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REVIEW

Hepatitis C virus, mitochondria and auto/mitophagy: Exploiting a host defense mechanism

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Abstract

Hepatitis C virus (HCV) is the major reason for liver transplantation and the main cause of liver-related morbidity and mortality in a great number of countries. As for the other viruses, this pathogen interferes in more than one process and in more than one way with host cell biology. A mounting body of evidence points, in particular, toward the drastic alterations of mitochondrial physiology and functions that virus is able to induce, albeit the mechanisms have partly remained elusive. Role of the mitochondria in immunity and in quality control systems, as autophagy, as well as the strategies that HCV has evolved to evade and even to manipulate mitochondrial surveillance for its benefit, highlights the importance of deepening the mechanisms that modulate this virus-mitochondrion interaction, not only to intensify our knowledge of the HCV infection pathogenesis but also to design efficient antiviral strategies.

Key words: Hepatitis C virus; Mitochondrial dysfunctions; Immunity; Autophagy; Mitophagy

Core tip: Among the strategies that host cells have evolved in the hard fight for their survival against viruses, auto/mitophagy processes have an emerging role. As preferential targets of hepatitis C virus (HCV) attack, mitochondria effectively establish themselves as an integral part of the host cell defense and mitophagy, as very recently unveiled, seriously impacts the course of hepatitis C infection. Aim of this review is to explore the current literature about the mechanisms that regulate the critical interplay between HCV and mitochondria, with particular regard to the strategies that the virus evolved to subvert and manipulate the auto/mitophagy pathways to its purposes.

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INTRODUCTION

Hepatitis C virus (HCV) is a leading cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma worldwide. It is a small-enveloped positive-strand RNA virus whose natural targets are hepatocytes and, possibly, B lymphocytes[1,2]. HCV belongs to the Hepacivirus genus of the family of Flaviviridae, that also contains the classical flaviviruses such as dengue virus and yellow fever virus, and chronically infects 120 to 180 million people worldwide[3,4]. The mechanisms of liver injury in chronic HCV infection are poorly understood though an emerging feature in the pathogenesis of the HCV-related disease is the presence of dysfunctions of mitochondria,

proved to be targets of viral proteins. A novel pathogenetic aspect about HCV-mitochondria interaction has recently emerged, disclosing HCV aptitude to hijack mitophagy pathway, a pivotal mitochondrial quality control system.

HCV: FROM MOLECULAR VIROLOGY TO PATHOGENESIS OF INFECTION

HCV genome, an uncapped RNA molecule of approximately 9.6 kb, is composed of an open reading frame encoding a polyprotein precursor of approximately 3000 amino acids, flanked at the 5' and 3' ends by non-translated regions (NTRs) that are highly conserved among different HCV isolates and contain essential structural motifs exerting critical regulatory functions in viral RNA translation and replication processes[5].

HCV entry into the host cell is mediated by complex processes including the timely coordinated interaction of virus particles with cell surface receptors and entry factors like the low density lipoprotein receptor, the tetraspanin CD81, and the tight junction proteins claudin 1 and occludin[1], the HCV clathrin-mediated endocytic uptake of the lipoviral particle within an acidic endosomal compartment, followed by the pH-dependent fusion of the viral and endosomal membranes, essential for the release of genome-containing viral nucleocapsid into the cytosol[6]. The positive-strand genomic RNA, free in the cell cytoplasm following decapsidation of viral nucleocapsids, serves together with newly synthesized RNA, as messenger RNA for synthesis of the HCV polyprotein precursor. HCV genome translation is under the control of an internal ribosome entry site within the 5' NTR and produces a large polyprotein that undergoes proteolytic cleavage catalysed by host and viral proteases[7].

The co- and post-translational processing of protein precursor leads to the mature structural core protein and the E1 and E2 envelope glycoproteins, as well as to the nonstructural proteins p7 viroporin, NS2, the NS3 serine protease, the NS4A, NS4B, NS5A proteins and the NS5B RNA-dependent RNA polymerase[3,5] involved in the enzymatic functions of viral replication and processing of the viral polyprotein.

The core protein is a RNA-binding protein involved in the viral nucleocapsid assembly. This highly basic membrane protein contains two domains and is able to interact with a growing list of cellular proteins and to regulate a variety of host cell biological processes such as apoptosis, cell trasformation and immune modulation[8]. The envelope glycoproteins E1 and E2 are type I transmembrane proteins with N-terminal ectodomains and short C-terminal transmembrane domains that heterodimerize to form a non-covalent complex, which presumably represents the building block for the viral envelope[9]. However, the structure of the HCV E1-E2 complex, as well as the processes that are involved in HCV attachment, entry and fusion remain virtually unknown[5]. It is known, instead, that p7 is a small ion channel protein, composed of two transmembrane domains, that is crucial for production of infectious viral progeny but not for RNA replication[10]. Another non-structural protein dispensable for RNA replication is NS2, the viral autoprotease essential for the cleavage of the polyprotein precursor at the NS2-NS3 junction, that is crucial for the viral assembly. NS3 is a multifunctional protein harbouring a N-terminal HCV serine protease, which mediates the downstream cleavage events in the nonstructural region and a C-terminal RNA helicase-NTPase, which is vital for HCV RNA replication[3]. NS3 forms a stable complex with its cofactor NS4[11], that plays a major role in regulating NS3 helicase activity and a variety of NS3-mediated processes[12]. NS4B is the least characterized HCV protein and its role is not well understood even if it is known that its expression triggers a specific membrane alteration, designated as "membranous web", probably forming the scaffolds necessary for virus multiplication[5]. NS5A is a membrane-associated phosphoprotein whose functions are strongly debated, although it has been demonstrated that its modifications affect HCV replication and that it is potentially involved in HCV response to interferon[11,13,14]. Finally, the NS5B RNA dependent RNA polymerase (RdRp) is a fundamental enzyme in the promotion of synthesis of new RNA genomes and it is considered a major target for anti-HCV intervention[1,4].

As for all positive-strand RNA viruses, HCV RNA replication occurs on modified intracellular membranes, forming the above-mentioned endoplasmic reticulum (ER)-derived membranous web, in a poorly understood process. Remodeled intracellular membranes serve as a scaffold for the assembly of the replication complex composed of viral proteins, cellular components, and nascent RNA strands[15]. HCV replication is thought to be a semiconservative and asymmetric process, catalyzed by the NS5B RdRp, in which the positive strand RNA genome serves as a template for the NS5B RdRp to generate the negative strand replicative intermediate, that will be used, subsequently, as a template to produce further positive sense genomes. Finally, the numerous strands of positive polarity produced can be translated to produce new viral proteins, or used for further RNA replication, or be packaged into new virus particles[16].

The HCV particles are composed of HCV RNA genome, core and the envelope glycoproteins, E1 and E2, and are characterized by an irregular and globular shape with a mean diameter of 100 nm. Typically, they also show a tight association with cellular lipoproteins and lipids that affect both morphology and biophysical properties of the virion[17]. Although the molecular events that regulate the assembly and the release of infectious HCV particles have yet to be understood, the interaction of viral core protein assembly with intracellular lipid droplet structures is presumable. In addition to viral factors, several host cell factors have been described as participating in HCV assembly, including components of the very-low-density lipoprotein synthesis and secretion pathway, like the apolipoprotein E[18]. Once a newly

produced HCV nucleocapsid is formed in the cytoplasm and it acquires an envelope by budding through an intracellular membrane, virus particle is released from the cell through the constitutive secretory pathway[16].

More than 10 trillion virion particles are produced per day, even in the chronic phase of infection and, as a small RNA virus with an error-prone RNA polymerase, HCV exhibits enormous genetic variability, strongly challenging the host immune-mediated control. Six distinct genotypes and multiple subtypes have so far been identified, associated to specific clinical aspects including response to antiviral treatment. Despite international research efforts, much remains to be defined regarding clinical course of HCV infection and pathogenesis, mainly due to the frequent silent onset of the acute phase, as well as the asymptomatic early stages of chronic infection. Moreover, although chronic liver diseases such as hepatitis, cirrhosis and hepatocellular carcinoma, are considered the archetypal HCV-associated diseases, HCV is also involved in the pathogenesis of a number of extrahepatic manifestations like autoimmune and rheumatic disorders as well B-cell lymphoproliferative diseases[19,20]. However, morbidity and mortality of persistent HCV infection are mainly due to hepatic fibrosis and to the subsequent occurrence of cirrhosis and hepatocellular carcinoma[21]. Both innate and adaptive immune responses are the first barrier against HCV infection and multiple and complex HCV interactions with the host immune system, including elusion mechanisms, have been described. As well as being a crucial line of defense in the fight against the virus, immune response, mainly based on T lymphocytes and helper T cells intervention, represents, on the other side, the main cause of liver collateral damage in a long-lasting inflammation and fibrosis context. A qualitatively insufficient $CD8^+$ T lymphocyte responses, as well as viral inhibition of host defense strategies and the presence of multiple viral species in the same patient are probable reasons for the HCV persistence[20,22]. Therefore, immunotherapeutic strategies designed to reinforce the cellular immune response against HCV are largely attractive, even if any step of the HCV life cycle is a potential novel target of antiviral therapy[16]. Several antiviral agents directly targeting HCV life cycle, including NS3/4A protease, NS5B and NS5A inhibitors, have been clinically approved whereas promising host-targeting antiviral agents, offering the benefit of virtually pangenotypic activity, like inhibitors of cyclophilin A and of miR122 have advanced to phase 2 or 3 clinical trials. Extraordinary progress in the molecular virology of HCV has been done so far, but a further effort in the understanding of the viral life cycle and pathogenesis should improve therapeutic and preventive strategies for HCV infection[23,24].

HCV AND MITOCHONDRIA: A LIFE AND DEATH STRUGGLE

As clinically observed and widely experimentally confirmed, mitochondria are one of the favorite targets of HCV attack. As sensors of the cell energy status, these organelles are involved in a myriad of cell physiological functions and are essential for crucial decisions like cell death, growth, proliferation and differentiation[25]. It is not surprising, therefore, that the HCV impact on mitochondria deeply affects different molecular pathways. Liver samples from patients with chronic hepatitis C typically show mitochondrial dysfunction along with ultrastructural abnormalities and oxidative stress associated to the presence of lipid peroxide-protein adducts and glutathione depletion, as well[26-28]. A correlation between the presence of oxidative protein derivatives and disease severity has also been shown and, concurrently, a decrease of oxidative stress markers has been found to be associated with viral eradication[29,30]. Chronic hepatitis C patients' oxidative stress phenotype has been successfully reproduced in transgenic mouse models for the hepatic disease with an increased lipid peroxidation and an oxidized mitochondrial glutathione pool reported[31]. Multiple and complex mechanisms of HCV interference with mitochondrial functions have been described and, although the consequences of this interplay remain to be elucidated, it has a considerable impact on the pathogenesis of infection, contributing to HCV evasion of the host innate immune response and infection persistence and, at the same time, potentially favoring both fibrogenesis and carcinogenesis processes[32]. The molecular mechanisms underlying the HCV-mitochondria interplay and how this may affect the viral lifecycle and translate in pathogenic effects for the host are not clear issues yet. As supported by increasing experimental data, HCV core protein is mainly responsible for the viral damaging action on mitochondria. Different phenomena, principally the widely observed increased mitochondrial reactive oxygen species (ROS) production seem to be, in fact, a direct consequence of core protein interactions with mitochondria, irrespectively of the context, either in infection, full-length replicon-bearing cells or in over-expression systems[33]. Even though core protein represents the paradigm of HCV-induced mitochondria impairment, other viral proteins have been shown to directly interact with mitochondria into the matrix or intermembrane space, or by binding to the outer membrane or to membrane sites closely associated with the ER (the mitochondria associated membranes, MAMs), supporting the idea that HCV proteins migrate to mitochondria by lateral diffusion from the ER *via* transient fusion of the membranous sub-compartments[34-36]. Although diverse, the molecular mechanisms that HCV adopts in disturbing mitochondria functions, generally concern redox state and calcium (Ca^{2+}) homeostasis. NS3 and E1 proteins, in fact, have been shown to induce enhanced ROS production and mitochondrial membrane potential ($mt\Delta\Psi$) reduction in a transient-expression system[37], as well as NS4A, that is able to provoke the release of cytochrome c from mitochondria into the cytosol[38] and NS5A, whose action on mitochondria most probably results from the drastic effects it produces on intracellu-

lar Ca^{2+} signaling[39-41]. Taking up substantial amounts of Ca^{2+} from the cytosol or the ER, mitochondria behave as large and dynamic Ca^{2+} buffers and mitochondrial Ca^{2+} uptake can be considered a real cellular mechanism which has a critical function in modulating physiological events like metabolic activity and cell fate[23,42]. The outward Ca^{2+} flux from the ER to the mitochondria is facilitated by MAMs that provide local microdomains where the high localized Ca^{2+} levels enable the functioning of the Deltapsi$_m$-driven Ca^{2+} uniport at the inner mitochondrial membrane, which, together with the voltage-dependent anion channel (VDAC)/porin of the outer mitochondrial membrane (OMM), is mainly responsible for the entrance of Ca^{2+} into the mitochondria[44,45]. The enhanced steady-state intramitochondrial Ca^{2+} concentration has different functional consequences, among which the allosteric activation of tricarboxylic acid cycle (TCA cycle) and oxidative phosphorylation (OXPHOS) enzymes, resulting in an overall stimulation of respiratory chain (RC) activity and higher ATP output, is one of the best characterized[46,47]. However, if excessive, Ca^{2+} accumulation by mitochondria could turn into a powerful trigger of the mitochondrial permeability transition pore (MPTP) permanent opening, resulting in osmotic swelling, breaking of the outer membrane, and finally, in the release of cytochrome c and other pro-apoptotic signalling molecules as a prelude to the programmed cell death activation[48]. Even though their relationship has not been completely clarified, several lines of evidence suggest that the directly induced oxidative stress is intimately connected to the alteration of mitochondrial Ca^{2+} homeostasis provoked by HCV infection. The interplay between Ca^{2+} and redox unbalance is far from being a linear cause-effect relationship, but novel outcomes resulting by our previous work[49] unequivocally identify the Ca^{2+} overload in the mitochondrial compartment as the primary event leading to the profound mitochondrial oxidative metabolism alterations induced by the coordinated expression of all HCV proteins in a well-defined cellular context.

To the aim to gather and reorganize the complex and abundant available data on the subject and to delineate a possible sequence of molecular events following HCV infection a comprehensive mechanistic working model was recently proposed by our group[50].

It was proposed that the HCV-related outward flux of Ca^{2+} from ER stores, following a direct interaction or an ER stress-mediated indirect effect of the virus[51-53], causes a reduction of the electrochemical transmembrane potential and thereby affects the efficiency of ATP synthesis. If timely limited, this effect may be counterbalanced by an adaptive stress-response consisting in the Ca^{2+}-mediated activation of TCA cycle and up-regulation of OXPHOS[45] but, in the case of a chronic viral infection, the persistent insult becomes very hard to counteract. High levels of mitochondrial Ca^{2+} may in fact severely impair OXPHOS, provoking electron leakage to O_2 with formation of the superoxide anion $O_2^{\bullet -}$ over the basal level[54]. Since the main source of ROS, under these conditions, is the RC complex I that is also one of the major targets of superoxide, a kind of self-inhibition mechanism is established[32,49]. In addition, enhanced intramitochondrial Ca^{2+} and ROS activate the MPTP whose opening causes flush out of low molecular weight metabolites comprising $NAD(P)^+$/NAD(P)H and the anti-oxidant molecule glutathione[55]. This further impairs the RC and the OXPHOS activity and reduces cellular ROS scavenging reserve with consequent worsening of the redox balance that strongly impacts on ER-mitochondria Ca^{2+} homeostasis[56] and triggers a detrimental self-nourishing cycle.

The pathological outcomes of this profound HCV-related subversion of mitochondrial physiology strictly depend on the severity of ROS and mitochondrial Ca^{2+}-related insult and may also diverge in virus load or viral protein expression and/or in clinical features of the host. Under conditions of low ROS and Ca^{2+}-dependent stress level, a pro-survival and proliferative adaptive response is induced by redox signaling and, in spite of the impairment of the RC activity, ATP levels are even higher in HCV protein-expressing cells, as we reported in a previous work[57]. This apparently inconsistent phenomenon may be explained with the HCV-linked activation of the hypoxia-inducible factor 1 and the resultant shift of the energetic metabolism of infected cells toward glycolysis[57,58]. If low production of ROS is not damaging to the infected cell, it may in turns favor HCV maintenance and lead to accumulation of mutagenic hits resulting in carcinogenic priming of the host cell and ultimately in hepatocellular carcinoma development. Under conditions of high concentrations of mitochondrial Ca^{2+} and ROS, instead, the MPTP permanent opening may lead, depending on the prevailing conditions, to selective removal of damaged organelles, apoptosis or necrosis[59]. If inadequate, apoptosis fails to remove cells carrying genetic alterations, promoting the development of hepatocellular carcinoma, that can be favored, at the same time, by chronic apoptotic stimulation and by the high rate of tissue regeneration induced that exposes cell to the risk of mitotic errors. However, the HCV struggle against mitochondria is not restricted to the injury of their cellular bioenergetic competence but it is also extended to hit the mitochondrial immune response. The recent identification of the MAVS protein (also known as CARDIF, VISA or IPS-1), which contains a C-terminal mitochondrial localization domain that targets it to the mitochondrial outer membrane, highlighted the so far underrated role of mitochondria in the antiviral innate immune response[60,61]. Recognition of early replication intermediates of HCV such as double-stranded RNA by the upstream sensors RIG-I and MDA5 induces a conformational change that results in their binding to MAVS that behaves as an essential adaptor propagating the signal downstream and finally inducing interferon-γ (IFN-γ) production[62]. Mitochondrial localization of MAVS is absolutely necessary for

its function, mistargeting of the protein away from mitochondria, in fact, completely abolishes this antiviral defense pathway[60]. Shortly after discovery of MAVS it was shown that the HCV NS3/NS4A complex avidly cleaves MAVS in a C-terminal anchor loop site, releasing the protein from the mitochondria. This mechanism enables HCV to paralyze the MAVS-downstream signalling pathway leading to IFNβ production, and to elude the host innate immunity in a continuously evolving fight for its survival[63].

HCV AND AUTO/MITO-PHAGY: HOW A VIRUS CAN BENEFIT FROM A HOST DEFENSE MECHANISM

By putting into action stress relief responses, induction of cytokines and apoptosis, as well as by functioning as the powerhouses of the cell, mitochondria are key players in the life of a eukaryotic cell. Accordingly, it is not surprising that mitochondrial dysfunctions can also potentially damage cells and have been implied in a wide range of age-related disorders and various forms of cancer[64]. Accurate removal of functionally compromised mitochondria is, therefore, crucial to prevent cellular damage and to sustain cellular well-being. Thus, the finely regulated process of lysosomal-mediated bulk degradation of cytosolic components and organelles, including mitochondria, known as autophagy (from Greek, meaning "self-eating"), represents an important protection mechanism of the cell against stressful conditions, promoting cellular survival, differentiation, development and homeostasis[65]. Originally characterized as an evolutionarily conserved cellular response to nutrient starvation, this "self-digestion" pathway not only contributes to maintain vital cellular functions in fasting conditions by mobilizing nutrients from macromolecular degradation, but also can rid the cell of unnecessary or damaged organelles, protein aggregates and even invading microorganisms[66]. Three types of autophagy, *i.e.*, macroautophagy, microautophagy, and chaperone-mediated autophagy, have been described to date, even if macroautophagy is the most extensively studied autophagy pathway that mediates the large-scale degradation of intracellular molecules. In the initial steps of this process, cytoplasmic material is engulfed by an isolation membrane, which is also called phagophore whose edges then fuse to form the double-membraned vesicles named autophagosomes. This is followed by fusion of the autophagosome with a lysosome to form an autolysosome where the captured material, together with the inner membrane, is degraded[67]. In addition to the master regulator of autophagy, the target of rapamycin, TOR kinase, which acts by inhibiting autophagy in response to insulin-like and other growth factor signals, other regulatory molecules of autophagy including Bcl-2, ROS, Ca^{2+}, and the AMP-activated protein kinase have recently been reported. In addition, more than 30 autophagy-related proteins, Atg, have been identified to date, in yeast, many of which are evolutionarily conserved, such as the mammalian Atg6/Vps30 ortholog Beclin 1[68,69]. The core machinery of macroautophagy and the various steps of this process are similar whether invoked for the clearance of bulk cytosol or for the selective elimination of mitochondria that are specifically removed by a dedicated pathway called "mitophagy[70]. Whether serving as a quality control mechanism to eliminate harmful damaged mitochondria, maintaining mitochondrial functional and genetic integrity, or to modulate the steady-state turnover of mitochondria and their number in response to developmental or physiological cues, mitophagy has established itself as an increasingly important process[71]. In many metazoan cell types, mitophagy is mediated by a pathway comprised of PTEN-induced putative protein kinase 1 (PINK1) and the E3 ubiquitin ligase Parkin, whose defects are associated with a form of autosomal recessive juvenile Parkinson's disease. Pink1 and Parkin physically interact with each other and, as suggested by genetic studies, Pink1 is upstream of Parkin. Pink1/Parkin pathway is triggered when PINK1, accumulated on the outer membrane of damaged mitochondria, facilitates recruitment of cytosolic Parkin, promoting mitochondria segregation from the mitochondrial network and targeting these organelles for their mitophagic clearance[70,72,73]. In mammalian cells, Parkin normally resides in the cytosol but it is selectively recruited to depolarized mitochondria, promoting the colocalization of mitochondria with the autophagy marker LC3[74]. How does Parkin promote mitophagy? Emerging evidence has suggested that Parkin plays a dual role in mitophagy process, priming the mitochondria through the ubiquitination of OMM proteins on the depolarized mitochondria[75], and promoting induction of autophagy by interacting with Ambra1 and activating class Ⅲ PI3K[76]. Initially supposed to be exclusively dedicated to the "recycling" of macromolecular material within the cell, the autophagy machinery is now emerging as a process that interfaces with most cellular stress-response pathways, including those involved in controlling immune responses and inflammation[77,78]. The immunological role of autophagy is, in fact, a newly recognized facet of innate and adaptive immunity against viral infection and certain viruses have developed strategies to counteract these antiviral mechanisms while others have become even able to co-opt the autophagy machinery as a proviral host factor favoring their own survival[79]. Recognition of viral RNA by innate immune sensors, as well as engagement of CD46, a cell surface receptor required for entry of a variety of pathogens[80], are only some of the different mechanisms of autophagy induction in infected cells. Liang *et al*[81] were the first to demonstrate the antiviral potential of autophagy by showing that the overexpression of Beclin1 by a recombinant Sindbis virus (SV) decreases SV replication and SV-induced neuronal apoptosis, protecting mice from fatal SV encephalitis. The innumerable strategies that viruses,

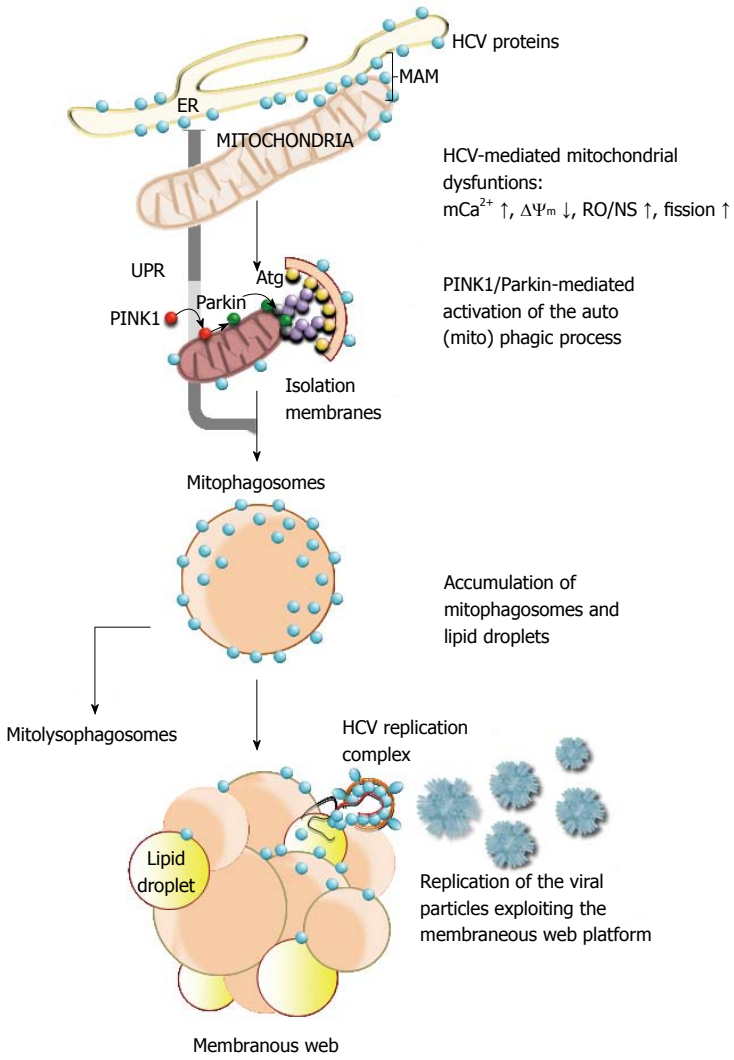

Figure 1 Schematic representation of the hepatitis C virus-induced auto(mito)phagy in infected host cell. The cartoon illustrates the suggested sequential steps leading to activation of the macro-autophagic process by the hepatitis C virus (HCV) proteins, which are shown as pale-blue circles localized at the endoplasmic reticulum (ER) membranes where translation and proteolytic processing of the HCV polyprotein take place. Through the mitochondria associated membranes (MAMs) the HCV proteins partly transfer to mitochondrial membranes inducing therein a number of alterations comprising enhanced influx of Ca^{2+}, decrease of the respiratory chain activity and of the transmembrane potential, increase of reactive oxygen/nitrogen species (RO/NS), promotion of the mitochondrial network fragmentation (fission). The dysfunctional mitochondria recruit on the their outer membrane the kinase PTEN-induced putative protein kinase 1 (PINK1), which phosphorylates and activates the ubiquitin ligase Parkin. Ubiquitinated mitochondrial proteins target the organelle to the nascent macrophagic vescicles (isolation membranes) through interaction with autophagy-related gene (Atg) factors thereby leading to progressive engulfment of mitochondria in the auto(mito)phagosomes. If the rate of macrophagosomes formation overwhelms that of their degradation, *via* fusion with lysosomes (mitolysophagosomes), this leads to accumulation of macrophagic vesicles that combines to lipid droplets developing a membranous web. This provides a structural/functional platform where the HCV replication complex assembles and releases viral particles. Although the scheme highlights a major role of the HCV protein-mediated mitochondrial dysfunctions in the induction of the autophagic process the participation of the HCV protein-induced ER stress-mediated unfolded protein response (UPR) is also shown. See text for further explanations and references.

as well as bacteria, developed to neutralise the host autophagic defense mechanisms highlight the importance of this process in immunity. These include the blockade of positive upstream regulators of autophagy, such as the IFN-inducible RNA-activated eIF2α protein kinase (PKR) signalling pathway, the activation of negative upstream regulators of autophagy, such as the nutrient-sensing TOR kinase signalling pathway or direct antagonism of the autophagy machinery. As mentioned above, certain viruses might manipulate the autophagic pathway or at least specific autophagy genes to foster their own self-serving purposes. It is the case of poliovirus, rotavirus, HIV, coronaviruses, dengue virus, and the hepatitis B and C viruses, which exploit autophagy proteins for their membrane formation and/or trafficking functions[82]. Interestingly, as cellular membranous structures, autophagosomes have been proposed to act as a scaffold for intracellular membrane-associated replication of certain cytoplasmic RNA viruses[83]. Actually, it has been demonstrated that negative inhibition and down-regulation of different regulators of the autophagy pathway strongly suppress productive HCV infection. Autophagy proteins (*i.e.*, Beclin-1, Atg4B, Atg5, and Atg12) behave, in fact, as proviral factors, required to initiate translation of the

incoming HCV RNA in *de novo* infected cells, but they are not required once infection is established[84]. Interestingly, several independent studies suggest that HCV is able to induce a cellular ER stress response, also termed the unfolded protein response (UPR), inducing the accumulation of autophagosomes in cells without enhancing autophagic protein degradation. Therefore, autophagosomes turn into sanctuaries for HCV replication and protection from host immune surveillance that favour chronic infection and liver injury. The inhibition of UPR signaling pathways in fact suppresses HCV-induced lipidation of LC3 protein, a necessary step for the formation of autophagosomes, suggesting a positive role of UPR and the partial autophagic response in HCV replication[85-87]. Moreover, Chu *et al*[88], also showed that HCV-induced ROS production, both in the cytosol and mitochondria of HCV protein-expressing hepatoma cell lines might contribute to the activation of autophagy.

Considering the above-mentioned multitude of HCV-induced pathophysiological insults leading to mitochondrial dysfunction, it might sound unsurprising that HCV also affects mitophagy. The first demonstration of the direct effect of HCV on a key mechanism responsible for mitochondrial turnover and quality control resulted from very recent studies by Kim *et al*[89], that revealed the ability of HCV to induce Parkin-dependent mitophagy. Using multiple strategies including confocal and electron microscopy, the authors observed a striking phenomenon of clustering of mitochondria in the perinuclear regions of the infected cells associated to mitochondrial translocation and aggregation of Parkin, irrespective of HCV genotypes. They also proved that HCV infection enhances Parkin-mediated ubiquitination of its known substrates Mfn2 and VDAC1 and that this process is attenuated by Parkin silencing. Furthermore, it was also shown that HCV infection induces an increase in both mRNA and protein levels of Parkin and PINK1. Significantly, increased Parkin protein levels were also found in liver tissues samples obtained from chronic HCV patients. An enhanced Parkin-mediated mitophagosome formation process also characterized HCV-infected cells in comparison to uninfected cells, followed by their later delivery to lysosomes to originate mitophagolysosomes. Once again, therefore, HCV usurps a physiological cell function to its own purposes, but which are these purposes? Kim *et al*[89] also highlighted that Parkin and PINK1 silencing negatively affects HCV RNA replication, suggesting a possible role of mitophagy in the viral life-cycle (Figure 1). Finally, it was demonstrated that the above-mentioned HCV-induced decrease in mitochondrial complex I activity, is surprisingly reversed by knockdown of Parkin that, similarly, also restores the number of mitochondria, usually reduced in HCV infected cells. Based upon these observations, the authors proposed a functional involvement of HCV-induced mitophagy in the impairment of oxidative phosphorylation and depletion of mitochondria that HCV typically provokes in the host cells.

CONCLUSION

Despite large research effort, knowledge about the intersection between the auto/mitophagy pathway and HCV infection is still quite rudimentary. However, among the diverse and complex physiological processes that can negatively or positively impact the course and natural history of the HCV-infection and the survival of both virus and host, these fundamental quality control systems undoubtedly play a pivotal role. Recent advances about autophagy functions are in fact reshaping our understanding of the pathogenesis of infective diseases. Since, further detailed analyses of the molecular mechanisms whereby virus exploits, for its benefit, the various components of the same auto/mitophagy machinery on which host relies to defend itself will greatly enhance our understanding of HCV associated liver disease pathogenesis and possibly, lead to the design of new selective antiviral therapeutic approaches.

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