Knockdown of Autophagy Inhibits Infectious Hepatitis C Virus Release by the Exosomal Pathway

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ABSTRACT
Hepatitis C virus (HCV) is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma in humans. We showed previously that HCV induces autophagy for viral persistence by preventing the innate immune response. Knockdown of autophagy reduces extracellular HCV release, although the precise mechanism remains unknown. In this study, we observed that knockdown of autophagy genes enhances intracellular HCV RNA and accumulates infectious virus particles in cells. Since HCV release is linked with the exosomal pathway, we examined whether autophagy proteins associate with exosomes in HCV-infected cells. We observed an association between HCV and the exosomal marker CD63 in autophagy knockdown cells. Subsequently, we observed that levels of extracellular infectious HCV were significantly lower in exosomes released from autophagy knockdown cells. To understand the mechanism for reduced extracellular infectious HCV in the exosome, we observed that an interferon (IFN)-stimulated BST-2 gene is upregulated in autophagy knockdown cells and associated with the exosome marker CD63, which may inhibit HCV assembly or release. Taken together, our results suggest a novel mechanism involving autophagy and exosome-mediated HCV release from infected hepatocytes.

IMPORTANT
Autophagy plays an important role in HCV pathogenesis. Autophagy suppresses the innate immune response and promotes survival of virus-infected hepatocytes. The present study examined the role of autophagy in secretion of infectious HCV from hepatocytes. Autophagy promoted HCV trafficking from late endosomes to lysosomes, thus providing a link with the exosome. Inhibition of HCV-induced autophagy could be used as a strategy to block exosome-mediated HCV transmission.

Hepatitis C virus (HCV) establishes chronic infection in more than 70% of infected individuals worldwide, and over 170 million people are currently infected with HCV. Persistent HCV infection is associated with a chronic inflammatory disease that ultimately leads to hepatic fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). HCV is a member of the genus Hepacivirus, which belongs to the family Flaviviridae. The virus is enveloped, and the single-stranded positive-sense RNA genome contains an open reading frame flanked by untranslated regions (1). Translation of the single open reading frame is driven by an internal ribosomal entry site (IRES) sequence present within the 5’ untranslated region (UTR), resulting in synthesis of a polyprotein approximately 3,000 amino acids in length. This polyprotein is processed by cellular and viral proteases into its individual viral proteins. The nonstructural proteins NS3, NS4A, NS4B, NS5A, and NS5B are sufficient to support efficient HCV RNA replication in the membranous compartments of the cytosol (1, 2). In infected individuals, HCV particles circulate as low-density lipoprotein (LDL)–virus complexes that are rich in triglycerides and contain HCV RNA, core protein, and apolipoproteins B and E (apoB and apoE), which are components of the beta-lipoproteins (very-low-density lipoproteins [VLDL] and LDL). The precise mechanisms of HCV assembly, budding, and release are currently under investigation.

There are different mechanisms by which cells release large biomolecules into the extracellular space, with the most common process being exocytosis (3). Exosomes are extracellular vesicles secreted upon fusion of endosomal multivesicular bodies (MVBs) with the plasma membrane (4). Endosomal sorting complex required for transport (ESCRT) proteins are involved in the generation of secretory MVBs. The ESCRT-0 component hepatocyte growth factor–regulated tyrosine kinase substrate (Hrs) and the ESCRT-I protein TSG101 are involved early in cargo recruitment. VPS4B is an ATPase that provides energy for the dissolution of ESCRT-III complexes at membrane fission, whereas apoptosis-linked gene 2-interacting protein X (ALIX) contributes to exosome biogenesis (5, 6). MVBS can either fuse with lysosomes to release vesicle contents for degradation or fuse with the plasma membrane to release extracellular exosomes (7). Virus budding is usually coupled tightly to virion assembly, and most viruses, therefore, use their structural proteins to recruit the ESCRT pathway. Enveloped viruses commonly assemble and release at the plasma membrane, although some are released into internal compartments (8, 9). In the latter case, the internal compartment must ultimately fuse with the plasma membrane to release the virus from the cell (8, 9).

The role of exosomes in HCV infection was first demonstrated by the presence of viral RNA in exosomes isolated from plasma of HCV-infected patients (10). The components required for MVB biogenesis, Vps4 and the ESCRT-III complex, are required for...
release of infectious HCV particles (11). Subsequently, an ESCRT-0 component and Hrs were reported to be involved in HCV release through the exosomal pathway (12). Further, the functional role of hepatocyte-derived exosomes in carrying viral RNA and transferring it to plasmacytoid dendritic cells was suggested for the activation and production of alpha interferon (IFN-α) (13). Exosome-mediated transmission of HCV also establishes productive infection in hepatocytes (14). Apart from HCV, the existence of infectious hepatitis A virus particles within extracellular vesicles has been observed and shown to be dependent on ESCRT-III-associated proteins, ALIX, and Vps4B of the MVB pathway (15). Interaction of ALIX with the ESCRT-III protein CHMP4B has been reported to facilitate the budding of human immunodeficiency virus (16).

The autophagy pathway, an evolutionarily conserved membrane system, is known to engulf damaged organelles and maintains cellular homeostasis through the formation of double-membrane vesicles called autophagosomes (17,18). Closed autophagosomes undergo a maturation process as they subsequently fuse with endosomes and lysosomes. Autophagosomes fuse with both early and late endosomes to form amphisomes prior to the lysosomal fusion step. The autophagosomal system extensively overlaps the endosomal system. The endosomal system provides membrane components for autophagosome biogenesis, and the amphisomes acquire machinery required for lysosomal fusion following the fusion of nascent autophagosomes with endosomes. Host cellular molecules, such as LC3, p62, and Rab7, are the substrates of autophagic degradation and may partly reflect the rate of endolysosomal degradation. LC3-I undergoes a posttranslational modification to form lipidated LC3-II, an autophagy pathway component and Hrs were reported to be involved in HCV release through the exosomal pathway (12). Further, the functional role of hepatocyte-derived exosomes in carrying viral RNA and transferring it to plasmacytoid dendritic cells was suggested for the activation and production of alpha interferon (IFN-α) (13). Exosome-mediated transmission of HCV also establishes productive infection in hepatocytes (14). Apart from HCV, the existence of infectious hepatitis A virus particles within extracellular vesicles has been observed and shown to be dependent on ESCRT-III-associated proteins, ALIX, and Vps4B of the MVB pathway (15). Interaction of ALIX with the ESCRT-III protein CHMP4B has been reported to facilitate the budding of human immunodeficiency virus (16).

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globulin conjugated to Alexa Fluor 647 (Molecular Probes). Images were superimposed digitally for fine comparisons.

**Western blotting.** Mock-infected, HCV-infected, or control siRNA- or BCN1 siRNA-treated virus-infected cells were lysed in a sample buffer, subjected to SDS-PAGE, and transferred onto a nitrocellulose membrane. The membrane was probed with specific antibodies to HCV core (C7-50; ThermoFisher); BST-2 (Novus Biologicals); Hsp70 (StressMarq; BD Biosciences); p62 (Abnova); and Beclin1, CD63, and Rab27a (Santa Cruz). The membrane was reprobed with actin or GAPDH (glyceraldehyde-3-phosphate dehydrogenase) for comparison of the protein loads. Proteins were visualized using an enhanced chemiluminescence (ECL) Western blot substrate (Pierce) and subjected to densitometric scanning by using an image analyzer and Quantity One software (Bio-Rad).

**RESULTS**

**HCV promotes a complete autophagy maturation process.** Autophagy is a dynamic multistep process and can be modulated at several levels. Accumulation of autophagosomes can either reflect an increase of autophagy flux, through the formation of de novo autophagosomes, or a reduced turnover of autophagosome recycling due to inhibition of their fusion with lysosomes. p62 (SQSTM1) serves as a substrate for autophagic degradation. We examined the expression of p62 in mock-infected and HCV (either genotype 1a or genotype 2a)-infected IHH or Huh7.5 cells. We observed lower expression of p62, suggesting that the lysosomal degradation process was not inhibited in HCV-infected cells (Fig. 1A and B). When we inhibited autophagy by gene silencing of either BCN1 or ATG7, followed by virus infection, we observed an accumulation of p62, suggesting impairment of the lysosomal degradation process. Similar observations were noted in autophagy knockdown HCV genotype 2a (JFH1)-infected Huh7.5 cells. Thus, HCV-infected cells undergo complete autophagy maturation, which is in agreement with previous reports (25, 26). We examined virus-infected cells for association of late endosomes and lysosomes and observed colocalization of CD63 and lysosome. On the other hand, reduced colocalization...
tion was noted in BCN1 knockdown virus-infected cells (Fig. 1C). Our results suggest that knockdown of the autophagy pathway can cause lysosomal dysfunction and may impair delivery of late endosome/exosome cargo to the lysosome in virus-infected cells.

**Autophagy in hepatocytes regulates secretion of infectious HCV.** To investigate whether autophagy is involved in the late stage of the HCV life cycle, IHH or Huh7.5 cells were transfected with siRNA against BCN1 or ATG7 and then infected with HCV. The infected cells and culture supernatants were collected at different time points postinfection. We observed increased expression of intracellular viral RNA in BCN1 knockdown virus-infected cells compared to control siRNA-treated virus-infected cells (Fig. 2A and B). Upregulation of intracellular viral RNA in ATG7 knockdown virus-infected cells was observed compared to control siRNA-treated virus-infected cells (Fig. 2C and D). We observed an increase in the expression of HCV protein in autophagy knockdown infected cells compared to parallel control siRNA-treated cells, indicating more virus may be present in autophagy knockdown infected cells (Fig. 2E).

We also analyzed the infectivity of isolated intracellular viral particles from either control or BCN1 siRNA-treated virus-infected cells after repeated freeze-thawing and measured the viral titer on naive Huh7.5 cells. HCV-infected cells were stained for NS5A protein, and the viral titer was calculated by TCID_{50}. The results are presented in log scale.

**FIG 2** Knockdown of BCN1 or ATG7 induces accumulation of intracellular viral particles. (A and B) IHH were treated with control siRNA or BCN1 siRNA and infected with HCV genotype 1a (H77) or genotype 2a (JFH1) at an MOI of 0.1. Cells were collected at different time points to measure the levels of intracellular HCV RNA and normalized to GAPDH by quantitative reverse transcription (qRT)-PCR. (C and D) IHH were treated with control siRNA or ATG7 siRNA and infected with HCV genotype 1a or genotype 2a at an MOI of 0.1. The intracellular HCV RNA level was measured by qRT-PCR at different time points and normalized with GAPDH. All experiments were performed three times, and the results are presented as the means of the results of the three experiments plus standard deviations. (E) IHH were treated with control siRNA or BCN1 siRNA and infected with GFP-tagged HCV genotype 2a (JFH1-GFP) at an MOI of 0.1. Representative photographs were taken 3 days postinfection. (F) IHH were treated with control siRNA or BCN1 siRNA and infected with HCV genotype 2a at an MOI of 0.1. Cells were collected 3 days postinfection, and infectious virus particles were isolated by repeated freezing and thawing. The titers of intracellular HCV particles were determined in naive Huh7.5 cells. HCV-infected cells were stained for NS5A protein, and the viral titer was calculated by TCID_{50}. The results are presented in log scale.
Upregulation of the infectivity of intracellular viral particles in BCN1 knockdown virus-infected cells was observed compared to control siRNA-treated virus-infected cells (Fig. 2F), suggesting HCV particles are retained in autophagy knockdown hepatocytes.

We also measured viral RNA in culture supernatants of autophagy knockdown virus-infected cells compared with control siRNA-treated HCV-infected cells. As expected, we observed a decrease in extracellular viral RNA in BCN1 or ATG7 knockdown virus-infected cells compared to control siRNA-treated virus-infected cells (Fig. 3A and B). We also examined the infectivity of virus particles in the culture supernatant by measuring the virus titer in naive Huh7.5 cells. We observed reduced infectivity of extracellular HCV particles generated from ATG7 knockdown virus-infected cells compared to those from control siRNA-treated virus-infected cells (Fig. 3C). We have shown previously that BCN1 knockdown impairs the infectivity of extracellular viral particles (19). A similar result was also observed in ATG7 knockdown HCV-infected Huh7.5.1 cells (27), although intracellular HCV RNA expression remained unchanged in that study. The discrepancy with the latter observation may be due to the use of a different cell line or subclone. The reduced infectivity in the supernatants and concomitant increased infectivity of intracellular virus in autophagy knockdown virus-infected cells compared to parallel controls suggested that secretion of virion particles was inhibited upon silencing of the autophagy machinery. Collectively, these results suggest that autophagy is required for HCV secretion into the cell culture medium.

Silencing autophagy impairs exosome-associated HCV release. HCV utilizes the exosome pathway for its release from host cells (13, 14). To examine whether autophagy is required for exosome-mediated viral release, we first isolated the exosomes from culture supernatants of control siRNA- or BCN1 siRNA-treated HCV-infected cells using a standard ultracentrifugation protocol (23, 28). Reduced expression levels of CD63 and Hsp70 (exosome markers) were observed in exosomes secreted from BCN1 knockdown cell culture supernatants compared to a mock-treated control (Fig. 4A). We did not observe expression of the early endosome marker EEA1 when used as a negative control, as expected (data not shown). Interestingly, we detected a higher level of expression of intracellular CD9 mRNA (an exosome marker) in autophagy knockdown virus-infected cells in comparison to the control (Fig. 4B). As expected, Beclin1 expression was reduced in cells treated with BCN1 siRNA compared to cells treated with control siRNA. However, intracellular CD9 expres-
sion was not altered in BCN1 knockdown naive hepatocytes (data not shown). We next examined the cellular localization of exosomes in the control or autophagy knockdown HCV-infected cells. We observed a significant increase in CD63 expression and higher colocalization of CD63 with HCV particles in BCN1 knockdown virus-infected cells than in control siRNA-treated virus-infected cells (Fig. 4C). In addition, Hsp70 expression was higher in BCN1 knockdown HCV-infected cell lysates than in

**FIG 4** Silencing of BCN1 accumulates exosomes within infected cells. (A) IHH were treated with control siRNA or BCN1 siRNA, followed by infection with HCV genotype 2a at an MOI of 0.1. After incubation with virus for 8 h, the cells were washed and incubated with medium containing exosome-depleted serum. At 3 days postinfection, the culture supernatants were collected to isolate exosomes. Western blot analysis for CD63 or Hsp70 was performed with exosomes isolated from culture supernatants of control siRNA- or BCN1 siRNA-treated HCV-infected cells. (B) Total RNA was isolated from control siRNA- or BCN1 siRNA-treated HCV-infected IHH. Intracellular CD9 or BCN1 was measured by qRT-PCR, and GAPDH was used for normalization. All experiments were performed three times, and the results are presented as the means of the results of the three experiments plus standard deviations. (C) Control siRNA- or BCN1 siRNA-treated IHH were infected with HCV genotype 2a (JFH1-GFP) at an MOI of 0.1. At 3 days postinfection, the cells were fixed, permeabilized, and stained with CD63 antibody (red). Nuclei were stained with DAPI (blue). Colocalization of CD63 and viral protein was examined by superimposing images in the Merge column by confocal microscopy, and representative images are shown. (D) Western blot analysis for Hsp70 was performed using specific antibody in cell lysates from control siRNA- or BCN1 siRNA-treated HCV-infected cells. The blots were reprobed with GAPDH to normalize the equal protein loads. Silencing of BCN1 enhanced Hsp70 protein in HCV-infected IHH in comparison to control siRNA-treated virus-infected cells. Densitometry scanning for quantitation is shown on the right. *, P < 0.05; ***, P < 0.001.
control siRNA-treated virus-infected cells (Fig. 4D), suggesting that HCV particles were retained inside the exosome in autophagy-impaired virus-infected cells. Together, our results demonstrate that exosome-associated HCV release is linked with autophagy in infected cells.

**Knockdown of Rab27a inhibits intracellular and extracellular HCV RNA expression.** Rab27a regulates exosome secretion by docking MVB at the plasma membrane (29). To examine the role of Rab27a in HCV infection, we treated cells with control siRNA or Rab27a siRNA and then infected them with HCV. Intracellular HCV RNA levels were significantly lower in Rab27a siRNA-treated cells than in control siRNA-treated virus-infected cells (Fig. 5A). Cell lysates treated with control siRNA or Rab27a siRNA were subjected to Western blot analysis for expression of Rab27a protein. As expected, Rab27a protein expression was inhibited in specific siRNA-treated cells (Fig. 5B). Interestingly, we did not observe a difference in expression of the exosomal marker CD9 at the mRNA level or of CD63 at the protein level (data not shown). We also observed that extracellular HCV RNA or infectivity was significantly lower (Fig. 5C and D) in Rab27a knockdown virus-infected cells than in control siRNA-treated virus-infected cells. Rab27a knockdown blocks HCV-induced autophagy (data not shown), although the precise mechanism remains to be determined. While our article was in preparation, Chen et al. (30) reported similar observations, although the relationship of Rab27a with exosome secretion in HCV-infected cells needs further investigation.

**Knockdown of autophagy enhances BST-2 expression and inhibits HCV release.** We next examined the presence of infectious HCV in exosomes. For this, exosomes isolated from mock- or HCV-infected cells were incubated with naive Huh7.5 cells, and HCV core protein was stained with a specific antibody to examine HCV infection. We observed the presence of HCV infectivity in Huh7.5 cells, as evident from viral protein expression. A representative image is shown in Fig. 6A, although we could not detect HCV protein in extracellular exosomes. We further measured viral RNA and infectivity of virus particles in exosomes isolated from control siRNA- or BCN1 siRNA-treated virus-infected cells. We observed variable HCV RNA levels in exosomes; however, the infectivity of HCV particles was significantly lower in exosomes isolated from autophagy knockdown virus-infected cells than in control siRNA-treated virus-infected cells (Fig. 6B). These results suggested that autophagy is required for exosome-mediated release of viral particles. BST-2 (also known as tetherin, CD317, or HM1.24) is encoded by an interferon-inducible gene that blocks the release of enveloped viruses, including retroviruses, filoviruses, arenaviruses, and herpesviruses, as well as paramyxoviruses and rhabdoviruses (31). We have previously shown that knockdown of autophagy enhances interferon-stimulated genes and the innate immune response in HCV-infected cells (19). To further elucidate the mechanism for restriction of exosome-mediated HCV release from the autophagy knockdown cells, we analyzed the expression of the BST-2/tetherin gene in autophagy knockdown HCV-infected cells. We observed increased expression of BST-2/tetherin in autophagy knockdown virus-infected cells compared to parallel controls (Fig. 6C). This result is in agreement with the recent finding that BST-2 inhibits HCV release (32). We next examined the intracellular location of BST-2 in the absence of BCN1 expression in HCV-infected cells. Immunofluorescence analysis suggested colocalization of BST-2 with CD63 in HCV-

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**FIG 5** Knockdown of Rab27a reduces HCV replication and secretion. (A) Huh7.5 cells were treated with control or Rab27a siRNA, followed by infection with HCV genotype 2a at an MOI of 0.1. At 3 days postinfection, RNA was isolated from the cell lysates to determine intracellular HCV RNA levels. Intracellular HCV RNA levels were measured by qRT-PCR and normalized with GAPDH. (B) Cell lysates transfected with control or Rab27a siRNA were subjected to Western blot analysis for expression of Rab27a protein. Rab27a protein expression was inhibited in specific siRNA-treated cells. (C) Culture supernatants were collected from control siRNA- or Rab27a siRNA-treated HCV-infected cells 3 days postinfection and used to infect naive Huh7.5 cells for virus titer determination. The results are presented as percentages relative to control cells (100%). (D) Extracellular HCV RNA was measured by qRT-PCR from control siRNA- or Rab27a siRNA-treated virus-infected cells as described for panel A. ***，$P < 0.001$. The error bars represent standard deviations.
infected cells (Fig. 6D). Accumulation of BST-2 with CD63 was significantly higher in BCN1 knockdown HCV-infected cells. CD63-BST2 colocalization was assessed by calculating the Pearson correlation coefficient on three images. Together, our results suggested that HCV particles traffic to the exosome with the help of autophagy machinery to be released from the cells.

**DISCUSSION**

In our present study, we demonstrated that autophagy knockdown in HCV-infected cells displays (i) higher intracellular HCV RNA and protein accumulation; (ii) increased intracellular exosomes, as evident by higher expression status of CD9, Hsp70, and CD63; and (iii) lower levels of infectious HCV in secreted (extracellular) exosomes. We also observed that the autophagy pathway regulates exosome-mediated virus transmission and promotes virus trafficking from late endosomes to lysosomes.

Many cell types continuously secrete a large number of exosomes. They have a diameter of approximately 50 to 150 nm and a buoyant density between 1.08 g/ml and 1.22 g/ml. Exosomes are released into the extracellular space from late endosomes/MVBs after fusion with the plasma membrane. Previous studies have indicated that cell culture (33–35) and patient serum-derived (36, 37) HCV particles have a broad range of buoyant densities (between 1.01 g/ml and 1.17 g/ml). Further, the low-density (1.1-g/ml) fraction displays exosome-like structures and also infectivity (34). HCV can also spread to neighboring cells with the help of exosomes carrying replication-competent viral RNA (38). Furthermore, exosomes carrying virus particles can establish productive infection in naive hepatocytes (14). Recently, both NS5A and core protein, along with the exosomal proteins CD63 and CD81, were detected in the low-density (1.083- to 1.098-g/ml) infective HCV
inhibits not noted (48, 49). We have observed a large accumulation of BST2 calization of BST-2/tetherin and HBV particles on MVBs has been generation of intracellular and extracellular infectious HCV par-
particles. Exosomal markers and NS5A or core protein were found to be colocalized at the plasma membrane, from which virus re-
lease occurs (39). We have also observed colocalization of HCV with the exosome marker protein CD63 in virus-infected cells, confirming the presence of virus particles in the exosomes. How-
ever, cross talk between autophagy and exosome release in HCV infection still remains unclear.

The endosomal and autophagy pathways are functionally con-
ected. Several studies implicated the components of endosomal trafficking, including ESCRT complexes, Rab GTPases (40), and vesicle fusion machinery SNAREs, in autophagosome maturation (41, 42). Recently, physical interaction between the core autophagy component ATG12-ATG3 and the ESCRT-associated protein ALIX was established (43). Autophagosomes fuse with MVBs before delivery to the lysosome. Autophagy components regulate MVB formation and exosome release in neurodegenera-
tive diseases. Toxic alpha-synuclein oligomers can be secreted through exosomes when the autophagic machinery fails (44). On the other hand, in age-related disorders, the decline in autophagy-related proteins leads to the accumulation of deleterious mole-
cules, promoting exosome secretion to remove harmful materials (45). Autophagy impairment is linked with lysosomal dysfunc-
tion, and we observed accumulation of p62 in autophagy knock-
down HCV-infected cells. Virus infection goes through a com-
plete autophagy maturation process, as reported by us and others (20, 25, 26). However, in the absence of autophagy proteins, the number of autolysosomes is reduced, leading to accumulation of virus particles within the exosomes.

Exosomes facilitate the budding of human immunodeficiency virus and hepatitis A virus (15, 16, 46). Infectious hepatitis A virus particles were observed within extracellular vesicles and were de-
pendent on host proteins associated with endosomal sorting com-
plexes required for transport (ESCRT), namely, VPS4B and ALIX (15). Interaction of ALIX with the ESCRT-III protein CHMP4B has been reported to facilitate the budding of human immune-
deficiency virus (16). We observed that silencing of the autophagy pathway impairs the exosome-mediated release of infectious HCV particles. We also observed that knockdown of Rab27a inhibits generation of intracellular and extracellular infectious HCV particles. Silencing of Rab27a decreases exosome release in the culture supernatant without altering the exosome protein content (29), thus implying a role in exosome-mediated HCV secretion. In-
creased expression of the BST-2/tetherin gene in autophagy knockdown HCV-infected cells was also noted. BST-2 is a trans-
membrane protein that contains a short N-terminal cytoplasmic domain, a membrane-spanning alpha-helix, a coiled-coil ectodo-
main, and a C-terminal glycosylphosphatidylinositol (GPI) an-
chor (31). This antiviral protein localizes at the plasma mem-
brane, as well as the membranes of multiple intracellular vesicles, including endosomes and the trans-Golgi network. The overex-
pression of BST-2 did not affect the efficiency of dengue virus (DENV) infection and intracellular replication but significantly reduced the virion yield from infected cells (47). A recent study demonstrated that BST-2/tetherin and HCV core protein colocal-
ize on lipid droplets, where the assembly and maturation of HCV particles take place (32). The IFN-induced BST-2/tetherin protein inhibits hepatitis B virus (HBV) release from cells, and the colo-
calization of BST-2/tetherin and HBV particles on MVBs has been noted (48, 49). We have observed a large accumulation of BST2 with CD63 in BCN1 knockdown HCV-infected cells. Therefore, we postulate that this could be one of the possible mechanisms of BST-2/tetherin-mediated restriction of infectious HCV release from autophagy knockdown cells. Further study is necessary to understand the cross talk between autophagy and exosomes in HCV infection and the role of BST-2/tetherin in restricting HCV release. Exosomes containing HCV might fuse with uninfected cells in a mechanism independent of viral envelope proteins and may contribute to natural infection. Our studies provide the role of exosomes in the spread of HCV infection, which can be blocked by the use of autophagy inhibitors.

ACKNOWLEDGMENTS
We thank Curt H. Hagedorn for providing GFP-tagged JFH1 virus and Charles Rice for the HCV NS5A antibody.

This work was supported by research grants DK081817 (R.R.) and DK080812 (R.R.) from the National Institutes of Health.

FUNDING INFORMATION
HHS | NIH | National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) provided funding to Ratna B. Ray under grant number DK081817. HHS | NIH | National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) provided funding to Ranjit Ray under grant number DK080812.

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