Published in final edited form as:

Hepatology. 2019 June ; 69(6): 2696–2698. doi:10.1002/hep.30427.

MicroRNAs: Unfolding the link between inositol-requiring enzyme 1 α and fatty liver

Xiaoying Liu, PhD, Richard M. Green, MD

Northwestern University Feinberg School of Medicine

Nonalcoholic fatty liver disease (NAFLD) is currently the most common cause of abnormal liver chemistry tests in the United States and western world. The mechanisms underlying hepatic steatosis and the progression to nonalcoholic steatohepatitis (NASH) remain incompletely understood. Multiple cellular stress pathways are believed to contribute to the pathogenesis of fatty liver, including endoplasmic reticulum (ER) stress and its adaptive unfolded protein response (UPR). Numerous studies have demonstrated the important role of ER stress and UPR activation in a host of liver diseases including viral hepatitis, alcohol and drug hepatotoxicity, cholestatic liver disorders and genetic liver diseases. Similarly, studies of human NAFLD and NASH and experimental animal models of fatty liver further support the importance of ER stress and the UPR in fatty liver disease pathogenesis.

The UPR signaling pathways are initiated through three transmembrane ER stress sensor proteins, including inositol-requiring enzyme 1 α (IRE1 α), PKR-like ER kinase (PERK) and activating transcription factor 6 (ATF6). All three UPR pathways, especially PERK and IRE1 α pathways, have been well implicated in the pathogenesis of NAFLD and NASH. The IRE1 α pathway is the evolutionally most conserved. In the presence of ER stress, IRE1 α autophosphorylates and activates its endonuclease activity to induce X-box binding protein 1 (XBP1) splicing and the cleavage of select mRNAs through regulated IRE1 α -dependent decay of mRNA (RIDD) (1). In addition to its function as a cell fate executor in response to ER stress, IRE1 α is essential in maintaining metabolic homeostasis, including lipid metabolism. In particular, hepatic IRE1 α appears to be protective from ER stress-induced lipid accumulation. Under acute ER stress conditions induced by pharmacologic stressors such as tunicamycin, hepatic deficiency of IRE1 α causes enhanced liver steatosis through increasing lipogenic genes, including CCAAT-enhancer-binding protein β (C/EBP β), C/EBP δ and peroxisome proliferator-activated receptor gamma (PPAR γ); and reducing apolipoprotein B-containing lipoprotein secretion (2). In a study of chronic ER stress induced by a high fructose diet, hepatocyte-specific IRE1 α deletion aggravates lipid accumulation in the liver, which is associated with down-regulation of very low-density lipoprotein (VLDL) assembly and secretion (3). Furthermore, hepatic RIDD can regulate lipid synthesis and metabolism. It has been shown that a number of lipid metabolism genes (such as *Pcsk9* and *Dgat2*) are direct substrates of RIDD. In the hepatocyte-specific XBP1 knockout mouse, the feedback hyperactivation of IRE1 α and resultant RIDD activation contributes to the hypolipidemic phenotype (4). In addition, precursor microRNAs (pre-miRNA) have emerged to be a new class of RIDD substrate, through which IRE1 α regulates apoptosis and other biological functions.

In a recent issue of *Science Signaling*, Wang *et al* (5) further confirm the protective role of IRE1 α in fatty liver using a chronic, high fat dietary model of NAFLD that causes the metabolic syndrome and hepatic steatosis. In this study, the authors demonstrate that the endonuclease activity of IRE1 α is suppressed in the livers of high fat diet-fed mice, and patients with hepatic steatosis, through a post-translational modification (S-nitrosylation) of IRE1 α . Liver-specific ablation of IRE1 α results in worsened hepatic steatosis and insulin resistance in mice fed a high fat diet. The authors further illustrate an important and novel regulatory mechanism of lipid homeostasis by IRE1 α . IRE1 α -mediated RIDD activity is shown to inhibit the maturation of miR-200 and miR-34 families of miRNA by targeting their pre-miRNAs, which contain potential G/C IRE1 α cleavage sites in their stem-loop structures. This inhibition occurs both *in vivo* and *in vitro* and is independent of XBP1. Furthermore miR-200 and miR-34 bind to the 3'-UTR region and attenuate the expression of PPAR α and SIRT1, which are key regulators in fatty acid oxidation and triglyceride lipolysis. This regulatory pathway is impaired in IRE1 α deficient mice, predisposing them to develop severe steatosis upon high fat feeding due, at least in part, to the reduced expression of PPAR α and SIRT1. Attenuated expression of these genes can cause a decrease in fatty acid oxidation and triglyceride lipolysis. These intriguing findings identify a new mechanistic connection between ER stress and lipid metabolism, independent of XBP1. Of note, IRE1 α is also known to regulate the expression of several miRNAs through XBP1. In addition, all three UPR pathways can regulate miRNA expression, and miRNAs in turn modulate UPR components and contribute to the adaptive or apoptotic signaling upon ER stress under various pathological conditions.

Multiple studies indicate that miRNAs have an important role in the pathogenesis of NAFLD and NASH. A large number of miRNAs are differentially expressed in NAFLD/NASH patients and in animal models of NAFLD. These miRNAs are involved in glucose metabolism, lipid metabolism, cell proliferation, fibrosis and apoptosis and may be important in biomarker development. Wang *et al* (5) demonstrate that miR-200 and miR-34 are upregulated in the livers of patients with hepatic steatosis. Inhibiting miR-200 and miR-34 via antagomir increases PPAR α and SIRT1 expression and reduces lipid accumulation in IRE1 α -deficient hepatocytes treated with fatty acids, thus suggesting that targeting miRNAs could be of therapeutic potential in patients with NAFLD and NASH. Interestingly, miR-200 and miR-34 have also been implicated in the pathogenesis of hepatic fibrosis and hepatocellular carcinoma, further emphasizing the importance of these miRNAs in liver diseases. In addition to the miR-200 and miR-34 families, several other miRNAs such as miR-146, miR-155 and miRNA-223 are also upregulated in the livers of human diabetic patients with hepatic steatosis. Moreover these three other miRNA are also increased in IRE1 α deficient mouse hepatocytes and livers, suggestive of them being potential RIDD targets. Although PPAR α and SIRT1 are potential targets of these miRNAs, further investigations are needed to delineate how they are regulated by IRE1 α and the precise role of these miRNAs in hepatic steatosis.

IRE1 α -mediated, RIDD-dependent, degradation of pre-miRNA has been previously shown to suppress the biogenesis of select miRNAs in order to regulate the translation of the proapoptotic protein Caspase-2 (6). The degradation of miRNA by RIDD is also implicated in maintaining the function of bone marrow-derived progenitor cells during wound healing

under diabetic conditions (7). The investigations by Wang *et al* (5) delineate the important molecular mechanisms of the protective function of IRE1 α in hepatic steatosis under a chronic physiologic metabolic stress condition, linking RIDD with lipid metabolism through miRNA regulation. Interestingly a recent study published in *Hepatology* (8) using a Bax-Inhibitor-1 deficient mouse model suggests that IRE1 α activation promotes the progression from NAFLD to NASH through IRE1 α endonuclease activity-dependent activation of the inflammasome and enhanced cell death. The exact role of hepatic IRE1 α in NAFLD and NASH is context-dependent. It appears that during the progression of NAFLD to NASH, IRE1 α plays a protective role in simple steatosis, whereas it promotes inflammation and cell injury in later stages of NASH. It would be interesting to investigate the functions of the miRNAs identified by Wang *et al* in the context of NASH progression. Nonetheless, ER stress and its downstream UPR signaling pathways are key factors in the development of fatty liver diseases, and the novel cell signaling pathways identified in Wang *et al* provide a new mechanism that may be important in the pathogenesis of NAFLD. A thorough understanding of their functions may identify specific targets for novel therapeutic approaches to treat fatty liver diseases.

References

1. Maurel M, Chevet E, Tavernier J, Gerlo S. Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem Sci* 2014;39:245–254. [PubMed: 24657016]
2. Zhang K, Wang S, Malhotra J, Hassler JR, Back SH, Wang G, Chang L, et al. The unfolded protein response transducer IRE1 α prevents ER stress-induced hepatic steatosis. *EMBO J* 2011;30:1357–1375. [PubMed: 21407177]
3. Wang S, Chen Z, Lam V, Han J, Hassler J, Finck BN, Davidson NO, et al. IRE1 α -XBP1s induces PDI expression to increase MTP activity for hepatic VLDL assembly and lipid homeostasis. *Cell Metab* 2012;16:473–486. [PubMed: 23040069]
4. So JS, Hur KY, Tarrio M, Ruda V, Frank-Kamenetsky M, Fitzgerald K, Koteliensky V, et al. Silencing of lipid metabolism genes through IRE1 α -mediated mRNA decay lowers plasma lipids in mice. *Cell Metab* 2012;16:487–499. [PubMed: 23040070]
5. Wang JM, Qiu Y, Yang Z, Kim H, Qian Q, Sun Q, Zhang C, et al. IRE1 α prevents hepatic steatosis by processing and promoting the degradation of select microRNAs. *Sci Signal* 2018;11.
6. Upton JP, Wang L, Han D, Wang ES, Huskey NE, Lim L, Truitt M, et al. IRE1 α cleaves select microRNAs during ER stress to derepress translation of proapoptotic Caspase-2. *Science* 2012;338:818–822. [PubMed: 23042294]
7. Wang JM, Qiu Y, Yang ZQ, Li L, Zhang K. Inositol-Requiring Enzyme 1 Facilitates Diabetic Wound Healing Through Modulating MicroRNAs. *Diabetes* 2017;66:177–192. [PubMed: 27634225]
8. Lebeaupin C, Vallee D, Rousseau D, Patouraux S, Bonnafous S, Adam G, Luciano F, et al. Bax inhibitor-1 protects from nonalcoholic steatohepatitis by limiting inositol-requiring enzyme 1 α signaling in mice. *Hepatology* 2018.