

Review

The Role of Mitochondrial Dysfunction in the Development of Acute and Chronic Hepatitis C

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Abstract

Currently, the issue relating to the discussion raised in this article appears to be for what purposes the hepatitis C virus (HCV) modulates cellular processes, such as antiviral defense, metabolism, apoptosis, and mitochondrial dynamics, by inhibiting the activity or expression of mitochondrial proteins and a number of cellular proteins. Additionally, to what pathological changes do these alterations lead? Thus, the aim of this review is to propose potential protein mitochondrial targets of HCV for the future development of new drugs aimed at inhibiting its interaction with cellular proteins. Considering current analyses in the literature, promising targets for the acute and chronic phases of HCV are proposed which include mitochondrial antiviral signaling (MAVS) (antiviral response protein), Parkin (mitophagy protein), Drp1 (mitochondrial fission protein), subunits 1 and 4 of the electron transport chain (ETC) complex (oxidative phosphorylation proteins), among others. This review illustrates how viral strategies for modulating cellular processes involving HCV proteins differ in the acute and chronic phases and, as a result, the complications that arise.

Keywords: hepatitis C; mitochondria; mitochondrial dysfunction; reactive oxygen species

1. Introduction

Hepatitis C virus (HCV) is the leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma worldwide. According to a World Health Organization study, HCV has been serologically diagnosed in 100 million people worldwide, with over 70 million possessing chronic hepatitis C [1]. The acute phase of HCV mostly has mild symptoms or is completely asymptomatic. At the same time, it is known that persistent viral infections can manifest either in a latent or chronic form [2]. For more than half of patients infected with HCV, the infection acquires a chronic form, whereby HCV strongly replicates, thereby significantly reducing the likelihood of its elimination [3]. Chronic HCV is one of the major risk factors for fibrosis and cirrhosis of the liver. The most dangerous consequence of HCV infection is the high risk of developing hepatocellular carcinoma (HCC), which, ultimately, is the main indication of the need for liver transplantation worldwide [3].

HCV is highly mutagenic, which leads to frequent genomic mutations that allow it to “hide” from the immune system, which contributes to the development of a chronic disease. In addition, due to the high heterogeneity of HCV, there are great difficulties in creating an effective vaccine [4,5]. The existing problems are also associated primarily with the lack of effective and safe drugs to cure this dis-

ease. Thus, the development of new drugs for the treatment of HCV infection is a promising task facing modern medicine. Identification of altered cellular mechanisms directly related to the destruction of the liver by HCV will allow the discovery of new molecular pathological targets for new medicines against HCV. One of these mechanisms can be the development of mitochondrial dysfunction. It is known that mitochondria are an attractive target for different viruses [6]. One of the most common mechanisms is the blocking of the innate antiviral response signaling by binding to the mitochondrial antiviral signaling (MAVS) protein [7,8]. Another mechanism of action on mitochondria is associated with providing the optimal space required for viral replication to occur in a cell. This mechanism is performed by forming a complex of elongated mitochondria with the endoplasmic reticulum (ER). In this case, viruses such as the Dengue virus, Zika virus, and SARS-CoV can initiate mitochondrial elongation by blocking mitochondrial division [9]. To spread virions, viruses can also modulate cellular apoptosis. Thus, the PB1-F2 protein in the influenza virus causes a rupture in the inner mitochondrial membrane, which initiates apoptosis [10].

As the described examples highlight, viruses are able to change cellular homeostasis for their own needs by influencing various mitochondrially mediated functions. When



studying the pathogenesis of HCV, its ability to influence mitochondria has been described since the 1990s. Moreover, evidence of the relationship between the degree of the development of mitochondrial dysfunction and the severity of the disease has been found. Additionally, it has been shown that mitochondrial abnormalities correlate with disease progression in patients with chronic HCV infections. At the same time, changes in cells and tissues mediated by mitochondrial dysfunction appeared within a short time after diagnosis. These changes were associated with ultrastructural rearrangements in the mitochondria [11], an increase in the level of detectable oxidative stress markers in the liver, and the appearance of these markers in the blood of patients [12]. The mechanisms through which HCV affects mitochondria have been analyzed in a number of early reviews and research articles. Thus, in the study [13], it was noted that the HCV core protein initiated the development of oxidative stress in the liver. In the study [14], it was found that the HCV E2 protein initiated mitochondria-dependent apoptosis in hepatocytes. In the study [15], it was shown that HCV proteins, E1 and NS3, mediated DNA damage through the induction of oxidative stress, which increased the risk of cancer development.

The conducted studies allow for a comprehensive assessment of the impact of HCV-induced mitochondrial dysfunction on the pathogenesis of the disease. In this review, we have shown the time effects of HCV on mitochondria by identifying mitochondrial targets of HCV in acute and chronic hepatitis. The identification of these targets is the first and most important step in the development of inhibitors that prevent HCV proteins from binding to their identified target proteins. This therapeutic mechanism will potentially contribute to blocking the action of the virus in a cell at the early stages of an infection.

2. Importance of Proper Mitochondria Functioning for the Vital Activity of Hepatocytes

The main function of mitochondria, which determines the vulnerability of cells due to mitochondrial dysfunction, is the production of adenosine triphosphate (ATP since mitochondria are the main “energy guardians” in the cell and provide energy for most cellular processes. The liver is one of the richest organs in terms of the number and density of mitochondria [16]. In this regard, liver cells (hepatocytes) are highly vulnerable to the development of mitochondrial dysfunction. This may be considered as one of the explanations as to why mitochondrial dysfunction plays an important role in the pathogenesis of HCV. This section defines the main features of the mitochondria in cell vitality, with an emphasis on why they are important to hepatocytes.

2.1 Oxidative Phosphorylation

ATP is formed during oxidative phosphorylation (OXPHOS), which is carried out as a result of redox reactions

that occur when an electron passes through the electron transport chain (ETC) on the inner mitochondrial membrane [16]. Electrons are delivered to the ETC by reducing the equivalents of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH₂) [17]. In the ETC, electrons are transferred between large protein complexes (complexes I–V), while protons are simultaneously transported into the intermembrane space of the mitochondria, which leads to the accumulation of the electrochemical membrane potential (ψ_m) [18]. The last protein complex is V, which is an ATP synthase enzyme, whose function is associated with pumping protons into the mitochondrial matrix. As a result of this process, energy ψ_m is released, due to which the catalytic synthesis of adenosine diphosphate (ADP) into ATP occurs. ATP plays an important role in many energy-dependent processes in hepatocytes, such as gluconeogenesis, ureagenesis, and bile acid synthesis, among others [18].

It is known that during the passage of electrons through the ETC, reactive oxygen species (ROS) are also formed as by-products of the main process [19]. Their primary formation reaction is based on the interaction of electrons with oxygen. ROS in high concentrations are able to oxidize mitochondrial proteins and DNA, which is a main cause of mitochondrial dysfunction. Mitochondrial DNA is highly vulnerable to oxidative damage since mitochondria lack enzymes that perform the repair of nucleic acids, while an additional danger is associated with the fact that ROS are formed in close proximity [18].

2.2 Mitochondrial Permeability

The integrity of the mitochondrial membrane is critical to maintaining proper mitochondrial and hepatocyte functions in general. However, under certain conditions, the permeability of the mitochondrial membrane can be disturbed. The reason for this may be the opening of the mitochondrial permeability transition pore (MPTP) [20].

The opening of the MPTP can greatly affect the production and accumulation of ATP and calcium, which can eventually lead to necrosis [21]. In addition, the opening of the MPTP promotes mitochondrial swelling, which leads to a rupturing of the mitochondrial membrane and an initiation of apoptosis [21]. As a result of stress factors (for example, breakage of DNA strands), MPTP occurs with a further release of proapoptotic factors [21]. An increase in apoptosis and necrosis has been noted in a number of liver diseases, including various forms of hepatitis [22].

2.3 Innate Immunity

Mitochondria may be involved in the activation of innate immunity [23]. Mitochondria are able to activate the triggering of the immune response through metabolic signals, such as changes in the ratio of AMP/ATP, the concentration of ROS, and the number of several tricarboxylic acid cycle (TCA cycle) metabolites. Additionally, an immune

response can be elicited when mitochondrial DNA is damaged during oxidative stress. Thus, modifications of the mitochondrial function can cause an activation of the immune response. The mitochondrial antiviral signaling (MAVS) protein is located on the outer mitochondrial membrane and is responsible for initiating the innate antiviral immune response [24]. In view of the fact that the liver can be affected by many pathogenic microorganisms, in addition to HCV [25], the implementation of this function is extremely important for the vital activity of hepatocytes.

3. Hepatitis C Immunopathogenesis

3.1 Host–Virus Interaction

HCV passes through the endothelium and attacks target cells, the main one of which is hepatocytes. Due to glycosaminoglycans and low-density lipoprotein receptors, HCV virions are concentrated on the cytoplasmic membrane of the cells selected for invasion [26]. It is known that glucosaminoglycans act as a platform on which HCV virions can land. The HCV proteins E1 and E2 are involved in this process [26]. In addition to glucosaminoglycans, HCV also interacts with other cellular receptors, such as the epidermal growth factor receptor, claudin-1, scavenger B1 receptor, CD81, etc. [27,28]. After the contact of the HCV E1/E2 proteins with the cellular receptor CD81, Rho GTPases are activated, which re-modulate actin filaments and allow the E2 protein complex with CD81 to bind to claudin-1. The newly formed complex allows the virion to undergo clathrin-mediated endocytosis [26]. After the internalization, HCV viral virions bind to the endosome, which facilitates the release of viral RNA into the cytoplasm to initiate the replication. In addition, the E2/CD81 interaction activates the secretion of RANTES (Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted) and MMP2 (matrix metalloproteinase-2), which contribute to the modulation of the migration of immunocompetent cells [29].

3.2 Innate Immune Response to Hepatitis C Infection

The innate immune response plays a required role in limiting HCV spread by inducing the apoptosis of infected hepatocytes and the activation of an antigen-specific adaptive immune response. During the phase of acute HCV infection, viral RNA is present in the cytoplasm of hepatocytes, where it interacts with the intracellular receptors in hepatocytes: Toll-like receptor 3 (TLR3), retinoic acid-inducible gene-1 (RIG-1), and melanoma differentiation-associated gene 5 (MDA5), which trigger an immune response by activating the production of interferon (IFN)- γ and IFN-1. Circulating HCV RNA is also detected by plasmacytoid dendritic cells (pDCs), which activate natural killer (NK) cells [30]. NK cells play an important role in the innate immune response to HCV infection: they stimulate the cytolysis of infected hepatocytes, activating their apoptosis, and promoting the release of proinflammatory

cytokines, which, in turn, activate lymphocytes and prevent HCV replication [31]. As a result of increased HCV replication, inflammatory signaling in hepatocytes is disrupted, which causes a chronic inflammatory response and the subsequent progression of the infection [29].

3.3 Adaptive Immune Response and Progression to Chronic Hepatitis C

It is considered that the cellular adaptive immune response, represented by CD8+ and CD4+ Th1-lymphocytes, plays a crucial role in the fight against HCV infection. T-lymphocytes persist for a long time in patients with HCV [32]. However, only 1% out of all the CD4+ T-lymphocytes circulating in the liver acquire specificity for HCV, which is one of the reasons for the progression of the disease [26]. Another reason for the transition of the infection into the chronic phase is a decrease in the CD4+ T cell response after the acute phase of the infection [26]. When the chronic HCV infection occurs, persistent viremia occurs for more than 6 months. At the same time, the adaptive immune response is activated, yet its action has already been impaired. In addition, chronic infection is promoted by the mechanisms involved in the escape from the immune defense, through HCV [33]. In particular, HCV RNA mutates heavily due to the lack of an ability to repair, which can lead to the emergence of many mutant forms of HCV, not all of which are recognized by T-lymphocytes [4]. Another potential mechanism in HCV evading immune surveillance may be through the activation of Tregs, which suppress the immune function of the organism [34].

4. Influence of HCV Infection on Mitochondria

The hepatitis C virus causes significant functional changes in mitochondria. The most frequent of these are associated with an increase in the release of mitochondrial ROS, a decrease in the functionality of the antioxidant systems, the suppression of the innate immune response mediated by mitochondrial proteins, changes in metabolism, and the modulation of mitochondrial dynamics [35]. A detailed analysis of these impacts can make it possible to identify the key HCV mitochondrial targets, by acting on those that the virus can change to manipulate cellular homeostasis for its own needs. It should also be considered that HCV may have different effects on mitochondria in the acute and chronic phases of infection. This allows the mitochondrial targets that are affected by HCV at different stages of the disease to be determined. Subsequently, these found targets can be used to develop new anti-HCV drugs that will inhibit the interaction between the HCV proteins and their molecular targets.

4.1 Development of Acute Hepatitis C Based on Mitochondrial Dysfunction

Acute hepatitis C is a short-lived viral infection. Hepatocytes are able to quickly respond to an infection by controlling various cellular mechanisms. Mitochondria are important protective units in cells, alongside being the central organelle where several significant antiviral molecular mechanisms converge. The mitochondrial antiviral signaling (MAVS) protein acts as an adapter for the transcription and production of interferons (IFNs), which are the most potent antiviral cytokines, in response to viral infections [36]. In this situation, the HCV strategy consists of the primary fixation into the hepatocytes and the creation of offspring for the subsequent expansion of the liver. To do this, the actions of HCV are aimed at preventing recognition by the immune system. The longer HCV remains undetected, the more likely it is to spread further throughout the liver tissue and develop into a chronic infection.

4.1.1 Effect of Hepatitis C Virus on Antiviral Response

As a result of the recognition of HCV RNA by the MAVS protein, an innate antiviral response is activated, which is associated with the increased production of interferon and proinflammatory cytokines [37]. These events create an inflammatory environment that attracts innate immune cells (primarily natural killer cells), which destroy infected cells. To avoid being destroyed in the earliest stages of infection, HCV proteins can inhibit antiviral signaling into mitochondria. This signal is initiated by RNA viruses in response to the recognition of dsRNA by the RNA helicase RIG-I, and the subsequent chain of reactions, one of the central roles that is played by the MAVS membrane protein is the directing of the signal to the expression of interferon β . However, the cleavage of MAVS from the mitochondrial membrane, caused by the NS3/4A HCV protein, disrupts its function and blocks the production of interferon [38]. Thus, blocking the interaction of NS3/4A with MAVS will potentially prevent the development of HCV infection at the earliest stage.

4.1.2 Effect of Hepatitis C Virus on Autophagy and Mitophagy

Autophagy is a regulated process of the destruction of damaged or dysfunctional cell organelles. Unutilized damaged cellular structures can act as inducers of inflammation, which is not beneficial for HCV pathogenesis in the acute phase of the infection since the probability of the immune cells detecting the virus and the elimination of infected cells increases. Therefore, the viral apparatus is aimed at enhancing autophagy. Some HCV regulatory proteins are able to induce autophagy through an interaction with autophagy proteins. Thus, it was found that the function of the HCV p7 protein, when bound to the beclin-1 protein, was associated with the initiation of autophagy [39]. Another HCV protein, NS3/4A, is able to interact with mitochondria-associated

immune-associated GTPases M (IRGM), which also play a regulatory role in the autophagy process. It is known that HCV proteins activate IRGM-mediated phosphorylation of ULK1, which is one of the initiating factors for triggering autophagy [40]. Other regulatory interactions between HCV proteins and autophagy proteins include the binding of HCV nonstructural protein 5B (NS5B) to autophagy related 5 (ATG5) and autophagy related 12 (ATG12) [41], and of HCV nonstructural protein 5B (NS4B) to Ras-related protein 5 (Rab5), vacuolar protein sorting 34 (Vps34), and beclin-1 [42]. It has also been shown that an increase in the expression of the HCV NS5A protein leads to an increase in the expression of the beclin-1 protein [42].

Mitophagy is a particular variant of autophagy and is aimed at removing damaged and dysfunctional mitochondria. Since “unhealthy” mitochondria are a source of ROS that initiate inflammation and apoptosis, their removal is a favorable factor for maintaining the initial population of HCV virions in infected hepatocytes. Mitophagy has been shown to increase with HCV infection [38]. It was demonstrated that HCV activates the expression of Parkin and Pink1, which are key enzymes in mitophagy, and also induces mitochondrial translocation of Parkin, which was the initial stage of mitophagy [43]. It is also known that NS5A HCV proteins cause the mitochondrial translocation of Parkin [44]. The HCV proteins responsible for increasing the expression of mitophagy proteins have not yet been identified, which also forms a promising direction for future research.

4.1.3 Effect of Hepatitis C Virus on Mitochondrial Fusion and Fission

The processes of mitochondrial fission and fusion in hepatocytes are aimed at redistributing mitochondrial content and establishing a balance in the number of mitochondria. Both processes are aimed at maintaining a high-quality composition of mitochondria, however, in the case of fission, two smaller mitochondria are formed from one mitochondrion, and in the case of fusion, one elongated mitochondria is formed from two individuals. HCV enhances mitochondrial fission, and since mitochondrial fission often precedes mitophagy, its increase during acute HCV infection was discussed in the previous section. Furthermore, in addition to evading the immune response, the activation of mitochondrial fission can be caused by an increase in the intensity of metabolism. An increased number of mitochondria can “work for the virus”, supplying energy for the assembly of new virions. It was shown that HCV induced the phosphorylation and translocation of the Drp1 (mitochondrial fission protein) to the mitochondrial membrane, which was necessary to initiate mitochondrial fission [45]. HCV is able to inhibit mitochondrial fusion, which is associated with mtDNA depletion [46]. Importantly, the balance between fission and fusion is critical for stabilizing mtDNA copy number and maintaining a healthy liver function [47].

Table 1. Mitochondrial and other cellular targets of HCV during the acute phase of infection.

HCV proteins	Cellular process modified by HCV	Target proteins or genes for HCV*	HCV aims
NS3/4A	Antiviral response	MAVS	Hiding from an immune response
NS3/4A		IRGM	
NS5A, p7	Autophagy	<i>Beclin-1</i> (the protein for p7 and the gene for NS5A)	Hiding from an immune response and inhibiting apoptosis
NS5B		ATG5 and ATG12	
NS5A	Mitophagy	Parkin	Hiding from an immune response and inhibiting apoptosis
?		<i>Pink1 and Parkin</i> (genes)	
?	Mitochondrial fission	Drp1	Hiding from an immune response; Apoptosis inhibiting;
?	Oxidative phosphorylation	<i>MT-ND1</i> , <i>MT-ND3</i> , <i>MT-ND4</i> , and <i>MT-ND4L</i> of complex I and <i>MT-CO2</i> of complex IV, ATP synthase (genes)	Supplying energy for HCV production Supplying energy for HCV production
?	Glycolysis	Hexokinase	Supplying energy for HCV production
E2	Apoptosis	Cytochrome C	Increasing viral progeny within the infected cell

*Protein (if not indicated otherwise). ?Precise HCV protein is unknown.

HCV, hepatitis C virus; MAVS, mitochondrial antiviral signaling; IRGM, immune-associated GTPases M.

Drp1 is a target for HCV but the question remains regarding whether HCV proteins can directly affect this target.

4.1.4 Effect of Hepatitis C Virus on Metabolism

Like many other viruses, HCV redirects energy metabolism from oxidative phosphorylation to glycolysis. This is performed so that it can quickly use the cell's energy resources, in a short time, for processes associated with RNA replication, translation of HCV proteins, and virion assembly. Thus, it was found that the expression of the key protein subunits in the ETC complexes: mitochondrially Encoded NADH: Ubiquinone Oxidoreductase Core Subunit 1,3,4,4L (MT-ND1), (MT-ND3), (MT-ND4), and (MT-ND4L) of complex I and MT-CO2 of complex IV decreased in 6 days after the onset of HCV replication [48]. ATP synthase was also found to be downregulated in 4 weeks after the onset of the infection [48]. An increase in the expression and activity of key glycolysis enzymes during HCV infection has also been noted [48]. Thus, the activity of hexokinase increased as a result of an interaction between this enzyme and the HCV NS5A protein [49]. Therefore, inhibition of this metabolic shift by creating drugs that prevent the interaction between HCV proteins and the enzymes involved in oxidative phosphorylation and glycolysis can potentially slow down the reproduction of HCV.

4.1.5 Effect of Hepatitis C Virus on Apoptosis

In the initial phase of HCV infection, it is advantageous for the virus to maximize progeny within the infected

cell by using its resources. To do this, HCV implements a variety of ways to block cell apoptosis from being initiated. One of these ways is to enhance mitophagy, as described above. Furthermore, HCV is able to directly inhibit apoptosis. Thus, in the study [50], it was shown that the HCV E2 protein reduced the level of cytochrome C and blocked its release from mitochondria, which reduced apoptosis in HCV-infected cells. Hence, reducing the interaction of these proteins may be an effective strategy to reduce the persistence of HCV. Mitochondrial and other cellular targets of HCV during the acute phase of infection are summarized in Table 1.

4.2 Development of Chronic Hepatitis C Based on Mitochondrial Dysfunction

If HCV persists for more than 6 months, the infection enters the chronic phase. Here, HCV no longer “hides” from the immune system; instead, it actively infects new hepatocytes, thereby increasing the area of damage in the liver. Leukocytes migrate to the liver, where they cause an uncontrolled inflammatory reaction, during which both infected and cells die.

4.2.1 Effect of Hepatitis C Virus on Apoptosis and Further Liver Damage

In the chronic phase, HCV actively spreads and infects new hepatocytes. In the newly infected cells, HCV can also inhibit apoptosis to create a larger number of virions; however, for HCV to spread further, contrastingly, activates

Table 2. Mitochondrial and other HCV targets during the chronic phase of infection.

HCV proteins	HCV-modified cellular processes	HCV target proteins or genes*	HCV aims	Liver complications
Core protein E2 NS5B	Apoptosis	Caspase 8 ?	Virions spreading into the liver	Increasing inflammation; fibrosis; cirrhosis
E2 ?	Cell proliferation	Rb MAPK/ERK (unknown precise target) Wnt/ β -catenin (unknown precise target)	Increasing the number of viral virions and their spreading into the liver	HCC
?	Glycolysis	HIF-1	Supplying energy for HCV production	HCC; steatosis
Core protein Some NSP proteins	Lipid metabolism	<i>ANGPTL-3</i> (gene) <i>PPAR-α</i> (gene)	Assembly and release of HCV virions	HCC

*Protein (if not indicated otherwise). ?Precise HCV protein is unknown.

NSP, non-structural; HCC, hepatocellular carcinoma; *ANGPTL-3*, angiopoietin-related protein 3; *PPAR- α* , Peroxisome proliferator-activated receptor.

apoptosis of the hepatocytes. It should be noted that the death of hepatocytes during HCV infection occurs mainly as a result of exposure to leukocytes and not the cytopathic effect of HCV. In a study [51] using hepatocyte cell culture, it was demonstrated that the hepatitis C core protein activated the mitochondrial apoptosis pathway through the opportunistic activation of caspase 8. Another study that used a cell culture model demonstrated that the HCV structural protein E2 was able to induce mitochondrial-dependent apoptosis, which is the opposite of the effect shown in Section 4.1.5., and can be associated with both the level of E2 expression and the cellular factors that can modulate its activity in different ways [14]. HCV infection is associated with an increase in oxidative stress and with changes in intramitochondrial Ca^{2+} (mt- Ca^{2+}) homeostasis. The overexpression of specific HCV proteins (mainly the core protein) leads to an increase in ROS production, which accompanies other mitochondrial dysfunctions [52]. The disease development in patients with chronic hepatitis C is modeled on the level of oxidative change and the mt- Ca^{2+} load. Thus, low levels of ROS and oxidative stress, which are dependent on mt- Ca^{2+} , activate a survival-promoting adaptive response and a proliferative adaptive response via redox signaling [53]. It was shown that various HCV proteins could inhibit ETC complex I, which led to an increase in ROS production and an increase in oxidative stress in the cell [54]. Elevated levels of ROS are initiating signals that trigger apoptosis. Thus, an increase in the apoptosis of hepatocytes leads to the growth of DAMPs (damage-associated molecular patterns), which form as a result of the destruction of cells and biomolecules under oxidative stress. This further stimulates inflammation and the subsequent healing reaction, which, when spreading throughout the damaged liver, can lead to the development of fibrosis and cirrhosis.

4.2.2 Effect of Hepatitis C Virus on Metabolism and Further Complications

As it was described in Section 4.1.4., the main metabolic change in acute HCV infection is associated with a shift in energy production, from oxidative phosphorylation to glycolysis. In the chronic phase, HCV continues to use cellular resources, including its energy substrates, which are necessary for RNA replication, the translation of HCV proteins, and capsid assembly. Rapid glycolysis, in this situation, remains the preferred way of obtaining energy. In the chronic phase of infection, the number of cells with preferential glycolysis increases, which is a similar situation to the Warburg effect in cancer cells. Additionally, HCV proteins are able to activate the HIF-1 hypoxia transcription factor, which increases the expression of glycolytic enzymes [55]. Plastic metabolism is also activated and occurs through the pentose phosphate pathway, whose increase is also observed during tumor development [48]. In addition, HCV proteins can directly affect cell proliferation. Thus, the HCV E2 protein can activate the MAPK/ERK signaling pathway, which leads to increased hepatocyte proliferation [56]. The HCV NS5B protein is able to bind to the retinoblastoma (Rb) protein, which also leads to increased hepatocyte proliferation [57]. It is known that HCV proteins are able to modulate the transcription of 15 genes associated with the regulation of cell proliferation, including genes involved in the Wnt/ β -catenin signaling pathway [58]. All these factors increase the risk of developing hepatocellular carcinoma (HCC), which is one of the most severe complications of HCV infection. The presence of mitochondrial dysfunction in HCC cells, revealed in the form of the deactivation of ETC enzymes [59], can serve as evidence of a metabolic shift toward glycolysis, via the HCV protein effect on mitochondrial ETC proteins. A change in carbohydrate metabolism leads to the accumulation of a number of metabolites, such as acyl-CoA, which

is also an intermediate participant in triglyceride synthesis reactions, leading to the progressive formation of lipid droplets [60]. In addition, the formation of lipid droplets is directly enhanced by HCV proteins: Core and NS5A. This process is important for the assembly and release of HCV virions from infected cells [61]. Other factors, such as the activation of HIF-1 and sterol regulatory element binding protein (SREBP-1c), whose activity increases during HCV infection, may enhance the de novo lipogenesis. Other factors causing lipid metabolism disorders occur through the modulation of ANGPTL3 transcription, via the HCV core protein and a decrease in PPAR- α transcription that occurs as a result of an exposure to the non-structural (NSP) HCV proteins, which leads to a decrease in the fatty acid β -oxidation [61]. Overall, this may explain the development of steatosis, which is also one of the complications of a chronic HCV infection [55]. Mitochondrial and other cellular targets of HCV that occur during the chronic phase of infection are summarized in Table 2.

4.3 Future Directions

As it can be seen from the analysis of the potential HCV protein targets, the action of HCV is based on either the inhibition of the activity of the proteins or the inhibition of their expression, by modulating the activity of their genes. Future research should be directed to the selection of mimetic proteins that, like HCV proteins, will bind to their cellular protein targets without exerting an inhibitory effect. The second strategy is related to the search for suitable transcriptional activators and enhancers, which can compete with HCV proteins for binding to the promoter of a key target gene. It is also necessary to elucidate the clear mechanisms through which some HCV proteins modulate cellular processes. For example, under what conditions does the E2 protein initiate apoptosis, and under what conditions does E2 inhibit it? In our opinion, the third task for future research is the creation of a complete interactome, which will be a detailed scheme of interactions between HCV proteins and cellular proteins. Fourth, it is worth paying attention to ways that normalize the metabolism in T cells since their metabolic dysfunction has been shown in chronic HCV infections [62]. Another important direction for future research, in our opinion, is the discovery of new potential epitopes of HCV proteins by studying the interaction between HCV and cellular proteins. It would be helpful for the development of HCV vaccines, which can be initially tested through bioinformatic methods, as demonstrated in the study by [63].

5. Conclusions

HCV proteins are able to interact with mitochondrial proteins and their genes to inhibit their activity and reduce their expression. This leads to the modulation of several cellular processes, which are associated with the action of mitochondria, such as metabolism, antiviral immune re-

sponses, mitophagy, mitochondrial division, and apoptosis. The modulation, induced by HCV proteins, may differ between the acute and chronic phases of the infection. During the acute phase, HCV hides from the immune system and “protects” the infected cells from death. However, when the chronic phase begins, HCV infects large areas of the liver, thereby initiating apoptosis. Furthermore, in the chronic phase of the infection, the shift in energy metabolism from oxidative phosphorylation to glycolysis and the initiation of hepatocyte proliferation represent the factors that are involved in the development of hepatocellular carcinoma. The resulting glycolysis leads to the disruption of lipid metabolism, which can lead to steatosis. The induction of oxidative stress by HCV leads to an increase in inflammation and the risk of cirrhosis. To combat HCV, a strategy has been proposed to develop drugs that will inhibit the binding of the HCV proteins to the mitochondrial proteins or genes, which can help block disturbances in normal cellular homeostasis and promote a rapid elimination of HCV, even in the acute phase of the infection.

Availability of Data and Materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Author Contributions

AB, VS and AO designed the review plan. VO analyzed the data. AB, AP, MP and AO wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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