Endoplasmic Reticulum Stress Response in Non-Alcholic Fatty Liver Disease: Sophisticated Pathways

Alkol Dışı Karaciğer Yağlanmasında Endoplazmik Retikulum Stress Yanıtı: Sofistike Yolaklar

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ABSTRACT

Non-alcoholic fatty liver disease comprises a broad spectrum of fat-associated liver conditions that can result in end-stage liver disease and the need for liver transplantation. The multiparallel hypothesis suggests that steatohepatitis is the result of numerous conditions acting in parallel, including form genetic susceptibility, lipotoxicity, disturbed gut microbiata to mitochondrial dysfunction, and endoplasmic reticulum(ER) stress. The unfolded protein response as the ER stress response is coordinated primarily by ER transmembrane stress transducers which is a defensive response initially activates the cell to recover from stress or adapt to stress. It reduces the secretory protein load, enhances protein folding and increases clearance capacity by promoting autophagy and ER-associated degradation. However, if these attempts fail or the ER stress gets prolonged, it will induce cell death programs to eliminate the stressed cells. In recent years, ER stress response has been identified as a crucial mechanism in steatohepatitis by leading improper lipid biosynthesis, inflammation, and autophagy or apoptosis.

Keywords: Steatohepatitis, Endoplasmic reticulum stress, Unfolded protein response

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ÖZET

Alkol dışı karaciğer yağlanması, yağ ile ilişkili karaciğer patolojilerini içine alan geniş çerçevede pek çok hastalığı kapsar. Bu hastalıklar son dönem karaciğer hastalığı ile sonuçlanıp karaciğer nakli dahi gerektirebilir. Multiparalel hipotezi steatohepatitin pek çok sayıda durumun birlikte hareket ederek etki ettiğini önerir. Bunlar genetik duyarlılık, lipotoksisite, bozulmuş barsak mikrobiatası, mitokondrial disfonksiyon, endoplazmik retikulum (ER) stresi gibi durumlardır. ER stresi olarak katlanmamış protein yanıtı, esas olarak ER membranı stres dönüştürücüleri ile ilişkilidir. Bu aracılar ile hücre savunma yanıtı ya hücreninin aktivasyonu ile bu stresden kurtulmasını ya da bu strese adapte olmasını sağlar. ER stres yanıtı, sekretuvar protein yükünün azalması, protein katlanmasının artması, otofaji ve ER aracılı protein yıkımının uyarılması ile protein klerensinin artmasıdır. Fakat, eğer bu stres yanıtı yetersiz kalır yahut uzarsa, stres altındaki hücrenin hücre ölüm programları devreye girerek stresli hücreyi yok eder. Son yıllarda ER stres yanıtının, uygunsuz lipid biyosentezi, inflamasyon, otofaji, apoptosis ile oluşan steatohepatit için önemli bir mekanizma olduğunu tanımlanmıştır.

Anahtar Sözcükler: Steatohepatit, Endoplazmik retikulum stressi, Katlanmamış protein yanıtı

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INTRODUCTION

Due to the epidemic of obesity across the world, nonalcoholic fatty liver disease (NAFLD) as the hepatic manifestation of the metabolic syndrome has become one of the most prevalent chronic liver disorders (1). NAFLD comprises a broad spectrum of fat-associated liver conditions that can result in end-stage liver disease and the need for liver transplantation (2).

Simple steatosis, or fatty liver, occurs early in NAFLD and may progress to nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis with increased risk of hepatocellular carcinoma (3). The development of hepatocellular injury and then fibrosis is a critical circumstance in the natural course of NASH (Figure 1). That's why, NAFLD is considerable health problem, with an estimated prevalence of 25%, and understanding of its pathogenesis improves the management of treatment and prevention (4).

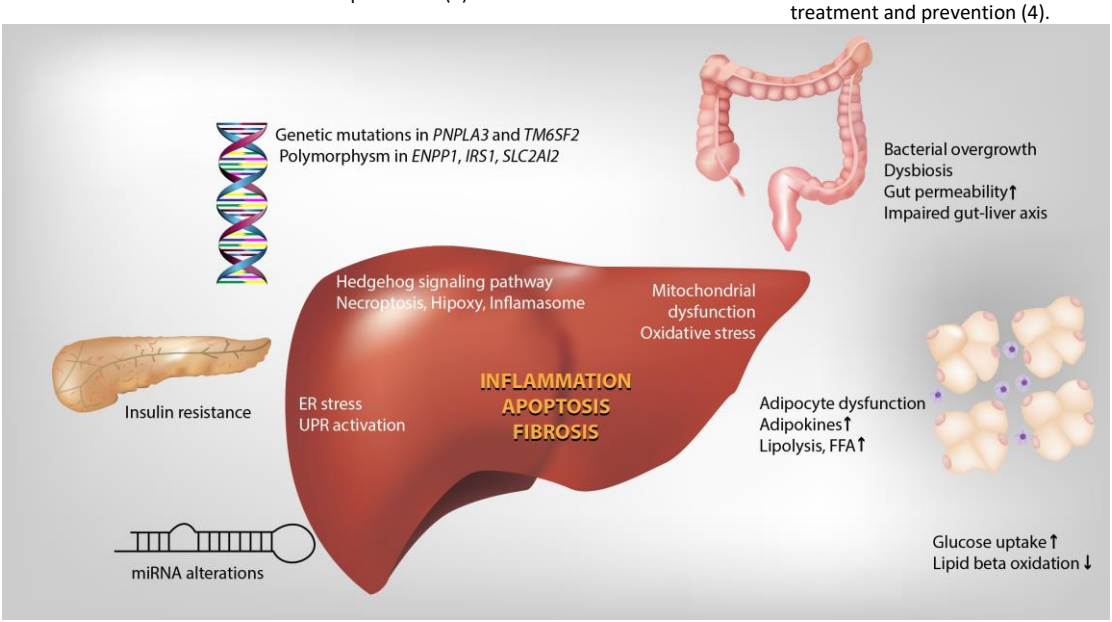

Figure 1. Natural course of non-alcoholic steatohepatitis

While many contributing factors in the pathogenesis of NASH are continuously identified, this sophisticated picture has not been unraveled yet. The multiparallel hypothesis suggests that NASH is the result of numerous conditions acting in parallel, including genetic susceptibility, lipotoxicity, oxidative stress, mitochondrial dysfunction, altered production of cytokines and adipokines, disturbed gut microbiota and endoplasmic reticulum stress (5-8) (Figure 2).

At the organ level, crosstalk between the liver, adipose tissue, muscle, and the gut plays an important role in the pathogenesis of NASH. At the cellular level, alterations of organelle function, such as mitochondria, peroxisome, endoplasmic reticulum (ER), and lysosome, have been shown to have key molecular mechanisms (9).

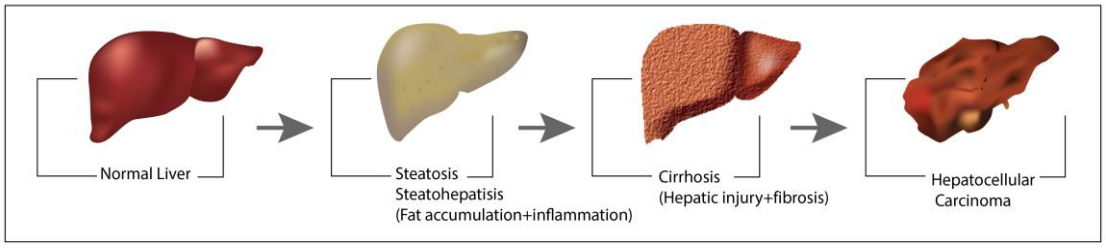

Figure 2. Non-alcoholic steatohepatitis is the result of numerous conditions acting in multiparallel hypothesis.

Hepatocytes are enriched in both smooth and rough ER to achieve a myriad of metabolic functions. Indeed, ER is responsible for vital cellular functions, including correctly folding of proteins, post-translational modification of secretory and membrane proteins, lipid biosynthesis, intracellular calcium homeostasis. The ER maintains a high-quality control system to provide that only the properly folded proteins are transported out of ER (10). The unfolded or misfolded proteins are retained in the ER and they are either refolded or degraded ultimately. The accumulation of unfolded or misfolded proteins in ER lumen is referred as ER stress. ER has evolved its own specific signaling pathway called the unfolded protein response (UPR) to overcome the stress. ER stressors emerge as a potential cause of damage in many disorders. Several pharmacologic and pathophysiological stimuli, such as glucose deprivation, aberrant calcium signaling, viral infection, falling in energy, lipid accumulation might impose stress on the ER, which leads to the activation of this UPR signaling pathway (11). This defensive response initially activates the cell to recover from stress or adapt to stress. The UPR reduces the secretory protein load, enhances ER protein folding (transcription of chaperones and foldases), and increases clearance capacity by promoting autophagy and ER-associated degradation (ERAD).

However, if these attempts fail or the ER stress gets prolonged, the UPR will induce cell death programs to eliminate the stressed cells (12) (Figure 1). UPR pathways prevent hepatic steatosis under physiologic conditions. In recent years, ER stress response has been identified as a crucial mechanism in NASH by leading to improper lipid biosynthesis, inflammation, autophagy, and apoptosis (13).

Initiation of ER stress in NAFLD

Hepatic lipid homeostasis is regulated by multiple mechanisms such as de novo lipogenesis, fatty acid oxidation, lipid storage, lipolysis, dietary fat consumption, and hepatic assembly and secretion of lipoprotein particles (14).

All potential pathological processes in NAFLD begins with excessive fat deposition. At this point, it should be highlighted that fat accumulation and lipotoxicity are not synonymous. Lipidomics studies have shown that, although most hepatic fat deposition in NAFLD is in the form of triglycerides, NASH is characterized by the accumulation of toxic lipid species resulting from complex lipid imbalance and hepatocellular damage (Table 1). Lipotoxicity can be defined as the accumulation of toxic lipid composition leading to organelle dysfunction, cell injury, and chronic inflammation (15).

Table 1. List of lipids having lipotoxicity in hepatocyte and their metabolic intermadiates

Toxic lipids and their intermediates
Saturated fatty acids (palmitic acid and stearic acid)
Ceramides and diacylglycerols
Lysophosphatidylcholine
Free cholesterol
Oxidized low density lipoproteins

Hepatic lipotoxicity both triggers ER stress and amplifies other pathways

Increased free fatty acids, and particularly saturated fatty acids (SFA), are defined as potential toxic lipids. The extent of the accumulation of SFA, such as palmitic acid (PA) and stearic acid, in the steatotic liver parallels liver disease severity and, consistently, these SFAs in lipotoxic properties have been linked to ER stress (16). PA is the most prevalent circulating SFA along with the total free fatty acid pool and is elevated in patients with obesity and NAFLD. The covalent attachment of PA to substrate proteins, palmitoylation, is recognized as an important post-translational modification in the ER (8,17). Palmitoylation is reversible, thereby allowing it to dynamically alter a range of protein functions including trafficking, localization, stability (18). In the hepatocyte, SFAs bind and activate plasma membrane receptors, especially death receptors, such as tumor necrosis factor (TNF)-related apoptosis-inducing ligand receptor (TRAIL-R2) and Toll like receptor (TLR4) (19, 20). The TRAIL-2 signaling pathway triggers caspase-8 to stimulate the effector caspases 3, 6, and 7, and directly induce apoptosis. In the intrinsic–mitochondrial pathway, active caspase cleaves the BH3-interacting domain death agonist proteins. These pro-apoptotic proteins rapidly drive permeabilization of the outer mitochondrial membrane and yield releasing mitochondrial cytochrome c and mitochondria-derived activator of caspase. Once in the cytosol, cytochrome c conjugates with ATP and apoptotic peptidase-activating factor-1 to recruit the initiator caspase-9 into a signaling complex called the apoptosome. Activated caspase-9 then also cleaves and activates the effector caspases-3, -6, and -7 to induce apoptosis (19, 20). TLR4 pathway activation triggers nuclear factor (NF)-kB–mediated synthesis of proinflammatory cytokines TNF-α, interleukin (IL)-6, pro–IL-1, and pro–IL-18 and activates stress kinases, including c-Jun N-terminal kinase (JNK) (19, 20). JNK, a member of the stress activated kinase family, is phosphorylated and activated by some stimuli such as sugars and lipids. JNK promotes caspase-induced apoptosis via Bax/PUMA-Bim signaling. JNK impairs mitochondrial respiration and enhances reactive oxygen species (ROS) generation in hepatocytes. JNK also reduces mitochondrial and peroxisomal beta oxidation and activates the proapoptotic proteins (19, 20). Moreover, SFAs activate TLR-4 on hepatic stellate cells to secrete the chemokine monocyte chemoattractant protein-1 and activate JNK in Kupffer cells and macrophages to induce proinflammatory chemokines secretion, chemotaxis, and secretion of pro-fibrogenic factors (21).

On the contrary, polyunsaturated fatty acids (PUFAs) contribute to fat removal from hepatocytes and have protective effects on NAFLD. Accordingly, decreased levels of omega 3 polyunsaturated fatty acid (n-3 PUFA), like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been found in patients with NASH and supplementation with this lipid class has been tested as a therapeutic approach in several studies (22). Similarly, the n-6 PUFA, namely linoleic acid (LA) and alpha linolenic acid (ALA), has been shown to protect hepatocytes from apoptosis and reduce JNK activation, resulting in less expression of proinflammatory mediators (23).

Suboptimal fatty acid oxidation in NAFLD accompanied by dysfunctional tricarboxylic acid cycle activity results in the accumulation of toxic lipid intermediates, like ceramides and diacylglycerols, inevitably leads to increase in ROS with inflammation and fibrosis (24). Ceramides may be generated de-novo from serine and palmitoyl-CoA or catalyzed from membrane sphingomyelin. Proinflammatory cytokines, including interleukin (IL)-1, and IL-6 lead to the production of ceramides which, in turn, contribute to the cell toxicity with proinflammatory actions via TNFα (24). However, the link between ceramides and apoptosis is still controversial. Furthermore, interference in ceramide synthesis was found to ameliorate steatosis and insulin sensitivity (25).

Lysophosphatidylcholine (LPC) is released from phosphatidylcholine (PC) is directly one of the downstream effectors of PA toxicity and is indirectly responsible for the induction of ER stress and activation of apoptotic pathways downstream of JNK (26). Small changes in the lipid composition in ER membrane, especially decreased ratio of PC to phosphatidylethanolamine (PE), have been linked to deterioration in hepatic ER calcium homeostasis and yields calcium sequestration in the cytoplasm due to inactivation of sarco-endoplasmic reticulum calcium ATPase (SERCA2b). This condition induces ER stress as the majority of ER chaperones are calcium dependent (26,27). The depletion of membrane PC concurrently disrupts hepatocyte membrane functional integrity, resulting in the release of proinflammatory cytokines and hepatocyte apoptosis (27).

Sterol regulatory element-binding proteins (SREBP) are master regulators of hepatic lipid homeostasis. SREBP-1 controls triglyceride biosynthesis, while SREBP-2 plays a critical role in cholesterol metabolism and LDL receptor expression (28). In NASH, SREBP-2 yields to upregulation of 3-hydroxy-3 methyl glutaryl coenzyme A (HMGCoA) reductase, the rate-limiting step of cholesterol synthesis, resulting in accumulation of free cholesterol. This elevated free cholesterol levels aggravates ER stress and then induces apoptosis and triggers JNK-dependent proinflammatory pathways (15, 16).

Lipoprotein metabolism, which is responsible for the transport of free and esterified cholesterol, is also dysregulated in NAFLD (6, 29). The scavenger receptors CD36 and SR-BI promote uptake of oxidized low-density lipoproteins (ox-LDLs) to the liver. Due to the activation of Kupffer cells and hepatic stellate cells (HSC) with ox-LDLs, dysregulated scavenger receptors lead to cholesterol deposition in NAFLD. The hepatic LDL-R expression was decreased, and plasma LDL concentration was increased in NAFLD patients (6). On the other hand, atherogenic dyslipidemia, which is characterized by high concentrations of plasma triglycerides, LDL and decreased high-density lipoprotein (HDL) levels stimulates insulin-induced hepatic lipid synthesis and is associated with NAFLD severity (29,30).

Remarkably, NAFLD has a complex background with both lipotoxicity and glucotoxicity (31). The process of 'glucotoxicity' involves alterations in carbohydrate metabolism. Excess carbohydrates, especially fructose, contribute to steatosis via activation of several lipogenic enzymes, including acetyl- CoA carboxylase, Stearoyl-CoA desaturase, and fatty acid synthase (32).

The Unfolded Protein Response in NAFLD

The UPR is coordinated primarily by three ER transmembrane stress transducers, protein kinase RNA –like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol requiring enzyme 1 (IRE1) (31,33) (Figure 3). Under physiological conditions, these proteins are maintained in an inactive state, bound to a molecular chaperone glucose-regulated protein 78 (GRP-78) which is also known as a master regulator of ER stress. Upon ER stress, GRP-78 dissociates from these stress sensors and leads to their activation (31, 33).

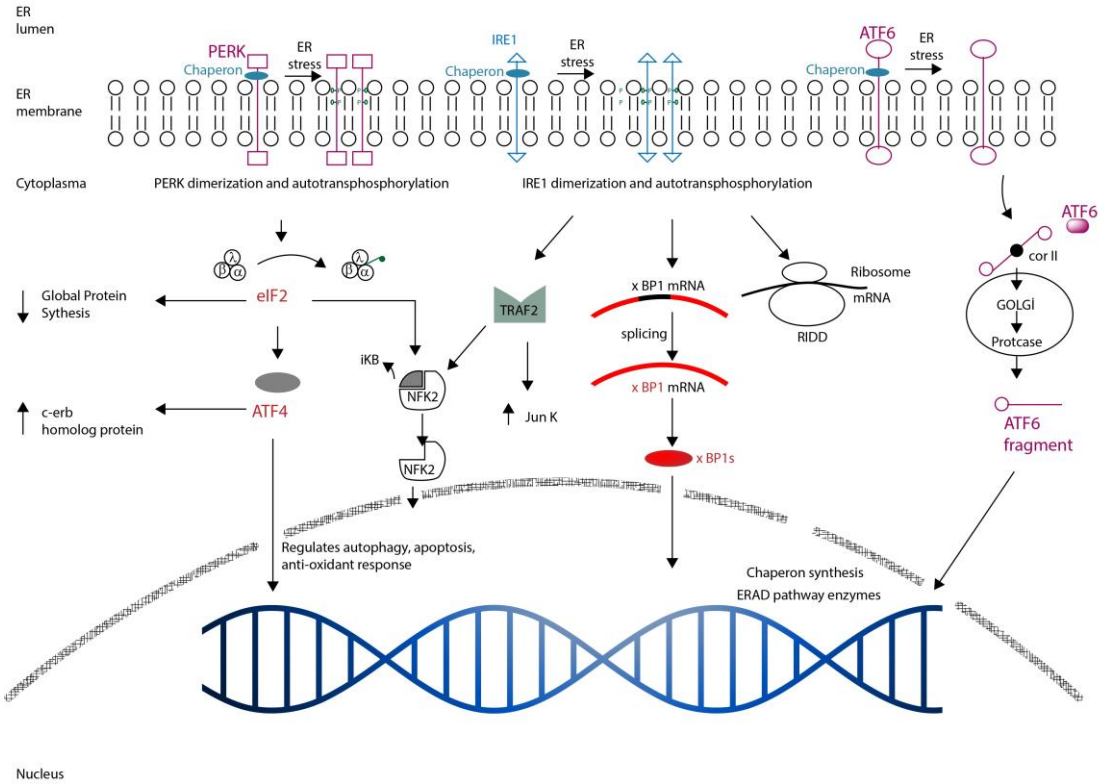

Figure 3. Unfolded protein response components and their downstream

The PERK pathway in NAFLD

PERK is a transmembrane protein with an N-terminal stress-sensing domain and a cytosolic kinase domain. Activation of PERK promotes phosphorylation of eukaryotic initiation factor (eIF2α) at Serine 51 in order to global attenuation of protein translation (34). eIF2α mediated inhibition of translation leads to activation of nuclear factor kappa B, which regulates antioxidant defenses and regulates antioxidant enzymes and cytoprotective proteins (36).

eIF2α phosphorylation decreases global protein synthesis and reduces protein uptake into the ER, meanwhile it induces the translation of selected proteins, including activating transcription factor 4 (ATF4), which controls specific transcriptional programs of the UPR (35). ATF4 activation leads to the production of molecular chaperones and ERAD for ameliorating ER protein folding or eliminating misfolded proteins by the ubiquitin-proteasome system, respectively (6, 11, 33). ATF4 also induces activation of C/EBP homologous protein (CHOP). Normally, the expression of CHOP is extremely low. The overexpression of CHOP promotes apoptosis in several cell lines. The down-regulation of Bcl-2 and the induction of the pro-apoptotic proteins Bim, Puma and Bax, a member of the death-receptor protein family are considered to related with CHOP-mediated apoptosis (36).

The ATF6 pathway in NAFLD

ATF6 is a transmembrane protein with a cytosolic Basic Leucine Zipper (bZip) transcription factor domain and two mammalian isoforms: ATF6α and ATF6β. GRP-78 dissociation results in the unmasking of two Golgi localization sequences within ATF6 (6,11,33). Once translocation to Golgi, ATF-6 is activated by proteolytic cleavage. Activated ATF6 fragments translocate into nucleus where it transcriptionally induces ER chaperones and upregulates the genes associated with ERAD machinery such as foldases (6, 11, 33).

The IRE1 pathway in NAFLD

IRE1 is the most conserved ER stress transducer, possessing two isoforms in mammals: IRE1α and IRE1β (37). IRE1α has dual enzymatic activities by means of its serine/threonine kinase and endoribonuclease (RNase) domains on its cytosolic tail. Sensing ER stress through its luminal domain, IRE1 dimerizes and oligomerizes by trans-autophosphorylation and conformational changes.

The activated RNase domain of IRE1 induces splicing of the mRNA encoding X-box binding protein 1 (XBP1) (6,11,33). XBP1s controls genes involved in protein folding, secretion, ERAD, and lipid synthesis.

In addition, XBP1s forms functional dimers with ATF6f to control distinct gene-expression patterns. Beyond its role in XBP1 mRNA splicing, IRE1 RNase is also involved in the direct degradation of mRNAs via IRE1-dependent decay (RIDD). Through RIDD, IRE1 cleaves substrate RNAs including mRNAs, ribosomal RNA, and microRNAs (38). In sustained conditions of ER stress, enhanced RIDD leads to cell death. IRE1α has an important role in ER stress-induced apoptosis (38). Activated IRE1α interacts with the adaptor protein tumor necrosis factor (TNF) receptor-associated factor 2 (TRAF2) and triggers phosphorylation cascade events that ultimately activate JNK. IRE1 also directly interacts with the pro-apoptotic effector proteins Bax and Bak, and then forms a common platform for pro-apoptotic signaling components (6, 11, 33).

The knockout of either IRE1 α or XBP1 results in embryonic lethality with major liver defects in mice (39). Although PERK and ATF6 are dispensable for liver development, studies related to their knockouts have shown that both are required to facilitate recovery from dietary or pharmacological challenges (37, 39).

If the adaptive UPR is overwhelmed by chronic or acute ER stress, it may become unable to preserve the normal function of the ER, even so an apoptotic cascade may be activated. ER stress signaling eventually appears to switch from pro-survival to pro-apoptosis (40).

UPR induces other pathways in NAFLD

Certain ER membrane-bound proteins like SERCA2b serve to pump calcium back into the ER lumen, thus it plays an important role in both overall cellular calcium homeostasis and maintenance of ER lumen calcium stores. It has been suggested that obesity-mediated reduction in SERCA2b protein or activity may lead to a reduced ER calcium store, decreased capacity for chaperone-mediated protein folding, and inactivation of the UPR (41). Palmitoylation can modulate ER proteostasis and ER-mitochondrial calcium crosstalk (18,41). Although it is not clear whether obesity increases palmitoylation of calnexin, this modification might have hints for obesity-associated changes in SERCA2b and hence obesity-associated ER stress and UPR (26).

The ER-mitochondrial coupling in the context of the dynamic nature of these two organelles may be relevant to obesity. Higher diacylglyceride, phospholipid, free cholesterol and FFA levels activate cellular ER stress. ER stress is inevitably concurrent processing with mitochondrial remodeling. The ER makes close physical contact with the mitochondria via mitochondrial-associated-membranes (MAMs) allowing calcium and lipid transfer between these two organelles (42). Inositol triphosphate receptors1-3 (IP3R1-3) are the major channels of calcium flux from the ER to mitochondria under physiological conditions. Voltage dependent anion channels 1-2 (VDAC1-2) are central players in the crosstalk between the cytoplasm and mitochondria in terms of the transport of various ions. Under prolonged ER stress, disturbed ER-mitochondrial communication causes the perturbation in mitochondrial Ca^{2+} homeostasis and induction of mitochondrial-dependent apoptosis through IP3R and VDAC. The changes in the levels of mitofusin2 (mfn2) and opacity outer membrane protein 1(OPA1) bring impaired energy production with cell damage (9,13).

The UPR signaling and inflammatory responses can interact and be integrated into NAFLD. ER stress activates NF-κB and JNK, leading to the induction of inflammatory genes, which in turn increase neutrophil recruitment to the liver and exacerbate both oxidative stress and inflammation (43). The PERK pathway of the UPR can activate an antioxidant program to limit the accumulation of ROS in response to ER stress via phosphorylation of NF-κB. Phosphorylated Nrf2 translocates to the nucleus and activates the transcription of a set of antioxidant and oxidant detoxifying enzymes. Therefore, Nrf2 deletion results in the rapid onset and progression of steatohepatitis. IRE1α binds to the adaptor protein TRAF2. The IRE1α-TRAF2 complex activates NF-κB and JNK, which in turn induce the synthesis of proinflammatory cytokines (20).

The UPR signaling pathway components themselves play a role in regulating hepatic lipid synthesis, export and oxidation, which are distinct from their well-defined roles in the canonical UPR (44, 45). Of these, the PERK-eiF2α-ATF-4 signaling pathway activates the expression of lipogenic enzymes. ATF-4-knockout mice showed protection against diet-induced obesity, hypertriglyceridemia, and hepatic steatosis due to reduced induction of PPARγ and other lipogenic genes (19). In hepatocytes, the IRE1α-XBP-1 couple modulates hepatic lipogenesis by directly binding to the promoters of a subset of lipogenic genes. The IRE1α-XBP-1 pathway deletion in mice livers showed decreased lipid biosynthesis (45). On the other hand, liver-specific XBP1 overexpression in dietary or genetic obesity models had an antisteatotic effect due to reduced fatty acid synthesis, and increased macrolipophagy (45,46). On the contrary, hepatocyte-specific XBP1 knockout mice exhibit greater liver injury, apoptotic and inflammatory signaling, and fibrosis (46). Although there remain multiple unanswered questions and incompatible results, negative regulation of steatosis by XBP1 and RIDD appears to be beneficial in reducing hepatic steatosis, whereas inactivation of IRE1α endoribonuclease activity promotes hepatic steatosis and hyperactivation of IRE1α promotes NASH (45,46). ATF-6 interacts with the nuclear SREBP-2, antagonizes transcription of SREBP-2-regulated lipogenic genes and decreases lipid deposition in cultured liver cells. ATF-6 knockout mice improve hepatic steatosis in response to an ER stress inducer in consequence of reduced fatty acid oxidation and decreased VLDL production (15).

CONCLUSION

In summary, except for regulating the protein synthesis and degradation, the UPR is involved in lipid metabolism, inflammation, autophagy and apoptosis in steatohepatitis. UPR reveals multiple distinct signal pathways, and the superposition of these signals determines the ultimate development of NAFLD. Although existing evidence is not enough to fully clarify the impact of regulating UPR in NAFLD, promising studies on ER stress initiation and the UPR signaling pathways in NAFLD might provide possible novel therapeutic targets in the prevention and treatment of NAFLD.

Conflict of interest

No conflict of interest was declared by the authors.

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