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TOPIC HIGHLIGHT

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Autophagy in hepatitis C virus-host interactions: Potential roles and therapeutic targets for liver-associated diseases

Po-Yuan Ke, Steve S-L Chen

Po-Yuan Ke, Steve S-L Chen, Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan
Po-Yuan Ke, Department of Biochemistry and Molecular Biology, Graduate Institute of Biomedical Sciences, and Molecular Medicine Research Center, College of Medicine, Chang Gung University, Taoyuan 33302, Taiwan
Po-Yuan Ke, Liver Research Center, Chang-Gung Memorial Hospital, Taoyuan 33305, Taiwan
Steve S-L Chen, PhD Program in Translational Medicine, Kaohsiung Medical University, Kaohsiung 80708 and Academia Sinica, Taipei 11529, Taiwan
Author contributions: Ke PY and Chen SSL wrote the paper.
Correspondence to: Steve S-L Chen, PhD, Research Fellow, Institute of Biomedical Sciences, Academia Sinica, Nangang District, Taipei 11529, Taiwan. schen@ibms.sinica.edu.tw
Telephone: +886-2-2652-3933 Fax: +886-2-2652-3073
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Abstract

Autophagy is a lysosome-associated, degradative process that catabolizes cytosolic components to recycle nutrients for further use and maintain cell homeostasis. Hepatitis C virus (HCV) is a major cause of chronic hepatitis, which often leads to end-stage liver-associated diseases and is a significant burden on worldwide public health. Emerging lines of evidence indicate that autophagy plays an important role in promoting the HCV life cycle in host cells. Moreover, the diverse impacts of autophagy on a variety of signaling pathways in HCV-infected cells suggest that the autophagic process is required for balancing HCV-host cell interactions and involved in the pathogenesis of HCV-related liver diseases. However, the detailed molecular mechanism underlying how HCV activates autophagy to benefit viral growth is still enigmatic. Additionally, how the autophagic response contributes to disease progression in HCV-infected cells remains largely unknown. Hence, in this review, we overview the interplay between autophagy and the HCV life cycle and propose possible mechanisms by which autophagy may promote the pathogenesis of HCV-associated chronic liver diseases. Moreover, we outline the related studies on how autophagy interplays with HCV replication and discuss the possible implications of autophagy and viral replication in the progression of HCV-induced liver diseases, *e.g.*, steatosis and hepatocellular carcinoma. Finally, we explore the potential therapeutics that target autophagy to cure HCV infection and its related liver diseases.

Key words: Autophagy; Hepatitis C virus; Steatosis; Cirrhosis; Hepatocellular carcinoma

Core tip: Hepatitis C virus (HCV) is a major cause of chronic liver disease and is associated with over 170 million infected individuals worldwide. However, a successful strategy for completely eradicating HCV infection is still limited. Autophagy is a catabolic process that delivers cytosolic components to lysosomes for breakdown. HCV has been shown to activate autophagy to promote viral growth *in vitro*. In this review, we outline the recent findings on the physiological significance of autophagy in the HCV life cycle and propose a potential role of autophagy in the development of HCV-related liver diseases as well as a perspective on therapeutics targeting autophagy to cure HCV infection.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a global public health burden. Approximately 170 million people are infected with HCV worldwide, and most of these patients become persistently infected. Furthermore, HCV infection in some patients may progress into chronic liver diseases, such as steatosis, cirrhosis, and hepatocellular carcinoma[1,2]. The successful rate of curing HCV infection using the current therapy that combines interferon (IFN) and ribavirin is still limited due to its low efficacy, drug resistance, and severe side effects in a particular population of infected individuals[3]. Additionally, preventive vaccines against HCV are not yet available[4,5]. Hence, a new antiviral drug with high potency and/or a protective vaccination against HCV infection are urgently needed. Autophagy is an evolutionarily conserved, catabolic pathway by which eukaryotic cells degrade unnecessary cytoplasmic compartments to recycle nutrients and maintain cellular homeostasis[6,7]. Recent studies collectively indicate that HCV activates autophagy to promote viral growth through regulating different steps of the viral life cycle by affecting different host cellular signaling processes[8-11]. Because autophagy has widely been shown to contribute to the progression of human diseases[12,13], HCV-activated autophagy could be physiologically significant in the pathogenesis of HCV-associated liver diseases. Most importantly, interference with the autophagic process can suppress HCV replication[8-11], suggesting that inhibition of autophagy can serve as a novel therapeutic strategy against HCV infection. Therefore, in this review, we outline the current findings on the functional roles of autophagy at each stage of the HCV life cycle and the molecular mechanism by which HCV activates the autophagic response. Lastly, we also discuss the possible impacts of the autophagic response on the development of HCV-related liver disorders as well as provide a perspective on the implications of modulating autophagy to control HCV infection.

HEPATITIS C VIRUS INFECTION

An unknown infectious agent that caused non-A, non-B post-transfusion hepatitis was first discovered in the mid-1970s[14]. In 1989, the nucleic acid sequence of this unidentified virus was cloned, reported, and formally named HCV[1]. It is estimated that over 3% of the human population is infected by HCV. Most of the infected individuals become chronically infected, and HCV infection often progresses to severe, liver-associated diseases, such as cirrhosis, steatosis, and hepatocellular carcinoma[2]. To date, due to the low efficacy of the combined therapy of pegylated IFN-α and ribavirin[3], options for complete eradication of HCV infection and a preventive strategy are still absent[4,5]; therefore, HCV infection is a global public health problem.

HCV is a membrane-enveloped, positive-sense, single-stranded RNA virus belonging to the *Hepacivirus* genus and the *Flaviviridae* family[2]. Until now, the known isolates of HCV were classified into seven genotypes, *i.e.*, genotypes 1 through 7, with 20%-30% sequence divergence, and an array of subtypes could be grouped within each genotype[15]. The genetic heterogeneity of these HCV genotypes could result in the variable degree of risk for progressive liver diseases and the different treatment outcomes of IFN-based therapy[16]. For instance, a higher prevalence of progression into hepatosteatosis and cirrhosis occurs in cases infected with HCV genotype 3[17,18]. Regarding the efficacy of standard IFN/ribavirin treatment, the successful rates of patients infected with genotypes 2 and 3 are higher than those of patients infected with genotypes 1 and 4[19-22].

Hepatocytes in the liver are the predominant targets of HCV infection, and the entry of HCV into hepatocytes is a stringently coordinated process that relies on successive and concerted interactions between the envelope glycoproteins E1 and E2 and host cellular factors that are present on the cell surface, *i.e.*, the so called "entry (co)receptors" (Figure 1). The known (co)receptors, including the tetraspanin CD81[23-25], scavenger receptor class B member I (SCRAB-I)[26,27], Claudin 1 (CLDN1)[28], and Occludin (OCLN)[29], have been shown to mediate HCV entry into hepatocytes (Figure 1). In addition, the low-density lipoprotein receptor (LDLR)[30], highly sulfated heparin[31], and the dendritic cell-specific intercellular adhesion molecule three grabbing non antigen[32] were reported to be involved in attachment to and concentration of lipoprotein-associated viral particles on the cell surface of infected cells (Figure 1). After attachment to the cell surface, the virions bind to CD81 and SCRAB-I on the plasma membrane through the interaction between the E2 protein and these two entry (co)factors[33-35]. Subsequently, the association of CD81 or SCARB-I with CLDN1 on the basolateral surfaces of hepatocytes facilitates the formation of entry complexes[36-38] (Figure 1), thus promoting the internalization process of viral particles *via* the clathrin-mediated and pH-dependent endocytosis pathway[39,40]. Following internalization into cells, the envelopes of the virions fuse with the endosomal membrane, allowing uncoating and release of the viral genomes into the cytoplasm, where the translation of viral proteins and replication of viral RNA occur[39,40] (Figure 1). The exact physiological role of OCLN in the entry of the HCV virion is still unclear, although the second extracellular loop of this protein, along with CD81, has been shown to determine the host tropism of HCV infection[29]. In addition to CLDN1 and OCLN, other tight junction proteins such as CLDN6 and CLDN9 have been reported to participate in the entry of HCV into peripheral blood mononuclear cells, which lack CLDN1 expression[41,42]. This represents an alternative route for HCV infection in extrahepatic compartments[42].

In addition to these entry (co)receptors, epidermal growth factor receptor (EGFR) and ephrin receptor A2 (EphA2) have recently been identified as additional

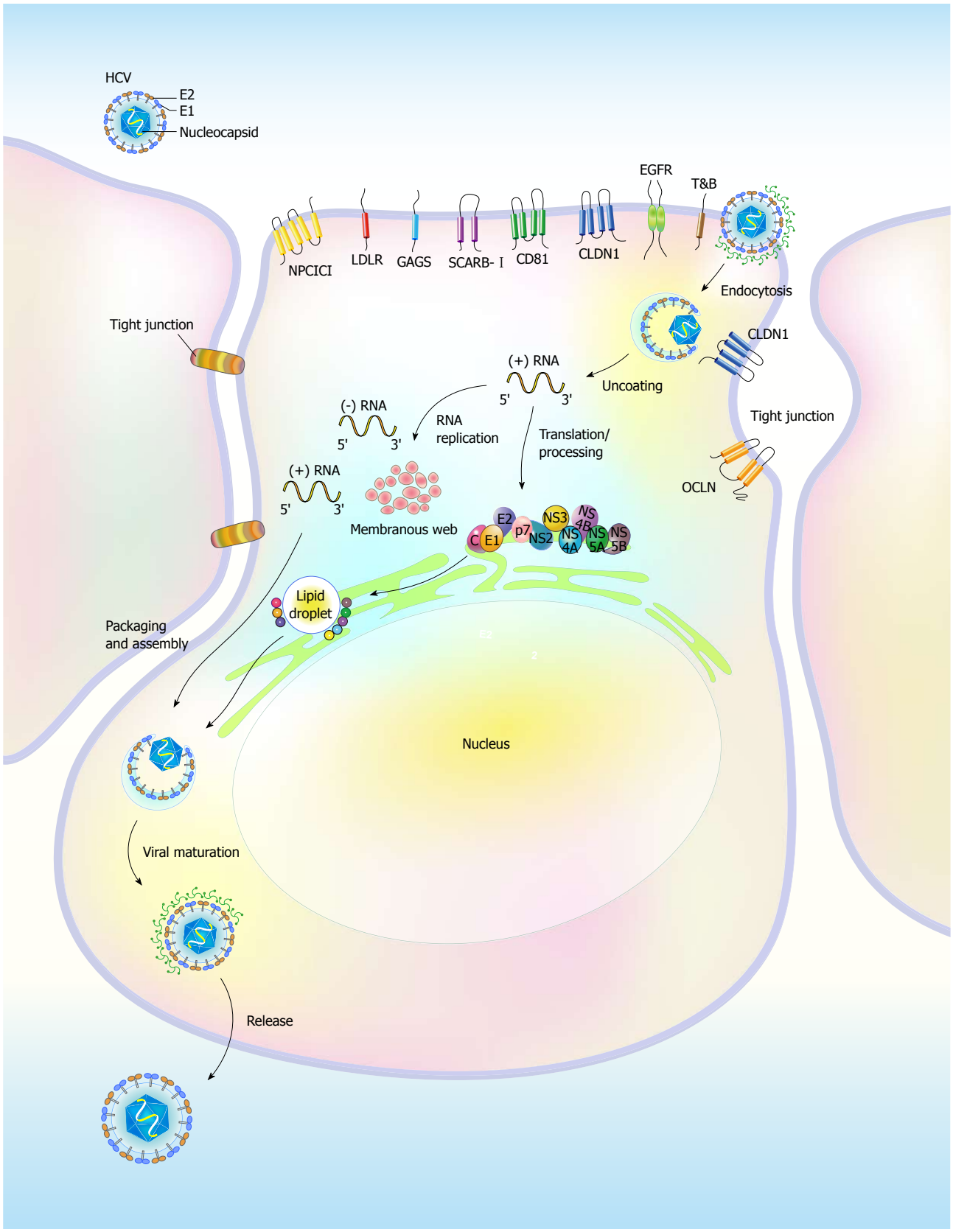

Figure 1 Life cycle of hepatitis C virus. Hepatitis C virus (HCV) replication involves binding of the virion to the entry molecules on the cell surface of host hepatocytes; endocytosis; viral genome uncoating and translation; polyprotein processing; RNA replication on the ER-associated membrane structure, called the "membranous web"; and virion packaging, assembly, maturation, and release. Glycosaminoglycans (GAG), low-density lipoprotein receptor (LDLR), scavenger receptor class B member Ⅰ (SCARB-Ⅰ), two tight junction molecules Claudin 1 (CLDN1) and Occludin (OCLN), epidermal growth factor receptor (EGFR), ephrin receptor A2 (EphA2), Niemann-Pick C1-like L1 (NPC1L1), and transferrin receptor (TfR) constitute the entry receptors. In addition to participating in viral RNA replication, the NS proteins are recruited onto the surface of lipid droplets (LDs) to promote viral RNA replication and the assembly of infectious viral particles, which are composed of the core, E1, and E2 proteins.

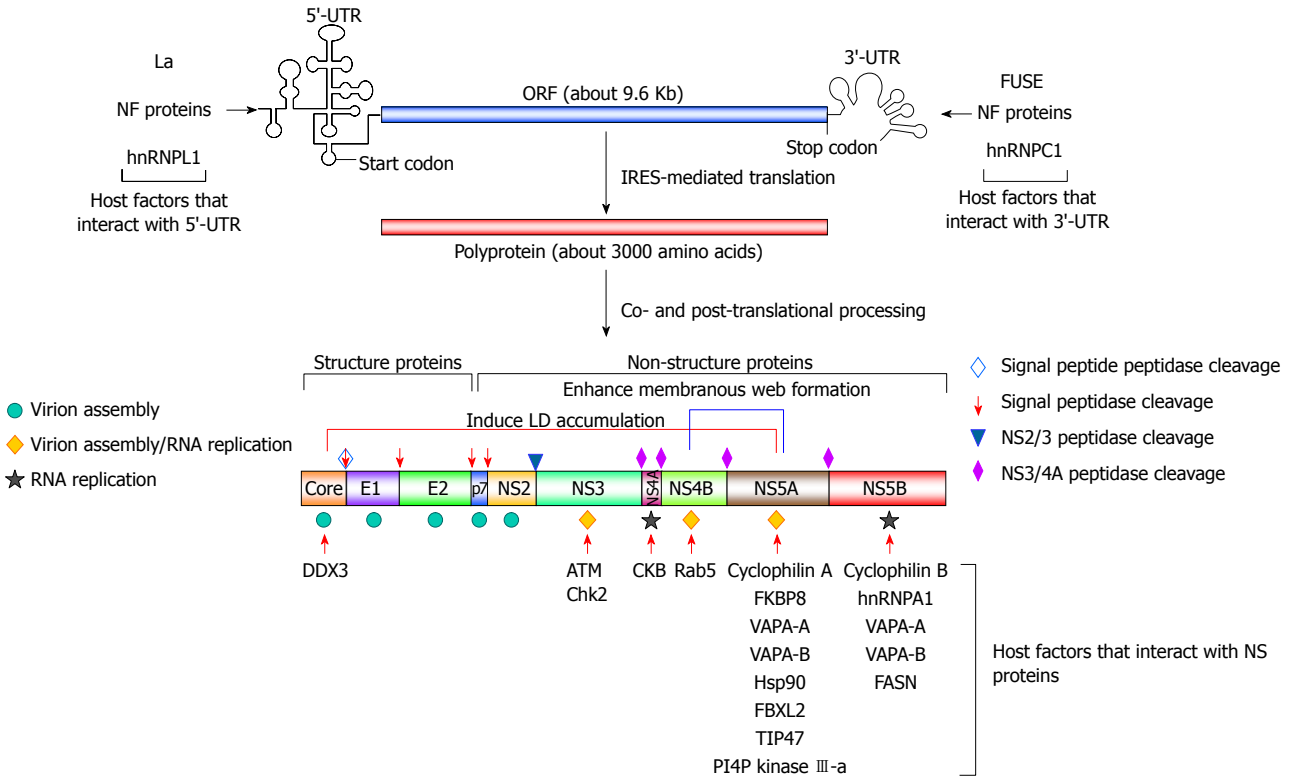

Figure 2 Genome organization of hepatitis C virus and cellular proteins that modulate viral RNA replication. The positive-sense, single-stranded hepatitis C virus (HCV) genome, containing an open reading frame (ORF) of 9.6 Kb that is flanked by the 5'- and 3' untranslated regions (UTR), is translated using an internal ribosome entry site (IRES) to a polyprotein, which is processed by cellular and viral proteases to mature into three structural proteins (core, envelope glycoproteins E1 and E2) and seven nonstructural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B). The role of each viral protein in virus assembly, RNA replication, or both is indicated by the symbol shown on the left. The cellular proteins that modulate HCV replication and their interacting viral counterparts are shown. Host factors that interact with the 5'-UTR and 3'-UTR, as indicated, regulate IRES-mediated translation and viral RNA replication, respectively. Membrane-associated proteins, as shown, modulate HCV replication through interactions with NS5A and NS5B. Other cellular factors, such as DDX3, ATM and Chk2, CKB, and Rab5, promote the replication of HCV viral RNA *via* interacting with core, NS3, NS4A, and NS4B, respectively.

(co)factors for HCV entry by facilitating the CD81-CLDN1 interaction[43] (Figure 1). Additionally, the receptor tyrosine kinase activities of these two molecules were shown to enhance the membrane fusogenic activity of HCV envelope glycoproteins[43]. In addition, the entry of HCV virions into host cells can be mediated by an association with cholesterol *via* the Niemann-Pick C1-like L1 (NPC1L1) cholesterol uptake receptor[44]. Likewise, transferrin receptor (TfR), which is an iron absorption receptor, was recently demonstrated to be involved in the internalization of HCV virions into hepatocytes[45] (Figure 1). These studies collectively indicate that infection of target cells by HCV is a highly regulated process.

INTERPLAY OF HCV AND HOST CELLULAR PROTEINS

The viral genome of HCV is a positive-stranded RNA of approximately 9.6 Kb in length[46] that contains two untranslated regions (UTRs) located on the 5' and 3' termini, which flank a major open reading frame (ORF) region[46] (Figure 2). The ORF of the HCV genome can be translated into a polypeptide of approximately 3300 amino acids using an internal ribosome entry site (IRES) that is located within the 5'-UTR. The large polyprotein is then co-translationally processed into structural (core and the envelope glycoproteins E1 and E2) and nonstructural (NS) proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) *via* a combination of viral and cellular proteases[46] (Figure 2). The structural proteins constitute the viral particle[2,46], whereas the NS proteins, in conjunction with cellular proteins, participate in viral RNA replication by organizing the replication complex within a multi-vesiculated, membranous web[47,48] (Figure 2). The NS proteins are also concertedly localized on the surface of lipid droplets (LDs), which is a process that is required for virion assembly[49,50] (Figure 2).

In addition to the involvement of viral proteins that function as cis-acting elements for HCV replication, many host cellular proteins have been known to regulate viral RNA replication through their interaction with viral proteins and RNAs. Playing a pivotal role in the IRES-mediated translation of a precursor polypeptide that is subsequently processed into individual viral proteins, the eukaryotic translational initiation factors and RNA-binding proteins, such as the La autoantigen[51,52], nuclear factors (NF) NF45, NF90, and NF110[53], the far upstream element-binding proteins[54], and heterogeneous nuclear ribonucleoproteins[55-57], have been implicated in viral

RNA translation and replication *via* interactions with the 5'- and 3'-UTRs (Figure 2), thus promoting HCV replication. In addition to these host factors that modulate viral translation, other host factors, such as cyclophilin B and hnRNP A1[57,58], may directly modulate viral RNA replication by forming a protein complex with the RNA-dependent RNA polymerase (RdRp), NS5B, and regulating replicase activity (Figure 2). The other cyclophilin family proteins, including cyclophilin A and FK506-binding protein 8 (FKBP8), have been reported to interact with NS5A, and this association recruits heat shock protein 90 to form a protein complex that promotes the efficiency of viral RNA replication[58-62] (Figure 2). On the other hand, a variety of vesicle-associated membrane proteins (VAPA), such as VAPA-A and VAPA-B, have been reported to positively regulate HCV replication by interacting with NS5A and NS5B[63,64] (Figure 2). Additionally, the geranylgeranylated protein F-box/LRR-repeat protein 2 is a host protein that interacts with NS5A to promote HCV replication[65,66] (Figure 2). In addition, other host factors, such as DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked[67], ataxia telangiectasia mutated (ATM), checkpoint Chk2 kinases[68], creatine kinase B (CKB)[69], and the small Ras GTPase-binding protein 5 (Rab5)[70], may regulate HCV replication *via* binding to core, NS3, NS4A, and NS4B, respectively (Figure 2).

In addition to cellular proteins that interact with viral proteins, several host cellular proteins exert their trans-acting function in the HCV life cycle by altering lipid metabolism. The findings that support this notion originate from observations suggesting that the expression of sterol regulatory element-binding proteins can be enhanced by HCV infection and the ectopic expression of individual viral proteins, *e.g.*, core, NS2, and NS4B[71-73] (Figure 3). The SREBP-mediated transactivation of lipogenic genes enhances cholesterol biogenesis and biosynthesis of fatty acids, which, in turn, promote the storage of neutral lipids within LDs[74]. Therefore, these results indicate that HCV infection may activate the gene expression of lipogenes, thus modulating the metabolic pathways of lipids to support the HCV life cycle. In line with this, the gene expression of fatty acid synthase, which is involved in the synthesis and transport of fatty acids, was reported to be upregulated and required for HCV viral RNA replication[75]. Recently, FASN was demonstrated to interact with NS5B to enhance RdRp replicase activity, thereby promoting HCV replication[76] (Figure 2).

Apart from these proteins that directly function in the modulation of lipid biosynthesis, a new subset of host cellular factors has emerged based on their roles in HCV replication *via* altering the expression and subcellular distribution of phosphatidylinositol-4-phosphate (PI4P). HCV infection was reported to increase the intracellular level of PI4P *via* PI4P kinase (PI4PK) Ⅲα and PI4PKⅢβ[77-83]. Interference with the gene expression of these two PI4P kinases dramatically inhibits HCV replication[77-83]. Furthermore, recent studies indicated that the NS5A protein can recruit (PI4PK) Ⅲα to the membranous web, which is a multi-vesiculated structure that supports efficient replication of HCV viral RNA, thus upregulating the level of PI4P to maintain the membranous web architecture[77,78,81,84,85]. Reciprocally, (PI4PK) Ⅲα can modulate the phosphorylation status of NS5A, thus regulating the morphogenesis of viral replication compartments[86]. On the other hand, annexin A2 and proline-serine-threonine phosphatase-interacting protein 2 (PSTPIP2), two host membrane-associated proteins, were also shown to regulate HCV replication *via* facilitating the formation of the membranous web[87,88]. In addition, the tail interacting protein of 47 kDa (TIP47), which is an LD-associated protein, has recently been shown to positively modulate HCV RNA replication by interacting with NS5A[89,90] (Figure 2). Collectively, these studies indicate that host cellular factors may regulate HCV replication *via* directly facilitating the reconstitution of the membranous web or modulating LDs by interacting with viral NS proteins.

HCV-ASSOCIATED LIVER DISEASES

The disease progression in HCV-infected patients reveals a nonlinear and long-term period mode. At the beginning of infection, most infected patients are non-symptomatic, although hepatitis, jaundice, and fulminant hepatic failure can be detected in some cases of acute infection[16,91] (Figure 3). However, more than 50% of HCV infection cases develop viral persistence, which often leads to liver steatosis, fibrosis, cirrhosis, and ultimately to hepatocellular carcinoma (HCC)[16,91-95] (Figure 3). In the majority of infected individuals, the disease progression to end-stage HCV-related diseases, such as liver cirrhosis and HCC, often occurs 20-30 years after acute infection, and approximately 20%-30% of the infected patients will progress to end-stage liver diseases[16,91-95] (Figure 3). In addition to the end-stage liver diseases, chronic HCV infection in some patients is also highly associated with extrahepatic diseases such as mixed cryoglobulinemia vasculitis, which is an inflammatory symptom of small blood vessels, due to the precipitation of cryoglobulin-containing immune complexes in blood vessels of the skin and other tissues[96,97]. Additionally, chronic HCV infection is associated with metabolic syndromes such as diabetes and insulin resistance[98,99]. The development of these diseases often requires long periods of time, thus affecting the health quality of patients and imposing a heavy burden on medical care for the treatment of HCV-related diseases. Therefore, it is urgent to design a new, efficacious therapeutic strategy and/or to develop prophylactic vaccines.

IMPLICATIONS OF HCV IN LIVER DISEASES

Many HCV proteins are known to participate in the pathogenesis of HCV-related diseases. For instance, the HCV core protein was reported to coat the surface

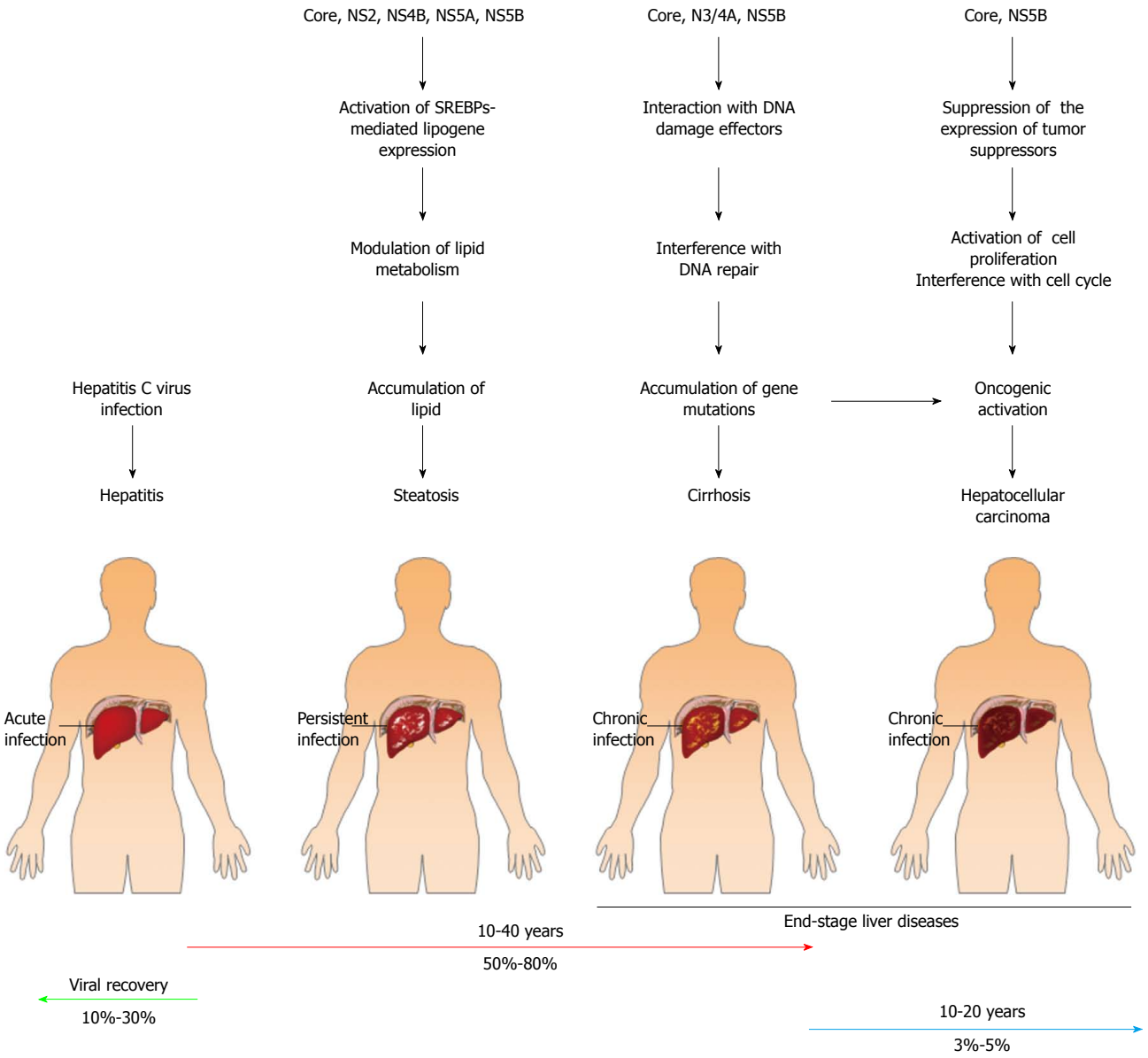

Figure 3 Contribution of viral proteins to the pathogenic changes leading to hepatitis C virus-related liver diseases. After acute hepatitis C virus (HCV) infection, approximately 10%-30% of cases will spontaneously recover, whereas the majority of HCV infection cases (approximately 50%-80% of infected cases) become persistently infected. Chronically HCV-infected hepatocytes in the livers of these patients progress into hepatosteatosis and liver cirrhosis. Ultimately, some (approximately 3%-5%) of the chronically infected individuals develop end-stage liver disease, *i.e.*, hepatocellular carcinoma. The potential contributions of the viral proteins to the development of HCV-associated liver diseases are shown. The HCV core protein and the NS2, NS4, NS5A, and NS5B proteins can promote hepatic steatosis by mediating the clustering of LDs and regulating the SREBP-mediated transactivation of lipogenes, respectively. The binding of core and NS5B to tumor suppressors downregulates the expression of these tumor suppressors, thus activating cell proliferation and interfering with cell cycle progression. Moreover, HCV core, NS3/4A, and NS5B can bind to DNA damage response-associated effectors, thereby inhibiting the DNA repair mechanism and inducing genome instability. Deregulated cell growth, cell cycle progression, and genome instability may, in turn, trigger oncogenic activation in infected cells, which finally leads to the development of hepatocellular carcinoma.

of LDs and induce the emergence of LDs from the endoplasmic reticulum (ER) and the clustering of LDs in a cultured cell model[100-102]. On the other hand, accumulating lines of evidence have shown that the HCV core protein can induce hepatic steatosis in an *in vivo* transgenic model[103-106]. Most importantly, the genetic variation within the core sequences of different HCV genotypes has been reported to critically determine the status of hepatic steatosis. The prevalence and severity of hepatic steatosis are higher in patients infected with HCV genotype 3 than in those infected with other genotypes[107-109]. This greater extent of steatosis in genotype 3-infected individuals may be due to the substitution of phenylalanine for tyrosine at amino acid residue 164 of the core protein in the genome of HCV genotype 3[110]. Despite this amino acid variation, several specific polymorphisms in the core protein of different HCV genotypes have been shown to increase the intracellular lipid levels and, thus, contribute to hepatic steatosis[111,112]. Cumulatively, these studies suggest that HCV infection may lead to hepatic steatosis through core-induced LD accumulation. Apart from the impacts of altering intracellu-

lar lipids, HCV core was shown to suppress the expression of the tumor suppressors p53 and cyclin-dependent kinase (CDK) inhibitor p21, thereby enhancing CDK2 activity and increasing the phosphorylation status of retinoblastoma, RB, in cells[113] (Figure 3). In turn, phosphorylated RB stimulates the DNA binding ability of E2F transcriptional factor 1 and activates the expression of downstream genes, such as S phase kinase-interacting protein 2, which is an initiating signal for cell proliferation[113] (Figure 3). Moreover, HCV NS5B has also been demonstrated to interact with RB and target it for proteolysis, thus activating downstream E2F-responsive promoters and cell proliferation[114] (Figure 3). Due to their suppression of the expression of tumor suppressors and promotion of cell proliferation, the HCV core and NS proteins may contribute to the progression of uncontrolled hepatocyte growth, thereby increasing the occurrence of hepatocellular carcinoma.

The interaction between HCV NS3/4A with ATM kinase has been shown to lead to the cytoplasmic retention and dephosphorylation of ATM, thus interfering with the activation of the DNA repair mechanism and desensitizing Huh7 cells to ionization[115] (Figure 3). In addition, the ATM and Chk2 kinases bind to the HCV NS5B protein to promote HCV viral RNA replication[68] (Figure 3). On the other hand, recent studies indicate that HCV infection may interfere with multiple signaling pathways of DNA repair *via* interactions between HCV core and the Nijmegen breakage syndrome protein 1 (NBS1), which is a downstream effector of the ATM-associated DNA damage response[116] (Figure 3). Taken together, these findings imply that HCV infection may interfere with the host DNA damage/repair response, thus benefiting viral growth. Interference with the integrity of the DNA repair mechanism may introduce error-prone effects on DNA replication, which leads to the accumulation of gene mutations, gene instability, and oncogenic activation in infected cells and, eventually, promotion of the progression of infected cells into hepatocellular carcinoma.

TOOLS USED IN HCV RESEARCH

Innovation of new therapeutic strategies against HCV infection relies on a comprehensive understanding of the entire viral life cycle and HCV-host interactions. Although HCV was identified more than two decades ago, our knowledge of how HCV infection leads to a homeostatic balance with host cells is still limited due to the lack of an *in vitro* cell culture model that can support the complete HCV cycle. The replication assay utilizing a subgenomic replicon that harbors only the HCV nonstructural genome in human hepatoma Huh7 cells was established in the late 1990s. This model allows one to study HCV RNA replication and the biological functions of each of the viral NS proteins[117-120]. The advent of a replicon system also facilitates the identification of adaptive mutations in the HCV viral genome and promotes the discovery of potent anti-HCV agents[118,121]. On the other hand, the establishment of an HCV pseudoparticle system (HCVpp), in which the HCV E1 and E2 glycoproteins are incorporated onto retro- or lentiviral particles, provides an efficient system to study HCV entry, identify entry (co)receptors, and screen for neutralizing antibodies[122-125]. In 2005, the robust production of infectious HCV in a cell culture system (HCVcc) based on the entire genome of the JFH1 strain, which is an HCV genotype 2a virus that was isolated from a fulminant hepatitis patient in Japan, was developed[126-128], thus marking a great achievement in the HCV research field. The generation of infectious HCVcc allows one to investigate each step of the viral life cycle and HCV-host cell interactions *in vitro* and will be useful for the screening and testing of new antiviral drugs. Nevertheless, the availability of an *in vivo* model for HCV research was limited to chimpanzee, which has been used as a model for studying viral replication kinetics, the immune response, and vaccine development[129-135]. The immune-deficient mouse system transplanted with human hepatocytes serves as an additional research tool to analyze the HCV life cycle in a humanized, small animal model[135,136]; however, the study using these two animal models is circumscribed by their high cost and the inconvenience of the experimental manipulations. Recently, a model that allows investigation of the complete HCV life cycle in an immune-competent mouse system was successfully developed by genetically engineering human CD81, SCARB- I , CLDN1 and/or OCLN into mice[137,138]. This system provides a new research platform for studying HCV infection *in vivo* and screening anti-HCV drug and vaccine candidates. Nevertheless, the low level of viral replication and virion production in this HCV-rodent model hampers the use of this *in vivo* model. Thus, further efforts are needed to develop an improved version of this rodent system.

AUTOPHAGY

Autophagy is considered a "self-eating" process in eukaryotic cells that engulfs unwanted cytoplasmic components within double-membranous vacuoles and delivers these cargos to lysosomes for breakdown. The autophagic process promotes the turnover of damaged organelles and aggregated proteins through lysosomal degradation to ensure the recycling of cellular constituents, thus maintaining cellular homeostasis[6,7]. The concept of "self-eating" was originally described in the mid-1950s in Christine de Duve's work on biochemical characterization of the lysosome in liver tissue[139,140]. Soon after this study, she and other researchers independently utilized transmission electron microscopy to show that dense bodies similar in size to mitochondria are present in the cytosol of renal and hepatic cells[141-146]. These observed dense bodies formed unique single- and double-membranous vesicle structures that were associated with lysosomes and contained mitochondria and endoplasmic reticulum (ER)[141,144,145]. Based on these observations, de Duve proposed a new term, "autophagy", to illustrate this de novo

process of sequestrating cytoplasmic organelles within a double membrane-enclosed vesicle termed an "autophagosome". Per Seglen's group then investigated the process prior to autophagosome formation in autophagy and identified the expansion of the "phagophore", which is an initial, membrane-rearranged structure, into the autophagosome[147-149]. Additionally, Mortimore and Schworer showed that amino acid deprivation activates autophagy and proteolysis in rat liver, and they were the first to suggest that energy imbalance and/or an insufficient nutrient supply can stimulate the initiation of the autophagic process[150-153]. In the early 1990s, the detailed molecular basis of autophagy began to be uncovered through Yoshinori Ohsumi's study using the yeast *Saccharomyces cerevisiae*[154,155]. Using the advantage of well-established genetic manipulation techniques and the well-known genomic background in the yeast model system, Ohsumi's and Klionsky's groups began to identify the genes involved in the autophagic process (ATGs)[156-168]. Most homologues of the yeast ATG genes also exist in humans and other eukaryotes, and the human orthologs of the yeast ATG genes can carry out similar functions[161,169]. Finally, a unified nomenclature for all ATGs in the different model systems was denoted[170-172]. These significant breakthroughs enhanced the understanding of the mechanism underlying how autophagy initiates and terminates and provided a crucial foundation for further investigation of autophagy-related processes.

MOLECULAR EVENTS LEADING TO AUTOPHAGY

Autophagy is the concerted action of the core molecular machineries that are involved in the initiation stage of the isolation membrane (IM)/phagophore, the middle step of elongation and enclosure of the autophagosome, and the late stage of autophagosome fusion with a lysosome and the subsequent degradation of the sequestrated components within the autolysosome by acidic proteases[6,7] (Figure 4). The completion of autophagy requires a dynamic membrane rearrangement process and multiple signaling pathways[171-174] (Figure 4). Upon nutrient deprivation, autophagy begins with the suppression of mammalian target of rapamycin (mTOR), which is a serine/threonine protein kinase that is required for cellular metabolism. Inhibition of mTOR leads to translocation of the downstream unc-51 like-kinase (ULK) complex, *i.e.*, ULK1-ATG13-FIP200-ATG101, from the cytoplasm to the unique ER membrane-associated compartments[175,176] (Figure 4). The class Ⅲ phosphatidylinositol-3-OH kinase (class Ⅲ-PI3K) complex, *i.e.*, PI3K-Vps15-Beclin-ATG14, is then recruited to the ER-derived nucleation site, where the P3K complex catalyzes the formation of phosphatidylinositol-3-phosphate (PI3P)[177] (Figure 4). The newly synthesized PI3P, in turn, recruits downstream effectors, including the double-FYVE-containing protein 1 (DFCP1) and the WD-repeat domain PI3P-interacting (WIPI) family proteins, resulting in the formation of an ER-associated Ω-like structure called the "omegasome"[178]. After the nucleation step, elongation and enclosure of the IM/phagophore to form an autophagosome require two ubiquitin-like conjugation complexes, *i.e.*, ATG12-ATG5-ATG16L and ATG4-ATG3-LC3Ⅱ (Figure 4). The ATG12-ATG5 conjugate is first produced through the concerted action of the ATG7 (E1-like) and ATG10 (E2-like) enzymes, and it then binds to ATG16L, which forms the ATG12-ATG5-ATG16L trimeric complex. The conjugation of microtubule-associated protein 1 light chain 3 (LC3), *i.e.*, the ATG8 homologue in mammals, to phosphatidylethanolamine (PE) begins with the proteolytic cleavage of its C-terminus by ATG4. Subsequently, the cleaved form of LC3 is conjugated to PE through the catalytic cascade of ATG7 and ATG3, thus generating the lipidated form of LC3, *i.e.*, LC3-Ⅱ[179]. Finally, the autophagosome fuses with the endosome and lysosome to form a mature autophagolysosome, named an "autolysosome", in which the engulfed materials are broken down and recycled for further use by cells[7]. Despite the emergence of an IM/phagophore from the ER[180,181], a variety of organelles, such as the plasma membrane (PM)[182], mitochondria[183], and Golgi apparatus[184], can also serve as membrane sources for the initiation of autophagy in mammalian and yeast cells (Figure 4). Nonetheless, what and how signaling pathways regulate each step of the membrane rearrangement processes must be further investigated.

AUTOPHAGY AND DISEASES

Apart from nutrient starvation, multiple stresses, including ER stress, accumulation of aggregated proteins and damaged organelles, and pathogen infections, can activate the autophagic response[7,185,186]. Upon infection by bacteria and viruses, the autophagic process is triggered in cells to directly engulf incoming pathogens and deliver them to the lysosome for degradation, *i.e.*, xenophagy[187-189]. In addition, autophagy may induce the innate immune defense to repress microbial infection, such as enhancing Toll-like receptor-mediated innate immune signaling and promoting the presentation of antigens derived from viruses such as vesicular stomatitis virus and Epstein-Barr virus onto a major histocompatibility complex class Ⅱ molecule[6,190-193]. Thus, autophagy can function as a restrictive route to eliminate pathogens. In addition, autophagy was shown to be exploited by many RNA viruses, such as mouse hepatitis virus, poliovirus, and rhinovirus, to promote their life cycle by serving as a membranous compartment for RNA replication[194]. On the other hand, autophagy also plays critical physiological roles in the pathogenesis of various diseases, including neurodegenerative disorders[12,13], inflammatory diseases[195,196], liver-associated diseases[197], and cancers[198]. Because of its impacts on a wide array of physiological and pathological conditions, autophagy has become an attractive field of biomedical research. Nevertheless, further studies are needed to better understand the regulation of autophagy and its exact physiological significance

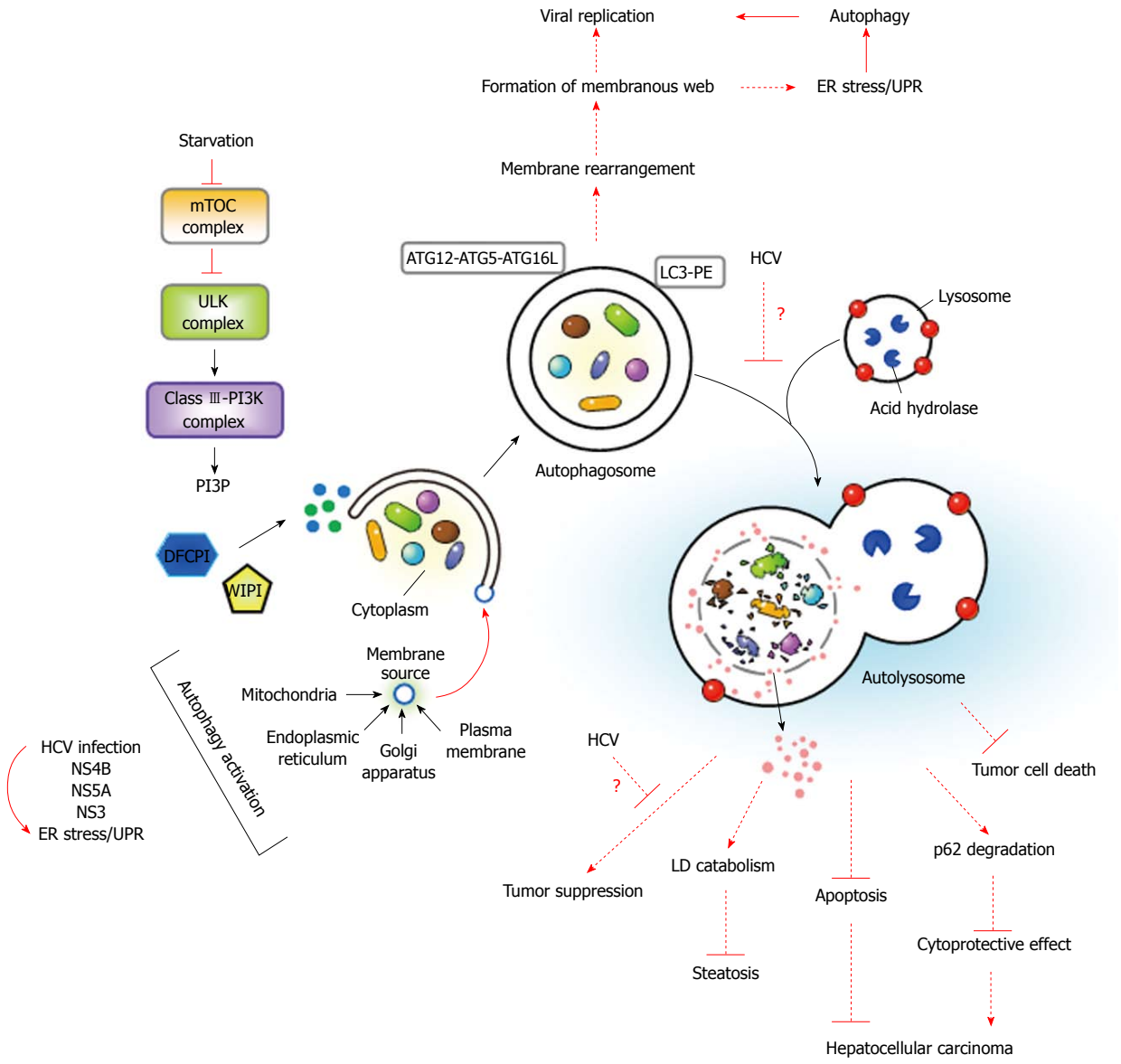

Figure 4 Sequential and coordinated events of autophagy and their impact on viral replication and hepatitis C virus-associated liver diseases. When cells undergo nutrient starvation, the activity of the mammalian target of rapamycin (mTOR) complex is inhibited, resulting in dephosphorylation, activation, and translocation of the unc-51 like-kinase (ULK) complex to the ER, where the ULK complex activates the class Ⅲ phosphatidylinositol-3-OH kinase (class Ⅲ-PI3K) complex to generate PtdIn(3)P (PI3P). PI3P, in turn, recruits double-FYVE-containing protein 1 (DFCP1) and WD-repeat domain PI3P-interacting (WIPI) protein into the isolation membrane, which may originate from the ER, mitochondria, plasma membrane, or Golgi apparatus. Two ubiquitin-like conjugation systems, the ATG12-ATG5-ATG16L and LC3-PE conjugation cascades, coordinate the elongation and enclosure of the autophagosome. Finally, the autophagosome fuses with a lysosome to degrade the sequestrated cytoplasmic components. Hepatitis C virus (HCV) infection, which activates ER stress/the unfolded protein response (UPR), as marked by a curved arrow, and ectopic expression of NS4B, NS5A, and NS3 are known to activate autophagy. The dashed lines indicate the potential implications of autophagy in HCV replication, HCV-related steatosis, and hepatocellular carcinoma. Activated autophagy may contribute to the rearrangement of the membrane as a resource of the membranous web for viral RNA replication. It is surmised that the HCV-induced blockade in autolysosome formation, as shown by a "?" during fusion of the autophagosome with a lysosome, may disrupt the effect of autophagy on LD and p62 degradation, thus contributing to the development of steatosis and hepatocellular carcinoma. On the other hand, interference with the effects of autophagy on tumor suppression by HCV, as indicted by a "?", or the inhibitory effects of autophagy on the apoptotic signaling and killing of tumor cells in HCV-infected patients may also enhance the progression to hepatocellular carcinoma.

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HCV AND AUTOPHAGY

Infections of flaviviruses such as HCV, Dengue virus, and Japanese encephalitis virus were shown to activate autophagy *in vitro*[8-11,199-202]. Many studies have reported that HCV induces the autophagic response in HCV viral RNA-expressing and HCVcc-infected cells[8-11]. By analyzing the viral expression of HCV genotype 1a H77 strain in immortalized human hepatocytes (IHH), Ait-Goughoulte's group demonstrated that autophagic vesicles and GFP-LC3-labeled punctate structures accumulate in viral RNA-transfected cells[8] (Table 1). These authors also found that the H77 HCV-induced autophagic response is accompanied by increases in Beclin expression

Table 1 Roles of autophagy in hepatitis C virus-host cell interactions

HCV genotype	Expression	Model	Analysis of autophagy activation	Physiological significance	Ref.
HCV-H77 (1a)	Transfection of viral RNA	IHH cells	1 Detection of GFP-LC3 punctate structure formation 2 Accumulated autophagosome in TEM analysis 3 Upregulation of Beclin expression and ATG5-ATG12 conjugate	Promotion of viral RNA replication	Ait-Goughoulte *et al*[8]
HCV-JFH1 (2a)	Transfection of viral RNA	Huh7.5 cells	1 Upregulated expression of LC3-II 2 No overlapping signal of GFP-LC3 punctate with lysosome 3 Autophagic activation by UPR 4 An incomplete autophagic process lacking enhanced autophagic degradation of long-lived proteins and p62	Promotion of viral RNA replication	Sir *et al*[11]
HCV-JFH1 (2a)	HCVcc infection	Huh7 cells	1 Upregulation of LC3-II 2 Accumulation of GFP-LC3 dot-like vesicles 3 No colocalization of autophagic vacuoles with viral proteins	Enhanced translation of the incoming viral RNA	Dreux *et al*[9]
HCV-JFH1 (2a)	HCVcc infection	Huh7.5-1 cells	1 Increase of GFP-LC3 dot-like structures 2 No colocalization of autophagic vacuoles with viral proteins	Promotion of virion assembly	Tanida *et al*[207]
HCV-JFH1 (2a)	HCVcc infection	Huh7 cells	1 Transient interaction of ATG5 with NS5B and NS4B 2 Association of ATG5 with membranous web	Promotion of viral RNA replication by organizing membranous web	Guévin *et al*[205]
HCV-JFH1 (2a) infection	HCVcc infection	Huh7 cells	1 Detection of early- and late-stage autophagic vacuoles by TEM analysis 2 Colocalization of autophagic vacuoles with lysosome 3 Accumulated LC3B-II expression by interference with autolysosome maturation 4 Complete autophagic process by HCV infection	Promotion of viral RNA replication by suppressing antiviral immunity	Ke *et al*[10]
HCV-H77 (1a); HCV-JFH1 (2a)	HCVcc infection	IHH	1 Activated IFN response in the HCV-infected cells by silencing of Beclin and ATG7 2 Increased caspase-dependent apoptosis by knockdown of Beclin and ATG7 in the HCV-infection cells	Promotion of viral RNA replication by suppressing antiviral immunity	Shrivastava *et al*[203]
HCV-Con1 (1b) and JFH1 (2a)	Replicon viral RNA transfection	Huh7 cells; Huh7.5-1 cells; Liver biopsy	1 An inverse correlation between hepatic steatosis and activation of autophagy in liver biopsy samples of infected patients 2 Colocalization of autophagic vacuoles with LDs	Promotion of catabolism of LDs	Vescovo *et al*[208]
HCV-JFH1 (2a)	Replicon viral RNA transfection	Huh7 cells; HCV-transgenic mice	1 Enhanced ROS level in mitochondria in HCV viral RNA-transfected cells 2 Activated autophagy by expression of HCV NS proteins 3 Alteration of antioxidant response by upregulation of antioxidant enzymes in HCV NS protein-expressing cells	Regulation of oxidative response in mitochondria	Chu *et al*[210]
HCV-JFH1 (2a)	HCVcc infection	Huh7 cells; Huh7.5-1 cells	1 Accumulation of mito autolysosome in HCV-infected cells 2 Stimulation of Parkin and Pink 1 expression in HCV-infected cells 3 Activation of mitophagy *via* a Parkin-dependent pathway	Elimination of damaged mitochondria and promotion of viral RNA replication	Kim *et al*[209]
HCV-JFH1 (2a) HCV-N (1b)	HCVcc infection; Replicon viral RNA transfection	Huh7 cells	1 Activation of autophagy through AKT1-TSC-mTORC1 signaling 2 Activation of autophagy *via* UPR	Promotion of viral RNA replication	Huang *et al*[214]
HCV-JC1 (2a) infection; HCV NS4B	HCVcc infection; Ectopic overexpression	Huh7.5 cells	1 Activation of autophagy by HCV NS4B amino acid 1-190 2 Requirement of Rab5 and PI3K for autophagic activation	Organization of virus replication site	Su *et al*[216]
HCV-JFH1 (2a)	HCVcc infection	IHH cells	1 Transcriptional activation of Beclin gene expression 2 Autophagy activation in a Bcl2-Beclin dissociation- and mTOR inhibition-independent manner	Promotion of viral RNA replication	Shrivastava *et al*[215]
HCV-JFH1 (2a)	HCVcc infection	Huh7.5 cells	1 Activation of autophagy through IRGM 2 Interaction of HCV NS3 with IRGM	Promotion of viral RNA replication; modulation of innate immunity (?)	Grégoire *et al*[237]

HCV: Hepatitis C virus; ROS: Reactive oxygen species; NS: Nonstructural; IRGM: Immunity-associated GTPase family M; UPR: Unfolded protein response; ATG: Autophagy-related gene; GFP-LC3: Green fluorescence protein-conjugated microtubule-associated protein 1 light chain 3; LDS: Lipid droplets.

and the ATG12-ATG5 conjugate[8]. Shortly after their study, Sir *et al*[11] reported that HCV triggers autophagosome formation in full length, JFH1 (genotype 2a) viral RNA genome-transfected Huh7 cells (Table 1). They further showed that HCV triggers incomplete autophagy, *i.e.*, autophagolysosome maturation is interrupted. This conclusion was based on the observation that the degradation of long-lived proteins and p62, which is a substrate of autophagic degradation, was inhibited despite increased numbers of LC3-Ⅱ and GFP-LC3-labeled dot-like vesicles in JFH1 RNA-transfected cells[11] (Table 1). Their study also demonstrated, for the first time, that autophagy positively regulates HCV growth *via* promoting viral replication because knockdown of ATG7 and LC3 dramatically suppressed viral RNA expression in HCV RNA-transfected cells[11] (Table 1). Whether autophagy is analogously activated by HCV in the HCV infection system had not been determined until Druex and her colleagues investigated autophagic activation in Huh7.5 cells infected with the JFH1 HCVcc virus in 2009[9] (Table 1). These authors provided first-line evidence demonstrating that HCV infection can induce the autophagic response to enhance the translation of the incoming viral RNA, rather than regulate viral growth, when virus RNA replication is established[9].

Utilizing the JFH1 infection system, we demonstrated that HCV infection of Huh7 cells enhances the autophagic flux and triggers the complete autophagic process throughout the formation of the mature autophagolysosome[9,10] (Table 1). Several lines of evidence supported this conclusion, including (1) the detection of early and late-stage autophagic vacuoles in the TEM analysis of HCV-infected cells; (2) the accumulation of LC3-Ⅱ expression by blocking the fusion of the autophagosome with a lysosome; (3) the predominant expression of RFP, but not GFP, fluorescence of a mRFP-GFP-LC3 reporter in the infected cells; and (4) the high degree of colocalization of GFP-LC3 puncta with lysosomes in infected cells[9,10] (Table 1). Moreover, we showed that silencing of the ATG genes and treatment with pharmacological inhibitors of autophagolysosome maturation repress HCV viral RNA replication[9,10]. However, interfering with autophagy had no detectable effect on virus entry or the translation of viral RNA[9,10]. Most importantly, inhibition of the HCV-activated complete autophagy drastically upregulated the IFN response that was mediated by the HCV pathogen-associated molecular pattern (PAMP), which is located within the poly-U/UC region of HCV 3'-UTR[9,10]. Consistent with our study, Shrivastava *et al*[203] reported that gene silencing of Beclin or ATG7 inhibits HCV growth and activates IFN and interferon-stimulated gene expression in HCV-infected human IHH cells (Table 1). Together, these two studies imply that autophagy may represent a repressive mode to protect HCV-infected cells against an excessive IFN antiviral response, thereby promoting viral RNA replication.

FUNCTIONS OF AUTOPHAGY IN HCV INFECTION

In addition to its suppressive effect on antiviral immunity, autophagy was reported to promote viral RNA replication through other mechanisms[204,205]. Guevin and colleagues showed the transient interaction of ATG5 with NS4B and NS5B as well as the detection of ATG5 in the HCV-induced membranous web, which suggested a proviral role of the autophagic machinery in the formation of the HCV replication complex[205] (Table 1 and Figure 4). Sir *et al*[204] further showed that NS5A, NS5B, and nascent viral RNA were colocalized with the autophagosome and argued that the HCV-induced autophagic membrane can be used as a membrane-associated compartment for the replication of viral RNA (Table 1). In addition to its pivotal role in viral RNA replication, autophagy was shown to regulate the assembly of infectious virions and protection of infected cells from death[206,207] (Table 1). Tanida and colleagues first reported that knockdown of ATG7 and Beclin gene expression moderately downregulates the extracellular titer of HCV virions without showing an apparent effect on the intracellular level of viral proteins and RNAs[207] (Table 1). This study suggests that HCV-activated autophagy may modulate the egress of HCV virions into infected cells. Additionally, HCV was shown to activate autophagy to protect infected cells from cell death[206] (Table 1). In this study, severe cytoplasmic vacuolation and cell death accumulated in Con1-HCV (genotype 1b)-transfected cells through interference with autophagy *via* ectopic expression of a protease-inactive mutant ATG4B^{C47A}[206], which implied that HCV may exploit autophagy as a cellular surveillance machinery to counteract the overloaded stress that is triggered by viral replication.

The HCV-induced autophagic process was also shown to regulate host cellular metabolism, including eliminating excess lipids and degrading damaged mitochondria[208,209] (Table 1 and Figure 4). Vescovo *et al*[208] studied the correlation of autophagy markers with the clinical parameters of lipid metabolism in liver biopsies of patients chronically infected with HCV and found an inverse relationship between autophagy activation and the extent of steatosis in those patients (Table 1). The authors further showed that autophagy participates in the catabolism of LDs in cells transfected with the HCV subgenomic RNA replicon[208], implying that HCV may utilize autophagic degradation to promote LD breakdown and circumvent virus-triggered lipid accumulation in host cells (Figure 4). In addition to its degradation role in lipid metabolism, a unique form of autophagy, termed "mitophagy", was recently shown to eliminate damaged mitochondria in HCV-infected cells in a Parkin-dependent manner[209,210] (Table 1). Knockdown of Parkin and Pink gene expression suppresses HCV viral RNA replication[209], suggesting a critical role of mitophagy in HCV replication.

INDUCTION OF AUTOPHAGY IN HCV INFECTION

The molecular mechanism for how HCV initiates autophagy is not fully understood, although several studies have shown that ER stress and the unfolded protein response (UPR) can stimulate autophagy activation[211-213] (Table 1 and Figure 4). Remarkably, two independent reports showed that the UPR is required for activation of autophagy by HCV[10,11]. Recently, Huang *et al*[214] showed that HCV can inhibit the protein kinase B (PKB)-tuberous sclerosis (TSC)-mTOR complex 1 (mTORC1) signaling pathway *via* virus-induced ER stress, thus activating autophagy. Nevertheless, Shrivastava *et al*[215] demonstrated that HCV induces autophagy by transcriptionally activating the expression of the Beclin mRNA and triggering mTOR signaling. In addition to virus-triggered ER stress and the UPR, viral protein expression seems to be another signal for HCV-activated autophagy (Figure 4). Su *et al*[216] showed that HCV NS4B can trigger incomplete autophagy *via* an interaction with Rab5 and Vps34 (Table 1). Moreover, HCV NS5A was reported to be sufficient to trigger the autophagic response[215] (Figure 4). On the other hand, Gregoire and colleagues demonstrated that several RNA viruses, including HCV, could modulate autophagy *via* the interaction of the immunity-associated GTPase family M (IRGM) with ATG5 and LC3[217] (Table 1). They also showed that HCV NS3 is sufficient to activate IRGM-mediated autophagy[217]. Collectively, these studies reveal that multiple signaling pathways may be involved in the HCV-activated autophagic response. However, further investigations are necessary to determine how HCV RNA or proteins cooperate with those cellular signaling pathways to modulate autophagy.

POTENTIAL ROLE OF AUTOPHAGY IN INHIBITING HCV-RELATED LIVER DISEASES

Although HCV infection is shown to positively induce the autophagic process in the cultured human hepatocyte system[8-11], the evidence for autophagy activation in an *in vivo* animal model and liver specimens from infected patients is still limited. Autophagy activated by HCV infection has been demonstrated to promote HCV growth in host cells *via* regulating RNA replication, the translation of incoming viral RNA, and the assembly of infectious viral particles[9-11,207]. In addition to its proviral role in the HCV life cycle, upregulation of autophagy functions in suppressing innate immunity[10,203,218], altering the apoptosis pathway[215] (Figure 4), and maintaining the surveillance of infected cells[206]. In addition, recent studies provide a new horizon for autophagy and its role in protection of host cells from excess LDs due to HCV infection and the elimination of damaged mitochondria *via* the degradative process[208-210]. These studies also suggest that the autophagic response is utilized to maintain cell homeostasis *via* promoting the breakdown of excess lipids and damaged organelles that are induced by HCV[208-210] (Figure 4). However, how these cell-signaling pathways, in turn, affect cellular metabolism or alter cell homeostasis, which lead to the development of HCV-associated liver diseases, still remains to be investigated (Figure 4).

The potential role of autophagy in the progression of HCV-induced steatosis and fibrosis emerges from the recent findings of Singh *et al*[219]. This group showed that autophagy regulates lipid metabolism in hepatocytes *via* a selective degradation process, *i.e.*, lipophagy[219] (Figure 4). Lipophagy represents a new mode of autophagy in lipid metabolism that catabolizes LDs in the liver[219]. Moreover, Singh *et al*[220] proposed another function of autophagy in the control of body lipids through regulating the differentiation of adipose tissues. Collectively, their studies imply that modulation of autophagy in the liver may affect the metabolic cycle of lipids. In line with their findings, Vescovo's group reported that HCV might subvert the degradative process of autophagy to promote the catabolism of LDs[208]. Based on the *in vitro* HCV replicon study and *in vivo* investigation of liver biopsies from patients chronically infected with HCV[208], Vescovo *et al*[208] concluded an inverse interrelationship between the extent of autophagy activation and the level of steatosis in HCV patients (Figure 4). This notion was based on their observations that the autophagic process facilitates LD breakdown in HCV replicon cells and that interference with autophagy leads to an elevated cholesterol level in HCV JFH1-infected cells. Although HCV-activated autophagy acts as a counteracting mechanism to prevent excessive accumulation of lipids that are induced by virus infection, it remains to be determined whether virus-induced autophagy affects the cell metabolism balance during the enhanced catabolism of LDs in liver cells, such as by altering the homeostatic levels of related lipids or interfering with the balance between the lipogenesis and lipolysis pathways. Moreover, whether activation of lipophagy by HCV infection affects the regular cellular functions and leads to pathological changes in the infected hepatocytes warrants further investigation. Nevertheless, the autophagy-mediated regulation of lipid metabolism may represent a mechanism of deregulation that interferes with metabolic homeostasis, thus promoting the progression of HCV-associated metabolic syndrome.

POSSIBLE LINK BETWEEN AUTOPHAGY AND THE HCV MEMBRANOUS REPLICATION COMPLEX

A recent study has shown that ectopic expression of HCV NS4B is sufficient to activate incomplete autophagy by interacting with Rab5 and Vps34 in human hepatoma cells[216], which suggests that activation of autophagy by the NS4B protein may be related to membranous web formation (Figure 4). Reciprocally, the HCV NS4B-induced membranous web accumulation could trigger a

stress response, such as ER stress, which was indicated to be an inducer of HCV-triggered autophagy[10,11] (Figure 4). It is still unknown whether HCV NS4B can utilize the autophagy-mediated membrane rearrangement process to generate double-membrane vesicles (DMV) within the membranous web, which is required for HCV replication. Notably, several studies indicated that HCV activates incomplete autophagy[11,216], which may serve as a means of inducing the accumulation of DMV. Moreover, the autophagosomal membrane has recently been demonstrated to be a site for HCV viral RNA replication[204]. In addition to NS4B, HCV NS5A was shown to activate autophagy *via* enhancing phospho-mTOR expression and its downstream target 4EBP1 in IHH cells[215]. HCV NS5A being a critical regulator for modulating the local concentration of PI4P, which is a critical component of the membranous web[77,78,81,84,85], implies again that HCV may exploit autophagy to regulate the formation of the membranous web (Figure 4). These studies collectively imply that the extent of host cellular autophagy may affect the pathogenesis of HCV-associated liver diseases through modulating the status of HCV replication and membranous web formation (Figure 4).

AUTOPHAGY AND HCV-RELATED LIVER CANCER

In addition to the possibility of participating in the development of liver-associated diseases by altering lipid metabolism, HCV-activated autophagy may contribute to the development of hepatocellular carcinoma (Figure 4). The role of autophagy in inhibiting tumor development originated from the investigation of the functional impact of knocking out the ATG genes in mice[221-225]. The heterozygous loss of the Beclin gene with a repressed autophagic process in mice promoted tumorigenesis and increased the occurrence of spontaneous malignancies, which indicated, for the first time, that Beclin may serve as a tumor suppressor in tumor progression[221]. Likewise, mosaic knockout of ATG5 and the conditional depletion of ATG7 in mice also resulted in spontaneous formation of benign liver cancer[223]. Additionally, inhibition of tumor suppressor genes, such as phosphatase and tensin homolog (PTEN) and p53, suppresses the basal autophagic response[223,226,227], suggesting an association of autophagy with tumor formation. On the other hand, recent studies from Komatsu's group showed that the accumulation of p62, which is a substrate of autophagy, by interference with autophagy promotes the formation of hepatocellular carcinoma[224,228] (Figure 4). This process occurs through direct interaction of p62 with Kelch-like ECH-associated protein 1 (Keap1), which is a component of Cullin3-associated ubiquitin E3 ligase, and ablation of the Keap1-mediated degradation of activating nuclear factor (erythroid-derived 2)-like factor 2 (Nrf2) and, therefore, leads to persistent activation of the expression of Nrf2 downstream cytoprotective genes[224,228]. These studies collectively unveil a novel role of autophagy in tumor suppression (Figure 4). Along with these findings, it would be interesting to investigate whether HCV-activated autophagy interferes with the suppressive effect of basal autophagy in tumor progression, thus promoting the development of liver cancer. Currently, how HCV-induced autophagy interacts with and/or affects basal autophagy *in vivo* to promote tumor formation is still unclear and needs to be studied.

On the other hand, accumulated evidence has indicated that cancer cells may activate autophagy to alter the tumor microenvironment and promote cell surveillance, which would, therefore, protect tumor cells from cell death[198,229-235] (Figure 4). The tumor microenvironment often faces stringent stress conditions of hypoxia and restricted nutrients; thus, tumor cells activate autophagy to counteract these stress responses[198,232,235]. Additionally, activation of autophagy is exploited by cancer cells to trigger resistance against anti-cancer therapy[229-231]. Hence, suppression of autophagy has been shown to synergistically enhance the efficacy of anti-cancer drugs to kill cancer cells[229,230]. The potential role of the HCV-activated autophagic response in the establishment of the tumor microenvironment and chemo-resistance of hepatocellular carcinoma has not yet been determined, but such a hypothesis is conceivably reasonable. For instance, the autophagy that is triggered by the virus may protect chronically HCV-infected cells from stress-induced cell death, such as through apoptosis, which would promote cell survival and possibly result in the development of tumors (Figure 4). Nevertheless, further investigations on the relationship between autophagy and the pathogenesis of HCV-related liver diseases and tumor progression are urgently needed. Without a convenient small animal model that supports the entire HCV life cycle and allows the monitoring of the HCV-associated disease progression, a large gap must be crossed before investigation on the *in vivo* relevance of autophagy in the development of end-stage HCV-associated liver diseases becomes feasible.

AUTOPHAGY AS AN ANTIVIRAL TARGET

Suppression of autophagy has emerged as a means to inhibit HCV replication[9-11,207]; therefore, the implications of repressed autophagic activity in anti-HCV therapy can be envisioned. Our recent studies indicated that pharmacological inhibitors of autophagy, such as chloroquine (CQ) and bafilomycin A1 (BAF-A1), can specifically inhibit HCV infection through activation of type I IFN antiviral immunity in the *in vitro* HCVcc model[10]. CQ and BAF-A1 were also shown to inhibit HCV entry *via* inhibiting the endocytosis pathway[39], and CQ has been demonstrated to inhibit the development of pancreatic tumor formation in a rodent model[233,234]. Recently, *in vivo* gene transfer of transcriptional factor EB, which is a master gene that regulates autophagy in the livers of mice, can promote clearance of mutant, hepatotoxic alpha-1-anti-trypsin, which is a protein aggregate that commonly causes liver injury[236]. This finding implicates

that modulation of cellular autophagy may provide an innovative and feasible therapeutic strategy for curing liver-associated diseases. Therefore, it is anticipated that these autophagic inhibitors, along with small molecule of inhibiting autophagy, could be therapeutically applied in the treatment of HCV infection and possibly HCV-associated liver diseases. Again, an *in vivo* small animal model for studying HCV infection and the progression of liver-related diseases is required for screening and testing the efficacy and safety of a potential therapeutic strategy.

CONCLUSION

Autophagy has emerged as an important topic in HCV research. However, the detailed mechanistic action of how HCV activates the autophagic process and comprehensive knowledge of the physiological significance of autophagy at each step of the HCV life cycle still remain to be investigated. Moreover, autophagy may contribute to the pathogenesis of HCV-associated liver diseases. In the future, studies on the exploration of the clinical relevance of autophagy in HCV-infected patients and *in vivo* investigations using small animal models that can support the complete HCV replication cycle shall provide mechanistic insights into the functional impacts of autophagy-HCV interactions in the pathogenesis of HCV-derived liver diseases. The results of these studies will benefit the development of new therapeutic strategies that are capable of curing HCV infection and elucidate the pathogenesis of HCV-associated liver diseases. These results will also facilitate the design of an efficacious vaccine that can protect the human population against HCV infection.

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