Alcohol and fat promote steatohepatitis: a critical role for fat-specific protein 27/CIDEC

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Abstract

Alcoholic liver disease (ALD) is a major public health problem worldwide and is the leading cause of end-stage liver disease. While the ultimate control of ALD will require the prevention of alcohol abuse, better understanding of the mechanisms of alcohol-induced liver injury may lead to treatments of fatty liver, alcoholic hepatitis, and prevention or delay of occurrence of cirrhosis. The elucidation and the discovery of several new concepts in ALD pathogenesis have raised our understanding on the complex mechanisms and the potential in developing the new strategies for therapeutic benefits. In this review, we provide the most up-to-date information on the basic molecular mechanisms focusing on the role of fat-specific protein 27/CIDEC in the pathogenesis of ALD.

INTRODUCTION

Alcoholic liver disease (ALD) represents a spectrum of liver disorders with clinical and pathological changes in individuals after chronic excessive alcohol consumption.[12] Patients may have minimal abnormalities from steatosis or may develop more severe signs and symptoms of liver disease seen in alcoholic hepatitis (AH) or cirrhosis.[23] While the ultimate control of ALD will require the prevention of alcohol abuse, better understanding of the mechanisms/pathogenesis may lead to treatments of ALD.

Fatty liver, the accumulation of triglyceride droplets in the liver, is the most common and earliest response of the liver to excessive alcohol use.[4] Synthesis of fatty acids and

triglyceride in excess of the capacity to oxidize it or export it in very low-density lipoprotein particles results in hepatic steatosis.[56] The effect of ethanol was initially attributed to the changes in the redox state, generated from alcohol metabolism by alcohol and aldehyde dehydrogenase; however, recent evidence suggested a complex molecular regulation of ALD. Its pathogenesis involves the dysregulation of transcription factors and metabolic regulators, protein adduct formation, activation of inflammatory cytokines and Kupffer cells, elevation of lipopolysaccharide, and endoplasmic reticulum (ER) stress response (see review by Gao and Bataller[2]). In this report, we will focus on an emerging new mechanism on the role of fat-specific protein 27 (FSP27)/cell death-inducing DFF45-like effector C (CIDEC) in the pathogenesis of ALD.

FSP27/CIDEC

The mouse *Fsp*27 gene is the human homolog of CIDEC, belonging to the CIDE family of proteins. Three CIDEs have been reported in mouse (Cidea, Cideb, and FSP27/Cidec) and human (CIDEA, CIDEB, and CIDEC).[78] FSP27/CIDEC proteins play an important role in the development of metabolic disorders as well as regulation of cell apoptosis.[79–11] The *Fsp*27 gene has two isoforms, *Fsp*27α and *Fsp*27β.[12] *Fsp*27α is highly expressed in white adipose tissues, the major organ for triacylglycerol (TAG) storage, whereas *Fsp*27β is highly expressed in brown adipose tissue and fatty liver.[12] FSP27/CIDEC is a lipid droplet (LD) protein that plays an important role in droplet formation.[13]

LDs, intracellular organelles, are composed of a core of neutral lipids (TAG) covered by a monolayer of phospholipids, free cholesterol and specific proteins.[1415] The ability to store neutral lipids in the form of LDs is evolutionarily conserved across species.[14] LDs in the adipose tissues serve as the reservoirs for fatty acids (in the form of TAG), which can be released during starvation[1416] and can be used for energetic substrates to high-demand tissues such as liver and muscle.[17] There are several proteins, in addition to FSP27/CIDEC, which are involved in LD formation, notably the PAT (*p*erilipin, *a*dipophilin, and the *t*ail-interacting protein of 47 kDa) family proteins, perilipin (PLIN 1–5).[141819]

In the adipose tissues, FSP27/CIDEC stimulates formation of TAG droplets and inhibits β-oxidation of non-esterified fatty acids.[13] It is significantly upregulated[101620–2223] and is important for expansion of LD size during adipogenesis.[13] Recent studies found that FSP27/CIDEC plays an important role in lipolysis through its interaction with adipose tissue triglyceride lipase (ATGL) and regulates insulin sensitivity in human adipocytes.[1718] FSP27/CIDEC facilitates the inhibitory effect of early growth response protein 1 (Erg1) on the transcription of ATGL,[18] leading to reduced lipolysis, and enhancing lipid storage capacity in the adipocytes.[17] The optimal fat storage is important in maintaining the overall metabolic phenotypes in the adipose tissues.[1724] High levels of free fatty acid can inhibit protein kinase B (AKT) phosphorylation and impair insulin sensitivity.[25] It is interesting that insulin-stimulated AKT activation is inhibited by siRNA-mediated FSP27 silencing. Using the gain of function approach, FSP27 overexpression protects the adipocytes from free fatty acid (FFA)-induced insulin resistance. These data suggest that FSP27 might protect adipocytes from the deleterious effects of FFAs via suppression of ATGL-mediated lipolysis.[17]

In mouse liver, *Fsp27* expression is significantly induced during early fasting and in the presence of hepatic steatosis.[9][26][27] Knockdown of *Fsp27* ameliorates hepatic steatosis,[9] while overexpression of *Fsp27* induces the accumulation of LDs and TAG contents.[16] The regulation of hepatic *Fsp27* expression is complex. Fasting-induced *Fsp27* expression was completely obliterated in cyclic AMP-responsive element binding protein H (CREBH) knockout mice.[12] Interestingly, the expression of other LD proteins in the PAT family was largely unaffected by the loss of CREBH,[12] suggesting the role of CREBH in regulating *Fsp27* expression. Overexpression of the constitutively active CREBH strongly induced *Fsp*27β in mouse hepatocytes and promoted LD enlargement and TAG accumulation in the liver, while the loss of CREBH decreased hepatic *Fsp*27β expression in fasted mice.[12]

In summary, FSP27/CIDEC, a lipid protein, plays an important role in lipolysis, insulin sensitivity, and TAG accumulation in steatotic liver.

Animal models for ALD

Currently, the most widely used model for alcohol-induced liver injury is *ad libitum* feeding with the Lieber-DeCarli liquid diet containing ethanol for 4–6 weeks;[28] however, this model, without additional secondary insult, only induces mild steatosis, slight elevation of serum alanine transaminase (ALT) and little or no inflammation.[28] It is not an ideal mouse model to study the mechanism of ALD beyond the hepatic steatosis stage.

AH, a severe form of ALD, can occur in patients with ALD, especially in those with recent excessive alcohol consumption.[23] In addition to the presence of steatosis, the typical findings of AH demonstrate neutrophilic infiltration, hepatocyte ballooning, and hyaline inclusions.[23] Slow progress in the field of ALD has resulted partly from a lack of experimental models of advanced ALD and AH. A model of short-term (10-day) plus binge ethanol feeding in mice (the National Institute on Alcohol Abuse and Alcoholism (NIAAA) model) was developed, which showed significant elevations of transaminases (AST and ALT), mild steatosis, and neutrophil infiltration, yet no fibrosis.[28] When chronic ethanol feeding is extended to a period to 8 weeks, followed by gavage administration of single or multiple doses of ethanol, mice developed the phenotypic features of severe alcoholic steatohepatitis (ASH) and mild fibrosis.[7] Alteration in gene expression profiles, determined by microarray analyses, in this model was found to be similar to those in human AH, suggesting that this is a very useful model to study the mechanism of AH.[7]

FSP27/CIDEC promotes development of ASH in mice

Among the most highly upregulated genes based on the array data, the *Fsp27* gene was 13-fold upregulated in mice chronically fed with ethanol for 8 weeks followed by 1 binge (E8W +1B).[7] The array data were confirmed by realtime PCR (RT-PCR) analysis of the liver tissues which showed that hepatic expression of *Fsp27* was upregulated by 10-fold in mice after E8w+1B feeding.[7]

The gene as well as protein expression of both isoforms of *Fsp27* gene were highly upregulated in mice fed with E8w+1B, when compared with controls.[7] However, it is important to note that ethanol does not directly upregulate hepatic FSP27 protein expression, as the expression of this protein was not observed when primary hepatocytes were treated

with ethanol (100 mM) in vitro.[7] Further studies suggest that the upregulation of *Fsp*27 is, in fact, secondary to the activation of ER by ethanol.[7]

The role of *Fsp27* in the pathogenesis of AH was also examined by using the 'loss of function' approach with Ad-*Fsp27* short hairpin RNA (shRNA) and hepatocyte-specific *Fsp*27 deletion (*Fsp27*$^{Hep-/-}$). Interestingly, the levels of serum transaminases (aspartate aminotransferase (AST) and ALT), hepatic steatosis, and degree of hepatic apoptosis (as measured by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay) were reduced in Ad-*Fsp27* shRNA-treated mice as well in *Fsp27*$^{Hep-/-}$ mice compared with those in pair-fed controls.[7] Further, the hepatic levels of malondialdehyde and 4-hydroxynonenal (oxidative stress/lipid peroxidation markers), which were highly elevated in E8w+1B mice, were significantly reduced after treatment with Ad-*Fsp27* shRNA, suggesting that FSP27 promotes ethanol-induced hepatic oxidative injury.[7]

FSP27 protein is found in the cytoplasm of adipocytes. Recent studies demonstrated that FSP27 protein is present in the cytoplasm and mitochondria from steatotic hepatocytes from ethanol-fed mice.[7] Overexpression of FSP27 protein via the injection of Ad-FSP27 exacerbated the elevation of serum ALT and AST levels and decreased mitochondrial contents and mitochondrial complex I activity in E8W+1B treated mice.[7] The combination of FSP27 overexpression and ethanol exposure synergistically increased mitochondrial reactive oxygen species (ROS) generation in hepatocytes in vitro.[7] Finally, FSP27 overexpression also induces hepatocyte death in the presence of ethanol by induction of Bax translocation and cytochrome C release, the two important early events in apoptotic pathway.[7]

Role of FSP27/CIDEC in human ASH

Recent evidence also suggests an important role for FSP27 in the pathogenesis of human ASH. First, microarray data revealed that CIDEC, the human homolog of *Fsp27,* was upregulated by fivefold in human liver samples from patients with AH compared with healthy controls.[7] RT-PCR analyses demonstrated that the expression of *CIDEC* mRNA was more than 40-fold increase in these samples. Second, the upregulation of hepatic *CIDEC* was closely associated with the severity of hepatic steatosis as well as the prognostic models for AH such as model for end-stage liver disease and age, serum bilirubin, international normalized ratio, and serum creatinine (ABIC) scores.[7] It is also positively correlated with hepatic venous pressure gradient and is an independent predictor for 90-day mortality in patients with AH.[7] The schematic diagram on the mechanism of FSP27/CIDEC and ASH is shown in figure 1.

FSP27/CIDEC contributes to the synergistic effect of obesity and acute ethanol-induced ASH

Obesity and alcohol consumption often coexist and synergistically promote the development and progression of liver injury, fibrosis, and hepatocellular carcinoma in patients.[29][30] The studies from several animal models also revealed that alcohol feeding and high-fat diet (HFD) feeding synergistically promote steatohepatitis in rodents.[31] Interestingly, a simple model of mixed steatohepatitis by feeding mice an HFD followed by gavage with a single

dose of ethanol was recently developed.[32] The most striking finding from this model was that feeding mice an HFD for as little as 3 days, which has been shown to impair hepatic insulin sensitivity,[33] significantly aggravated the acute ethanol binge-induced neutrophilia, hepatic neutrophil infiltration, and liver injury.[32] Long-term (3 months) HFD feeding plus gavage of a single dose of ethanol caused severe steatohepatitis with severe steatosis, massive neutrophil infiltration, and marked elevation of serum ALT and AST. Mechanistic studies revealed that hepatic expression of chemokine (C-X-C motif) ligand 1 (CXCL1) was highly upregulated (up to 20-fold and 30-fold) in the liver after 3-day HFD+ethanol and 3-month HFD+ethanol feeding, respectively.[32] Genetic deletion of the *Cxcl1* or blocking CXCL1 with a neutralizing antibody ameliorated HFD+acute ethanol-induced liver inflammation and injury. In addition, it is known that hepatic *Fsp27* mRNA is highly elevated after HFD feeding[34] and that overexpression of FSP27 increases the sensitivity of hepatocytes to ethanol-induced ROS production and injury.[7] Thus, it is plausible to speculate that upregulated hepatic FSP27 expression is another important mechanism by which HFD-fed mice are very sensitive to acute alcohol-induced acute ASH.

CONCLUSION

The use of chronic ethanol feeding for 8 weeks followed by gavage administration of a single dose of ethanol can induce hepatic histology mimicking ASH. This model, therefore, will be useful for the future study to identify and investigate other important mediators that may contribute to the pathogenesis of ASH. Using this mouse model, the researchers have found the important role of hepatic FSP27/CIDEC in promoting ASH in mice as well as in patients with ASH. However, further studies investigating the role of FSP27/CIDEC notably in the adipose tissues and the cross talk between adipose tissue and liver on the pathogenesis of hepatic steatosis and ASH in mouse model of obesity and alcohol feeding will be needed.

Acknowledgments

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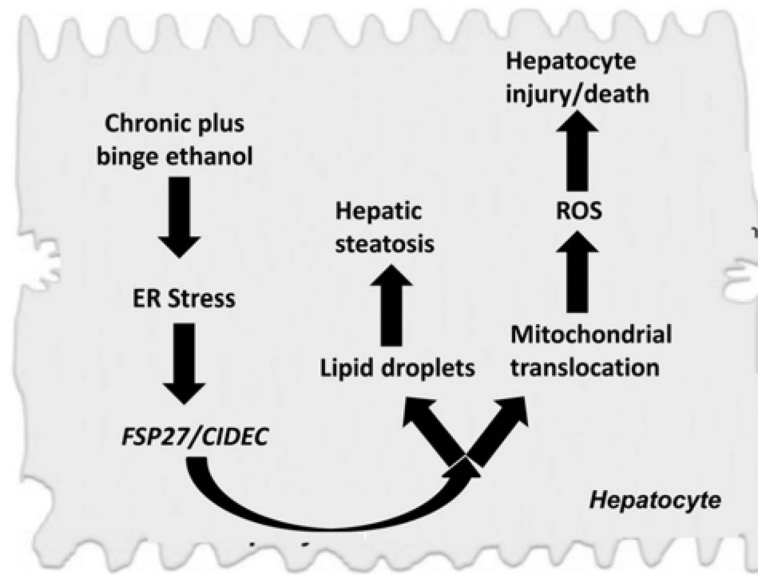
Figure 1.
Schematic diagram on the potential role of FSP27/CIDEC in the pathogenesis of AH
(modified from Xu *et al*[7]). AH, alcoholic hepatitis; CIDEC, cell death-inducing DFF45-like
effector C; ER, endoplasmic reticulum; FSP27, fat-specific protein 27; ROS, reactive oxygen
species.