD complex? Does ER stress-associated LD formation provide survival or devastating pathways in the progression of type II diabetes? Is it possible to modulate LD formation by targeting ER stress signaling? Answering these questions will benefit and direct the future understanding and treatment of type II diabetes and the other types of metabolic disease.

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Review Article

ER Stress and Lipid Metabolism in Adipocytes

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The role of endoplasmic reticulum (ER) stress is a rapidly emerging field of interest in the pathogenesis of metabolic diseases. Recent studies have shown that chronic activation of ER stress is closely linked to dysregulation of lipid metabolism in several metabolically important cells including hepatocytes, macrophages, β-cells, and adipocytes. Adipocytes are one of the major cell types involved in the pathogenesis of the metabolic syndrome. Recent advances in dissecting the cellular and molecular mechanisms involved in the regulation of adipogenesis and lipid metabolism indicate that activation of ER stress plays a central role in regulating adipocyte function. In this paper, we discuss the current understanding of the potential role of ER stress in lipid metabolism in adipocytes. In addition, we touch upon the interaction of ER stress and autophagy as well as inflammation. Inhibition of ER stress has the potential of decreasing the pathology in adipose tissue that is seen with energy overbalance.

1. Introduction

In the last two decades, the complexity of adipose tissue has finally become apparent. Investigations surrounding the biological impact of obesity, insulin resistance, and the metabolic syndrome have surged, resulting in a more intricate understanding of "fat." Adipose tissue (AT) is not only highly specialized to store long-term energy, but is also a central endocrine organ. Therefore, AT is inherently involved in the interplay of inflammatory cascades and energy metabolism, which are important players in metabolic disorders. Even more, sick fat, or adiposopathy, has now been coined an independent endocrine disease [1].

Adiposopathy can occur environmentally through overnutrition. Adipocytes store extra energy in the form of triglycerides (TG) inside cytosolic organelles (lipid droplets, or LD). When there is a continuous need to store TGs, adipocytes must expand in size while continuously being stressed to synthesize more proteins for LD formation. There is an inherent threshold at which adipocytes become too stressed, secrete multiple cytokines, and can no longer expand. The cytokines released activate resident macrophages and call in circulating macrophages, which begin to attempt to engulf these cells, forming the signature "crownlike structures" found in obese tissue [2].

During this cascade, increased cytokines can increase adipocyte lipolysis. Increased lipolysis leads to an increase of circulating free fatty acids (FFA) that are deposited in muscle and liver ("lipid dumping") and results in a decreased insulin sensitivity in these tissues (reviewed in [3]). Particularly, FFA from visceral AT is directly deposited into the portal vein, increasing the risk of fatty liver disease. This may be the underlying basis of current clinical understanding that increased visceral fat is a high-risk factor for cardiovascular disease [4, 5].

An increase in FFA release is not only induced by an inflammatory state in AT, but also cellular insulin insensitivity. For this reason, most literature focusing on adipocyte dysregulation in metabolic disease concentrates on the nutrient sensing pathways. However, another important pathway involved in adipocyte pathology is the induction of endoplasmic reticulum (ER) stress. In the past, overstimulation of ER stress has been linked to diseases of genetics and aging (reviewed in [6]), but may in fact be involved in more environmentally induced diseases as well. This paper

discusses the recent understanding regarding the role of ER stress in regulating lipid metabolism in adipocytes and the clinical consequence therein.

2. ER Stress in the Adipocyte

Numerous cellular pathways can be altered in times of stress, leading to cellular aberrations and dysfunction. However, in the realm of overnutrition, ER stress is arguably the most common and important [7–10]. The ER is central for protein folding, secretions (e.g., cytokines), calcium homeostasis, and lipid synthesis. In the adipocyte, the ER is directly involved with LD formations and maintenance of lipid homeostasis.

Inducing ER stress is relatively effortless *via* depletion of ER calcium stores, changes in ER lipid membrane composition, reactive oxygen species (ROS), or accumulation of misfolded and/or unfolded proteins. When triggered, the ER signals to the cell through the unfolded protein response (UPR) to aid in increased production of proteins needed for protein folding, while decreasing transcription and increasing degradation of other nonessential proteins. If the UPR is unable to return the ER to homeostatic conditions, it will trigger apoptosis.

A central component of the UPR is an ER chaperone protein, BiP/GRP78. In homeostatic conditions, BiP/GRP78 is bound to three ER membrane resident proteins. An insult that alters ATP in the lumen decreases calcium, or increases a demand for protein folding causes GRP78 to unbind. These three proteins, ER transmembrane kinase/endoribonuclease **IRE1**, double-stranded RNA-activated protein kinase-like ER kinase (**PERK**), and activating transcription factor 6 (**ATF-6**), trigger a cascade upon their release, which ultimately leads to the activation of transcription factors that upregulate protein chaperones, proteasome components, and with continuous activation, turns on GADD-153/**CHOP** (C/EBP homologous protein), a major transcriptional factor responsible for ER-stress-induced apoptosis.

2.1. IRE1. Upon release from GRP78, IRE1 transautophosphorylates, activating its RNase activity. The activated IRE1 specifically acts on its downstream target X-box-binding protein 1 (**XBP1**) and removes a 26 base pair intron sequence of XBP1 resulting in the formation of spliced XBP1 (XBP1^s). There are multiple targets of XBP1^s, such as ER protein chaperones and proteins involved in ER-associated degradation (ERAD) [11–13]. However, beyond the traditional genes it activates, the biological function of XBP1^s has now been shown to be more diverse.

In fact, XBP1's ability to induce many ER proteins, and increase expansion of the rough ER [14] has demonstrated its necessity in ER biogenesis. Specific and elaborate knockout models have demonstrated this further; when the ER was poorly developed, secretory cells subsequently failed to function [15, 16]. Sriburi et al. have found that overexpression of XBP1^s in preadipocytes induces upregulation of the rate-limiting enzyme in phosphatidylcholine synthesis (CTP: phosphocholine cytidylyltransferase or CCT) [14, 17]. As this is the major phospholipid found in the ER membrane, it follows that XBP1 increases ER biogenesis by both stimulation of ER proteins and membrane components.

This activity of XBP1 is most likely not cell specific, due to the already described centrality of this transcription factor in secretory cell types and hepatocytes. What is of interest in adipocytes, however, is the close interplay of ER biogenesis and LD formations. LDs, as mentioned previously, are a central organelle in adipocytes, though they also are found to a much lesser extent in other cells such as hepatocytes and macrophages. LDs are known to contain a core of triacylglycerols and cholesterol, but the multiple proteins found in their phospholipid monolayer are only beginning to be understood [18]. Although it is already known that the ER assembles and processes the lipids and proteins needed for LD formation, it is not fully known how they are transferred. The formation of a naïve LD is hypothesized to occur when neutral lipids accumulate at the ER membrane and then bud off. However, others propose LDs form as a bicelle or vesicular budding. In addition, the ER may in fact remain linked to LDs, allowing free exchange of proteins [19, 20].

Beyond the debate on whether these two organelles are physically linked, there is no dispute on the centrality of CCT. When CCT is limited, LDs begin to fuse due to less phosphatidylcholine on their surface [21]. Even more, when one gene of CCT was knocked down 60% in drosophila, there was a significant increase of triacylglycerol content [21]. This may be a compensation in which diacylglycerols normally utilized in the CCT pathway are now channeled to neutral lipids in the LDs. Nonetheless, the main end is larger and denser LDs with less active CCT.

The link between CCT, LDs, and the UPR is most likely the foundation of the essential nature of the IRE1-XBP1 pathway in adipogenesis. XBP1-shRNA-treated preadipocytes fail to differentiate, and only transduction of the XBP1^s rescued cells [22]. *In vivo* mouse models are more difficult to handle, as the full XBP1 knockout die *in utero* [15]. To circumvent this, one group has placed a liver-specific XBP1 gene into this model, but even these mice die during the neonatal starvation period [16]. These mice are smaller with a negligible white adipose mass, even compared to their heterozygous counterparts.

The mechanism underlying XBP1's significant role may be due to the upregulation of CCAAT/enhancer-binding protein-α (C/EBPα) [22]. CCAAT/enhancer-binding proteins are essential transcription factors in adipogenesis, with β and δ being major players in early differentiation and α essential in mid- to late differentiation. Sha et al. found that XBP1^s upregulates C/EBPα, and C/EBPβ increases transcription of XBP1 [22]. Therefore, XBP1 is integral in the loop of transcriptional activation of adipocyte differentiation as well as the functional maturation of LD formation.

2.2. PERK. The PERK-eIF2α pathway is another UPR leg involved in adipogenesis. When released, PERK transautophosphorylates leading to activation of its kinase domain. The major result of this is phosphorylation of eukaryotic translation initiation factor 2α (eIF2α). In the phosphorylated state, this essential component of the translational machinery cannot recycle GTP, inhibiting general translation

but at the same time increasing the translation of mRNAs which contain internal ribosome entry sites, such as ATF-4, BiP/GRP78, and SREBP-1 [23–25].

Activating transcription factor (**ATF**)-4 is a well-studied protein involved in the UPR (reviewed in [26]). This transcription factor is heavily involved in increasing amino acid metabolism and protein transport [27, 28]. Importantly, ATF-4 also upregulates stress-related transcription factors ATF-3 and CHOP. CHOP is a central transcription factor involved in cellular perturbations, including inhibition of adipocyte differentiation [29–31], and ultimately inducing apoptosis. However, there is still necessity of balance as although high induction of ATF-4 will lead to CHOP activation, complete absence will affect AT lipogenesis [32]. More studies are needed to fully understand the role of ATF-4 in lipogenesis in adipocytes.

In contrast, more is understood about **SREBP**s (sterol regulatory element-binding proteins). SREBPs are additional transcription factors found in the ER membrane. There are three isoforms- SREBP-1a, -1c, and -2. SREBP-1c is involved in fatty acid synthesis and lipogenesis, -2 in cholesterol synthesis, and -1a in both pathways. The SREBPs are retained in the ER *via* insulin-induced gene (Insig) binding to SREBP-cleavage-activating-protein-(SCAP-) bound SREBP. At times of sensed decreases in cholesterol or fatty acids, SCAP-SREBP dissociates from Insig and relocates to the Golgi where SREBP is cleaved by two site proteases (S1P and S2P). The mature form of SREBP further translocates to the nucleus, activating genes involved in cholesterol and lipid metabolism, such as 3-hydroxy-3-methylgutaryl-CoA (HMG-CoA) synthase, HMG-CoA reductase, squalene synthase, acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS). Therefore, disruption of ER homeostasis not only alters protein production, but also affects cholesterol and fatty acid synthesis.

Normally, SREBPs are released when there is a sense of depletion of cholesterol or lipids in the ER membrane. However, SREBP1 processing is also regulated through PERK-eIF2α. In fact, knockout of PERK substantially decreases active SREBP1 in mammary glands [33]. This is most likely a result from the recent finding that SREBP1 contains an internal ribosome entry site [23]. Therefore, activation of ER stress will redundantly lead to active SREBP1 through both upregulation of translation and release of protein from the membrane.

In adipocytes of the SREBPs isoform, -1c is the most highly expressed. SREBP1c is an essential transcription factor during adipogenesis (and thus has a dual name of adipocyte determination and differentiation 1/ADD1). Likewise, the PERK pathway has also been found to be important during differentiation of adipocytes *in vitro* [33]. Overexpression of ADD1/SREBP1c leads to an increase of LD formation in preadipocytes, while conditional overexpression in mouse AT inhibits normal mass growth [34]. In addition, SREBP1c has been shown to directly activate C/EBPβ [35], further supporting its role in adipogenesis. The contradictory results demonstrated with the above mouse models may demonstrate the balance needed by all transcription factors for functional and normal AT.

2.3. ATF-6. There are two genes encoding ATF-6, α, and β. The α isoform is a strong transcriptional activator [36], and the form classically studied during UPR activation. When ATF-6 is released from GRP78, it is translocated to the Golgi *via* a localization signal that was hidden when in the bound form. In the Golgi, ATF-6 is cleaved by the same proteases that process SREBPs, releasing the active cytoplasmic domain, which is a transcription factor. Here, ATF6α heterodimerizes with XBP1 and upregulates genes with the ER stress response element (ERSE) in their promoters, including GRP78 [37] and other ER chaperone proteins, CHOP, and even XBP1 (reviewed in [38]).

In the realm of UPR activation altering lipid metabolism in adipocytes, not much has been noted in the literature concerning ATF6. Knockout mouse models of either ATF6α or β do not show any striking physiological changes, but have allowed for the clarification that ATF6α is the more essential isoform for the ER stress pathway [39], though β is also involved [36]. Some work has recently demonstrated that ATF6 activation plays a role in the liver to control lipid deposition [40, 41] through inhibition of SREBP-2 [42]. What is of more importance in the adipocyte is the direct function of ATF6 to upregulate XBP1, described above as central in adipogenesis.

ATF6α heterodimerizes with XBP1^s in the nucleus to activate genes downstream of UPR activation. However, it is currently not shown if this relationship is also required for upregulation of C/EBPα, or CCT activity. More investigations are needed to completely elucidate the direct function of ATF6 in adipocyte lipid metabolism.

3. Autophagy, the UPR, and Lipid Metabolism Dysregulation

Autophagy is a self-protective cellular pathway activated by multiple stimuli including viral infection, perceived starvation, organelle dysfunction, and ER stress (discussed below). However, just as in the case of UPR, autophagy has the ability to increase cellular damage or cell death when overstimulated. The multifaceted autophagic pathway is continuously being studied, as is the capacity of this process to help regulate metabolism in mammalian cells. In the past few years, an expanding area of research has unfurled around autophagy and lipid metabolism regulation. In hepatocytes, autophagosomes aid in the control of lipid accumulations by delivering LDs to lysosomes [43]. Similarly, in neurons altered autophagy leads to lipid accumulation [44]. Due to its obvious role in lipid metabolism, Singh and colleagues have now coined this leg of autophagy as lipophagy, in which lipid droplets are degraded through autophagy rather than lipolysis [45].

Further, components of the autophagosome may be necessary for lipid droplet formations [46]. This link was found through the microtubule-associated protein 1A/1B light chain 3 (**LC3**), an essential protein in the autophagy pathway. At induction of autophagy, a double membrane sequesters components of the cytoplasm through the coordination of multiple proteins and membrane expansion. During the initial stages, cytosolic LC3-I is activated through

other autophagic-specific proteins by cleavage and lipidation, converting it to membrane-bound LC3-II. Shibata et al. have found that LC3-II does not only colocalize to autophagosomes (the specific autophagy sequestering vacuoles), but also to LDs in hepatocytes and cardiac myocytes [46]. This same group has also demonstrated that LC3 colocalizes to LDs in differentiating adipocytes by using LC3-siRNA [47]. The siRNA of LC3 drastically decreased the ability of adipogenesis [47]. LC3-II has been shown to have tethering capacity to help the fusion of autophagosomes to lysosomes [48]. Therefore, there is a hypothesis that LC3-II is acting to bring LDs into the autophagosome pathway for downstream lipid breakdown [43, 49]. This would provide another pathway of lipid flux beyond lipases acting directly on the LD.

Knockout models have demonstrated how essential autophagy is in adipogenesis. Baerga et al. were able to establish this by first showing the significant increase of autophagosome formations during induction of adipogenesis, followed by the inhibition of differentiation in a knockout *atg5* mouse model [50]. *Atg5* encodes a protein that is required similarly to LC3 for the maturation of the preautophagosome. Using this model, Baerga et al. saw both *in vitro* and *in vivo* that inhibition of autophagy restrained maturation of preadipocytes, resulting in a marked reduction of WAT in neonatal mice (this mouse model is not able to survive the neonatal starvation period). Of most interest, in the knockout mouse embryonic fibroblasts induced to differentiate, cells that began to mature died through apoptosis, while those in the same culture that did not begin to differentiate remained alive. This study was followed by another with adipose-specific deletion of *atg7* [51], the gene encoding an essential protein upstream of Atg5. Interestingly, WAT tissue of this knockout model was more characteristic of BAT in both morphology (smaller cells and LDs) and enzyme levels. The importance of Atg7 in adipogenesis was confirmed by Singh et al. who knocked down the same gene, but used slightly different cell lines and mouse model [43]. However, both groups came upon the same finding that the autophagic pathway is essential in adipogenesis.

The trigger of autophagy activation during adipogenesis is currently not known. However, PPARγ, an essential transcription factor of adipogenesis, may be involved. In one cancer cell line, it was found that PPARγ agonists can activate the autophagy pathway [52]. Yet, there is another study that contradicts these findings [53], and such investigations have not yet been repeated in an adipocyte model. Nonetheless, the summation of above experiments does demonstrate that autophagy is essential in adipogenesis, and without, may cause a transdifferentiation of WAT to BAT. On the other hand, a decrease of autophagy in the liver leads to lipid overload in hepatocytes. Intuitively, the difference lies in the biology of the two cell types, where adipocytes are normally storing lipids and hepatocytes are not. In metabolic disease states, such as the metabolic syndrome, it is easy to conceive how dysregulation of autophagy could ultimately lead to fatty liver with increased TG storage in the liver and decreased storage in AT.

4. Autophagy and ER Stress

Autophagy and ER stress pathways are not disconnected from one another as previously assumed. In contrast, activation of both can aid in cell survival at times of stress. For one example, autophagy offers an alternative pathway for degradation of proteins when ER-activated proteasomes can no longer handle the load [54–58]. In addition, activation of cell death of each pathway may be interlinked. While classic knowledge is based on ER stress activating apoptosis through CHOP upregulation and autophagy-mediated cell death via a completely separate process, recent findings demonstrate that these two cell death pathways are interlinked.

In more noxious circumstances, it has been shown that cell death through prolonged UPR activation can occur through autophagy-induced cell death [55]. Likewise, inhibition of autophagy increases cell viability with prolonged ER stress [59–61]. However, in nutrient overload and metabolic disorders, impaired autophagy can increase ER stress [62], perhaps due to decreased aberrant protein degradation and energy turnover needed to maintain ER homeostasis. This complex crosstalk of ER stress with the autophagy pathway is not yet well understood. Recently, it was found that ER stress activation can inhibit Akt phosphorylation, the upstream inducer of autophagy at times of perceived starvation [63]. However, the responsible protein(s) are still not known and may even be cell-type specific [64].

Another link is hypothesized to occur through the PERK pathway of the UPR [65, 66]. Some studies have shown that PERK phosphorylation of eIF2α leads to an upregulation of LC3 [58]. Yet, it has not been shown if this is directly from eIF2α phosphorylation inducing LC3 translation, or through ATF-4 activation increasing *Atg12* transcription [67, 68]. In fact, our current studies suggest that HIV Protease inhibitor (PI)-induced activation of autophagy is closely linked to ER stress *via* the ATF-4 pathway. We have found that those HIV PIs that induce metabolic side effects in the clinic also induce ER stress and autophagy in hepatocytes and adipocytes. The corresponding activation of autophagy seems to be one of the underlying factors by which HIV PIs induce dysregulation of lipid metabolism.

Recent studies have shown a strong link between activation of ER stress, increased autophagy induction, and increased SREBP activity leading to lipid overload in hepatocytes [69], although a mechanism remains to be determined. One group of investigators has demonstrated the capability of SREBP-2 to directly upregulate the expression of autophagy essential proteins [70], giving significance to a previous finding that cholesterol depletion leads to autophagy induction in multiple cell lines [71]. Additionally, knockdown of SREBP-2 decreased LC3 association with LDs in hepatocytes [70]. Although SREBPs are not a current forefront of proposed activators of autophagy, it is probable that in times of cellular lipid depletion, LDs are processed for more essential cellular requirements, and this pathway can be activated through ER stress-induced activation of SREBPs. Although these investigations have not been completed in adipocytes, our laboratory has found that in addition to HIV PIs inducing ER-stress and autophagy in adipocytes,

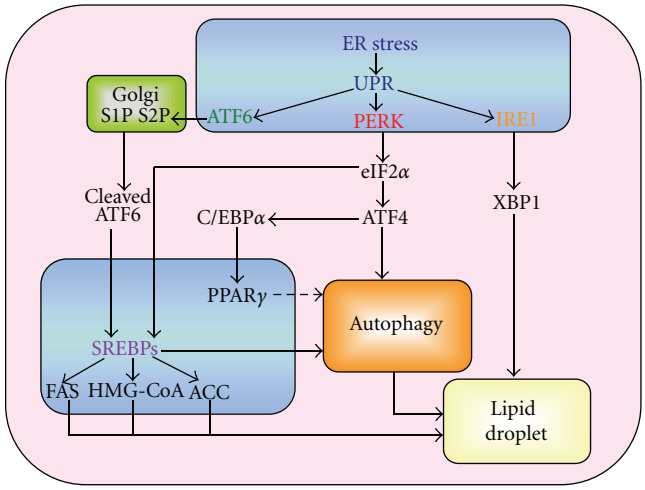

Figure 1: Potential link between ER stress signaling pathways and lipid droplet formation in adipocytes.

SREBP-1c activation is also altered. Until more investigations are completed, the exact stream can only be hypothesized (Figure 1).

5. ER Stress and Inflammation

Obesity and resulting metabolic diseases such as insulin resistance are now known to be strongly associated with chronic inflammation, a substantial risk factor for further complications, most notably atherosclerosis. Increased plasma concentrations of IL-6 and TNF-α have been repeatedly noted in obese individuals [72–74]. Investigations into mechanisms underlying obesity and diabetes has demonstrated that inflammation in AT can detrimentally alter human physiology.

With increasing overload, adipocytes begin to hypertrophy. Cells become stressed from the actual expansion and from exceeding an adequate oxygen diffusion distance in tissue [75, 76]. Adipocytes then signal with a release of proinflammatory IL-6 and TNF-α cytokines, which activate resident macrophages as well as induce infiltration of circulating macrophages. Stressed adipocytes are subsequently engulfed, resulting in the formation of characteristic crown-like structures.

During this process, released IL-6 and TNF-α from stressed adipocytes and activated macrophages can inhibit adipogenesis [77]. In fact, TNF-α alone is enough to inhibit induction of PPARγ and C/EBPα [78]. Even more, the induction of inflammation can also lead to insulin resistance in AT, already well known and continuously investigated [79–82]. Taken together, the ability to store excess energy in AT is drastically decreased with the decrease of mature adipocytes and the death of cells.

Even more, ER stress has been shown to be activated at times of overnutrition [8]. In adipocytes, ER stress can be activated due to the need of LD synthesis, enzyme production, and conversion of energy to TG at times of overnutrition. Importantly, ER stress has repeatedly been shown to induce the cellular inflammatory cascade through the c-Jun N-terminal kinase (**JNK**) pathway, and JNK has been shown to be upregulated in AT of obese individuals [83, 84]. Additionally, ER stress may trigger the adipocyte inflammatory cascade through PERK activating IKB kinase β (**IKKβ**) when cells are stimulated with free fatty acids [85]. This pathway is also known to be a heavy regulator of inflammatory cytokine release and, together with JNK activation, would lead to the proinflammatory state seen in AT in metabolic disease states.

Proinflammatory profile at times of overnutrition is not unique to AT, but occurs throughout the body. However, AT is unique in that it is solely responsible for the subsequent decrease of adiponectin secretion. Adiponectin is an adipocyte-specific anti-inflammatory cytokine that negatively correlates with cardiovascular disease and fatty liver disease [86–88], with a decrease of secretion in overexpanded or stressed tissue [89]. It has been found that adiponectin can alleviate ER stress [90]. Zhou et al. have shown that ER stress initiation is sufficient to decrease adiponectin release. In animal models, they demonstrated that stabilization of adiponectin protein can decrease obesity-induced ER stress in AT [90]. *In vitro*, induction of autophagy could alleviate ER stress responses and subsequently stabilize adiponectin secretions [91]. These are promising findings, and more studies are needed to determine if upregulation of autophagy could ultimately lead to therapeutic options for metabolic diseases (Figure 2).

6. Future Directions

We have provided ample references demonstrating that ER stress can induce lipid metabolism dysregulation in adipocytes. Such an assertion is not only important for interested molecular biologists, but for clinicians as well. It has been shown that fat depots of obese patients have increased ER stress [84, 92, 93]. What is more, there may be a link between ER stress upregulation, the inflammatory state of this tissue, and insulin resistance [84, 92, 94, 95].

The cycle of overnutrition, ER stress, and AT pathology is complex. With the information provided here and our own findings, we support the hypothesis that inhibiting ER stress activation may be therapeutically beneficial in the treatment of metabolic diseases. Chaperones, which enhance ER-protein-folding capacity, have shown potential in the laboratory.

Two chaperones already FDA approved have been studied in hepatocytes, adipocytes, and β-cells for their ability to relieve ER-stress-induced dysfunctions, namely, 4-phenylbutyric acid (PBA) and taurine-conjugated ursodeoxycholic acid (TUDCA). Both were shown to relieve insulin resistance in adipocytes at times of ER stress [96, 97]. In addition, they were able to decrease JNK and IKKβ activity when cells were stimulated with ER stress inducers, including free fatty acids [85, 96]. *In vivo*, PBA and TUDCA were able to relieve ER stress activation in obese mice [96]. However, further studies are needed to confirm these beneficial effects and elaborate on the extent that chaperone treatment may aid in nutrition overload-induced ER stress and downstream alterations.

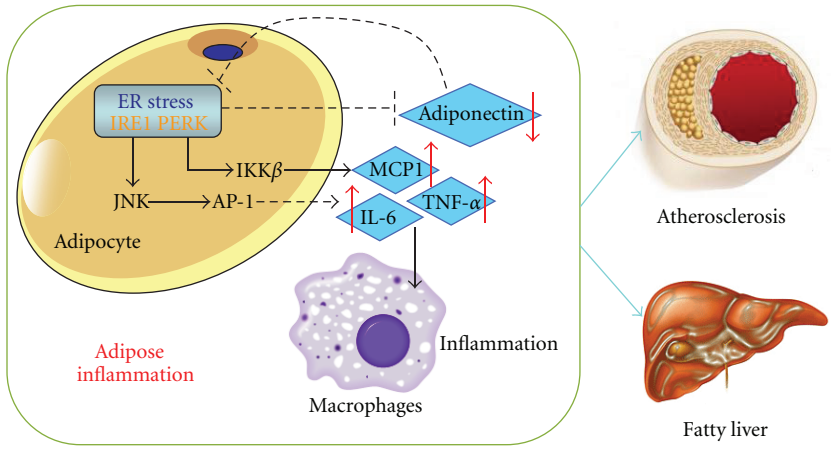

Figure 2: ER-stress-induced inflammation in adipocytes and macrophages contributes to atherosclerosis and fatty liver diseases.

Inhibiting ER stress activation may be the key to an approach for metabolic syndrome therapy. However, more questions remain in this field. Namely, the role of all parts of the UPR in adipocyte lipid metabolism needs to be uncovered, and the mechanism intertwining ER stress and autophagy needs to be further elucidated. Understanding these missing components will allow not only further understanding of key lipid pathways in a central metabolic cell type, but also help determine the best approach that can be utilized for clinical metabolic dysfunctions in patients with altered AT physiology.

Abbreviations

ACC: Acetyl-CoA carboxylase
Add1: Adipocyte determination and differentiation 1
AT: Adipose tissue
ATF: Activating transcription factor
CHOP: C/EBP homologous protein
CCT: CTP phosphocholine cytidylyltransferase
eIF2α: Eukaryotic translation initiation factor
ER: Endoplasmic reticulum
FAS: Fatty acid synthase
FFA: Free fatty acids
Insig: Insulin-induced gene
HMG-CoAR: 3-Hydroxy-3-methylgutaryl-CoA reductase
IKKβ: IKB kinase β
IRE1: Inositol requiring enzyme 1
IRS1: Insulin response substrate
JNK: cJun N-terminal kinase
LC3: Microtubule associated light chain protein
LD: Lipid droplet
PBA: Protein-1 namely 4-phenylbutyric acid
PERK: PKR-like eukaryotic initiation factor 2α kinase
SCAP: SREBP cleavage activating protein
SREBP: Sterol regulatory element binding protein
TUDCA: Taurine-conjugated ursodeoxycholic acid
TG: Triglyceride
UPR: Unfolded protein response
XBP1: X-box binding protein.

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Review Article

Endoplasmic Reticulum Stress and Lipid Metabolism: Mechanisms and Therapeutic Potential

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The endoplasmic reticulum (ER) plays a crucial role in protein folding, assembly, and secretion. Disruption of ER homeostasis may lead to accumulation of misfolded or unfolded proteins in the ER lumen, a condition referred to as ER stress. In response to ER stress, a signal transduction pathway known as the unfolded protein response (UPR) is activated. UPR activation allows the cell to cope with an increased protein-folding demand on the ER. Recent studies have shown that ER stress/UPR activation plays a critical role in lipid metabolism and homeostasis. ER-stress-dependent dysregulation of lipid metabolism may lead to dyslipidemia, insulin resistance, cardiovascular disease, type 2 diabetes, and obesity. In this paper, we examine recent findings illustrating the important role ER stress/UPR signalling pathways play in regulation of lipid metabolism, and how they may lead to dysregulation of lipid homeostasis.

1. Introduction

The liver plays a central role in whole body lipid homeostasis. Metabolic signals such as carbohydrates and dietary fatty acids regulate hepatic gene expression leading to glycolytic and lipogenic signalling pathways. In addition, the pancreatic hormones, insulin and glucagon, play a pivotal role in the transcriptional and posttranslational regulation of lipogenesis and lipid oxidation [1]. Lipogenesis, the process of *de novo* lipid biosynthesis, occurs when an excess of carbohydrates is consumed, or when circulating insulin levels are high. Carbohydrates undergo glycolysis to generate acetyl-CoA molecules which are the building blocks for fatty acid (FA) synthesis. Following esterification, one glycerol molecule and three FA chains produce triacylglycerol (TG) molecules which are transported in apoB containing very low-density lipoprotein (VLDL) particles [2] to the adipose tissue for long-term storage. Under fasting conditions when insulin levels are low and glucagon levels are high, FA oxidation or lipolysis occurs which allows for mobilization of FA and uptake by the liver [3]. However, disruption in these homeostatic mechanisms may lead to the development of dyslipidemia, insulin resistance, fatty liver, and excess adipose mass, ultimately causing cardiovascular disease and diabetes.

In recent years, increasing evidence suggests that ER stress and UPR activation can regulate cellular processes beyond ER protein folding and can play crucial roles in lipid metabolism [4–10]. ER stress, which occurs due to disruption in ER protein-folding capacity, leads to activation of an evolutionarily conserved UPR signalling system in order to restore ER homeostasis [11]. Accumulating evidence suggests that activation of the UPR pathways can modulate lipid metabolism by controlling the transcriptional regulation of lipogenesis. Excess adipose mass and obesity are a direct consequence of increased *de novo* lipogenesis and TG storage in the adipose tissue. The presence of ER stress has been observed in various tissues from obese mice [12, 13] and humans [14–17]. UPR activation has also been linked to fatty liver disease where lipid droplets accumulate in hepatocytes. The role of ER stress and UPR pathways in the development of fatty liver disease has been under intense investigation (reviewed in [18]). Here, we aim to examine the evidence regarding the role of UPR pathways in modulating

the transcriptional regulation of lipid metabolism. Furthermore, potential therapeutic approaches targeting the ER stress response in obesity and dyslipidemia will be discussed.

2. Transcriptional Regulation of Lipid Metabolism

A number of key transcription factors have been identified which regulate hepatic lipogenesis and fatty acid oxidation. These include sterol-regulatory-element-binding protein-1c (SREBP-1c), liver X receptor (LXR), peroxisome-proliferator-activated receptors (PPARs), and carbohydrate-responsive-element-binding protein (ChREBP). Enzymes such as glucokinase (GK), liver pyruvate kinase (LPK), acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl CoA desaturase-1 (SCD-1) are critical for the biochemical conversion of glucose into fatty acids and TG [19, 20].

SREBP-1c, a member of the SREBP family of transcription factors, is thought to be the main driving force for hepatic lipogenesis and development of fatty liver disease known as hepatic steatosis [21, 22]. There are three isoforms, SREBP-1a, -1c and -2. SREBPs are synthesized as inactive precursors bound to the ER membrane [23]. While SREBP-1a/-2 upregulate cholesterol synthesis genes, SREBP-1c is responsible for the regulation of genes involved in FA and TG synthesis pathways [24]. Under sterol-replete conditions, SREBPs are held in the ER through their interaction with SCAP, an anchoring molecule, and Insig, an ER transmembrane protein. The SREBP-SCAP complex is released from Insig upon sterol-deplete conditions. SCAP assists in the transport of SREBP from the ER to the golgi for cleavage by site 1 and 2 proteases [25]. Following proteolytic cleavage, the active mature form of SREBP translocates into the nucleus where it induces genes required for lipid biosynthesis and uptake [26].

SREBP-2 is the main transcription factor responsible for regulating the cholesterol biosynthetic pathways [27]. Cholesterol is the precursor for steroid biosynthesis and plays an important role in membrane biology. Excess unesterified intracellular cholesterol can lead to membrane disruptions and cellular toxicity and hence must be tightly regulated [28]. Therefore, under sterol-deplete conditions, SREBP-2 is cleaved and translocates to the nucleus allowing for expression of its target genes, including HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis [29, 30].

SREBP-1c is the predominant isoform and the main regulator of lipid synthesis in the liver [31]. Overexpression of the active form of SREBP-1 in the liver leads to hepatic steatosis due to increased lipid synthesis, uptake, and TG accumulation [32], while loss of SREBP-1 has been linked to marked reduction in both lipogenesis and hepatic steatosis [22, 33]. Interestingly, the proteolytic cleavage of SREBP-1c is not affected by sterol depletion [34]. Proteolytic cleavage and activation of SREBP-1c is stimulated by insulin [35]. Insulin-mediated SREBP-1c activation occurs through insulin receptor substrate-1 (IRS1) and activation of its downstream targets protein kinase B (PKB/Akt) and mammalian target of rapamycin complex 1 (mTORC1) [34]. Although the exact mechanism by which insulin stimulates SREBP-1c cleavage is not entirely understood, it has been shown that insulin leads to phosphorylation of the ER-bound inactive SREBP-1c, increasing its posttranslational processing [36]. Furthermore, insulin represses Insig2 mRNA which is thought to enhance SREBP-1c activation [37, 38].

SREBP-1c activity may also be induced through the nuclear hormone receptor peroxisome-proliferator-activated receptor-γ (PPARγ) [39] as well as liver X receptor (LXR) activity [20], both of which play a critical role in lipogenesis. Ligand-activated nuclear PPARγ heterodimerizes with retinoid X receptors (RXRs) resulting in expression of its target genes such as CD36, a fatty acid transport protein involved in the transport and metabolism of intracellular FA [40]. Ultimately, PPARγ activity allows for transcription of genes involved in promoting lipogenesis [41]. In addition, positive feedback loops have been identified where SREBP-1c activity increases the formation of PPARγ ligands which lead to its activation [42]. PPARγ also leads to LXRα gene expression which is a potent activator of SREBP-1c target genes [40]. PPARα activity, on the other hand, regulates peroxisomal, microsomal, and mitochondrial FA oxidation pathways by transcriptionally regulating enzymes involved in these pathways [40]. Interestingly, LXR competes with PPARα for RXRα heterodimerization, thereby repressing RXRα-PPARα signalling. This in turn suppresses LXR-SREBP-1c activity [40]. This crosstalk would ensure that lipogenic and lipolytic pathways are not simultaneously activated. Finally, lipogenic and glycolytic gene expression may also be regulated by ChREBP, a transcription factor responsive to high glucose levels and important in regulating the expression of LPK, an enzyme required for hepatic glycolysis [43].

In addition to regulation of lipogenic and lipolytic pathways, fatty acid uptake and lipoprotein secretion are also important for lipid homeostasis. Expression of PPARα, for example, leads to mobilization and transport of catabolized fatty acids by inducing expression of enzymes such as fatty-acid-binding protein (FABP) and fatty-acid translocase (FAT) [40]. Fatty acids undergo esterification to form TG which can be exported out of the liver as VLDL particles. ApoB is the key component of VLDL particles and microsomal triacylglycerol transfer protein (MTP) allows for the addition of TG to apoB, forming the VLDL particle. However, the overall rate of VLDL assembly depends on the rate of apoB synthesis in the ER [40].

3. The ER and UPR Activation

The ER is a membranous organelle with several critical cellular functions. First, it is the site where nascent polypeptides fold into their proper conformation and any necessary posttranslational modifications such as glycosylation and disulphide bond formation take place. This task is accomplished by ER resident chaperones and foldases and protein disulphide isomerases (PDI) [11]. Second, phospholipid synthesis takes place in the ER which allows for expansion of

lipid bilayers in the cell [1]. Third, the ER is a major storage site for calcium ions which are required for cellular signalling processes [44]. Fourth, enzymes such as cytochrome p450 in the ER allow for efficient metabolism of drugs [45].

A number of physiological, pharmacological, and pathological conditions are known to disrupt ER homeostasis and affect its protein-folding capacity. The inability of the cell to efficiently fold and secrete proteins is defined as ER stress. Cells have evolved mechanisms to adapt to adverse conditions in order to maintain homeostasis and survive. One such coping mechanism is UPR activation in response to ER stress conditions [46, 47]. Activation of the UPR ultimately results in (i) enhancement of ER protein-folding capacity through expansion of the ER and increased expression of chaperones and foldases, (ii) inhibition of protein translation, and (iii) ER-associated protein degradation (ERAD) of misfolded proteins [48]. If ER stress conditions are not resolved, ER-stress-induced cell death may ensue. Generally, ER-stress-associated cell death occurs through caspase activation [49, 50]; however, caspase-independent necrosis and autophagy have also been observed [51].

The UPR in mammalian cells is composed of three signalling branches which are initiated by three ER transmembrane sensors, inositol-requiring protein 1 (IRE1), double-stranded RNA-dependent protein kinase-like ER kinase (PERK), and activating transcription factor 6 (ATF6). Activation of these sensors is dependent on the dissociation of the ER-resident chaperone glucose-regulated protein of 78 kDa (GRP78), also known as BiP, from their luminal domains [52]. This occurs during ER stress conditions when GRP78 is required for the folding of proteins in the ER and thus is recruited away from IRE1, PERK, and ATF6, thereby activating the UPR. Activation of the UPR pathways is often used as an indicator of ER stress due to the technical difficulties in directly measuring compromised ER integrity or protein aggregates in the ER [1]. Figure 1 depicts an overview of mammalian UPR signalling pathways.

Homodimerization and autophosphorylation of PERK following dissociation of GRP78 leads to its kinase activity. PERK phosphorylates the α subunit of eukaryotic initiation factor 2 (eIF2) resulting in translational attenuation [53]. Translation of certain mRNAs with short open reading frames in the 5′-UTR is enhanced by phosphorylation of eIF2α. ATF4 is an example of such mRNA and its expression results in activation of C/EBP homologous protein (CHOP) which is a proapoptotic transcription factor [54]. GADD34 (growth-arrest and DNA-damage-inducible protein 34) is induced by CHOP, which acts to dephosphorylate eIF2α as a negative feedback loop and relieve the cell of the translational repression during prolonged ER stress [55].

Similar to PERK, IRE1 is a type 1 transmembrane serine/threonine receptor protein kinase/endonuclease which upon dissociation of GRP78 homodimerizes leading to autophosphorylation and activation of its kinase and endoribonuclease functions [48]. Unfolded proteins may also directly bind to IRE1 promoting its homodimerization and autophosphorylation [56–58]. Activation of IRE1 results in splicing of *XBP1* mRNA, a process by which a 26-nucleotide sequence of *XBP1* mRNA is excised leading to a shift in its reading frame. Unlike the unspliced XBP1 protein, which is rapidly degraded, spliced *XBP1* (*XBP1s*) encodes a bZIP transcription factor with a potent transactivation domain [59]. *XBP1s* translocates to the nucleus where it leads to expression of a number of UPR target genes including genes involved in protein folding and secretion, protein degradation and ER translocation [1, 60]. Consistent with its transcriptional target genes, *XBP1* is required for the secretory function of certain highly secretory cell types such as antibody-producing plasma cells [61].

ATF6, the third arm of the UPR, is comprised of two transmembrane bZIP transcription factors, ATF6α and ATF6β, which under normal conditions are held in the ER in a complex with GRP78 [62]. ER stress and dissociation of GRP78 from ATF6 leads to its translocation to the Golgi where it is cleaved by site 1 and site 2 proteases, a process similar to that of the SREBPs. The sequential proteolysis by S1P and S2P leads to the release of the N-terminal cytosolic domain of ATF6 which then upon entry into the nucleus activates UPR target genes [63]. Among these target genes are XBP1, CHOP, and ER chaperones such as GRP78 which allow the ER to cope with the increased protein-folding demand [62, 64]. Interestingly, ATF6 and XBP1 possess very similar DNA-binding specificity [60] and can heterodimerize suggesting that they may have common target genes [65].

4. ER Stress and Lipid Metabolism

It is has been known for about a decade that ER stress can lead to altered lipid metabolism and hepatic steatosis. A study by our group demonstrated that homocysteine-induced ER stress can lead to hepatic steatosis and altered cholesterol and TG biosynthetic pathways, both in cultured cells and in livers of hyperhomocysteinemic mice [66]. Overexpression of GRP78, which attenuates ER stress and UPR activation, has been shown to decrease hepatic steatosis by reducing SREBP-1c activity [5]. More recently, specific arms of the UPR and their downstream signalling molecules have been examined in cell culture and animal models to decipher their function and role in lipid metabolism. It is now well established that various components of the UPR signalling network play a role in the regulation of lipid metabolism [4–10]. Figure 2 summarizes the interactions between various components of UPR signalling and lipid metabolism.

4.1. PERK Pathway. Activation of PERK is transient and has often been difficult to detect [10], but recently the Phos-tag gel approach has proven to be a successful tool for detection of PERK phosphorylation [67]. Furthermore, the phosphorylation status of eIF2α, a downstream target of PERK, is often assessed to monitor PERK activity. Changes in nutritional status such as fasting and feeding result in altered phosphorylation status of eIF2α. Fasting followed by 4 hours of feeding leads to an increase in phospho-eIF2α levels in the liver, which were even greater in high-fat-diet-fed mice [10]. To study the effects of compromised PERK-eIF2a-dependent UPR signalling, transgenic mice with enforced expression of GADD34 were generated [10]. GADD34, by associating with

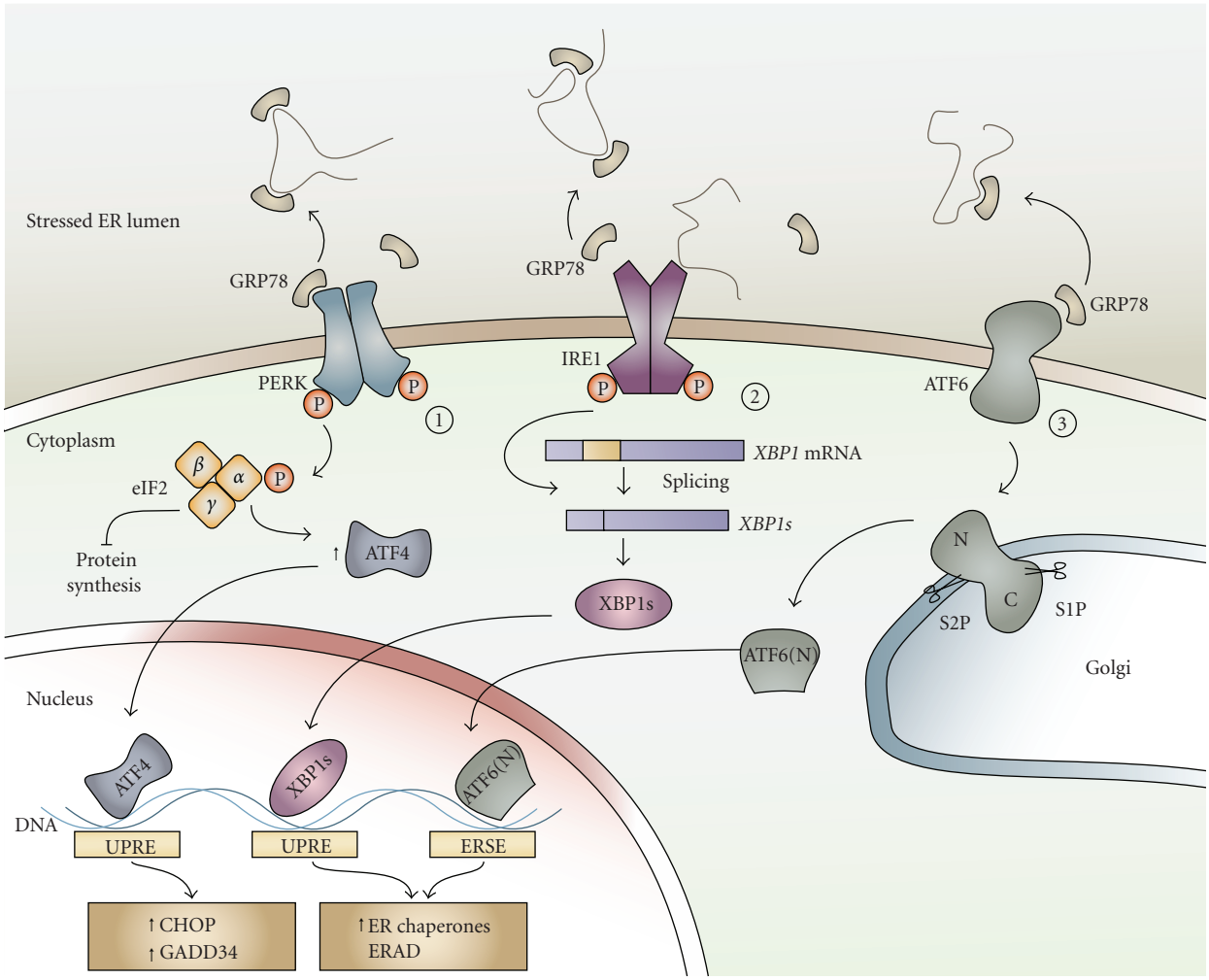

Figure 1: ER stress and activation of the UPR signalling pathways. Accumulation of misfolded or unfolded protein aggregates in the ER lumen, a condition known as ER stress, leads to activation of three ER transmembrane proteins, PERK, IRE1, and ATF6. GRP78, a ubiquitous ER chaperone that is normally bound to these ER stress sensors and keeps them inactive, dissociates from them in order to assist with the folding of proteins in the ER lumen. However, this dissociation leads to activation of the 3 UPR pathways. (1) PERK homodimerization and autophosphorylation results in the subsequent phosphorylation of the α subunit of eIF2 which by inhibiting global protein synthesis reduces the ER protein load. ATF4 expression, however, increases upon eIF2α phosphorylation which translocates to the nucleus allowing for transcription of UPR target genes by binding to the UPR response element (UPRE). These genes include CHOP, a proapoptotic transcription factor that results in cell death if ER stress conditions persist, and GADD34, which acts as a negative regulator of the PERK pathway by dephosphorylating eIF2α. (2) IRE1 is activated in a similar manner to PERK by homodimerization and autophosphorylation. Additionally, interaction of misfolded or unfolded proteins with the luminal domain of IRE1 can also further promote its activation. *XBP1* mRNA is an IRE1 substrate that undergoes splicing to produce *XBP1s*, encoding a transcription factor that can lead to upregulation of ER chaperones and other UPR target genes. (3) ATF6 activation leads to its translocation to the Golgi where it is sequentially cleaved by site 1 and site 2 proteases. This leads to the release of the N-terminal ATF6 fragment which translocates to the nucleus, binds to the ER stress response element (ERSE) thereby activating UPR target genes.

protein phosphatase 1, acts to specifically dephosphorylate eIF2α. Therefore, these mice were defective in activating the gene expression program downstream of eIF2α phosphorylation upon feeding and under severe ER stress conditions [10]. Close examination of the metabolic changes in the transgenic mice indicated that defective eIF2α-mediated signalling results in fasting hypoglycemia, reduced liver glycogen stores, and enhanced insulin sensitivity. Additionally, under dietary stress of a high-fat diet, the transgenic mice exhibited reduced hepatosteatosis and greater insulin sensitivity as compared to wild-type mice [10]. Expression of PPARγ and its lipogenic target genes was reduced in the transgenic mice with the eIF2α phosphorylation defect only when fed a high-fat diet. Repressed expression of C/EBPα and C/EBPβ proteins was also observed in livers of transgenic mice [10].

Rutkowski et al. generated mice harbouring a S51A mutation in eIF2α rendering them unable to phosphorylate

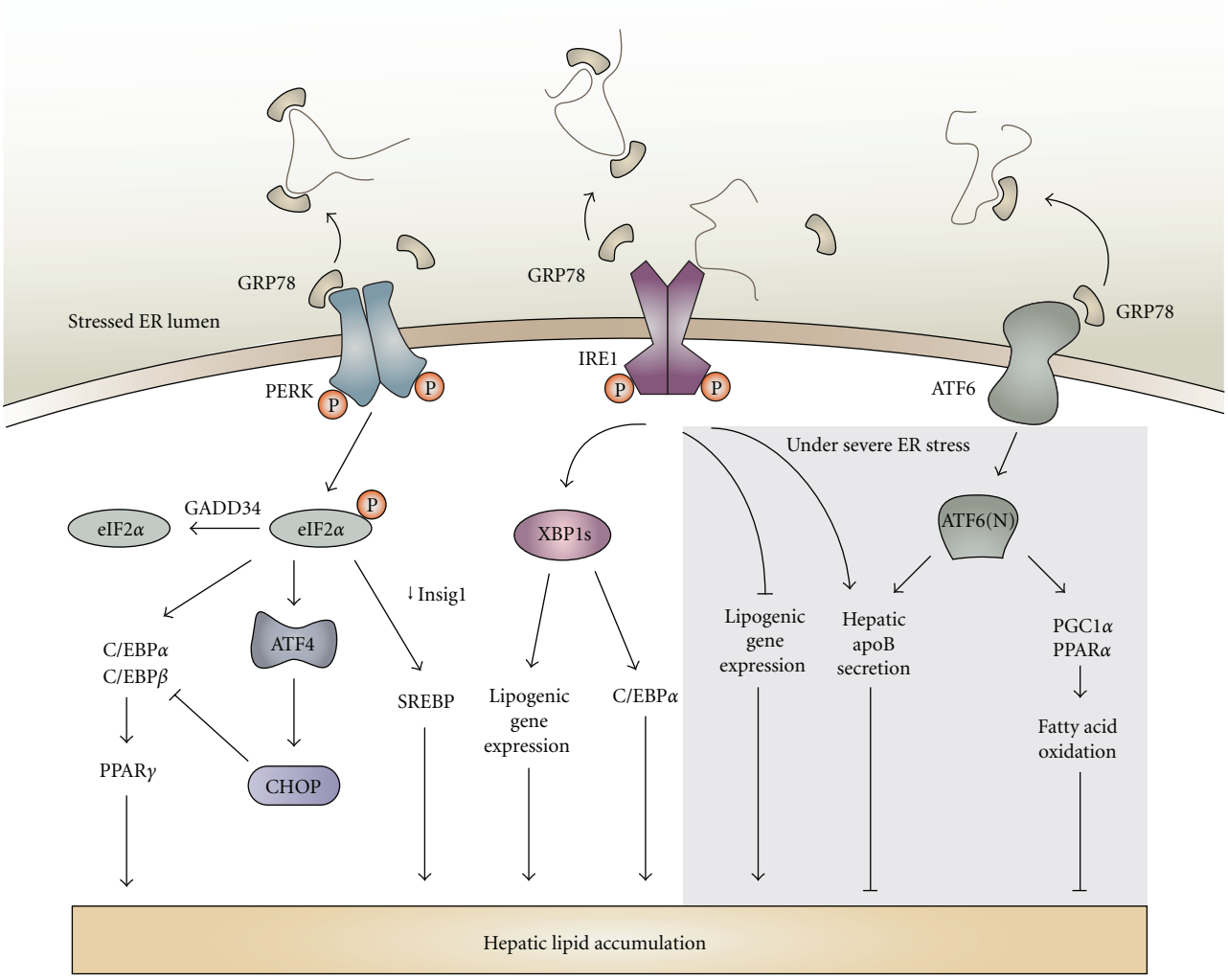

Figure 2: Crosstalk between UPR signalling pathways and lipogenesis. Phosphorylation of eIF2α and activation of the PERK pathway under high-fat diet-induced ER stress conditions allow for enhanced lipogenesis by inducing C/EBPα and decreasing Insig1 protein translation which increases activation of SREBP. However, under severe or prolonged ER stress conditions, CHOP expression may lead to dysregulation of the C/EBPs. Similarly, high-carbohydrate-diet-induced ER stress conditions depend on XBP1 for expression of lipogenic genes and increase of C/EBPα activity, both of which promote lipogenesis. However, severe ER stress conditions, imposed by tunicamycin, lead to XBP1-mediated inhibition of lipogenic gene expression. Furthermore, both XBP1 and ATF6 are important for apolipoprotein B secretion from hepatocytes and activation of fatty acid oxidation pathways (PPARα, PGC1α) under such conditions. These pathways culminate in attenuation of lipogenesis and prevention of fatty liver disease under severe ER stress.

eIF2α and therefore allowing constitutive expression of the unphosphorylated form of eIF2α [7]. This transgenic mouse model was also utilized to examine the PERK/eIF2α arm of the UPR and its role in ER-stress-mediated hepatic lipogenesis [7]. Similar to the findings by Oyadomari et al., mice with constitutive eIF2α expression also exhibited suppressed hepatic C/EBPα protein expression. In contrast, however, after a tunicamycin challenge, these mice developed fatty liver [7]. These differences point to the source and severity of ER stress (chronic and adaptive dietary stress versus direct and acute ER stress challenge) as important factors in the regulation of lipid metabolism.

Another recent study examined the role of PERK in the regulation of lipogenesis in adipocytes and mammary epithelial cells [4]. Absence of PERK in mouse embryonic fibroblasts differentiating into adipocytes and in mammary epithelium attenuated lipogenesis and expression of genes such as *SREBP1*, *SCD1*, *FAS*, and *ACL* [4]. As a result, the mammary glands from PERK-deficient mothers had lower TG and FA content which lead to growth retardation in the pups. This study also demonstrated that SREBP1 activation is dependent on decreased Insig1 translation which occurs due to PERK and eIF2α-dependent translational attenuation [4].

Due to its upstream open reading frames, the *ATF4* mRNA is among the transcripts that escape the global translational attenuation that occurs upon phosphorylation of eIF2α. ATF4-knockout mice exhibit smaller white adipose tissues relative to total body weight [68], which prompted

a closer examination of these mice. Yoshizawa et al. revealed that ATF4 alters glucose metabolism by decreasing insulin sensitivity in the liver, adipose and muscle tissue [68]. Wang et al. reported decreased expression of lipogenic genes and increased beta-oxidation in the white adipose tissue of ATF4-knockout mice [69]. Interestingly, these observations were not reproducible in primary cell cultures which led to the identification of osteoblastic ATF4 expression as the regulator of whole-body energy homeostasis [68]. Taken together, these results suggest that the PERK-eIF2α pathway plays an important role in promoting lipogenesis both in the liver and other tissues.

4.2. IRE Pathway. A recent study by Zhang et al. demonstrated that IRE1α has an important role in preventing ER stress-induced hepatic steatosis [9]. Given that *Ire1α*-null mice die during embryogenesis, hepatocyte-specific *Ire1α*-null ($Ire1\alpha^{Hepfe/-}$) mice were generated to understand the function of IRE1α in hepatocytes. These mice appeared phenotypically normal in the absence of a stress challenge. However, treatment of $Ire1\alpha^{Hepfe/-}$ mice with tunicamycin, an ER-stress-inducing agent that inhibits protein N-glycosylation [46], led to identification of a defective adaptation to ER stress and altered lipid metabolism in the absence of IRE1α [9]. Expression of ER stress-induced proapoptotic transcription factors ATF4, CHOP, and ATF3 were increased in tunicamycin treated $Ire1\alpha^{Hepfe/-}$ mouse livers as compared to control mice. The number of TUNEL-positive apoptotic cells and cleaved caspase-3 expression was also higher in $Ire1\alpha^{Hepfe/-}$ livers [9]. Furthermore, evaluation of hepatic fat content and plasma lipids revealed that $Ire1\alpha^{Hepfe/-}$ livers have enhanced hepatic steatosis and reduced plasma lipids due to suppressed apoB-containing lipoprotein secretion. Increased expression of key lipogenic transcription factors such as PPARγ, C/EBPβ, ChREBP, and LXRα and greater expression of mRNA encoding lipogenic enzymes such as SCD1, DGAT2, DGAT1, and ACC1 were observed in $Ire1\alpha^{Hepfe/-}$ livers, in particular after tunicamycin treatment [9]. Taken together, these findings suggest that IRE1α is required to suppress hepatic lipid accumulation, particularly under severe ER stress conditions.

A report by Iqbal et al. demonstrated that IRE1β may also have an important role in lipid metabolism primarily in intestinal cells [70]. *Ire1β*−/− mice fed a high-fat and high-cholesterol diet developed hyperlipidemia due to enhanced microsomal triglyceride transfer protein (MTP) expression in enterocytes which led to increased chylomicron secretion [70].

Interestingly, XBP-1 a transcription factor downstream of IRE1 activation has a role in hepatic lipid regulation independent of being an ER stress-response mediator [6]. *Xbp1*-null mice die during embryogenesis; however, deletion of XBP1 in the liver led to hypodyslipidemia and reduced expression of genes encoding lipogenic enzymes such as DGAT2, SCD1, and ACC2 [6]. Livers from mice with an XBP1 deletion had diminished hepatic TG secretion and lipid synthesis but the rate of apoB protein turnover was not affected [6]. These findings indicated that XBP1 is required for *de novo* lipid synthesis in the liver. While liver XBP1 deficiency did not itself cause ER stress or any obvious liver or body abnormalities, there was evidence for increased activation of its upstream kinase IRE1, likely due to a regulatory feedback mechanism [6]. This may in part explain why absence of hepatic IRE1α led to increased lipid accumulation, while deficiency in its downstream target XBP1 did not affect steatosis. Increased activation of IRE1 which is required to suppress lipogenesis may be influencing the phenotypic outcome in the mice deficient in hepatic XBP1. XBP1 likely does not regulate ER stress-mediated steatosis as tunicamycin-induced fatty liver occurred both in the presence and absence of spliced XBP1 [7]. There is evidence suggesting that in adipocytes, XBP1 binds to the promoter region of C/EBPα which promotes adipogenesis and lipid deposition [71]. XBP1 also plays an important role in phosphatidylcholine synthesis, the main ER membrane phospholipid which allows for ER biogenesis and expansion under ER stress conditions [72].

4.3. ATF6 Pathway. ATF6 and SREBPs are ER membrane-bound transcription factors and their activation is dependent on cleavage by the same proteases in the Golgi, followed by nuclear translocation of the N-terminal fragment to the nucleus [63, 73]. ER stress has been linked to the activation and cleavage of both ATF6 and SREBP2 [64, 74, 75]. A close examination of the relationship between ATF6 activity and SREBP2-mediated lipogenesis revealed that nuclear ATF6 interacts with the nuclear form of SREBP2 and thereby antagonizes SREBP2-regulated transcription of lipogenic genes and lipid accumulation in cultured liver and kidney cells [73]. The authors suggest that this negative regulation of SREBP2 activity by ATF6 accumulation in the nucleus would allow the cell to cope with ER stress conditions and save on cellular energy resources.

Several recent studies have examined the role of ATF6 *in vivo* by studying the role of ER stress on fatty liver disease and lipid droplet formation in ATF6α-knockout mice [7, 76, 77]. Interestingly, similar to $Ire1\alpha^{Hepfe/-}$ mice, ATF6α-knockout mice exhibited no apparent phenotype under physiological conditions; however, when given an ER stress insult by injection of tunicamycin, the livers in the knockout mice were unable to recover [76, 77]. Livers from tunicamycin-injected ATF6α-knockout mice showed signs of dysfunction as measured by serum ALT, protein content, and albumin levels [76]. Furthermore, the livers in the knockout mice had greatly reduced expression of ER chaperones following tunicamycin injection and increased numbers of TUNEL-positive apoptotic cells, suggesting that ATF6 protects hepatocytes from ER stress-induced damage and apoptosis [76]. The differences observed in tunicamycin-injected ATF6α-knockout mice as compared to wild-type mice are likely not due to increased cytotoxicity of tunicamycin in the ATF6α-knockout mice as no significant differences were noted in the upregulation of cytochrome P450 isoforms and cleavage of nuclear PARP between the groups of mice [7].

The phenotypic outcome of the ER stress insult in ATF6α-knockout mice was hepatic steatosis caused by induction of lipid droplet formation due to reduced β-oxidation of FA and attenuated VLDL formation [76]. Specifically,

there was sustained expression of CHOP in the livers of ATF6α-knockout mice compared to wild-type mice as well as a decrease in PPARα expression and apoB-100 protein levels, favouring the accumulation of lipids in the liver [7, 76]. *De novo* lipogenesis was ruled out as a mechanism for the increased lipid droplet accumulation in livers from these mice as expression of lipogenic genes (*SCD1*, *FASN*, and *DGAT2*) was suppressed in tunicamycin-injected ATF6α-knockout mouse livers [7]. In addition, while steatosis was the most evident phenotype, upon closer examination it was discovered that after 48 hours of tunicamycin treatment, ATF6α-knockout mice became profoundly resistant to exogenous insulin [7]. This finding is intuitive given that ER stress can lead to insulin resistance [12]. Taken together, the findings from these studies suggest that loss of ATF6 predisposes the liver to stress-induced insulin resistance and lipid accumulation.

The studies to date suggest that lipogenic genes and lipid metabolism are differentially regulated under physiological conditions such as high-carbohydrate or high-fat diet feeding in comparison to acute or unresolved ER stress conditions that arise when mice are injected with tunicamycin. For example, while XBP1 increases hepatic *de novo* lipogenesis, its upstream kinase IRE1α, or ATF6 which shares DNA-binding sites with XBP1, was protective against hepatic lipid accumulation. Indeed, a recent study by Rutkowski et al. demonstrated that it is the acute or unresolved form of ER stress that leads to hepatic steatosis [7]. Injection of tunicamycin in mice deficient in one of the UPR signalling components led to chronic upregulation of CHOP, defective eIF2α phosphorylation, and decreased C/EBPα gene expression [7]. CHOP was reported to be at least partially responsible for the suppression of gene expression seen in tunicamycin-injected mice with compromised UPR signalling [7]. While wild-type mice exhibited rapid but transient CHOP induction, ATF6-knockout and *Ire1*$\alpha^{\text{Hepfe/}-}$ mice presented with persistent upregulation of CHOP and nuclear localization [7]. CHOP can heterodimerize with the C/EBP family of transcription factors in the nucleus repressing their target gene expression [78]. Negative regulation of C/EBPα by prolonged nuclear CHOP expression due to unresolved ER stress appears to play a key role in the profound metabolic disruption under severe ER stress conditions which results in fatty liver disease. Indeed, the promoter region of both *Srebp1* and *Ppar*α possesses potential binding sites for C/EBPα [7]. The differential effects of acute/unresolved ER stress conditions in comparison to diet-induced adaptive ER stress conditions also explain why phosphorylation of eIF2α can lead to hepatic steatosis in one study model while defective eIF2α phosphorylation can accelerate lipid accumulation and steatosis in another study.

5. The Impact of Lipids on ER Stress

The relationship between ER stress and lipid metabolism is bidirectional. While activation of ER stress pathways can result in lipogenesis and altered lipid homeostasis, lipids and aberrant lipid metabolism can also cause ER stress [79–82]. Saturated fatty acids such as palmitate and stearate are known inducers of ER stress in various cell types and can modulate survival and apoptotic signals in the cell [81, 82]. A recent study carried out comparative proteomic and lipidomic analysis of fractionated ER from lean and obese liver tissues [79]. The results suggested enrichment of metabolic enzymes involved in lipid metabolism and a downregulation of ER-associated protein synthesis genes in the obese ER proteome. These findings implied that the ER in obese liver cells shifts from being the major site of protein synthesis to carrying out lipid synthesis and lipid metabolism functions [79]. Furthermore, the analysis revealed that there is a greater proportion of *de novo* synthesized saturated fatty acids incorporated into hepatic ER lipids than dietary polyunsaturated fatty acids. Another interesting finding was the increased proportion of phosphatidylcholine (PC) in comparison to phosphatidylethanolamine (PE), both abundant ER membrane phospholipids, in the liver ER from obese mice [79]. The increased PC/PE ratio led to perturbation in the calcium transport activity of the SERCA pump resulting in impaired ER calcium retention. Since ER calcium is important for ER homeostasis and chaperone function, such changes in calcium concentrations would lead to protein misfolding and ER stress. This appears to be a plausible mechanism for hepatic ER stress in obesity [79]. Hepatic ER stress can promote *de novo* lipogenesis and insulin resistance as described above which then in turn may lead to further exacerbation of the ER stress situation, creating a vicious cycle.

6. Therapeutic Potential Targeting ER Stress in Dyslipidemia and Obesity

ER stress and UPR activation have been implicated in the pathogenesis of a number of diseases such as diabetes, obesity, cancer, renal, cardiovascular, and neurodegenerative diseases as well as fatty liver disease [48, 83–86]. As such, potential ways of attenuating ER stress and UPR activation would provide opportunities in pharmacological intervention in a wide array of diseases. A recent study revealed for the first time in humans that obese insulin-resistant subjects express markers of ER stress in their white adipose tissue [17]. Similarly, an association between ER stress and obesity was also found in obese nondiabetic subjects [15]. Gastric bypass surgery-mediated weight loss in obese patients was effective at reducing ER stress in adipose and liver tissues and improved insulin sensitivity [16]. Furthermore, when ER stress was reduced by hepatic overexpression of GRP78 in *ob/ob* mice, hepatic TG and cholesterol content was reduced and insulin sensitivity improved [5]. These findings together with data in rodents indicating the presence of ER stress in tissues of obese animals [12, 13] suggest a strong association between ER stress and obesity. Therefore, the ER serves as an important new treatment target against obesity and its metabolic complications.

The use of small molecules called chemical chaperones has been examined in a number of disease models as potential tools for lowering ER stress and preventing the activation of UPR pathways. These chaperones similar

to molecular chaperones nonselectively stabilize mutant proteins and assist in their folding and translocation across membranes [87]. Most chemical chaperones are osmolytes and equilibrate cellular osmotic pressure. These can be categorized into 3 classes: carbohydrates (such as glycerol and sorbitol), amino acids (such as glycine and taurine), and methylamines (such as betaine) [87, 88]. The drawback to the use of most chemical chaperones is their nonspecificity and high-dose requirement for effective protein folding properties. However, two such chemical chaperones, 4-phenylbutyric acid (4-PBA) and tauroursodeoxycholic acid (TUDCA) have been approved by the US Food and Drug Administration (FDA) and are used in humans. Currently, 4-PBA is approved for use in children with urea-cycle disorders as an ammonia scavenger, while TUDCA is being tested for its liver-protecting properties in cholestatic liver disease in humans [87].

The low-molecular-weight fatty acid 4-PBA has been tested in a number of disease models for its ability to facilitate protein folding and trafficking, ultimately relieving ER stress [13, 89–98]. The chaperoning property of 4-PBA was first identified when investigating its effect on the translocation and trafficking of a mutant cystic fibrosis transmembrane conductance regulator protein (CFTR). Addition of 4-PBA to the cells allowed for stabilization of the mutant CFTR protein and facilitated their translocation to the cell membrane [99]. In addition to its chaperone properties, 4-PBA also possesses HDAC inhibitor activity and is under investigation as an anticancer drug [100–102].

Another effective reagent that has been shown to have chaperone properties is TUDCA, which can be classified as a hydrophilic endogenous bile acid [87]. TUDCA has antiapoptotic properties by reducing calcium efflux and blocking ER-stress-mediated caspase-12 activation [103]. Furthermore, TUDCA also activates cell survival pathways such as PI3K signalling, thereby inhibiting cell death [104]. Apart from these signalling properties, TUDCA can interact with the mineralocorticoid receptor and promotes its dissociation from cytosolic chaperones thereby preventing its translocation to the nucleus for transcriptional activity. In the case of primary neurons, addition of TUDCA was effective at preventing amyloid beta-peptide-induced apoptosis through its chaperoning properties [105].

In recent years, several studies have identified beneficial effects of 4-PBA and TUDCA supplementation on insulin resistance, obesity, and diabetes. Oral administration of 4-PBA and TUDCA to obese and insulin-resistant *ob/ob* mice normalized hyperglycemia, restored insulin sensitivity in the liver, muscle, and white adipose, and diminished fatty liver disease [92]. Our group examined the effect of 4-PBA supplementation on diet-induced obesity. For this purpose, C57BL/6 mice were placed on a high-fat diet with or without 4-PBA supplementation in the drinking water. Mice treated with 4-PBA gained significantly less weight, exhibited lower plasma glucose, TG, and leptin levels, and had smaller adipocytes as compared to mice on a high-fat diet alone [89]. Chemical chaperones also have chaperone activity within the central nervous system [13, 106]. Leptin, an adipocyte-derived hormone which acts on hypothalamic neurons to suppress appetite, is important in regulating energy expenditure and body weight [107]. ER stress may be one of the factors resulting in leptin resistance in the brain, as injection of tunicamycin, an ER-stressor-induced hypothalamic ER stress, increased food consumption and weight gain despite elevated blood leptin concentrations [13]. Both 4-PBA and TUDCA were shown to be effective at lowering hypothalamic ER stress and increasing the sensitivity of neurons to leptin, thereby reducing body weight in genetic and diet-induced obesity models [107]. In the context of atherosclerosis, 4-PBA was effective at protecting macrophages against palmitate-induced ER stress and apoptosis in culture [108]. A reduction in ER stress and apoptosis was also observed in the macrophages within the atherosclerotic lesions of mice treated with 4-PBA, which were smaller in size [108]. These findings indicate that 4-PBA treatment can protect cells from the deleterious effects of lipid accumulation on disease progression.

ER stress has been linked to fatty liver disease and liver injury [109, 110]. Lipid-induced ER stress inhibits apoB100 secretion in liver cells promoting the development of steatosis [111]. Treatment of hepatoma cells with 4-PBA leads to the inhibition of lipid-induced ER stress and enhanced apoB100 secretion [111]. Consistent with the studies on macrophages and progression of atherosclerosis, alleviating lipid-induced ER stress in hepatocytes also protects the cells from ER-associated apoptosis [112]. Since hepatocellular injury and damage can lead to progression of fatty liver disease into steatohepatitis [113, 114], blocking ER stress serves as an important treatment strategy [95]. A recent study examined the effects of oral administration of TUDCA on hepatic steatosis and hepatic gene expression in *ob/ob* mice [115]. Yang et al. found a significant decrease in liver fat content and reduced expression of genes involved in *de novo* lipogenesis with TUDCA treatment [115]. However, they did not find any differences in body weight or insulin sensitivity over the three-week duration of the study. Examination of the effects of orally administered TUDCA on insulin sensitivity in obese human subjects revealed a 30% improvement in insulin sensitivity in muscle and liver tissues but no alterations in hepatic TG content were observed [116]. The differences in the mechanism of action between oral treatment and intraperitoneally injected TUDCA may explain some of these contrasting outcomes [115].

The effectiveness of chemical chaperones such as 4-PBA and TUDCA as a treatment strategy for dyslipidemia, cardiovascular disease, diabetes, and obesity require further study in human subjects. Both 4-PBA and TUDCA have additional functions which may be directly or indirectly alleviating ER stress conditions. Investigation into the discovery of new chemical and biological approaches to enhance ER function and facilitate the trafficking of proteins would be useful for treating ER-stress-related diseases. Furthermore, ways of targeting specific UPR pathways would allow for better specificity in targeting ER stress in various disease states [87]. Currently, small molecules that can target IRE1α and alter its endonuclease activity offer hope for further study. These kinase-inhibiting RNase attenuators can also selectively enhance XBP1 mRNA splicing and lead to prevention

of apoptotic cell death, while attenuating IRE1α-mediated decay of mRNA such as those encoding ER chaperones [117]. The recent finding that unfolded peptides can directly bind to IRE1 and promote its oligomerization and activation suggests that compounds that can target its peptide-binding groove and oligomerization interface may be effective at regulating IRE1 activity [58]. Finally, given the challenges with directly measuring ER stress, assay systems which can assess actual cellular ER stress will prove to be useful [118].

7. Conclusions

A growing body of evidence links ER stress and UPR activation to diseases associated with lipid metabolism. The UPR signalling pathways and activation of transcription factors such as XBP1 and ATF6 have novel roles in controlling the transcriptional regulation of lipogenesis. While IRE1α itself is protective against ER-stress-induced lipogenesis and hepatic steatosis, its downstream mediator XBP1 promotes transcription of genes involved in fatty acid and cholesterol biosynthesis. Phosphorylation of eIF2α downstream of PERK affects the transcriptional activity of C/EBPs, PPARγ, and SREBP-1c thereby leading to lipid accumulation and hepatic steatosis under high-fat-diet conditions. Similar to IRE1α, ATF6α also protects against ER stress-induced steatosis and lipid droplet formation in mice. Furthermore, nuclear ATF6 attenuates SREBP2-mediated lipogenesis. The exact mechanisms by which ER stress signalling pathways affect lipid homeostasis are incompletely understood. Given the temporal differences in the activation of the three arms of the UPR, a closer examination of each branch of the UPR will allow for a better understanding of how various components of this signalling network impact on lipogenesis and disease progression. Such studies will further enhance our understanding of biological and pharmacological tools needed to effectively treat ER-associated diseases.

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Review Article

UPR-Mediated Membrane Biogenesis in B Cells

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The unfolded protein response (UPR) can coordinate the regulation of gene transcription and protein translation to balance the load of client proteins with the protein folding and degradative capacities of the ER. Increasing evidence also implicates the UPR in the regulation of lipid synthesis and membrane biogenesis. The differentiation of B lymphocytes into antibody-secreting cells is marked by significant expansion of the ER, the site for antibody synthesis and assembly. In activated B cells, the demand for membrane protein and lipid components leads to activation of the UPR transcriptional activator XBP1(S) which, in turn, initiates a cascade of biochemical events that enhance supplies of phospholipid precursors and build machinery for the synthesis, maturation, and transport of secretory proteins. The alterations in lipid metabolism that occur during this developmental transition and the impact of membrane phospholipid restriction on B cell secretory characteristics are discussed in this paper.

1. Introduction

Activated B lymphocytes proliferate and proceed along distinct developmental pathways that determine their function and fate. Specifically, responding B cells can rapidly differentiate in extrafollicular sites into short-lived antibody-secreting cells that predominantly secrete IgM antibodies [1]. Alternatively, responding B cells can enter germinal centers, undergo somatic hypermutation and isotype switching, and then become memory B cells or long-lived antibody-secreting cells [2]. Extrinsic factors, including the nature of the antigen and T cell help in the form of membrane-bound molecules and soluble cytokines, play key roles in regulating B cell responses. However, intrinsic signals are also pivotal in directing the fate of responding B cells as evidenced by the critical role of the unfolded protein response (UPR) transcription factor XBP1(S) in driving the differentiation of antibody-secreting cells [3, 4], the effectors of humoral immunity. Here, we discuss the current understanding of the relationship between the UPR, lipid biosynthesis and organelle biogenesis in activated B cells.

2. Lipid Supply and Demand

B lymphocytes proliferate and differentiate into antibody-secreting cells upon interaction with specific antigen or certain Toll-like receptor (TLR) ligands. When B cells are stimulated to enter the cell cycle and proliferate, the mechanisms that control the membrane phospholipid supply in rapidly dividing cells are engaged. The division of one cell into two daughter cells requires a doubling of membrane content during cell cycle progression [5]. Phosphatidylcholine (PtdCho) is the major membrane phospholipid in mammalian cells and is a precursor to the two other most abundant membrane phospholipids, sphingomyelin (SM) [6] and phosphatidylethanolamine (PtdEtn) [7]. PtdCho and the other phospholipids accumulate in a periodic manner during S phase, coincident with DNA synthesis. The net increase in membrane PtdCho results from an interaction between cell cycle-dependent oscillations in the rates of PtdCho biosynthesis and degradation. PtdCho synthesis is stimulated very early during G1 phase [8–10], but is accompanied by rapid PtdCho turnover. Two phospholipases have been implicated in the PtdCho turnover associated with

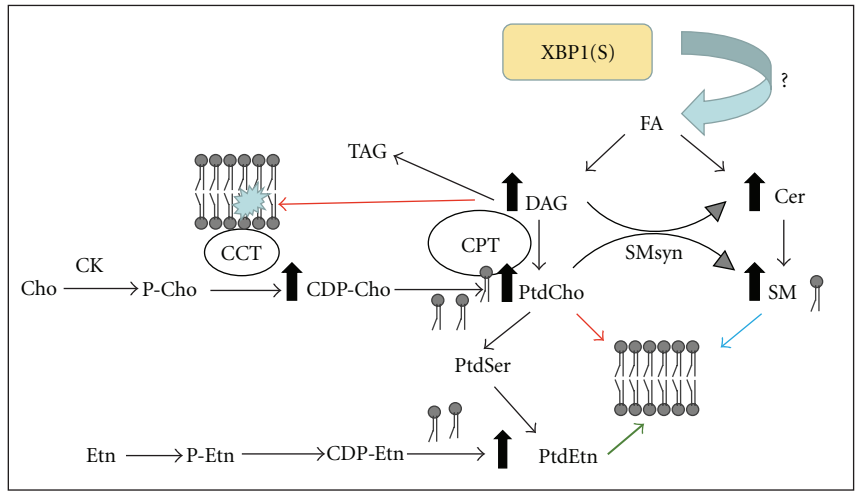

Figure 1: Activation of membrane phospholipid synthesis. Expression of XBP1(S) stimulates *de novo* fatty acid (FA) synthesis and the new FAs are incorporated into diacylglycerol (DAG) and ceramide (Cer), immediate precursors of phosphatidylcholine (PtdCho), and sphingomyelin (SM) phospholipids, respectively. The mechanism of stimulation by XBP1(S) has not yet been defined. Elevation of the DAG level alters the membrane lipid composition which leads to activation of the choline cytidylyltransferase (CCT) enzymes which produce CDP-choline (CDP-Cho). The DAG and CDP-Cho precursors are converted to PtdCho by the choline phosphotransferase (CPT) enzymes. Excess DAG which is not incorporated into phospholipid, is redirected and incorporated into triacylglycerol (TAG) which can accumulate in lipid droplets. PtdCho conversion to SM is mediated by sphingomyelin synthase (SMsyn). PtdCho conversion to phosphatidylethanolamine (PtdEtn) is routed through phosphatidylserine (PtdSer). PtdEtn can also be synthesized from ethanolamine (Etn) and DAG by the alternative CDP-ethanolamine (CDP-Etn) pathway. Elevation of all three phospholipids, PtdCho, SM, and PtdEtn, contributes to membrane biogenesis during B cell activation. Cho, choline; P-Cho, phosphocholine; CK, choline kinase; Etn, ethanolamine; P-Etn, phosphoethanolamine.

cell cycle progression, the group VIA calcium-independent phospholipase A_2 [11] and the neuropathy target esterase [12]. Near the G1/S transition, PtdCho turnover is diminished substantially, yielding a net increase in membrane PtdCho. Toward the latter part of the cell cycle, prior to cytokinesis, PtdCho synthesis is downregulated [5]. This cyclic variation in the supply of membrane phospholipid for cell proliferation is maintained in the absence of differentiation.

B cells are unique, however, and in addition to proliferation also undergo a subcellular membrane expansion as they differentiate into antibody-secreting cells after stimulation. There is a major increase in synthesis and secretion of immunoglobulin (Ig) heavy (H) and light (L) chains [13]. Nascent Ig chains are cotranslationally translocated into the endoplasmic reticulum (ER), an oxidizing, calcium-rich environment containing many resident molecular chaperones and folding enzymes [14]. Within this specialized protein folding compartment, H and L chains are assembled into functional antibodies. Induction of high-rate Ig synthesis during the differentiation process is accompanied by expansion of the rough ER membrane, at least 3- to 4-fold in surface area and volume [15, 16]. Thus, both proliferation and differentiation require an increased supply of phospholipids to fuel membrane and organelle biogenesis. To meet this demand, the synthesis of phospholipids, particularly PtdCho, increases when B cells are activated [15, 17].

3. Phosphatidylcholine Synthesis

The predominant means for PtdCho biosynthesis in mammalian cells proceeds via the three steps of the cytidine diphosphocholine (CDP-choline) pathway [18] (Figure 1). First, choline kinase (CK) phosphorylates choline in the presence of ATP to yield phosphocholine. CKα and CKβ are two isoforms which are soluble proteins found in the cytosol [19, 20]. Second, choline cytidylyltransferase (CCT) converts phosphocholine to CDP-choline in the presence of CTP, and this is the rate-limiting step in the pathway [21]. In every cell type examined thus far, including B cells [17], CCT catalyzes the slow step in the pathway and thereby determines the rate of PtdCho formation. Comparatively small amounts of CDP-choline are found in cells, in relation to other phospholipid precursors, as CDP-choline is utilized almost immediately after it is made. CCT, including all mammalian isoforms, transiently associates with the ER membrane and the lipid composition of the ER membrane governs CCT association and activity [22]. Elevated expression of CCT stimulates PtdCho synthesis but often does not result in an increased amount of cellular PtdCho in most proliferating cells due to compensatory elevation of PtdCho turnover mediated by phospholipases [23, 24]. Third, the phosphocholine moiety of CDP-choline is transferred to diacylglycerol (DAG), producing PtdCho. This final step can be catalyzed by either cholinephosphotransferase (CPT1) or choline/ethanolaminephosphotransferase (CEPT1), a bifunctional enzyme that can synthesize both

choline- and ethanolamine-containing phospholipids. The CPT enzymes are integral membrane proteins, and the CPT1 is found with the Golgi apparatus while the CEPT1 associates with the ER [25, 26]. Here, we refer to the activities of CPT1 [27] and CEPT1 [28] collectively as CPT activity. The locations of the CPT enzymes designate the subcellular sites of membrane biogenesis; however, enforced overexpression of CPT activity does not enhance PtdCho synthesis [29, 30]. Rather, the supply of CDP-choline and DAG determine the amount of PtdCho. Thus, elevated expression of the CPT enzymes can be considered as a marker for Golgi and/or ER membrane expansion, but not necessarily as a driver of membrane phospholipid synthesis.

In lipopolysaccharide- (LPS-) stimulated splenic B cells, CK activity remains fairly constant, CCT activity modestly increases ≈2-fold, and CPT activity increases ≈6-fold [15]. These modulations of the CDP-choline pathway enzymes in LPS-stimulated splenic B cells correlate with a 6- to 7-fold increase in PtdCho synthesis [15, 31]. Our studies using the CH12 B cell lymphoma indicate that increased CCT activity is pivotal for enhanced flux through the CDP-choline pathway in LPS-stimulated B cells [17]. In this system, the CCT expression and enzyme specific activity do not increase when assayed under optimal *in vitro* conditions following LPS stimulation. However, radiolabeling experiments of stimulated cells demonstrate that the formation of CDP-choline is substantially enhanced, indicating allosteric activation of CCT by membrane lipids. Indeed, microsomal lipids isolated from stimulated cells contain an elevated amount of DAG and significantly stimulate the activity of purified recombinant CCT, compared to lipids isolated from unstimulated cells. Thus, in this case, the formation of DAG is key to stimulation of PtdCho synthesis: first, by activating CCT, and second, by providing substrate for the CPT enzymes. The CCT, in turn, governs the fate of the DAG as DAG is incorporated either into phospholipid under permissive CCT conditions or into triacylglycerol (TAG) when the CCT activity is reduced [32] (Figure 1).

4. A "Physiologic" UPR

ER stress occurs when the load of client proteins exceeds the folding capacity of the ER, a condition that can be catastrophic if unresolved. To rebalance load with capacity in the ER, thereby relieving ER stress, the UPR can slow the flow of nascent polypeptides into the ER lumen and enhance the ER machinery needed for folding and/or disposal of client proteins [33, 34]. The mammalian UPR is orchestrated by a trio of signaling pathways that are separately initiated by three ubiquitously expressed ER transmembrane proteins: PERK (PKR-like ER kinase) [35, 36], ATF6 (activating transcription factor 6) α and β [37, 38], and IRE1 (first identified in a yeast mutant with inositol requiring phenotype) α and β [39, 40]. The activation status and role of each UPR pathway has been examined during the differentiation of antibody-secreting B cells.

The PERK protein possesses a serine/threonine kinase domain in its cytoplasmic region through which it mediates translational attenuation [35, 36]. Upon activation, PERK phosphorylates the α subunit of eIF-2 (eukaryotic initiation factor-2) on serine 51, thereby impeding formation of translation initiation complexes and slowing the flow of nascent polypeptides into the ER [41, 42]. PERK does not appear to be activated during the differentiation of antibody-secreting B cells [43, 44]. In support of this concept, studies of gene-targeted mice reveal that the PERK pathway is dispensable for antibody secretion [43].

ATF6α and ATF6β are type II ER transmembrane proteins [37, 38]. Upon UPR activation, ATF6 traffics from the ER to the Golgi complex where it is clipped by the Site-1 and Site-2 proteases [45, 46]. Once liberated from the membrane by this process of intramembrane proteolysis, the cytosolic N-terminal domain of ATF6 moves into the nucleus where it functions as a transcriptional activator of genes encoding ER resident molecular chaperones, folding enzymes and components involved in ER-associated degradation (ERAD) of misfolded proteins [37, 38, 47–49]. While ATF6α and β are both functional, only ATF6α appears essential for induction of ER stress responsive genes and survival of cells subjected to ER stress conditions [48, 49]. Overexpression of active ATF6α is sufficient to drive synthesis of fatty acids and phospholipids and to induce expansion of rough ER [50], suggesting that this UPR pathway might participate in the differentiation of antibody-secreting B cells. Indeed, ATF6α is activated in LPS-stimulated B cells [43, 51, 52]. However, recent studies of ATF6α-deficient mice indicate that ATF6α, like PERK, is dispensable for the differentiation of antibody-secreting B cells (Brewer et al., manuscript in preparation).

The IRE1 proteins contain a serine-threonine kinase module and a C-terminal endoribonuclease domain in their cytoplasmic regions [39, 40]. Upon activation, IRE1 executes site-specific cleavage of *Xbp1* (X-box binding protein 1) mRNA. A 26-nt intron is excised and an undefined mechanism then ligates the resulting 5′ and 3′ fragments, yielding a spliced *Xbp1* mRNA with an altered reading frame [53–55]. Both unspliced and UPR-spliced *Xbp1* transcripts encode basic leucine zipper (bZIP) transcription factors, XBP1(U) and XBP1(S), respectively. The XBP1(S) factor exhibits enhanced transactivating capacity and greater stability as compared to XBP1(U) [53–56]. Like ATF6α, XBP1(S) is sufficient to upregulate synthesis of fatty acids and phospholipids and to drive expansion of rough ER [30, 50]. *Xbp1* is essential for optimal induction of genes encoding proteins that function throughout the secretory pathway and for proper development of the ER in a variety of specialized secretory cell types [57, 58]. When B cells are stimulated to secrete antibody, *Xbp1* mRNA increases and undergoes UPR-mediated splicing to yield XBP1(S) [3, 52, 53], a factor required for the generation of antibody-secreting B cells [3, 4]. Thus, the physiologic UPR of activated B cells features the IRE1/XBP1 pathway.

5. XBP1(S), Lipid Synthesis, and ER Biogenesis

Xbp1 is required for embryonic development [59]; thus, the role of this UPR transcription factor in lymphocytes was

first investigated using the *Rag-2* complementation system [4]. Those experiments revealed that XBP1-deficient B cells are markedly defective in antibody secretion *in vivo* in response to immunization and *in vitro* in response to LPS. Importantly, it was shown that XBP1(S), but not XBP1(U), effectively restores the ability of XBP1-deficient B cells to secrete antibody in response to LPS *in vitro* [3] and is sufficient to drive ER expansion [30, 58]. More recently, the Cre-*loxP* system has been employed for selective deletion of *Xbp1* in B cells and studies using this system have corroborated the earlier findings [60, 61]. Using this system, the abundance of PtdCho was shown to increase in LPS-stimulated XBP1-deficient B cells, but to a lesser degree than in wild-type cells [62]. The levels of PtdCho, SM, and phosphatidylinositol were significantly reduced in activated XBP1-deficient B cells, but PtdEtn, phosphatidylserine, and phosphatidylglycerol were similar to corresponding amounts in wild-type activated B cells. In addition, a meager, but discernible, expansion of the rough ER was observed in LPS-stimulated XBP1-deficient B cells [62].

PtdCho is most drastically affected by XBP1 deficiency because it is the most abundant phospholipid of the ER membranes. SM is derived directly from PtdCho, where the phosphocholine headgroup of PtdCho is transferred to ceramide by the SM synthase [63] (Figure 1). Thus, a reduction in PtdCho availability would be reflected by a reduction in SM. The pathway for PtdCho conversion to PtdEtn is not as direct, however, and a second pathway of PtdEtn synthesis via CDP-ethanolamine can bypass a deficiency in PtdCho [64]. Thus, the amount of PtdEtn is less affected following activation of XBP1-deficient B cells and PtdEtn increases to almost the same extent as in activated wild-type B cells. On the other hand, the enforced expression of XBP1(S) in NIH-3T3 fibroblasts leads to a substantial increase in PtdEtn [30], augmenting the XBP1(S)-independent mechanism(s) of lipogenesis. The *de novo* synthesis of ceramides, key precursors in SM production, is upregulated upon LPS stimulation [65] and contributes to the increase in SM. Inhibition of ceramide formation impairs ER expansion and protein glycosylation in the ER lumen [65], suggesting a link among these processes. These data establish that XBP1 is required for maximal increases in PtdCho, SM, and rough ER in LPS-stimulated B cells, but the mechanisms by which XBP1 mediates these events remain to be elucidated. The scheme in Figure 1 shows a cascade of biochemical events which illustrates how XBP1(S) stimulation of fatty acid synthesis [50] is a key feature that drives membrane phospholipid expansion in B cells [17]. Furthermore, these data suggest that XBP1-independent mechanisms, as yet undefined, must also contribute to the regulation of PtdCho synthesis and ER biogenesis during the differentiation process.

It has been proposed that the escalation of Ig synthesis in differentiating B cells taxes the protein folding machinery of the ER and, consequently, triggers the UPR [3]. This model was supported by an experiment showing reduced induction of XBP1(S) in B cells that had undergone *ex vivo* Cre-mediated deletion of IgH chain prior to LPS stimulation [3]. In contrast, recent studies have shown strong induction of XBP1(S) in $\mu_s^{-/-}$ B cells stimulated with LPS [60, 62], indicating that increased synthesis of soluble μ H chains is not a prerequisite for UPR activation. In keeping with these data, we previously showed that synthesis of XBP1(S) precedes induction of maximal Ig translation in LPS-stimulated CH12 B cells [52], indicating that the IRE1/XBP1 pathway is activated at an earlier stage of the differentiation process. What then is the signal(s) for UPR activation in stimulated B cells? This remains a fundamental question, and its answer is integral to understanding the mechanisms that drive development of antibody-secreting B cells.

6. Phosphatidylcholine Synthesis and UPR Signaling

Mammals express three CCT isoforms that are similar in enzymatic activity and regulation. CCTα is encoded by the *Pcyt1a* gene whereas CCTβ2 and CCTβ3 are encoded by alternatively spliced transcripts from the *Pcyt1b* gene [66]. CCTα is predominantly expressed in most tissues, including B cells [17], and is required for early embryonic development [67]. Tissue-specific deletion of the *Pcyt1a* gene using the Cre-*loxP* system has revealed critical roles for CCTα in specialized secretory cells, including surfactant lipid production and secretion by alveolar epithelial cells [68], assembly and secretion of lipoproteins by hepatocytes [69], and cytokine secretion by activated macrophages [70]. We recently showed that selective deletion of CCTα significantly hampers the ability of B cells to upregulate PtdCho synthesis upon stimulation, and interestingly, this correlates with heightened induction of the IRE1/XBP1 branch of the UPR [31].

When challenged with a T cell-dependent protein antigen, the animals harboring CCTα-deficient B cells were unable to produce normal levels of IgG but secreted hyperlevels of IgM [31]. The correlation between the reduced PtdCho synthesis and elevated IgM secretion in the CCTα-deficient B cells was counterintuitive, however, based on the implied need for membrane PtdCho expansion during plasma cell differentiation. Investigation of the UPR components revealed that the impaired production of PtdCho triggers IRE-mediated splicing of *Xbp1* mRNA early after activation, thereby promoting differentiation of IgM-secreting cells. The inability of CCTα-deficient B cells to undergo isotype switching correlates with a proliferation defect. However, blocking proliferation by a different mechanism did not elicit XBP1(S) activation, supporting the idea that the early and potent induction of XBP1(S) by PtdCho deficiency in CCTα-deficient B cells accelerates and augments the transition into antibody secretion. From these observations, we propose that the IRE1/XBP1 branch of the UPR responds to increased demand for phospholipids as well as increased demand on the protein folding capacity of the ER (Figure 2). In agreement, restriction of either PtdCho [71] or fatty acid synthesis [72] has been shown to elicit activation of UPR components in other systems. It is intriguing to speculate that lipid supply might function as

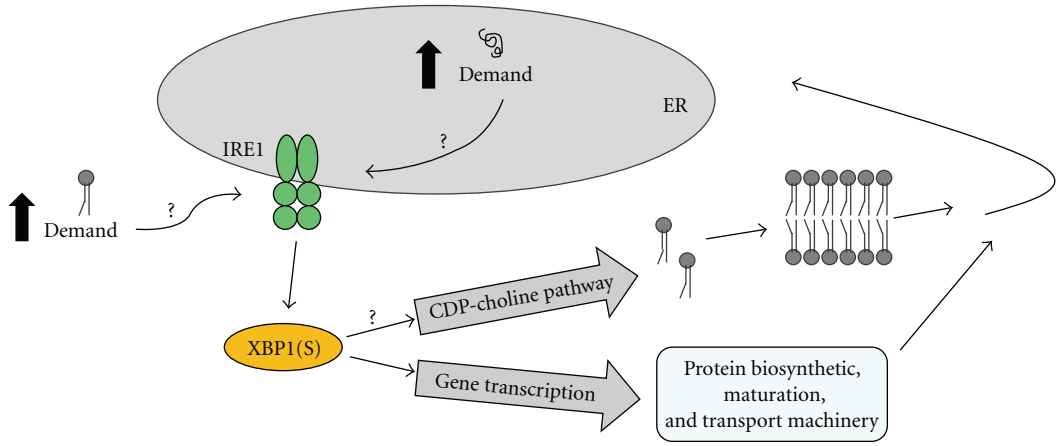

Figure 2: XBP1(S), lipids, and secretory pathway machinery in ER biogenesis. In activated B cells, we propose that increased demand for lipids as well as increased demand on the protein folding capacity of the ER promotes induction of the XBP1(S) transcriptional activator via the IRE1/XBP1 branch of the UPR. The means by which these demands are sensed by the IRE1/XBP1 pathway remain unclear. XBP1(S), via transcriptional control, upregulates expression of a large cohort of proteins involved in the synthesis, maturation, and transport of cargo proteins within the secretory pathway. Much of this secretory machinery localizes to the ER. XBP1(S), via mechanisms that are poorly understood, also drives lipid biosynthesis, including production of the major phospholipid PtdCho by the CDP-choline pathway. Thus, XBP1(S) coordinates mechanisms that supply both the lipid and protein components necessary for construction of the ER.

a metabolic cue for induction of the IRE1/XBP1 pathway in activated B cells.

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Review Article

Mechanisms of Alcohol-Induced Endoplasmic Reticulum Stress and Organ Injuries

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Alcohol is readily distributed throughout the body in the blood stream and crosses biological membranes, which affect virtually all biological processes inside the cell. Excessive alcohol consumption induces numerous pathological stress responses, part of which is endoplasmic reticulum (ER) stress response. ER stress, a condition under which unfolded/misfolded protein accumulates in the ER, contributes to alcoholic disorders of major organs such as liver, pancreas, heart, and brain. Potential mechanisms that trigger the alcoholic ER stress response are directly or indirectly related to alcohol metabolism, which includes toxic acetaldehyde and homocysteine, oxidative stress, perturbations of calcium or iron homeostasis, alterations of S-adenosylmethionine to S-adenosylhomocysteine ratio, and abnormal epigenetic modifications. Interruption of the ER stress triggers is anticipated to have therapeutic benefits for alcoholic disorders.

1. Introduction

Alcohol is the most socially accepted addictive drug. Alcohol abuse and dependence causes social problems such as domestic violence and loss of productivity in work place as well as traffic accident-related injuries and chronic organ disorders. Excessive alcohol use is the third leading cause of preventable death in the United States and is responsible for 3.8% of deaths worldwide [1–3]. Alcohol-related medical problems can be improved upon a good understanding of pathogenesis of alcohol-induced injuries. After its consumption, alcohol is readily distributed throughout the body in the blood stream and crosses biological membranes which affect virtually all organs and biological processes in the body. Most of the alcohol that enters the body is first oxidized to toxic acetaldehyde, which is catalyzed by the cytosolic alcohol dehydrogenase (ADH) (Figure 1). Acetaldehyde is then converted by acetaldehyde dehydrogenase (ALDH) to acetic acid, which occurs primarily in the liver [4]. Alcohol can also be oxidized to acetaldehyde by cytochrome P450IIE1 (CYP2E1) which generates hydrogen peroxide. Alcohol-related medical illness results directly or indirectly from the toxic alcohol metabolites in cells and tissues. Alcoholic injuries can be found in most organs including brain, gastrointestinal tract, immune system, kidney, lung, heart, pancreas, and most frequently liver (reviewed in [1, 5–13]). Alcohol-induced liver disease (ALD) is better characterized than in other organs. The progression of ALD includes a spectrum of liver diseases, ranging from steatosis, steatohepatitis, fibrosis, to cirrhosis and even cancer [1, 7, 13]. However, the underlying molecular mechanisms of ALD are not completely understood. Both primary factors and cofactors are involved in the pathogenesis of ALD. Primary factors include but are not limited to increased oxidative stress mainly from mitochondrial malfunction and CYP2E1, increased endotoxin production and TNF signaling, impaired innate and adaptive immunity, hypoxia, impaired methionine metabolism, and epigenetic modifications [7, 9, 10, 13–18]. Cofactors may include malnutrition or complications with diabetes, obesity, smoking, or HCV/HIV infections [1, 9, 10, 13]. Alcohol-induced perturbations of homeostasis in the endoplasmic reticulum (ER) have evolved as an important factor contributing to fatty liver disease, which has been reviewed by a few comprehensive reviews [19–22]. Evidence for the involvement of ER in the pathogenesis of alcoholic injury is now

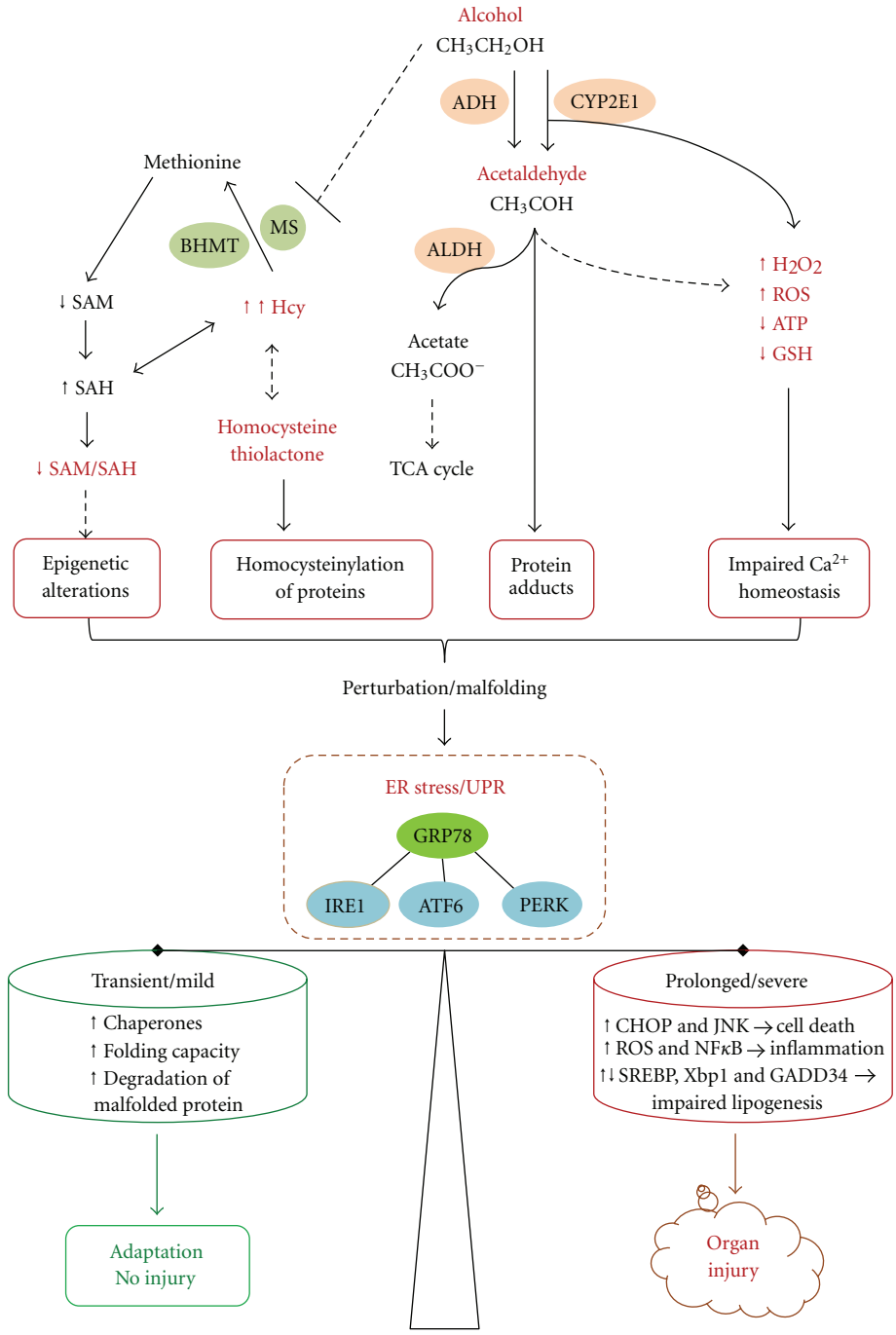

Figure 1: Mechanisms of alcohol-induced endoplasmic reticulum (ER) stress and organ injuries. ADH: alcohol dehydrogenase; ALDH: acetaldehyde dehydrogenase; CYP2E1: cytochrome P450 2E1; ROS: reactive oxidative stress; GSH: glutathione; BHMT: betaine-homocysteine methyltransferase; MS: methionine synthase; Hcy, homocysteine; SAM: S-adenosylmethionine, SAH: S-adenosylhomocysteine; TCA: tricarboxylic acid; UPR: unfolded protein response; GRP78: glucose-regulated protein 78; IRE1: inositol requiring enzyme; ATF6:activating transcription factor 6; PERK: protein kinase ds RNA-dependent-like ER kinase; CHOP: C/EBP-homologous protein; JNK, c-jun-N-terminal kinase; NFκB, nuclear factor κB; SREBP: sterol regulatory element binding protein; Xbp-1: X box binding protein 1; GADD34: growth arrest and DNA damage-inducible protein. See the context for details.

accumulating beyond the liver. The purpose of this review is to highlight phenomenological evidence for alcohol-induced ER stress in select organ disorders and to discuss potential molecular mechanisms causing alcoholic ER stress.

2. ER Stress and the Unfolded Protein Response (UPR)

The ER is an essential organelle for protein synthesis and modifications, for storing and releasing Ca^{2+}, for the

biosynthesis of lipids and sterols, and for detoxification of certain drugs. ER stress is a condition under which unfolded or malfolded proteins accumulate in the ER (reviewed in [18–21]). ER stress results from perturbations in ER homeostasis such as calcium depletion, inhibition of glycosylation, alterations of the redox state, or lipid overloading. ER stress triggers the unfolded protein response (UPR), which constitutes a series of ER-to-nucleus signaling mediated by three ER resident transmembrane sensor proteins, inositol requiring protein 1 (IRE1), ds-RNA-activated protein kinase (PKR) like ER kinase (PERK), and activating transcription factor 6 (ATF6) (Figure 1). The three sensors are activated upon dissociation from their inhibitory binding with the chaperone GRP78/BiP. IRE1, which has kinase and endoribonuclease activities, is activated by transautophosphorylation. The activated IRE1 processes the transcription factor X-box binding protein-1 (XBP1) mRNA via the unconventional splicing to form transcriptionally active spliced XBP1 (sXBP1). sXBP1 activates UPR target genes, including chaperones and ER-associated degradation (ERAD) pathway genes. The second sensor PERK phosphorylates the eukaryotic initiation factor 2α-subunit (eIF2α), leading to an inhibition of the initiation of translation and a global attenuation in protein translation. Phosphorylation of eIF2α selectively activates activating transcription factor 4 (ATF4), which regulates ER chaperone genes, ERAD pathway genes, amino acid metabolism genes, and the transcription factor C/EBP homologous protein (CHOP) [19–21]. The third sensor ATF6 is cleaved in the Golgi to form a transcriptionally active fragment that traffics to the nucleus to activate UPR target genes. In general the UPR results in reduced synthesis of nascent proteins, increased unloading of unfolded proteins, and increased capacity of folding, which lead to restoration of ER homeostasis.

However, prolonged or severe UPR provokes a complex network of interacting and parallel responses contributing to pathological consequences such as apoptosis, inflammation, and fat accumulation [19–24]. The ER stress-induced apoptosis is mediated by a few factors. CHOP regulates growth arrest and DNA damage-inducible protein (GADD34). GADD34 binds protein phosphatase-1 and enhances eIF2α dephosphorylation, leading to premature restoration of translation and enhanced ER stress. CHOP can also regulate expression of the TRAIL receptor DR5, pro- and antiapoptotic Bcl-2 family protein Bim, Bax and Bcl-2 modulating cell death [19–21]. Sustained activation of IRE1 recruits the adaptor protein TRAF2 and activates JNK and NF-κB, both of which mediate apoptosis [23]. In addition, alterations in ER calcium homeostasis, upregulation of ER oxidase 1 (ERO1) by CHOP, activation of caspase 12, and activation of GSK3β by tribbles 3 (TRB3) and AKT are other mechanisms underlying ER stress-induced inflammation and apoptosis [21, 23, 25]. Lipid accumulation is also a main pathological feature of prolonged ER stress, and each of the three ER sensor pathways has direct molecular effects on lipid synthesis. The IRE1α-XBP1 branch regulates C/EBPα and C/EBPβ that control directly the expression of genes involved in *de novo* fatty acid biosynthesis [26]. The ATF6 branch is involved in phospholipid biosynthesis as well as in fatty acid oxidation and lipoprotein secretion [27, 28]. The PERK-eIF2α branch influences expression of C/EBP family and PPARγ transcription factors via the eIF2α-specific phosphatase GADD34 and regulates SREBP1-related *de novo* lipid synthesis and accumulation [18–24, 29, 30].

3. ER Stress in Alcoholic Organ Injuries

3.1. Liver. Alcohol is mainly metabolized in the liver, and liver cells are rich in ER which assumes synthesis of a large amount of secretory and membrane proteins [19, 20, 29]. Partial role of ER in alcohol metabolism was initially realized decades ago as NADH from the hepatic oxidation of ethanol to acetaldehyde by ADH was found to support also microsomal ethanol oxidations [14, 15]. The inducible microsomal ethanol oxidizing system (MEOS) is associated with proliferation of the ER and a concomitant induction of cytochrome P4502E1 (CYP2E1) in rats and in humans. Free radical release as a consequence of CYP2E1 function in the ER and subsequent oxidative stress and lipid peroxidation generally contribute to ALD [14, 15]. However, alcohol-induced ER stress response was not recognized until recently. Molecular evidence for an impaired UPR was first found in the intragastric alcohol-fed mice using microarray gene expression profiling [18]. The alterations of selected ER stress markers were associated with severe steatosis, scattered apoptosis, and necroinflammatory foci. Moderate upregulation of expression of SREBP-1c and SREBP-2 and their responsive genes was detected by immuoblotting [18]. SREBP-1c knockout mice were protected against triglyceride accumulation [30–32]. Knocking out CHOP resulted in minimal alcohol-induced apoptosis in mouse liver [32–34]. In a setting of alcohol infusion and moderate obesity, there are synergistic effects of accentuated ER and mitochondrial stress, nitrosative stress mediated by M1 macrophage activation, and adiponectin resistance on hepatic necroinflammation and steatohepatitis [35]. In micropigs fed alcohol, liver steatosis and apoptosis were shown to be accompanied by increased mRNA levels of CYP2E1, GRP78 and SREBP-1c, and protein levels of CYP2E1, GRP78, activated SREBP and caspase 12 [36]. In addition, the ER stress response was correlated with elevated transcripts of lipogenic enzymes such as fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and stearoyl-CoA desaturase (SCD). Further, alcohol-induced lipopolysaccharide (LPS) is linked to impaired UPR and advanced hepatic injury [37–39]. In cirrhotic rat livers, only eIF2α was activated in the basal state. After LPS challenge, full UPR as indicated by activation of IRE1α, ATF-6, and eIF2α was detected [37]. However, LPS-induced accumulation of NF-κB-dependent antiapoptotic proteins was not observed, suggesting that the UPR sensitized the cirrhotic livers to LPS/TNFα-mediated apoptosis. Alcohol-induced hepatic ER stress response not only occurs in rodents but also in livers of baboon and human patients [40, 41]. In baboon fed alcohol orally, upregulation of calpain 2, calpain p94, and ERD21 and downregulation of eIF2α were among the genes of altered expression that was revealed by using cDNA array analysis

[41]. Gene expression profiling of cirrhotic liver samples from human alcoholics also revealed alterations of calpain and calreticulin that are indicative of ER malfunction.

3.2. Pancreas. The pancreas is one of the important digestive organs adversely affected by alcohol abuse. Pancreatitis is among the most common alcohol-related hospital diagnosis in USA [11]. The underlying mechanisms for alcohol-induced pancreatitis are not well understood. Similar to the liver, the pancreas has the capacity to metabolize alcohol via both the oxidative and nonoxidative pathways yielding toxic metabolites such as acetaldehyde and lipid esters. Fatty acid ethyl and cholesteryl esters are known to accumulate in the acinar cell after chronic alcohol consumption which decreases the stability of the membranes of zymogen granules and lysosomes [42, 43], which cause a premature activation of intracellular digestive enzyme and may predispose the gland to autodigestive inflammation and injury. In respect to the role of organelles in alcoholic pancreatic injury, the ER has been considered as the acinar cell has the highest rate of protein synthesis among all tissues in adult organism. In fact, perturbations of ER homeostasis are found in acute pancreatitis [44, 45], and all the three ER stress/UPR transducers (i.e., IRE1, ATF6, and PERK) and their downstream pathways are activated. However, chronic alcohol feeding alone causes minimal pancreatic tissue injury in animal models [45, 46]. Further studies demonstrate that alcohol feeding activates the UPR in pancreas with upregulation of the transcription factor XBP1 in the intragastric alcohol infusion model [47, 48]. This suggests that alcohol induces a physiologic adaptive UPR that may prevent pathophysiologic pancreatitis responses. Indeed, heterozygous deletion of the XBP1 gene prevents XBP1 upregulation and results in pathologic changes including extensive dilation of the ER with occasional dense luminal inclusions, hallmarks of ER stress, and significant accumulation of autophagic vacuoles in acinar cells [48]. Thus, impaired UPR in the pancreas can potentiate alcohol-induced toxicity and aggravate pancreatic damages.

3.3. Brain. Alcohol exposure during development has devastating effects on the loss of neurons in selected brain areas, which leads to profound damages to the central nervous system (CNS). Alcohol consumption during pregnancy causes fetal alcohol spectrum disorders (FASDs) [1, 49]. Microcephaly, abnormal cortical thickness, reduced cerebral white matter volume, ventriculomegaly, and cerebellar hypoplasia are the prominent CNS abnormalities in FASDs. Children with (FASD) have a variety of cognitive, behavioral, and neurological impairments [49]. What cause ethanol-induced neurodegeneration are not clear. Considering that ER stress plays a role in the pathogenesis of several popular neurological diseases such as Huntington's disease, brain ischemia, Alzheimer's disease, and Parkinson's disease [50–53], an involvement of ER stress in alcohol-induced neuron toxicity has been hypothesized [54]. Recent evidence from both *in vitro* and *in vivo* tests appears to support the assumption. Exposure of SH-SY5Y neuroblastoma cells or primary cerebellar granule neurons to ethanol alone had little effect on the expression of ER stress markers [54]; however, ethanol markedly increased the expression of GRP78, CHOP, ATF4, ATF6, and phosphorylated PERK and eIF2α in the presence of tunicamycin or thapsigargin, which was accompanied with increased cell death. Acute exposure of seven-day-old mice to ethanol by subcutaneous injection at a dose of 5 g/kg significantly increased ER stress response. Increase of ATF6, CHOP, GRP78, and mesencephalic astrocyte-derived neurotrophic factor as well as the phosphorylation of IRE1, eIF2α, PERK, and PKR were detected within 24 hours after the ethanol exposure. Further, the ethanol-induced increase in phosphorylated eIF2α, caspase-12 and CHOP was distributed in neurons of specific areas of the cerebral cortex, hippocampus, and thalamus. Since the age of the animals used in this experiment is equivalent to the third trimester of pregnancy in humans, the above evidence suggests that ethanol directly induce ER stress in the developing brain.

3.4. Heart. It is well documented that chronic heavy alcohol drinking is a risk factor for cardiovascular disorders including cardiac hypertrophy, myofibrillar disruption, reduced contractility, and decreased ejection fraction [55]. Alcohol may change the circulatory hemodynamics resulting in stress on the heart. The stressed heart demands more cardiac output which leads to compensative hypertrophic responses such as neurohormonal activation and increased growth factors and cytokines, resulting in enlarged cardiomyocytes and increased sarcomere assembly. ER stress may play a critical role in regulating protein synthesis in cardiac myocytes, and thereby produce cell enlargement and cardiac hypertrophy. Chronic alcohol consumption by FVB (Friend virus-B type) albino mice at 4% of diet for 12 weeks resulted in increased heart weight and heart-to-body weight ratio [56]. In the myocardium of the FVB mice chronically fed alcohol, GRP78, CHOP, and IRE1a protein expression levels were increased, indicative of the UPR. Class I alcohol dehydrogenase efficiently oxidizes alcohol resulting in increased production of acetaldehyde. Overexpressing alcohol dehydrogenase in the FVB mice during chronic ethanol treatment resulted in a greater UPR upregulation [56]. The finding indicates that acetaldehyde from alcohol metabolism may induce ER stress. Furthermore, overexpressing of the antioxidant protein metallothionein in FVB mice significantly reduced peak shortening and maximal shortening velocity of cardiac myocytes by LPS, which is often elevated in alcoholics [13–15, 39, 40]. In parallel, the transgenic FVB mice displayed decreased protein levels of GRP78, CHOP, PERK, and IRE1 whereas the wild type FVB displayed a significant increase in the protein levels of PERK, phospho-JNK, and phospho-p38 in the myocardium in response to LPS [56, 57].

4. Mechanisms of Alcohol-Induced ER Stress

4.1. Acetaldehyde Adducts and ER Stress. Alcohol-derived acetaldehyde is highly reactive [58–62]. At physiological temperature and pH, acetaldehyde reacts with nucleophilic

groups in proteins, such as α-amino groups of internal lysine residues and the ε-amino group on the N-terminal amino acid of unblocked proteins forming unstable Schiff base acetaldehyde adducts. In addition, ethanol abuse may also lead to the formation of other types of protein adducts, such as malondialdehyde-acetaldehyde hybrids and α-hydroxyethyl protein-adducts. The acetaldehyde adducts initiate immunogenic reactions, cause conformational changes and inactivation of the adducted targets, or trigger aberrant protein degradation, which contribute to the development of alcoholic organ diseases (Figure 1). Malondialdehyde–acetaldehyde adduct is found to be the dominant epitope after malondialdehyde modification of proteins in atherosclerosis [63]. Antibodies to the aldehyde adducts have been detected in the serum of patients with atherosclerotic lesions and correlate with the progression of atherosclerosis. It is known that atherosclerosis develops as a result of protein unfolding and modification of protein and/or macromolecular complex function at the cellular level [63]. In supporting this, evidence for ER stress response was found in transgenic mice with cardiac overexpression of ADH that increased acetaldehyde exposure [56, 57]. The ADH transgene increased induction of IRE1, eIF-2α, GRP78, and CHOP and exacerbated chronic alcohol ingestion-induced myocardial dysfunction and hypertrophy. Further, in a mouse model of acute ethanol intoxication, inhibition of ADH causes downregulation of GRP78 mRNA levels [64]. This suggests a causal relationship between ethanol metabolism and ER stress response. Acetaldehyde adducts also affect ER Ca^{2+} handling in rat ventricular myocytes [65, 66], which may disturb ER calcium homeostasis playing a critical role in stress-mediated cellular injury [67]. In response to alcohol dosing *in vivo,* the actin in Type I and Type II fibre predominant muscles of rats was found to form stable covalent adducts with acetaldehyde [68]. Histochemical analysis showed that unreduced-acetaldehyde-protein adducts were located within the sarcolemmal (i.e., muscle membrane) and subsarcolemmal regions, which perturbed the membranes and increased protein and enzyme activity of sarcoplasmic-ER Ca^{2+}-ATPase, resulting in muscle cell death and alcoholic myopathy. In addition, acetaldehyde adducts are found in the central nervous system which may be responsible for alcoholic ER stress response. In the brain of a heavy drinker who had died suddenly while drinking continuously, acetaldehyde adducts were immunologically identified [69]. In a mouse model administered with the Lieber-DeCarli liquid diet and alcohol, acetaldehyde adducts were readily detected in degenerated neurons in the cerebral cortex [70]. The neural region that alcoholic ER stress response occurred colocalized with the acetaldehyde adducts. In young mice, ethanol-induced increase in ER stress protein markers was found to be distributed in the immature neurons of specific areas of the cerebral cortex, hippocampus and thalamus [54]. Thus, while most organs of the body can be affected by alcohol-derived acetaldehyde, cardiac and skeletal muscle cells and neurons appear to be particularly susceptible to acetaldehyde adducts that cause ER stress and injury.

4.2. Homocysteine Toxicity and ER Stress. Homocysteine (Hcy) is a normal intermediate involved in the metabolism of the essential amino acid-methionine (Figure 1). Excessive Hcy is toxic to cells. An abnormally elevated level of Hcy in the blood, a medical condition termed hyperhomocysteinemia (HHcy), is an independent risk factor in cardiovascular, neurodegenerative diseases, diabetes, obesity, and hepatic steatosis [32, 71–73]. It is generally accepted that aminoacyl thioester homocysteine thiolactone (HTL) derived from Hcy editing during protein synthesis contributes to the most of Hcy toxicity [74, 75]. HTL undergoes not only nucleophilic, which can be facilitated in the presence of acetaldehyde, but also electrophilic reactions to form protein adducts or homocysteinylation of protein lysine side chains and/or other free amine groups [75]. These reactions cause malfolding of proteins and trigger ER stress response. Evidence linking HHcy to ER stress and alcoholic liver injury has well been established in cell and animal models [16, 18–20, 32]. The intragastric alcohol feeding exhibited a greater than 5-fold increase in mouse plasma Hcy [18, 34, 35]. Hcy is metabolized normally by remethylation to methionine which is catalyzed by methionine synthase (MS) using folate as a methyl donor and by betaine-homocysteine methyltransferase (BHMT) using betaine as a methyl donor. Chronic alcohol-induced disturbance of methionine metabolism appears to contribute to the alcoholic HHcy. Alcohol inhibits enzyme activity of MS in mice and rats and reduces mRNA expression of BHMT and MS in mice [16, 17, 34, 76–79]. Simultaneous betaine feeding in the intragastric alcohol-fed mice decreased alcoholic HHcy and abrogated ER stress response in parallel with decreased ALT and amelioration of alcohol-induced necroinflammation, apoptosis, and fatty liver [18]. In cultured HepG2 cells, BHMT overexpression inhibited Hcy-induced ER stress response, lipid accumulation, and cell death [77]. In primary mouse hepatocytes, suppression of BHMT by RNA interference potentiated Hcy-induced but not tunicamycin-induced ER stress response and cell injury [77]. Transgenic mice expressing human BHMT in organs peripheral to the liver are resistant to alcohol or a high methionine and low folate diet induced HHcy and fatty liver [78]. In intragastric alcohol-fed rats, BHMT is induced, which minimizes the effect of inhibited MS on Hcy levels and subsequent ER stress response and injury [79]. In a survey using 14 mouse strains, Ivan Rusyn has found that the alcoholic HHcy is correlated with alcohol-induced liver jury (personal communication, 2011). Therefore, the above several lines of evidence support Hcy toxicity as a pathogenic factor contributing to alcohol-induced disorders.

4.3. SAM/SAH Ratio, Epigenetic Alterations and ER Stress. There are two types of important epigenetic regulations of gene expression: DNA methylation of cytosines within CpG dinucleotides and histone modifications [80, 81]. Aberrant epigenetic changes are involved in the etiology of a growing number of disorders such as alcohol dependence. Both global hypomethylation of DNA in liver and hypermethylation of DNA from peripheral blood cells have been reported in animal models and in human subjects with alcohol

dependence [82–86]. This is because DNA methylation in general depends on the methyl donor S-adenosylmethionine (SAM) and is inhibited by S-adenosylhomocysteine (SAH). Both SAM and SAH are involved in methionine metabolism [87, 88]. Inside the cell, SAM is demethylated to SAH, which is readily converted to Hcy which is remethylated to methionine. Plasma Hcy is not metabolized and represents the cumulative export of Hcy from liver and other tissues. Alcohol consumption decreases levels of SAM and increases levels of SAH and/or Hcy resulting in a decrease in SAM to SAH ratio (Figure 1) [76, 78, 87–92]. Thus, alcohol has a marked impact on the hepatic methylation capacity. Evidence demonstrating epigenetic effects on alcoholic ER stress is emerging [17, 82]. In 66 male alcoholic patients with alcohol dependence, chronically elevated Hcy levels are associated with increased DNA methylation in the promoter region of homocysteine-inducible ER protein (HERP) and decreased expression of HERP mRNA in the blood [93, 94]. The decrease in HERP levels is followed by a lethal ER stress, mitochondrial dysfunction, and cell death in neurons of the developing and adult brain [94]. Thus it is conceivable that alcoholic Hcy regulates HERP and causes ER stress and injury through an epigenetic mechanism. In respect to the epigenetic modifications of histone, it is reported that alcohol causes a dose- and time-dependent selective acetylation of histone H3-K9 in cultured hepatocytes [95, 96]. Intragastric administration of ethanol increases the levels of acetylated H3-K9 by 2-3 folds in the liver of mice after 12 h [97]. Further analysis indicates that the increased acetylation is tissue specific as it is noted in liver, lung, and spleen but not in tissues from other organs tested. Thus, while other stress pathways such as the MAPK signaling may be involved, the alcoholic epigenetic effects on the ER stress pathways can be more relevant. For instance, in both cystathionine beta synthase heterozygous ($CBS^{+/-}$) and wild type (WT) mice fed ethanol diets by intragastric infusion for 4 weeks, steatohepatitis, reduction in liver SAM, elevation in liver SAH, and reduction in the SAM/SAH ratio were observed [17]. Hepatic ER stress markers including GRP78, ATF4, CHOP, caspase 12, and SREBP-1c were upregulated and negative correlated with the SAM/SAH ratio in response to alcohol. Further, trimethylated histone H3 lysine-9 (3meH3K9) protein levels in centrilobular regions revealed by immunohistochemistry were reduced in ethanol-fed mice. The levels of 3meH3K9 in the promoter regions of GRP78, SREBP-1c, and CHOP revealed specifically by a chromatin immunoprecipitation assay were decreased only in $CBS^{+/-}$ mice fed alcohol. Since CBS is involved in transsulfuration of Hcy, the findings imply that interactions of CBS ablation and alcohol feeding impair methionine metabolism, which leads to epigenetic modifications of ER stress signaling pathways. In addition, the key modulator of UPR, sXBP1 has recently been found to be a nonhistone protein target of acetylation mediated by p300 and deacetylation mediated by the NAD^+-dependent class III deacetylase SIRT1 (sirtuin 1) [98, 99]. SIRT1 is demonstrated to be one of the major targets of alcohol action which influences TNF-α production in macrophages and alters glucose and lipid metabolism in the liver leading to hepatic steatosis and inflammation [100–102]. SIRT1 may also play a role in alcohol-induced ER stress response and injury through an epigenetic mechanism.

4.4. Oxidative Stress and Disruption of Ca^{2+} or Iron Homeostasis and ER Stress. In the ER, proteins undergo oxidative protein folding. PDI is a critical oxoreductase that catalyzes disulfide bond formation with consequent generation of reactive oxygen species (ROS) during the oxidative protein folding [19, 103]. ROS is normally under control due to cellular glutathione that sustains PDI ability to regenerate and form disulfide bridges repeatedly [103–105]. However, chronic ethanol consumption increases the production of a variety of ROS, including superoxide, H_2O_2, lipid peroxides, and peroxynitrite [1, 13–15]. Alcoholic ROS reduce glutathione level and increase oxidized glutathione, which breaks the redox status of the ER (Figure 1). This loss of redox homeostasis perturbs the oxidative folding and makes PDI ineffective in the catalytic redox cycles leading to more utilization of reduced glutathione. Depletion of glutathione generates excessive ROS which triggers ER stress. Antioxidant treatment, CHOP deletion, or translation attenuation has been shown to reduce oxidative stress and preserve ER function [19–23]. Ethanol rapidly caused oxidative stress in cultured neuronal cells and antioxidants blocked alcoholic potentiation of ER stress and cell death [54]. An association of ER stress response with increased oxidized glutathione was found in the pancreatic acinar cell of the ethanol-fed rats [47]. In HepG2 cells, acetaldehyde impaired mitochondrial glutathione transport and stimulated mitochondrial cholesterol content, the latter of which was preceded by increased levels of CHOP and SREBP1 [106]. Chronic exposure of animals to alcohol or overexpression of cytochrome CYP2E1 in hepatocytes increases the expression of superoxide dismutase (SOD) and activates nuclear factor erythroid 2-related factor 2 (Nrf2), which is an ER stress responsive factor [14, 107–109]. These lines of evidence suggest an intimate relationship between ER stress and ROS production. Furthermore, alcoholic oxidative stress plays a critical role in possible interplays between ER stress and mitochondrial stress, which can be mediated either by intracellular calcium or iron. Alcohol or Hcy induces alterations of lipid composition in the ER and affected ratio of phosphatidylcholine (PC) to phosphatidylethanolamine (PE) [20, 78]. Alterations of the PC/PE ratio disrupt ER calcium homeostasis causing ER stress [110]. Under ER stress, abnormal release of intracellular Ca^{2+} from the ER via inositol 1,4,5-triphosphate receptor (IP3R) channels leads to excessive mitochondrial Ca^{2+} uptake, which in turn promotes ROS production and apoptosis via multiple effects on the mitochondria [67, 111, 112]. Elevated serum iron indices (transferrin saturation, ferritin) and hepatic iron overloading are often observed in patients with alcoholic liver disease [113–117]. Excessive iron damages mitochondrial iron–sulfur clusters that generate defects in heme-containing cytochrome c and cytochrome oxidase leading to excess mitochondrial ROS [118]. Iron homeostasis is regulated by hepcidin, a circulatory antimicrobial peptide synthesized in hepatocytes [119]. Critically, ER stress response can regulate expression of hepcidin

[19, 29, 120]. Thus a vicious cycle exists: alcoholic ROS and/or ER stress damage mitochondria through iron, which in return augments ROS and stresses the ER further, all of which probably act synergistically to cause severe alcoholic injury.

4.5. Synergistic ER Stress by Alcohol, Drugs, Viral Infection and Environments. Acute alcohol or chronic alcohol at moderate concentrations may not induce readily detectable ER stress response in some cell and animal models [29, 47]. This does not rule out the doomed potential of alcohol to induce ER stress. Indeed, ER stress can be synergistically induced by alcohol in the presence of environmental factors, genetic predispositions, drugs, or virus infection. First, it is recently noted that an accelerated development of pancreatitis in alcoholic patients who smoke may result from an additive or multiplicative effect that is mediated by ER stress response [47]. Second, in a mouse model with liver-specific deletion of Grp78, low-level oral alcohol feeding did not induce HHcy that is often seen in mice fed high doses of alcohol [29]. However, the low alcohol feeding activated SREBP1 and unconventional splicing of Xbp1 (sXbp1) and decreased Insig 1 and ATF6 and its downstream targets such as ERp57 and Derl3 in the liver GRP78 knockouts, leading to aggravated lipid accumulation in the liver. Thus, compared to the aforementioned Hcy-ER stress mechanism, Grp78 deletion represents a genetic predisposition that unmasks a distinct mechanism by which alcohol induces ER stress, one that normally is largely obscured by compensatory changes in normal animals or presumably in the majority of human population who have low-to-moderate drinking. Similarly, certain drugs potentiate alcoholic ER stress response. For instance, some HIV protease inhibitors (HIV PIs) used in anti-HIV therapeutics can cause adverse side effects such as dyslipidemia and liver injury [29, 121, 122]. Portion of HIV-infected patients often concomitantly consume or abuse alcohol leading to more severe liver injury. One of the underlying mechanisms is severe ER stress responses that are caused by both alcohol and the HIV drugs. It has been demonstrated that single gavage dosing for alcohol alone or ritonavir and lopinavir combined did not induce detectable liver injury in wild type [29]. However, the gavage treatment with alcohol plus the two HIV drugs caused significant increase in plasma ALT as well as activation of CHOP, ATF4, and sXbp1. Thus, alcohol exacerbates some HIV drug-induced ER stress and subsequent injury. Third, it is known that both alcoholic activation of the ER stress sensor-IRE1α and alcohol-induced accumulation of proinflammatory cytokines such as TNFα, IL-6, and MCP-1 activate JNK and/or NF-κB pathways that mediate tissue/organ injuries [9, 10, 23, 29, 37–39]. This pathway overlap may be a result of interactions between ER stress and inflammation. The likely scenario is that mild ER stress under moderate alcohol dosing has a negative impact on ER function, which makes cells more susceptible to inflammatory signals, which subsequently augments ER stress response and injury via the JNK pathway. Fourth, alcohol may sensitize virus-infected cells to ER stress and apoptosis. It is reported that hepatitis C (HCV) infection causes ER stress in cell and animal models as well as in patients with chronic HCV [123–125]. HCV directly induces steatosis and development of hepatocellular carcinoma (HCC), which is correlated with a state of oxidative stress in mice transgenic for the HCV core protein [126, 127]. There is clinical evidence indicating that alcohol metabolism increases HCV replication and modulates the host response to HCV [128, 129]. The HCV nonstructural protein 5A (NS5A) localizes to the ER and is part of the HCV replication complex that forms altered cytoplasmic membrane structures. The membrane structure triggers ER stress and the UPR, leading to a release of ER Ca^{2+} stores and subsequent oxidative stress [124]. In addition, interactions between HCV core and destabilization of the mitochondrial electron transport chain result in increased production of ROS [130, 131]. Since alcohol alone perturbs Ca^{2+} homeostasis and stimulates ROS generation, it is conceivable that ROS mediates the synergistic interactions between alcohol consumption and HCV infection.

5. Concluding Remarks

While a large number of different stress responses and pathological pathways have been implicated in ethanol-induced injury [1, 7, 13–15], the occurrence of ER stress in the major organs including liver, brain, pancreas, and heart firmly supports its contributing role to alcoholic disorders. Alcohol causes alterations in many specific steps involved in the ER stress and UPR. The potential causes for alcohol-induced ER stress are directly or indirectly related to alcohol metabolism, which include but may not be limited to toxic acetaldehyde and homocysteine modifying proteins, oxidative stress from impaired CYP2E1 function and perturbations of calcium or iron homeostasis, alterations of SAM to SAH ratio and subsequent biochemical or epigenetic modifications, and, most importantly, interactions between these factors. Each of the factors may contribute more or less to the induction of the ER stress depending on tissues/organs or experimental models, dosage and duration of alcohol exposure, and presence of other environmental factors. Current investigations and conclusions on alcoholic ER stress appear depending on positive identifications of selective molecular markers of ER stress response, conclusions from which can be misleading sometimes. For instance, the ER stress-induced UPR is dynamic. It can be protective when most of the ER markers are positively detected or detrimental when most markers are latent or disappearing. The timing and quantity of the protection cannot be defined currently. Thus, circumstantially negative observations of the ER stress markers may not necessary rule out an existence of alcoholic ER stress. Future research should be directed at developing sensitive markers, particularly epigenetic markers, for identifying the alcoholic ER stress, and at defining timing and dynamics of the alcoholic ER stress and injuries using both acute and chronic models. Another point is that the ER is a cytosolic network that communicates readily with other cellular loci such as mitochondria, lysosome, cytoplasm, and nucleus. Simultaneous appearance of alcoholic dysfunctions of the other loci such as ATP depletion, abnormal degradation of

the inside materials, oxidative stress, and numerous other stress responses could overshadow the role of ER stress in alcoholic diseases. Thus, the role of alcoholic ER stress in organ disorders can be defined precisely by studying complex interplays among the organelles and loci in disease pathogenesis, which could provide better therapeutic strategies targeting the ER. Finally, with respect to the therapeutic interventions at alcoholic ER stress, possible approaches include lowering homocysteine and raising SAM by nutritional support with betaine or folate [16, 20, 32], improving protein folding by using chemical chaperone PBA (sodium 4-phenylbutyrate) and TUDCA [19, 20, 29], blocking eIF2α dephosphorylation by using salubrinal [132], and ameliorating ROS production from the oxidative protein folding by using antioxidants. However, results of clinical trials are not available. Each of the individual approaches alone may not be a simple or universal cure as alcohol-induced pathogenesis is very complex. It is anticipated that properly combined treatments with all the beneficial agents can be effective.

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