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Impaired TFEB-Mediated Lysosome Biogenesis and Autophagy Promote Chronic Ethanol-Induced Liver Injury and Steatosis in Mice

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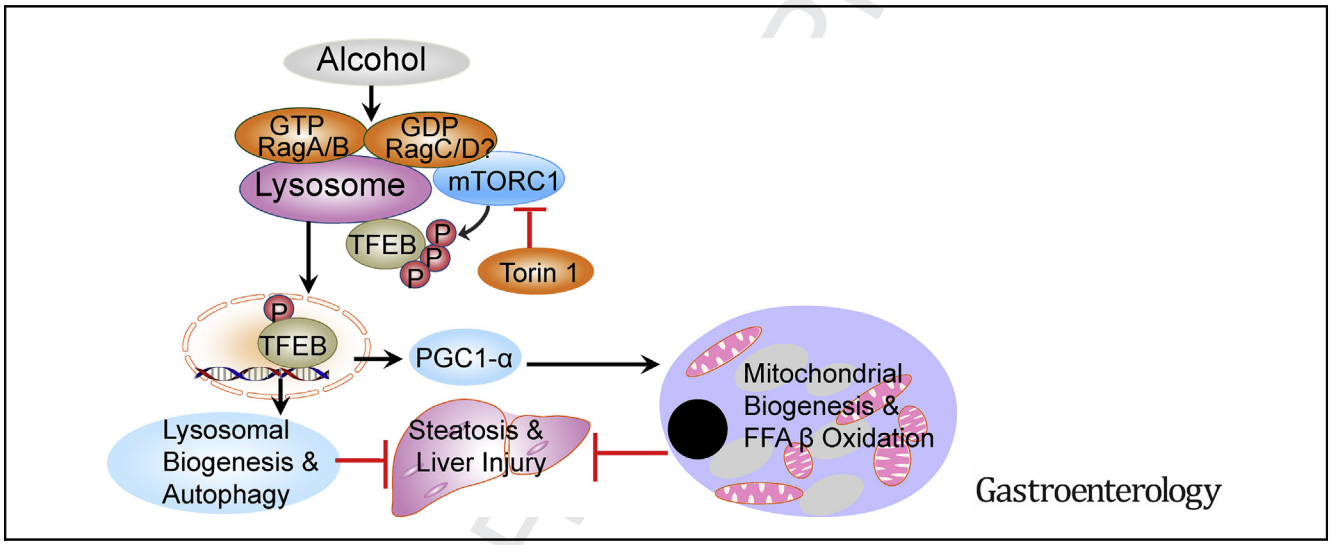

BACKGROUND & AIMS: Defects in lysosome function and autophagy contribute to the pathogenesis of alcoholic liver disease. We investigated the mechanisms by which alcohol consumption affects these processes by evaluating the functions of transcription factor EB (TFEB), which regulates lysosomal biogenesis. **METHODS:** We performed studies with GFP-LC3 mice, mice with liver-specific deletion of TFEB, mice with disruption of the transcription factor E3 gene (TFE3-knockout mice), mice with disruption of the *Tefb* and *Tfe3* genes (TFEB and TFE3 double-knockout mice), and *Tfeb*$^{flox/flox}$ albumin cre-negative mice (controls). TFEB was overexpressed from adenoviral vectors or knocked down with small interfering RNAs in mouse livers. Mice were placed on diets of regular ethanol feeding plus an acute binge to induce liver damage (ethanol diet); some mice also were given injections of torin-1, an inhibitor of the kinase activity of the mechanistic target of rapamycin (mTOR). Liver tissues were collected and analyzed by immunohistochemistry, immunoblots, and quantitative real-time polymerase chain to monitor lysosome biogenesis. We analyzed levels of TFEB in liver tissues from patients with alcoholic hepatitis and from healthy donors (controls) by immunohistochemistry. **RESULTS:** Liver tissues from mice on the ethanol diet had lower levels of total and nuclear TFEB compared with control mice, and hepatocytes had decreased lysosome biogenesis and autophagy. Hepatocytes from mice on the ethanol diet had increased translocation of mTOR into lysosomes, resulting in increased mTOR activation. Administration of torin-1 increased liver levels of TFEB and decreased steatosis and liver injury induced by ethanol. Mice that overexpressed TFEB in the liver developed less severe ethanol-induced liver injury and had increased lysosomal biogenesis and mitochondrial bioenergetics compared with mice carrying a control vector. Mice with knockdown of TFEB and TFEB-TFE3 double-knockout mice developed more severe liver injury in response to the ethanol diet than control mice. Liver tissues from patients with alcohol-induced hepatitis had lower nuclear levels of TFEB than control tissues. **CONCLUSIONS:** We found that regular ethanol feeding plus an acute binge decreased hepatic expression of TFEB, which is required for lysosomal biogenesis and autophagy. Strategies to block mTOR activity or increase levels of TFEB might be developed to protect the liver from ethanol-induced damage.

Keywords: Fatty Liver; Gene Regulation; Hepatic Protection; Mouse Model.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

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NEW FINDINGS

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LIMITATIONS

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Alcoholic liver disease (ALD) is a major cause of chronic liver disease worldwide, claiming 3.3 million deaths globally in 2012.[1] The pathogenesis of alcohol-induced liver injury is characterized by hepatic steatosis, inflammation, and fibrosis, which can progress to cirrhosis and liver cancer.[2–4] Despite major progress in understanding the mechanisms for ALD, no successful treatment for ALD is available.

Cells can adapt and protect themselves in response to stress by activating cellular protective mechanisms, including autophagy and lysosomal biogenesis. Autophagy is a catabolic process that degrades cellular proteins and subcellular organelles such as mitochondria, which is critical for prevention of and recovery from alcohol-induced liver injury.[5,6] However, it seems that acute and regular alcohol exposure can differentially regulate hepatic autophagy.[7,8] We previously reported that autophagy is activated in mouse livers and primary cultured hepatocytes in response to acute alcohol exposure.[5,9] Although 2 independent groups reported that regular ethanol exposure increased autophagic flux in mice and increased autophagosome numbers in rats,[10,11] previous studies indicated that regular ethanol exposure disrupts lysosome function.[12] Moreover, animals with regular ethanol exposure and heavy drinkers develop hepatomegaly with increased hepatic protein accumulation.[13] These observations suggest a defect in hepatic autophagy in long-term alcohol conditions. However, the mechanisms by which alcohol impairs lysosomal functions in the liver are largely unknown.

The lysosome contains more than 50 acid hydrolases and is the terminal component of autophagy. Transcription factor EB (TFEB) is a master regulator of lysosome biogenesis and autophagy-related gene transcription,[14,15] which is mainly regulated by the mechanistic target of rapamycin complex-1 (mTORC1). mTORC1 is a conserved serine-threonine kinase and acts as a nutrient and energy sensor to regulate cell growth by coordinating signals from nutrients and growth factors in addition to cellular energy levels.[16] Tumor suppressor protein tuberous sclerosis complex 1 (TSC1) and TSC2 form a complex and negatively regulate the guanosine triphosphate (GTP)-loading state of Rheb, a Ras-related GTP-binding protein. Rheb interacts with and activates mTORC1 when it binds with GTP.[16,17] However, amino acids activate mTORC1 by activating the Ragulator-Rag complex independent of the TSC1-TSC2 complex.[18] There are 4 Rag proteins in mammals, A–D, in which Rag A and Rag B are functionally redundant, whereas Rag C and Rag D also are functionally redundant.[18,19] Increasing evidence indicates that mTORC1 translocates to lysosomes and becomes activated by Rag GTPases,[16,20] which results in the inactivation of TFEB by phosphorylation.[14,21] TFEB overexpression enhances lysosomal and mitochondrial functions, which markedly attenuates steatosis induced by a high-fat diet in mice.[22] A previous descriptive study showed that mice fed with an ethanol diet for 35–62 days had decreased hepatic nuclear TFEB levels, which seemed to be associated with deceased autophagy.[7] However, the causal role and mechanisms by which TFEB regulates alcohol-induced liver injury was not investigated in this correlative study.[8] Regular feeding plus acute binge alcohol (hereafter referred to as "Gao-binge") in mice mimics consumption patterns of human alcoholics, and it causes greater liver injury and inflammation than other chronic Lieber-DeCarli alcohol mouse models.[4,23] However, autophagy status in this Gao-binge model in mouse livers has not been characterized. Moreover, whether Gao-binge alcohol affects TFEB and lysosomal biogenesis in the liver has not been studied. The aim of this study was to determine autophagic flux and TFEB-mediated lysosomal biogenesis in the liver in addition to the contributions of TFEB and its underlying mechanisms in Gao-binge–induced liver injury. We found that Gao-binge impaired TFEB-mediated lysosomal biogenesis by activation of mTORC1, resulting in insufficient autophagy in mouse livers. Overexpression or ablation of TFEB in mouse livers attenuated or exacerbated alcohol-induced liver injury in mice.

***Authors share co-first authorship.**

Abbreviations used in this paper: **Ad, adenovirus; AH, alcoholic hepatitis; ALD, alcoholic liver disease; ALT, alanine aminotransferase; ATP6V0E1, ATPase H^+ transporting V0 subunit E1; ATP6V1D, ATPase H^+ transporting V1 subunit D; ATP6V1H, ATPase H^+ transporting V1 subunit H; Ctrl, control diet plus maltose binge; DKO, double knockout; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; ERK1/2, extracellular signal-regulated kinase 1/2; EtOH, ethanol diet plus ethanol binge; GFP, green florescent protein; GTP, guanosine triphosphate; H&E, hematoxylin and eosin; KO, knockout; LAMP1, lysosomal-associated membrane protein-1; LC3, microtubule-associated protein light chain-3; LDs, lipid droplets; Leu, leupeptin; Ly6B, lymphocyte antigen B superfamily; mTORC1, mechanistic target of rapamycin complex-1; PGC1α, peroxisome proliferator-activated receptor-γ coactivator-1-α; PKCβ, protein kinase Cβ; TFE3, transcription factor E3 gene; TFEB, transcription factor EB; TG, triglyceride; Tor, torin-1; TSC1, tuberous sclerosis complex-1; WT, wild-type.**

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