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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Targeting the Enterohepatic Bile Acid Signaling Induces Hepatic Autophagy via a CYP7A1–AKT–mTOR Axis in Mice

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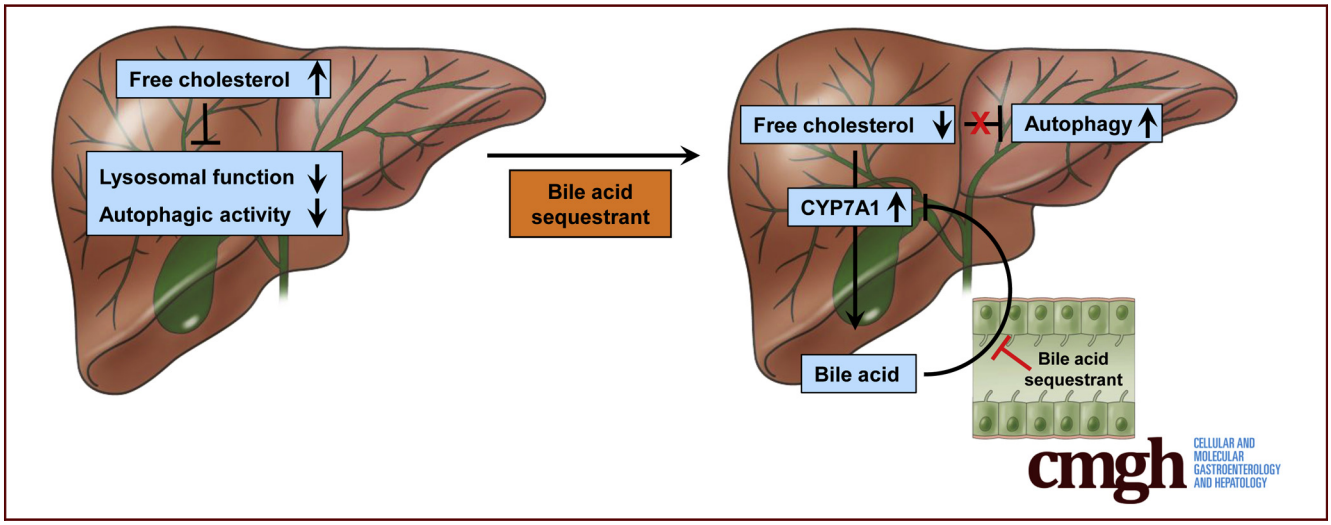

SUMMARY

Free cholesterol accumulation impairs lysosome function and inhibits autophagic activity in hepatocytes. Induction of bile acid synthesis attenuates AKT/mechanistic target of rapamycin (mTOR) signaling, leading to autophagy activation. Targeting the enterohepatic bile acid signaling by bile acid sequestrant induces hepatic autophagy in mice.

BACKGROUND & AIMS: Hepatic cholesterol accumulation and autophagy defects contribute to hepatocyte injury in fatty liver disease. Bile acid synthesis is a major pathway for cholesterol catabolism in the liver. This study aims to understand the molecular link between cholesterol and bile acid metabolism and hepatic autophagy activity.

METHODS: The effects of cholesterol and cholesterol 7α-hydroxylase (CYP7A1) expression on autophagy and lysosome function were studied in cell models. The effects and mechanism of disrupting enterohepatic bile acid circulation on hepatic autophagy were studied in mice.

RESULTS: The results first showed differential regulation of hepatic autophagy by free cholesterol and cholesterol ester, whereby a modest increase of cellular free cholesterol, but not cholesterol ester, impaired lysosome function and caused marked autolysosome accumulation. We found that CYP7A1 induction, either by cholestyramine feeding in mice or adenovirus-mediated CYP7A1 expression in hepatocytes, caused strong autophagy induction. Mechanistically, we showed that CYP7A1 expression markedly attenuated growth factor/AKT signaling activation of mechanistic target of rapamycin (mTOR), but not amino acid signaling to mTOR in vitro and in vivo. Metabolomics analysis further found that CYP7A1 induction not only decreased hepatic cholesterol but also altered phospholipid and sphingolipid compositions. Collectively, these results suggest that CYP7A1 induction interferes with growth factor activation of AKT/mTOR signaling possibly by altering membrane lipid composition. Finally, we showed that cholestyramine feeding restored impaired hepatic autophagy and improved metabolic homeostasis in Western diet–fed mice.

CONCLUSIONS: This study identified a novel CYP7A1–AKT–mTOR signaling axis that selectively induces hepatic autophagy, which helps improve hepatocellular integrity and metabolic homeostasis. *(Cell Mol Gastroenterol Hepatol 2017;3:245–260; http://dx.doi.org/10.1016/j.jcmgh.2016.10.002)*

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See editorial on page 133.

Emerging evidence supports that nonesterified free cholesterol (FC) accumulates in fatty livers and contributes to hepatocyte injury.[1–4] Impaired hepatic cholesterol homeostasis further contributes to dyslipidemia, atherosclerosis, and a higher risk of cardiovascular disease.[5] Hepatic cholesterol homeostasis is coordinately controlled by input and elimination pathways. The sterol response element binding protein-2 (SREBP-2)–mediated endoplasmic reticulum (ER) cholesterol-sensing mechanism regulates cellular cholesterol input pathways.[6] A decrease of ER cholesterol leads to proteolytic cleavage activation of SREBP-2, which induces the expression of 3-hydroxy-3-methylglutaryl-coenzyme A-CoA reductase (HMGCR) and low-density lipoprotein receptor (LDLR) to increase intracellular cholesterol levels. On the other hand, bile acid synthesis is the major cholesterol elimination pathway. The cholesterol 7α-hydroxylase (CYP7A1) is an ER resident enzyme that catalyzes the first and rate-limiting step in the conversion of cholesterol into bile acids.[7] Bile acid synthesis has a major impact on ER cholesterol content, and CYP7A1 induction can activate the entire SREBP-2–regulated transcriptional network to regulate cellular cholesterol metabolism in the liver.[8] This ER cholesterol-sensing mechanism is the molecular basis for the cholesterol-lowering drugs statins and bile acid sequestrants. In addition, a number of studies have suggested that nutrient signaling regulation of hepatic bile acid synthesis may be tightly linked to hepatic metabolic homeostasis.[9–11]

Autophagy is a well-conserved cellular self-degradation system that eliminates protein aggregates and damaged organelles to help maintain cellular integrity.[12] Autophagy is also a catabolic process that generates nutrients and energy by degrading macromolecules in response to nutrient deprivation.[13] The nutrient-sensing mechanistic target of rapamycin (mTOR) signaling plays a key role in regulating autophagy activity in response to changes of nutrient availability.[13] A hepatic autophagy defect exacerbated hepatocyte injury in fatty liver diseases.[14–16] Furthermore, obesity and fatty liver are associated with defective hepatic autophagy, possibly owing to hyperinsulinemia, lipid accumulation, and impaired vesicle fusion.[17–19]

Recent clinical and basic research have shown that targeting the enterohepatic bile acid signaling by bile acid sequestrants or the selective apical sodium-dependent bile acid transporter inhibitors represents a promising therapeutic strategy for decreasing plasma cholesterol and improving insulin sensitivity in diabetes and fatty liver disease, and attenuating bile acid toxicity in cholestasis.[20–23] However, the highly complex changes of hepatic and intestinal metabolic and signaling pathways caused by disrupting enterohepatic bile acid circulation still are incompletely understood. In this study, we show that free cholesterol accumulation is a major causal factor for liver autophagy impairment, and that targeting the enterohepatic bile acid signaling is an effective approach to induce hepatic autophagy via a novel CYP7A1–AKT–mTOR signaling cascade, which is potentially beneficial in improving hepatocellular integrity and metabolic homeostasis.

Materials and Methods

Reagents

Antibodies against microtubule-associated protein 1A/1B-light chain 3 (LC3B), phospho- and total tibosomal protein S6 (S6) (S240/244), eukaryotic translation initiation factor 4E-binding protein 1 (4EBP-1) (T37/46), AKT (S473), glycogen synthase kinase 3β (GSK3β) (S9), and histone 3 were purchased from Cell Signaling Technology (Danvers, MA). P62 antibody was purchased from Abnova (Walnut, CA). Actin antibody, water-soluble cholesterol (in methyl-β-cyclodextrin), cholestyramine (ChTM), acyl-CoA:cholesterol acyltransferase (ACAT) inhibitor Sandoz 58-035 chloroquine, and cholestyramine were purchased from Sigma (St. Louis, MO). Lysotracker red and anti-SREBP-2 antibody were from Thermo Scientific (Grand Island, NY). Highly purified human LDL was purchased from Lee Biosolutions (Maryland Heights, MO). The mCherry-galectin expression vector was described previously.[24] Lipoprotein-depleted serum was purchased from Kalen Biomedical, LLC.

Animals

Male 8- to 10-week-old C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). The Western diet (TD.88137; Harlan Teklad, East Millstone, NJ) contains 42% fat calories and 0.2% cholesterol. ChTM was mixed with diets (2%, wt/wt). For confocal microscopy, mice were injected with Ad-RFP-GFP-LC3 at 10^8 pfu/mouse via tail vein 3 days before death. All mice were fasted for 6 hours starting from approximately 6 AM and killed. All animals received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals. All protocols were approved by the Institutional Animal Care and Use Committee.

Cell Culture and Treatments

HepG2 cells were purchased from ATCC (Manassas, VA). Cells were maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum or in Dulbecco's modified Eagle medium containing lipoprotein-depleted serum as indicated. Experiments were initiated when cells

Abbreviations used in this paper: **ACAT, acyl-CoA:cholesterol acyltransferase; CE, cholesterol ester; ChTM, cholestyramine; CQ, chloroquine; CYP7A1, cholesterol 7α-hydroxylase; DIO, diet-induced obesity; ER, endoplasmic reticulum; FC, free cholesterol; 4EBP-1, eukaryotic translation initiation factor 4E-binding protein 1; GSK3β, glycogen synthase kinase 3β; HMGCR, HMG-CoA reductase; LC3, microtubule-associated protein 1A/1B-light chain 3; LDLR, low-density lipoprotein receptor; LMP, lysosome membrane permeabilization; mRNA, messenger RNA; mTOR, the nutrient sensing mechanistic target of rapamycin; PI, phosphatidylinositol; PM, plasma membrane; S6, tibosomal protein S6; SREBP, sterol response element binding protein.**

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