World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i20.2507

World J Gastroenterol 2011 May 28; 17(20): 2507-2514
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2011 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Natalia A Osna, MD, PhD, *Series Editor*

Involvement of autophagy in alcoholic liver injury and hepatitis C pathogenesis

Natalia A Osna, Paul G Thomes, Terrence M Donohue Jr

Natalia A Osna, Paul G Thomes, Terrence M Donohue Jr, University of Nebraska Medical Center, VA Medical Center, Omaha, NE 68105, United States
Author contributions: All authors contributed equally to this manuscript; Osna NA and Donohue TM Jr reviewed the current literature; Thomes PG provided much of the preliminary data on alcohol and autophagy mentioned in the text.
Supported by NIAAA, R21AA017232 and Dean's Reviewed Research Grant of the University of Nebraska Medical Center
Correspondence to: Natalia A Osna, MD, PhD, University of Nebraska Medical Center, VA Medical Center, 4101 Woolworth Ave, Omaha, NE 68105, United States. nosna@unmc.edu
Telephone: +1-402-9953735 Fax: +1-402-4490604
Received: January 7, 2011 Revised: March 23, 2011
Accepted: March 30, 2011
Published online: May 28, 2011

Abstract

This review describes the principal pathways of macroautophagy (i.e. autophagy), microautophagy and chaperone-mediated autophagy as they are currently known to occur in mammalian cells. Because of its crucial role as an accessory digestive organ, the liver has a particularly robust autophagic activity that is sensitive to changes in plasma and dietary components. Ethanol consumption causes major changes in hepatic protein and lipid metabolism and both are regulated by autophagy, which is significantly affected by hepatic ethanol metabolism. Ethanol exposure enhances autophagosome formation in liver cells, but suppresses lysosome function. Excessive ethanol consumption synergizes with hepatitis C virus (HCV) to exacerbate liver injury, as alcohol-consuming HCV patients frequently have a longer course of infection and more severe manifestations of chronic hepatitis than abstinent HCV patients. Alcohol-elicited exacerbation of HCV infection pathogenesis is related to modulation by ethanol metabolism of HCV replication. Additionally, as part of this mechanism, autophagic proteins have been shown to regulate viral (HCV) replication and their intracellular accumulation. Because ethanol induces autophagosome expression, enhanced levels of autophagic proteins may enhance HCV infectivity in liver cells of alcoholics and heavy drinkers.

© 2011 Baishideng. All rights reserved.

Key words: Autophagy; lysosome; Autophagosome; Hepatitis C virus; Hepatitis C virus replication cycle; Ethanol

Peer reviewers: Ekihiro Seki, MD, PhD, Department of Medicine, University of California San Diego, Leichag Biomedical Research Building Rm 349H, 9500 Gilman Drive MC#0702, La Jolla, CA 92093-0702, United States; Hui-Jie Bian, Professor, vice-director, Department of Cell Biology/Cell Engineering Research Center, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China

Osna NA, Thomes PG, Donohue TM Jr. Involvement of autophagy in alcoholic liver injury and hepatitis C pathogenesis. *World J Gastroenterol* 2011; 17(20): 2507-2514 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i20/2507.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i20.2507

INTRODUCTION

Alcohol is known to exacerbate the pathogenesis of hepatitis C virus (HCV) infection and makes chronic hepatitis C patients less sensitive to antiviral treatment. Recent investigations indicate that autophagy is involved in the regulation of HCV replication and infectivity. Here, we provide basic information about the role of autophagy in liver and how ethanol modifies the autophagic response in liver cells. In addition, to underline a link between autophagy and viral replication, we review the data about HCV structure, replication cycle and autophagy in HCV-infected liver cells. Since alcohol consumption exacerbates HCV pathogenesis, we propose possible mechanisms that lead to liver failure in HCV-infected patients who drink

excessively, which may in part be regulated *via* autophagy-mediated accumulation of HCV in liver cells.

AUTOPHAGIC MACROMOLECULAR DIGESTION IN THE LIVER

Lysosomes and autophagy

In most tissues, the majority of intracellular proteins are degraded by the proteasome, an integral component of the ubiquitin-proteasome system (UPS)[1]. In tissues such as liver that respond quickly to the composition of the plasma, the lysosome has a large part in degrading extracellular proteins (e.g. obsolete plasma proteins) brought by endocytosis into the hepatocyte. Lysosomes also degrade the remaining fraction of intracellular proteins that are not proteasome substrates and break down complex lipids (e.g. triglycerides), complex carbohydrates (e.g. glycogen) and nucleic acids. All the latter hydrolytic steps are done by autophagy, which literally means "self eating"[2]. While autophagy always terminates by degrading macromolecular substrates in lysosomes, it can begin *via* one of three distinct cellular pathways. The most well-characterized process by which autophagy is defined is macroautophagy, the vacuolar sequestration by endoplasmic reticulum (ER) membranes of bulk portions of cytoplasm, forming a double membrane-enclosed body known as an autophagosome or autophagic vacuole (AV). The nascent AV, which contains both soluble constituents and particulate organelles, fuses with existing lysosomes to form a hydrolytic organelle called an autolysosome. Here, the sequestered substrates are hydrolyzed and the degradation products are released. Microautophagy, which is less well characterized than macroautophagy, is the uptake of smaller cytoplasmic particles by the lysosomal membrane followed by their degradation in the lysosome interior. Chaperone-mediated autophagy (CMA) is the uptake and degradation by lysosomes of single protein substrates that bear a KFERQ peptide motif. The latter is recognized by soluble chaperonins of which the most prominent is the heat shock constitutive protein 70 (HSC 70), which binds to and "directs" the protein substrate to lysosome-associated membrane proteins (LAMP). Most significant is LAMP-2A, which recognizes substrate chaperonin complexes and facilitates entry of the protein substrate into the lysosomal matrix for degradation. CMA was formerly considered a minor autophagic pathway, but recent findings indicate its heightened importance in liver cell maintenance and hepatic performance. CMA activity declines with age due to a gradual loss of LAMP-2A on the lysosome surface. Artificial restoration of this protein in aged LAMP-2A transgenic mice significantly improves liver function[3]. CMA also has a compensatory function when macroautophagy in liver cells declines after artificial knockdown of macroautophagy gene products[4].

Autophagy is important for liver cell survival, particularly in times of nutrient deprivation, when autophagy swiftly responds to fasting. The short half-life of autophagosomes (< 10 min)[5] indicates that the protein components required for their formation are readily available for recruitment. Like the proteasome, autophagy has a key housekeeping and backup function as it degrades misfolded, aggregated proteins that are resistant to proteasome-catalyzed degradation[6]. The importance of removing such aggregated proteins is clearly illustrated by the association of aggregate accumulation with disease pathogenesis. Patients with α-1-antitrypsin (A-1AT) deficiency are more susceptible to cirrhosis and liver cancer because of a propensity of the mutated, unsecreted A-1AT protein to aggregate within hepatocytes[7].

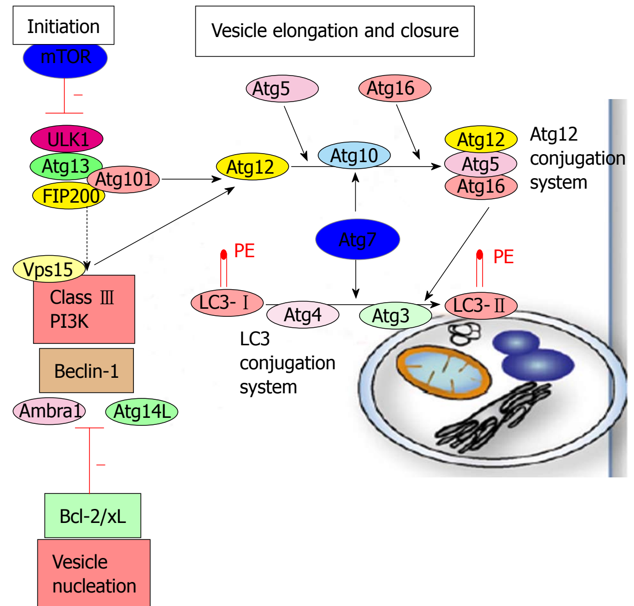

Figure 1 Molecular aspects of autophagosome formation. Reproduced with permission from[11].

Mechanism of autophagy

Identification of the major autophagy genes in yeast[7-10] designated "Atg" has contributed significantly to our understanding of how autophagy proceeds in mammals. In all eukaryotic cells, nutrient deprivation is an inducer of autophagy. The mechanism of autophagy is described below in three steps and depicted in Figure 1.

Inititation of autophagosome formation: Inititation of autophagosome formation is regulated by the class Ⅲ phosphoinositol-3-kinase (PI-3K) which triggers an upstream signaling cascade to inhibit the activity of the mammalian target of rapamycin (mTOR), a major anabolic kinase and a potent suppressor of autophagy. Inhibition of mTOR triggers the autophagic response by activating Atg proteins. In mammals, a complex is formed among the serine/threonine-protein kinase, ULK1 (a homolog of the yeast Atg1), the focal adhesion kinase family interacting protein 200, (FIP200), Atg17-like protein and Atg13, to begin the process of AV nucleation[11]. Autophagosome (autophagic vacuole) formation begins when Beclin-1 (Atg6) forms a complex with Atg14. This subsequently

brings about formation of a double membrane vesicle that is likely derived from the ER[12]. Coordinated complexes of Atg proteins catalyze vacuole (vesicle) elongation, notably the Atg12 ubiquitin-like conjugation system, consisting of Atg 5, 12 and 16, catalyze cleavage of Atg8, the microtubule light chain-3 (LC3I), and its conjugation to phosphatidylethanolamine (PE) to form the autophagic marker, LC3Ⅱ. The latter subsequently localizes to the autophagosome membrane, while its unlipidated precursor, LC3I remains in the cytoplasm (Figure 1).

Docking and fusion of cargo-filled autophagosomes: Docking and fusion of cargo-filled autophagosomes with lysosomes follows autophagosome formation to form the hydrolytic autolysosome. Fusion is believed to be highly dependent upon the lysosomal membrane protein, LAMP-2.

Breakdown of the autolysosome contents occurs: The mechanism that triggers this hydrolysis is incompletely understood but it is likely that the collective action of lysosomal hydrolases brings about autolysosome dissolution.

Lysosomal proteolysis during ethanol consumption: Liver enlargement (hepatomegaly) is common in alcoholics and in alcohol-fed laboratory animals. The rise in hepatic protein and fat each accounts for half the net increase in liver mass[13]. The net protein gain very likely contributes to the more severe alcohol-induced liver pathologies, because some of the accumulated proteins are damaged by oxidants generated from ethanol metabolism, from mitochondrial leakage and from secondary reactions that enhance production of reactive species[14,15]. In early studies, we showed that ethanol-induced protein accumulation reflects slower degradation of long-lived proteins, which are generally degraded in lysosomes[16]. We later confirmed that ethanol feeding diminishes the proteolytic capacity of liver lysosomes[17]. This is due to a reduced capacity for their acidification[18] and lower contents of cathepsins B and L[19]. The latter deficiency results from disruption of cathepsin precursor trafficking to lysosomes[20], owing to declines in the ligand-binding activity, content, and synthesis rate of the mannose-6-phosphate receptor[21,22]. This protein recognizes and binds cathepsin precursors for placement into the lysosomal compartment[23].

Because lysosomes degrade the contents of autophagic vacuoles, we considered it likely that ethanol suppresses the initial stages of autophagy. Others demonstrated that livers of ethanol-fed rats exhibit volume densities of autophagosomes and autolysosomes substantially lower than controls[24]. Other lines of evidence support autophagic suppression, including ethanol-induced down-regulation of AMP kinase, a catabolic regulator[25] and ethanol-elicited disruption of cytoskeletal proteins[26] that are essential for delivery of AV cargo to lysosomes.

Ethanol effects on intracellular autophagosome content in vitro and ex vivo

Using the marker, LC3-Ⅱ we quantified autophagosomes in recombinant HepG2 (VL-17A) cells which metabolize ethanol *via* ADH and CYP2E1. Parental HepG2 cells express neither enzyme[27]. Contrary to our expectations, exposure of VL17A cells to 25 or 100 mmol/L ethanol for 12 to 72 h enhanced autophagosome content over that of untreated cells. AV elevation by ethanol was blocked by simultaneous exposure to 4-methyl-pyrazole (4MP), an inhibitor of ethanol oxidation. Furthermore, exposure to ethanol of non-metabolizing HepG2 cells showed no differences from untreated controls in autophagosome content, to suggest that the initiation of autophagy depends on the generation of ethanol metabolites[28]. These results are consistent with those reported recently[29]. Interestingly, they contrast with another report[30] demonstrating that in CYP2E1-expressing E-47 (HepG2 recombinant) cells, ethanol exposure enhances steatosis but only slightly increases autophagy by 50%. In non-metabolizing C34 cells (similar to HepG2 cells) ethanol exposure causes a four-fold rise in autophagy but only a slight elevation of steatosis. The apparent disparity may be explained by differences in the duration of ethanol exposure (12 to 72 h reported here, compared with 120 h in the Wu *et al*[30] study) and the high degree of cellular steatosis, which is reciprocally regulated with autophagy in liver cells[31].

We further tested the autophagic response in control and ethanol-exposed precision-cut rat liver slices (PCLS)[32]. LC3 Ⅱ levels in PCLS exposed to 50 mmol/L ethanol were increased two-fold over controls after 24 h of incubation. Simultaneous exposure to 4MP blocked this response[28].

***In vivo* studies:** After acute ethanol administration to mice, we observed a 35% enhancement in autophagosome content. This coincided with a decline in reduced glutathione (GSH) and a significant elevation in hepatic lipid peroxides, both indicators of oxidant stress. Chronic ethanol feeding to transgenic LC3-green fluorescent protein (GFP) mice caused a five-fold increase over controls of fluorescent puncta, indicating enhanced autophagosome formation. It is noteworthy that ethanol-fed mice in this study exhibited hepatomegaly and elevated liver protein over pair-fed controls[28] consistent with our previous findings in rats[16,33].

Summary

Our results are consistent with those recently published[29] but are inconsistent with our hypothesis that ethanol suppresses the autophagic pathway. An obvious paradox emerges from these recent findings in view of our previous work, showing slower protein degradation in livers of ethanol-fed animals because of a disruption of lysosome function and biogenesis[17-22]. An explanation for this disparity is that our recent investigations focused on autophagosome formation while our earlier studies emphasized lysosome function. We now postulate that ethanol metabolism enhances autophagosome formation while it disrupts the distal, degradative step of autolysosome formation. Previous work in alcohol/endotoxin-induced

pancreatitis, revealed that LAMP-2 protein is severely reduced in pancreata of ethanol-fed/endotoxin-treated rats. This alteration reduces autolysosome formation and subsequent degradation of autophagosome cargo[34]. We postulate that this also occurs in liver during ethanol metabolism. Finally, we do not know the exact mechanism by which ethanol metabolism induces autophagosome content but we surmise that acetaldehyde, the primary oxidation product of ethanol metabolism and/or other reactive species generated by reactions secondary to ethanol metabolism, is/are probably responsible for this activation. Further, chronic alcohol consumption causes oxidant stress, which enhances the unfolded protein response (UPR) in the ER[35]. Because AV membranes are derived from this organelle[12], ER stress could enhance the formation of autophagosomes during ethanol oxidation.

HCV, ETHANOL AND AUTOPHAGY

Ethanol-induced oxidative stress and HCV infection

HCV is a well-established second hit, which drives the progression of alcoholic liver disease. Worldwide, 170 million people (about 2.2% of the world's population) are infected with HCV, and in about 80% of cases, infection persists for many years. Chronic viral hepatitis provides the potential risk for cirrhotic liver disease and life-threatening complications of portal hypertension and hepatocellular carcinoma[36]. The ability of HCV proteins to induce oxidative stress *via* generation of reactive oxygen species (ROS) by mitochondrial electron transport complex I plays an important role in HCV infection pathogenesis. Therefore, ROS release results in decreased mitochondrial GSH and mitochondrial depolarization, which can be augmented by simultaneous ER oxidative stress[37-39]. Cytochrome P450 (including CYP2E1) is involved in evolution of hepatitis to HCV-associated hepatocarcinoma[40]. Furthermore, alcohol abuse associated with CYP2E1 activation strongly accelerates the progression of HCV infection by increasing fibrosis as well as the risk of death from cirrhosis in HCV patients. It is believed that immunosuppressive effects of alcohol impair viral clearance[41,42]. The main mechanisms of HCV infection development, including immune dysfunction, apoptosis, steatosis and hepatic iron overload, can be triggered by heavy alcohol consumption in HCV patients[43,44].

Hepatitis C virus and viral proteins

HCV, an enveloped virus of Flaviviridae family, has a positive-sense and single-stranded RNA. Its genome is 9.6 kb, encodes a polyprotein of 3010 amino acids and consists of a single open reading frame (ORF) flanked by untranslated regions[45]. The NH_2-terminal part of the polyprotein includes three structural proteins: core (capsid protein) and two envelope proteins, E1 and E2[46]. The polyprotein is processed by cellular and viral proteases into distinct structural and non-structural viral proteins. The COOH-terminal portion of the polyprotein consists of the non-structural (NS) proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) that form the replication complex for synthesis of viral RNA and regulate virion assembly[47-49]. The release of structural proteins from the nascent polyprotein is catalyzed by cellular proteases, while NS proteins are produced by viral proteases[50].

The HCV replication cycle

To initiate the viral life cycle, HCV binds to specific cell receptors on the hepatocyte surface and is internalized by clathrin-dependent endocytosis. Although the exact mechanism of HCV cell entry is unknown, it is believed to be a multi-step process involving at least the tetraspanin CD81[51], tight junction molecules, claudin 1 and occludin 1, as well as scavenger receptor SR-B1[52,53]. Since extracellular HCV also circulates bound to lipids and very-low-density lipoproteins (VLDLs)[54], the low density lipoprotein receptor may have a role in facilitating viral attachment. Initial capture of HCV particles by lipoprotein receptors is followed by interactions with SR-B1, CD81 and tight junction receptors leading to uptake and intracellular penetration of HCV *via* low-pH endosomes[55]. Most receptors for HCV entry are species specific, which prevents infection of mouse tissue with human virus, thereby limiting or preventing cross-species infections. After attachment to the receptors and penetration inside the cells, HCV viral and cellular membranes are fused at the acidic compartment and an HCV RNA is released into cytoplasm. Viral RNA replication requires the formation of a complex contacting NS proteins, replicating RNA and cellular membranes, and an enveloped HCV virion carrying a newly-synthesized viral genome is formed by budding into the ER lumen. After assembly of the viral particle on lipid droplets (LDs), HCV leaves the cells through a secretory (VLDL) pathway[56].

Association of HCV with lipid droplets

HCV core protein released from the polyprotein after host signal peptidase cleavage undergoes further maturation by proteolytic processing by signal peptidase[57,58]. *In vitro* expression of core protein is associated with ER membranes and the surface of LD[59]. The immature form of core protein cannot attach to LD. After maturation, core protein is directed from the ER to LD, and it attaches *via* a D2 domain, which includes a 55 amino acid protein containing two amphipathic α-helices (HⅠ and HⅡ) separated by a hydrophobic loop[60]. D2 is important for core protein stability and its subsequent associations with cell membranes. The neighboring core segment, D1, has important functions in RNA binding and protein-protein interactions[46]. LDs are directly involved in the production of infectious HCV particles[61]. They are surrounded by unique membranes called "membraneous web", allowing a high amount of plus- and minus-strand HCV RNA as well as NS proteins (NS5A) to attach to LD covered with HCV core protein. NS5A-core interactions require the serine phosphorylation toward the C-terminal end of NS5A and are essential for the association of replication complex to the periphery of LDs, which is a pre-requisite for infectious virion assembly[56]. Importantly, disruption of the core protein-LD association causes defects in HCV RNA and NS localization

and results in a loss of infectious viral particle assembly[61], demonstrating that core protein is responsible for recruitment of NS proteins to LD. Attachment of core protein to LDs leads their aggregation toward the periphery of the nucleus, suggesting that the core modifies the microtubule-dependent mobility of LDs; it also displaces adipocyte differentiation-related protein (ADRP), the major LD surface protein[62]. The modulation of the microtubule network by core protein is extremely important, as microtubule disruption reduces virus production. Thus, core protein changes the intracellular localization of LDs, securing the contacts between the sites of RNA synthesis and LDs. Due to core-NS5A protein-protein complex formation, these two proteins form a bridge between LDs and the sites of HCV RNA replication[46]. The targeting of the viral components to LDs establishes the link between assembly and release of HCV virions, VLDL production and apoprotein B lipidation[63].

HCV and autophagy

Some viruses, including HBV and HCV, subvert autophagy and use it to their own benefit. The link between autophagy and HCV replication is supported by several studies. Knockdown of autophagic proteins, beclin-1, Atg4B, Atg5 and Atg12 suppresses HCV replication at the onset of infection[64]. Specifically, Atg7 knockdown decreases the production of infectious viral particles without affecting HCV viral protein expression[65]. By inducing ER stress, HCV infection stimulates autophagosome formation regardless of HCV genotype[64,66]. Recently, we have shown that this autophagosome formation and specifically, LC3 lipidation can be further enhanced by exposure to ethanol of JFH1-infected ethanol-metabolizing hepatoma cells (unpublished observations). Nevertheless, a co-localization of LC3-Ⅱ protein in autophagosome and HCV proteins has not been found[65,67]. However, a recent study of Guevin *et al*[68] demonstrated a transient link between Atg5 and HCV NS5B (RNA-dependent RNA polymerase) protein at a very early stage of infection. This study was conducted in the frame of yeast two-hybrid analysis on the cells co-expressing Atg5 and NS5B. Interaction between these proteins is required for the onset of viral replication. Atg5 is indeed involved in other positive-strand RNA virus replications *via* formation of a double membrane vesicle[69]. This Atg5-NS5B interaction suggests that autophagy proteins play some additional, autophagy-unrelated role. Induction of autophagosome formation in HCV infection does not necessarily mean activation of autophagic protein degradation because HCV induces an incomplete autophagic response by impairing autolysosome function[66,70]. One of the possible reasons is either defective autophagosome-lysosome fusion or impaired postfusion proteolytic degradation of autophagosome contents induced by alkalization of lysosome due to HCV p7 protein[71]. The reduced capacity for lysosomal digestion may further prolong HCV survival.

HCV replication and ethanol

As revealed from numerous studies, exposure of liver cells to ethanol affects HCV replication. Zhang *et al*[72] demonstrated that ethanol produces a concentration-dependent increase in HCV replication and related that effect to the activation of NF-κB promoter. In addition, exposure of Huh7 cells and primary human hepatocytes to increasing concentrations of ethanol up-regulates intracellular and extracellular HCV RNA[73]. However, the role of ethanol metabolism in HCV replication is still controversial. Recent studies in full genomic and subgenomic HCV replicon cells have shown that alcohol increases HCV replication 4-fold in a CYP2E1-dependent manner and these effects are blocked by N-acetyl cysteine[74]. As demonstrated by Seronello *et al*[75], replication of both HCV genotypes 1b and 2a depends on lipid metabolism, is enhanced by acetaldehyde and requires elevated NADH/NAD+ ratio. On the other hand, extracellular and intracellular ROS suppress HCV replication. In addition, the effect of ethanol on HCV RNA replication in ethanol-metabolizing JFH1-infected Huh7.5 CYP2E1-expressing (RLW 2-9) cells depended on when ethanol was applied to the cells; application of ethanol before infection of cells with JFH1 enhanced HCV RNA replication, while exposure of RLW 2-9 cells to ethanol 3 d after infection suppressed HCV RNA levels[76].

The mechanisms by which ethanol interferes with or activates the HCV replication cycle are not clear. While the literature on viral entry develops rapidly, very little is known about how ethanol affects the expression of receptors for HCV entry. These effects may depend on the ability of cells to metabolize ethanol. Thus, by using ethanol non-metabolizing cells, it has been shown that the expression of CD81 is up-regulated on monocytes of HCV-infected alcohol-consuming patients[77], while ethanol exposure decreases claudin1 expression on alveolar epithelial cells[78]. Recently, we showed that ethanol exposure did not affect CD81, but enhanced claudin 1 expression on ethanol-metabolizing CYP2E1$^+$ HCV replicon and JFH1-infected infected hepatoma cells[76,79]. Other mechanisms explaining the enhancement of HCV replication/expression by ethanol exposure to hepatocytes, including the regulation of autophagy, are under investigation.

HCV, fatty liver and autophagy

Expression of PPARα, a nuclear receptor that modulates the expression of oxidative enzymes and fatty acid import into mitochondria, is impaired by HCV[80]. HCV core protein reduces expression of PPARα as well as its transcriptional activity. In addition, HCV core protein activates fatty acid synthesis by affecting SREBP1c. Sterol response element-binding proteins (SREBPs) are transcription factors that are bound to ER and regulate the activity of enzymes that support cholesterol and fatty acid synthesis. Genes for these proteins are transcriptionally induced by HCV[81]. The phosphorylation and activation of these proteins *via* the MAP kinase or PI3-K-Akt pathways leads to induction of fatty acid synthase. Activation of SREBP1 by HCV core protein requires the participation of PA28γ, a nuclear protea-

some activator[82]. Lipid droplets accumulate fat and are the place of HCV full particle assembly[83]. Since lipid droplet content is regulated in part by autophagy[84] and autophagic degradation of the substrates (including lipids) is compromised by HCV and ethanol, this defect in autophagy may further promote fat deposition in the liver of HCV alcohol-consuming patients.

CONCLUSION

Autophagy is mediated by autophagosomes that "capture" autophagic substrates (proteins and lipids) and deliver them to the lysosome for further degradation. Ethanol up-regulates the upstream part of autophagy (autophagosome formation), but it suppresses lysosomal function, thereby negatively affecting protein degradation. By creating ER stress, HCV also enhances autophagosome formation; however, some HCV proteins (p7) interfere with normal lysosome function. In addition, autophagic proteins are involved in regulation of HCV replication. Autophagosome content hepatoma cells can be further increased by exposure to ethanol. The effects of ethanol on HCV replication are redox-dependent (require elevated NADH/NAD+ ratio). Thus, HCV- and alcohol-modified autophagy apparently plays a role in enhanced viral replication, intracellular virus accumulation and steatosis progression in HCV-infected alcohol-consuming patients.

REFERENCES

1 **Roos-Mattjus P**, Sistonen L. The ubiquitin-proteasome pathway. *Ann Med* 2004; **36**: 285-295

2 **Yin XM**, Ding WX, Gao W. Autophagy in the liver. *Hepatology* 2008; **47**: 1773-1785

3 **Zhang C**, Cuervo AM. Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function. *Nat Med* 2008; **14**: 959-965

4 **Wang Y**, Singh R, Xiang Y, Czaja MJ. Macroautophagy and chaperone-mediated autophagy are required for hepatocyte resistance to oxidant stress. *Hepatology* 2010; **52**: 266-277

5 **Mortimore GE**, Pösö AR. Intracellular protein catabolism and its control during nutrient deprivation and supply. *Annu Rev Nutr* 1987; **7**: 539-564

6 **Harada K**, Mitaka T, Miyamoto S, Sugimoto S, Ikeda S, Takeda H, Mochizuki Y, Hirata K. Rapid formation of hepatic organoid in collagen sponge by rat small hepatocytes and hepatic nonpar enchymal cells. *J Hepatol* 2003; **39**: 716-723

7 **Perlmutter DH**. The role of autophagy in alpha-1-antitrypsin deficiency: a specific cellular response in genetic diseases associated with aggregation-prone proteins. *Autophagy* 2006; **2**: 258-263

8 **Klionsky DJ**, Cregg JM, Dunn WA Jr, Emr SD, Sakai Y, Sandoval IV, Sibirny A, Subramani S, Thumm M, Veenhuis M, Ohsumi Y. A unified nomenclature for yeast autophagy-related genes. *Dev Cell* 2003; **5**: 539-545

9 **Klionsky DJ**. Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat Rev Mol Cell Biol* 2007; **8**: 931-937

10 **Klionsky DJ**, Cuervo AM, Seglen PO. Methods for monitoring autophagy from yeast to human. *Autophagy* 2007; **3**: 181-206

11 **Rautou PE**, Mansouri A, Lebrec D, Durand F, Valla D, Moreau R. Autophagy in liver diseases. *J Hepatol* 2010; **53**: 1123-1134

12 **Dunn WA Jr**. Studies on the mechanisms of autophagy: formation of the autophagic vacuole. *J Cell Biol* 1990; **110**: 1923-1933

13 **Baraona E**, Leo MA, Borowsky SA, Lieber CS. Alcoholic hepatomegaly: accumulation of protein in the liver. *Science* 1975; **190**: 794-795

14 **Bardag-Gorce F**, Li J, French BA, French SW. The effect of ethanol-induced CYP2E1 on proteasome activity: the role of 4-hydroxynonenal. *Exp Mol Pathol* 2005; **78**: 109-115

15 **Bardag-Gorce F**, French BA, Nan L, Song H, Nguyen SK, Yong H, Dede J, French SW. CYP2E1 induced by ethanol causes oxidative stress, proteasome inhibition and cytokeratin aggresome (Mallory body-like) formation. *Exp Mol Pathol* 2006; **81**: 191-201

16 **Donohue TM Jr**, Zetterman RK, Tuma DJ. Effect of chronic ethanol administration on protein catabolism in rat liver. *Alcohol Clin Exp Res* 1989; **13**: 49-57

17 **Donohue TM Jr**, McVicker DL, Kharbanda KK, Chaisson ML, Zetterman RK. Ethanol administration alters the proteolytic activity of hepatic lysosomes. *Alcohol Clin Exp Res* 1994; **18**: 536-541

18 **Kharbanda KK**, McVicker DL, Zetterman RK, MacDonald RG, Donohue TM Jr. Flow cytometric analysis of vesicular pH in rat hepatocytes after ethanol administration. *Hepatology* 1997; **26**: 929-934

19 **Kharbanda KK**, McVicker DL, Zetterman RK, Donohue TM Jr. Ethanol consumption reduces the proteolytic capacity and protease activities of hepatic lysosomes. *Biochim Biophys Acta* 1995; **1245**: 421-429

20 **Kharbanda KK**, McVicker DL, Zetterman RK, Donohue TM Jr. Ethanol consumption alters trafficking of lysosomal enzymes and affects the processing of procathepsin L in rat liver. *Biochim Biophys Acta* 1996; **1291**: 45-52

21 **Haorah J**, McVicker DL, Byrd JC, MacDonald RG, Donohue TM Jr. Chronic ethanol administration decreases the ligand binding properties and the cellular content of the mannose 6-phosphate/insulin-like growth factor II receptor in rat hepatocytes. *Biochem Pharmacol* 2002; **63**: 1229-1239

22 **Haorah J**, MacDonald RG, Stoner JA, Donohue TM Jr. Ethanol consumption decreases the synthesis of the mannose 6-phosphate/insulin-like growth factor II receptor but does not decrease its messenger RNA. *Biochem Pharmacol* 2003; **65**: 637-648

23 **Kornfeld S**. Structure and function of the mannose 6-phosphate/insulinlike growth factor II receptors. *Annu Rev Biochem* 1992; **61**: 307-330

24 **Pösö AR**, Hirsimäki P. Inhibition of proteolysis in the liver by chronic ethanol feeding. *Biochem J* 1991; **273** (Pt 1): 149-152

25 **You M**, Matsumoto M, Pacold CM, Cho WK, Crabb DW. The role of AMP-activated protein kinase in the action of ethanol in the liver. *Gastroenterology* 2004; **127**: 1798-1808

26 **Tuma DJ**, Sorrell MF. Effects of ethanol on protein trafficking in the liver. *Semin Liver Dis* 1988; **8**: 69-80

27 **Donohue TM**, Osna NA, Clemens DL. Recombinant Hep G2 cells that express alcohol dehydrogenase and cytochrome P450 2E1 as a model of ethanol-elicited cytotoxicity. *Int J Biochem Cell Biol* 2006; **38**: 92-101

28 **Thomes P**, Trambly, CS, Osna, NA, Clemens, DL, Thiele, GM, Duryee, MJ, Fox, HS, Haorah, J. Proteasome Activity and Autophagy in Liver are Reciprocally Affected After Ethanol Exposure. *Hepatology* 2010; **52**: A615

29 **Ding WX**, Li M, Chen X, Ni HM, Lin CW, Gao W, Lu B, Stolz DB, Clemens DL, Yin XM. Autophagy reduces acute ethanol-induced hepatotoxicity and steatosis in mice. *Gastroenterology* 2010; **139**: 1740-1752

30 **Wu D**, Wang X, Zhou R, Cederbaum A. CYP2E1 enhances ethanol-induced lipid accumulation but impairs autophagy in HepG2 E47 cells. *Biochem Biophys Res Commun* 2010; **402**: 116-122

31 **Singh R**, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ. Autophagy regulates lipid

metabolism. *Nature* 2009; **458**: 1131-1135
32 **Klassen LW**, Thiele GM, Duryee MJ, Schaffert CS, DeVeney AL, Hunter CD, Olinga P, Tuma DJ. An in vitro method of alcoholic liver injury using precision-cut liver slices from rats. *Biochem Pharmacol* 2008; **76**: 426-436
33 **Donohue TM Jr**, Zetterman RK, Zhang-Gouillon ZQ, French SW. Peptidase activities of the multicatalytic protease in rat liver after voluntary and intragastric ethanol administration. *Hepatology* 1998; **28**: 486-491
34 **Fortunato F**, Bürgers H, Bergmann F, Rieger P, Büchler MW, Kroemer G, Werner J. Impaired autolysosome formation correlates with Lamp-2 depletion: role of apoptosis, autophagy, and necrosis in pancreatitis. *Gastroenterology* 2009; **137**: 350-360, 360.e1-e5
35 **Kaplowitz N**, Ji C. Unfolding new mechanisms of alcoholic liver disease in the endoplasmic reticulum. *J Gastroenterol Hepatol* 2006; **21** Suppl 3: S7-S9
36 **Armstrong GL**, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; **144**: 705-714
37 **Wen F**, Abdalla MY, Aloman C, Xiang J, Ahmad IM, Walewski J, McCormick ML, Brown KE, Branch AD, Spitz DR, Britigan BE, Schmidt WN. Increased prooxidant production and enhanced susceptibility to glutathione depletion in HepG2 cells co-expressing HCV core protein and CYP2E1. *J Med Virol* 2004; **72**: 230-240
38 **Wang T**, Weinman SA. Causes and consequences of mitochondrial reactive oxygen species generation in hepatitis C. *J Gastroenterol Hepatol* 2006; **21** Suppl 3: S34-S37
39 **Korenaga M**, Okuda M, Otani K, Wang T, Li Y, Weinman SA. Mitochondrial dysfunction in hepatitis C. *J Clin Gastroenterol* 2005; **39**: S162-S166
40 **Tsunedomi R**, Iizuka N, Hamamoto Y, Uchimura S, Miyamoto T, Tamesa T, Okada T, Takemoto N, Takashima M, Sakamoto K, Hamada K, Yamada-Okabe H, Oka M. Patterns of expression of cytochrome P450 genes in progression of hepatitis C virus-associated hepatocellular carcinoma. *Int J Oncol* 2005; **27**: 661-667
41 **Khan KN**, Yatsuhashi H. Effect of alcohol consumption on the progression of hepatitis C virus infection and risk of hepatocellular carcinoma in Japanese patients. *Alcohol Alcohol* 2000; **35**: 286-295
42 **Nevins CL**, Malaty H, Velez ME, Anand BS. Interaction of alcohol and hepatitis C virus infection on severity of liver disease. *Dig Dis Sci* 1999; **44**: 1236-1242
43 **Safdar K**, Schiff ER. Alcohol and hepatitis C. *Semin Liver Dis* 2004; **24**: 305-315
44 **Singal AK**, Anand BS. Mechanisms of synergy between alcohol and hepatitis C virus. *J Clin Gastroenterol* 2007; **41**: 761-772
45 **Moradpour D**, Penin F, Rice CM. Replication of hepatitis C virus. *Nat Rev Microbiol* 2007; **5**: 453-463
46 **McLauchlan J**. Hepatitis C virus: viral proteins on the move. *Biochem Soc Trans* 2009; **37**: 986-990
47 **Ma Y**, Anantpadma M, Timpe JM, Shanmugam S, Singh SM, Lemon SM, Yi M. Hepatitis C virus NS2 protein serves as a scaffold for virus assembly by interacting with both structural and nonstructural proteins. *J Virol* 2011; **85**: 86-97
48 **Bartenschlager R**, Frese M, Pietschmann T. Novel insights into hepatitis C virus replication and persistence. *Adv Virus Res* 2004; **63**: 71-180
49 **Jones CT**, Murray CL, Eastman DK, Tassello J, Rice CM. Hepatitis C virus p7 and NS2 proteins are essential for production of infectious virus. *J Virol* 2007; **81**: 8374-8383
50 **Penin F**, Dubuisson J, Rey FA, Moradpour D, Pawlotsky JM. Structural biology of hepatitis C virus. *Hepatology* 2004; **39**: 5-19
51 **Pileri P**, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa D, Grandi G, Abrignani S. Binding of hepatitis C virus to CD81. *Science* 1998; **282**: 938-941
52 **Catanese MT**, Graziani R, von Hahn T, Moreau M, Huby T, Paonessa G, Santini C, Luzzago A, Rice CM, Cortese R, Vitelli A, Nicosia A. High-avidity monoclonal antibodies against the human scavenger class B type I receptor efficiently block hepatitis C virus infection in the presence of high-density lipoprotein. *J Virol* 2007; **81**: 8063-8071
53 **Evans MJ**, von Hahn T, Tscherne DM, Syder AJ, Panis M, Wölk B, Hatziioannou T, McKeating JA, Bieniasz PD, Rice CM. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 2007; **446**: 801-805
54 **Burlone ME**, Budkowska A. Hepatitis C virus cell entry: role of lipoproteins and cellular receptors. *J Gen Virol* 2009; **90**: 1055-1070
55 **Dreux M**, Dao Thi VL, Fresquet J, Guérin M, Julia Z, Verney G, Durantel D, Zoulim F, Lavillette D, Cosset FL, Bartosch B. Receptor complementation and mutagenesis reveal SR-BI as an essential HCV entry factor and functionally imply its intra- and extra-cellular domains. *PLoS Pathog* 2009; **5**: e1000310
56 **Fukasawa M**. Cellular lipid droplets and hepatitis C virus life cycle. *Biol Pharm Bull* 2010; **33**: 355-359
57 **Okamoto K**, Mori Y, Komoda Y, Okamoto T, Okochi M, Takeda M, Suzuki T, Moriishi K, Matsuura Y. Intramembrane processing by signal peptide peptidase regulates the membrane localization of hepatitis C virus core protein and viral propagation. *J Virol* 2008; **82**: 8349-8361
58 **Schröder B**, Saftig P. Molecular insights into mechanisms of intramembrane proteolysis through signal peptide peptidase (SPP). *Biochem J* 2010; **427**: e1-e3
59 **Barba G**, Harper F, Harada T, Kohara M, Goulinet S, Matsuura Y, Eder G, Schaff Z, Chapman MJ, Miyamura T, Bréchot C. Hepatitis C virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. *Proc Natl Acad Sci USA* 1997; **94**: 1200-1205
60 **Boulant S**, Montserret R, Hope RG, Ratinier M, Targett-Adams P, Lavergne JP, Penin F, McLauchlan J. Structural determinants that target the hepatitis C virus core protein to lipid droplets. *J Biol Chem* 2006; **281**: 22236-22247
61 **Miyanari Y**, Atsuzawa K, Usuda N, Watashi K, Hishiki T, Zayas M, Bartenschlager R, Wakita T, Hijikata M, Shimotohno K. The lipid droplet is an important organelle for hepatitis C virus production. *Nat Cell Biol* 2007; **9**: 1089-1097
62 **Boulant S**, Douglas MW, Moody L, Budkowska A, Targett-Adams P, McLauchlan J. Hepatitis C virus core protein induces lipid droplet redistribution in a microtubule- and dynein-dependent manner. *Traffic* 2008; **9**: 1268-1282
63 **Huang H**, Sun F, Owen DM, Li W, Chen Y, Gale M Jr, Ye J. Hepatitis C virus production by human hepatocytes dependent on assembly and secretion of very low-density lipoproteins. *Proc Natl Acad Sci USA* 2007; **104**: 5848-5853
64 **Dreux M**, Gastaminza P, Wieland SF, Chisari FV. The autophagy machinery is required to initiate hepatitis C virus replication. *Proc Natl Acad Sci USA* 2009; **106**: 14046-14051
65 **Tanida I**, Fukasawa M, Ueno T, Kominami E, Wakita T, Hanada K. Knockdown of autophagy-related gene decreases the production of infectious hepatitis C virus particles. *Autophagy* 2009; **5**: 937-945
66 **Ait-Goughoulte M**, Kanda T, Meyer K, Ryerse JS, Ray RB, Ray R. Hepatitis C virus genotype 1a growth and induction of autophagy. *J Virol* 2008; **82**: 2241-2249
67 **Dreux M**, Chisari FV. Autophagy proteins promote hepatitis C virus replication. *Autophagy* 2009; **5**: 1224-1225
68 **Guévin C**, Manna D, Bélanger C, Konan KV, Mak P, Labonté P. Autophagy protein ATG5 interacts transiently with the hepatitis C virus RNA polymerase (NS5B) early during infection. *Virology* 2010; **405**: 1-7
69 **Lee YR**, Lei HY, Liu MT, Wang JR, Chen SH, Jiang-Shieh YF, Lin YS, Yeh TM, Liu CC, Liu HS. Autophagic machinery activated by dengue virus enhances virus replication. *Virology* 2008; **374**: 240-248
70 **Sir D**, Chen WL, Choi J, Wakita T, Yen TS, Ou JH. Induction

of incomplete autophagic response by hepatitis C virus *via* the unfolded protein response. *Hepatology* 2008; **48**: 1054-1061

71 **Wozniak A**, Jones K, Weinman S. Hepatitis C virus p7 protein modulates autophagy by altering lysosomal pH. *Hepatology* 2010; **52**: A390

72 **Zhang T**, Li Y, Lai JP, Douglas SD, Metzger DS, O'Brien CP, Ho WZ. Alcohol potentiates hepatitis C virus replicon expression. *Hepatology* 2003; **38**: 57-65

73 **Ye L**, Wang S, Wang X, Zhou Y, Li J, Persidsky Y, Ho W. Alcohol impairs interferon signaling and enhances full cycle hepatitis C virus JFH-1 infection of human hepatocytes. *Drug Alcohol Depend* 2010; **112**: 107-116

74 **McCartney EM**, Semendric L, Helbig KJ, Hinze S, Jones B, Weinman SA, Beard MR. Alcohol metabolism increases the replication of hepatitis C virus and attenuates the antiviral action of interferon. *J Infect Dis* 2008; **198**: 1766-1775

75 **Seronello S**, Ito C, Wakita T, Choi J. Ethanol enhances hepatitis C virus replication through lipid metabolism and elevated NADH/NAD+. *J Biol Chem* 2010; **285**: 845-854

76 **Osna N**, Kharbanda, K, White, R, Mercer, D. Ethanol-induced oxidative stress promotes the spread of hepatitis C virus (HCV) in virally infected ethanol metabolizing cells. *Hepatology* 2010; **52**: A614

77 **Carrière M**, Rosenberg AR, Conti F, Chouzenoux S, Terris B, Sogni P, Soubrane O, Calmus Y, Podevin P. Low density lipoprotein receptor transcripts correlates with liver hepatitis C virus RNA in patients with alcohol consumption. *J Viral Hepat* 2006; **13**: 633-642

78 **Fernandez AL**, Koval M, Fan X, Guidot DM. Chronic alcohol ingestion alters claudin expression in the alveolar epithelium of rats. *Alcohol* 2007; **41**: 371-379

79 **Austin L**, Kharbanda, K, Beard, M, Osna, N. Ethanol affects expression of receptors for HCV viral entry in liver cells. *Hepatology* 2009; **50**: A1155-A1156

80 **Negro F**, Sanyal AJ. Hepatitis C virus, steatosis and lipid abnormalities: clinical and pathogenic data. *Liver Int* 2009; **29** Suppl 2: 26-37

81 **Waris G**, Felmlee DJ, Negro F, Siddiqui A. Hepatitis C virus induces proteolytic cleavage of sterol regulatory element binding proteins and stimulates their phosphorylation *via* oxidative stress. *J Virol* 2007; **81**: 8122-8130

82 **Moriishi K**, Mochizuki R, Moriya K, Miyamoto H, Mori Y, Abe T, Murata S, Tanaka K, Miyamura T, Suzuki T, Koike K, Matsuura Y. Critical role of PA28gamma in hepatitis C virus-associated steatogenesis and hepatocarcinogenesis. *Proc Natl Acad Sci USA* 2007; **104**: 1661-1666

83 **McLauchlan J**, Lemberg MK, Hope G, Martoglio B. Intramembrane proteolysis promotes trafficking of hepatitis C virus core protein to lipid droplets. *EMBO J* 2002; **21**: 3980-3988

84 **Fujimoto T**, Ohsaki Y. Proteasomal and autophagic pathways converge on lipid droplets. *Autophagy* 2006; **2**: 299-301

S- Editor Tian L **L- Editor** O'Neill M **E- Editor** Ma WH