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Hepatitis C virus manipulates humans as its favorite host for long term relationship

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Abstract

Chronic hepatitis C virus (HCV) infection associated liver disease is a global health problem. HCV often causes silent disease, and eventually progresses to end stage liver disease. HCV infects hepatocytes, however initial manifestation of liver disease is mostly displayed in hepatic stellate cells causing fibrosis/cirrhosis, and believed to be occurring from inflammation in the liver. It is still unclear why HCV is not spontaneously cleared from infected liver in the majority of individuals and develops chronic infection with progressive liver disease. Direct-acting antivirals (DAAs) show excellent results in controlling viremia, although beneficial consequence in advanced liver disease remains to be understood. In this review, we highlight the current knowledge that has contributed in our understanding on the role of HCV in inflammation, immune evasion, metabolic disorders, liver pathogenesis, and efforts in vaccine development.

HCV replication

HCV is hepatotropic, and belongs to the *Flaviviridae* family. HCV genome containing a positive strand RNA was identified almost 30 years ago (1). The genome is ~9.6 kb long with a single open reading frame, and 5' and 3' untranslated regions (UTR) flanking with specific RNA structure for viral RNA replication and translation (Fig. 1). HCV has been classified into 7 genotypes and several subtypes based on the genomic sequence variations (2). HCV genotypes 1, 2 and 3 are the most prevalent than other genotypes. HCV genome encodes a polyprotein of ~3,000 amino acids and is cleaved by cellular and viral proteases. Three structural proteins (Core, E1 and E2) are cleaved by cellular proteases, and the seven nonstructural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) are cleaved by virus encoded proteases (3, 4). HCV Core protein encapsidate the positive strand viral genome. In addition, several investigators have shown multiple functional properties, including oncogenic potential of Core protein (5). HCV envelope glycoproteins, E1 and E2, bind to the host proteins (designated as binding factors, co-receptors or receptors) and promote virus entry into cells. P7 is a viroporin and involved in HCV assembly. HCV non-structural proteins, NS2-NS5B, play a role in virus replication and assembly. Several host factors are

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involved in the virus replication and assembly processes, which are eloquently discussed in other reviews (3, 4, 6). HCV exploits autophagy machinery for virus growth (7–9). LC3 lipidation is a key event in autophagosome formation and is induced upon HCV infection. Autophagosomes also serve as a platform for HCV RNA synthesis (9), suggesting that the influence of autophagy on HCV replication is complex. Matured HCV particles are released from hepatocytes primarily through secretory pathways (4). Interestingly, exosomes - a component of microvesicles - carry viral genome and help in viral spread to naïve hepatocytes (10–12). Generation of infectious HCV is very much restricted in human hepatocytes, even though difficult to grow in culture. HCV genotype 2a growth in cell culture was first developed by Wakita et al (13), and limited HCV genotype 1a growth was also reported (14, 15).

Despite a number of obstacles, major global research effort from the scientific community has illuminated many aspects of HCV life cycle facilitating the development of DAA with a sustained virological response (16, 17). These antivirals consist of NS3/4A protease inhibitors (inhibit protease activity), NS5A phosphoprotein inhibitors and non-nucleoside polymerase inhibitors (inhibit biogenesis of membranous web and assembly), and nucleoside and nucleotide NS5B polymerase inhibitors (block viral RNA synthesis).

Innate immune response to HCV infection

INTERFERON RESPONSE

To understand the mechanism how HCV antagonizes IFN signaling, several viral proteins were studied. HCV E2 and NS5A proteins interact with PKR and disrupt eIF2 α phosphorylation (18). HCV infected hepatocytes display upregulation of total STAT1 without detectable phosphorylated STAT1, and modest activation of ISRE promoter (19). HCV Core protein induces suppressor of cytokine signaling 3 (SOCS3) and SOCS1 expression, which blocks STAT1 function. In early phase of infection, HCV NS3/4A cleaves host protein mitochondrial antiviral signaling protein (MAVS) and fails to transduce RIG-I/MDA5 signal for IRF3-IFN- β activation (18). Other study showed that interference of mitochondrial fission significantly increases ISRE activities (20), suggesting HCV evolved strategies, independent of NS3/4A, in modulating innate immune responses.

The level of IFN- α production in HCV infected patients varies. IRF-7, one of the IFN-stimulated genes (ISGs), plays a major role in IFN- α production. IRF-7 undergoes phosphorylation when activated and translocates into the nucleus. IRF-7 amplifies the type I IFN response by inducing expression of IFN- α , which also acts in both autocrine and paracrine manners through the IFN- α/β receptor. Interestingly, IRF-7 remains localized in the cytoplasm of HCV infected hepatocytes (19). The initial burst of IFN expression following HCV infection may occur from uncoating of the virus genome and RNA replication. However, this activation may not be sufficient to trigger high enough antiviral responses for HCV clearance, as virus infection fails to translocate IRF-7 and inhibits IFN- α synthesis. HCV infection also inhibits ISGF3 complex formation by targeting PP2A in IFN signaling pathway (21).

microRNAs (miRNAs) are small non-coding RNAs and play important role in several signal transduction pathways, including IFN signaling. miR-130a is elevated in HCV infected liver tissues and cells and interfere with IFN signaling by targeting IFITM1 (22). Further, HCV infection induces miR-373 in hepatocytes which directly targets JAK1 and IRF9, and negatively regulates type I IFN signaling pathway (23).

Pathogen recognition receptors (PRRs) recognize viral pathogen-associated molecular patterns (PAMPs) during acute virus infection (18, 24, 25). The RIG-I-like receptors (RLRs) are cytoplasmic RNA helicases that function as PRRs for the recognition of HCV RNA following virus infection. The length of the poly-uridine core (U-core) within the poly-U/UC tract of the 5'-triphosphate of HCV RNA is essential component for RIG-I recognition, and activates transcription factors and type I and type III IFN production, triggering innate antiviral immunity to HCV infection. Thus, HCV interferes with the IFN pathway at many different levels for establishment of persistent infection (Fig. 2).

AUTOPHAGY

HCV induced autophagy was shown by several investigators. The question is whether HCV induced autophagy is friend or foe for the virus for establishment of chronic infection and IFN signaling. Beclin-1 or ATG7 (key molecule of autophagy signaling) knocked-down hepatocytes when infected with HCV activates type I IFN signaling pathway, resulting in an inhibition of viral growth (26). A different study demonstrated that suppression of autophagy by depletion of ATG5 or chloroquine treatment of HCV infected cells enhance IFN signaling pathway. HCV-induced autophagy also depletes TRAF6, which in turn suppresses NF- κ B activation, induces proinflammatory cytokines (IL-6 and TNF- α), and enhances HCV replication (9). IFN- λ 1 treatment inhibits autophagic activity in Huh7 cells by downregulating the expression of ATG5 and aminobutyric acid receptor-associated protein through miR-181a and miR-214, and contributes to anti-HCV activity (27). Together these results support the notion that HCV induced autophagy favors virus growth by negatively regulating IFN response in host cell.

INFLAMMASOME ACTIVATION

Inflammasome is a multi-protein complex and a player of innate immune system. Inflammasome responds to PAMPs after exposure to microbial infection (28). IL-1 β and IL-18 are the important players in inflammasome processes. High level of IL-18 is detected in the acute phase of HCV infection. HCV induces secretion of IL-1 β /IL-18 in THP-1 cell line (a macrophage cell-culture model), human PBMC derived macrophages, and primary human Kupffer cells (liver resident macrophages) (29–31). Induction of these proinflammatory cytokines occurs via the NF- κ B signaling pathway, suggesting that HCV initiates inflammasome signal 1 pathway in macrophages. In fact, HCV p7 RNA alone can induce IL-1 β from macrophages which can be inhibited by KCl or amantadine- ion channel blocker (29). In agreement, HCV-incubated macrophages reduces IL-1 β maturation upon pretreatment with a potassium channel inhibitor (30). HCV poly(U/UC) RNA transfected into macrophages also induces IL-1 β secretion (24). The biological outcome of HCV induced inflammasome in macrophages is still under investigation. Induction of IL-1 β /IL-18 may have positive or negative influence in hepatic inflammation and disease outcome.

Interestingly, HCV induced IL-1 β from macrophages does not induce inflammation or activation in human hepatic stellate cells (32). However, HCV does establish chronic infection. Therefore, it is possible that the production of IL-1 β /IL-18 may not be enough for virus clearance, thus leading to chronicity. Together these observations implicate HCV employs multiple strategies for triggering inflammasome activation in macrophages.

NATURAL KILLER CELL REGULATION

Hepatic resident natural killer (NK) cells expand and/or recruit NK cells from the blood during infection. NK cells induce early innate immune response to infection from pathogens. These cells either directly target infected hepatocytes or act indirectly by influencing other immune cells, such as DCs or T cells for virus clearance. A human study suggests sustained NK cell activation for protection against HCV infection (33), although the role of NK cells in HCV infection, especially in the context of viral control, remains to be understood. A rapid and strong NK cell response at an early stage of infection may result in T cell responses for virus clearance. However, chronic HCV infection associates with dysfunctional NK cell phenotypes. NK cells secrete IFN- γ as a major cytokine, and the lack of IFN- γ production by NK cells impairs HCV clearance. Acute phase of HCV infection activates NK cells, while chronic infection impairs NK cell frequency, phenotype and function. A polarized NK cell phenotype is induced from chronic HCV infection and IFN- α production. This phenotype may contribute to liver injury by TRAIL expression and cytotoxicity. NK cells from HCV infected patients express inhibitory receptors and cytokines, such as TGF- β and IL-10, in attenuating adaptive immune response (33, 34). NK cells exposed to HCV infected hepatocytes fail to increase complement synthesis for inhibition of major histocompatibility complex class I-related chains A and B expression as a ligand and cell killing. NKG2D expression is lower in circulating NK cells from chronically HCV infected patients. Thus, impairment of NK cell function in chronic HCV infected humans may significantly impact on innate immune response.

COMPLEMENT REGULATION

The complement system is a group of proteins and works for the innate and adaptive immune responses. Complements play a role in inflammation by promoting dendritic cell mediated NK cell activation (35–37). Complement receptor gC1qR expression on CD4⁺T cells influences the outcome of HCV infection (34). The frequency of gC1qR⁺CD4⁺ T cells increases and maintains during HCV infection, while individuals resolving HCV infection do not. C3 complement component is an important mediator of the antibody and T-cell immune responses. C3 facilitates antigen uptake/presentation and immune cell priming. During a systemic viral infection, C3 activity is required for optimal CD8⁺T cell expansion. Serum C3 level is depleted in HCV-infected cirrhotic patients. HCV E2 envelope glycoprotein suppresses C3 expression and impairs macrophage and DC maturation for antigen presentation and CD4⁺T cell stimulation (37, 38).

HCV induces CD55 as a negative regulator of complement activation, and suppresses C3, C4 and C9 complement components of the membrane attack complex. Upregulation of CD55 expression on cell surface or secretion by HCV core protein inhibits complement dependent cytotoxicity (39). Antibodies against cancer cell surface proteins enhance

complement dependent cytolysis or antibody-dependent cell-mediated cytotoxicity. HCV induced cell surface CD55 and secretory CD55 expression in the tumor microenvironment limit complement-mediated damage of HCV infected cells favoring cell survival and growth promotion. Together, the information highlight the co-operative approach of individual HCV proteins in the control of host NK cells and complement components or complement associated CD55 protein functions for protection of tumor cell killing.

Regulation of adaptive immune response by HCV

HCV has developed strategies to escape immune responses in most infected humans. Hepatic antigen-presenting cells, primarily Kupffer cells and DCs, present virus derived epitopes to both CD4⁺T and CD8⁺T cells in the context of MHC class II and MHC class I, respectively, in orchestrating adaptive immune responses. HCV induces IL-10 in monocyte derived DCs, and inhibits DC-mediated antigen-specific T-cell activation. HCV clearance by individuals display broad CD4⁺T-cell responses, stronger T-cell proliferation, and IL-2, IFN- γ , TNF- α production than individuals developing chronic infection dominated by Treg and IL-10 production (40–42). Strong HCV-specific CD8⁺T-cell responses are generated in patients with a self-limited infection unlike chronic infection (43). Increased HCV-specific memory T-cells persist in spontaneously resolved infections. Success of DAA treatment for HCV eradication and the nature of host immune status associated with virus recovery were recently reported (44–46). Available information suggests baseline innate immune response contributes to a successful DAA therapy. DAA treatment may result in expansion of lymphocytes and redifferentiation towards effector-like phenotype. This effect, particularly on CD4⁺T cells, may have impact on CD8⁺T cells. Specific restoration of proliferative HCV-specific CD8⁺T cells under DAA therapy was observed. A recent observation suggests that functional virus-specific T-cell proliferation may even predict sustained virologic response for treatment among individuals (47). HCV alters antigenic sites/epitopes to avoid recognition by T cells and antibodies to escape immune surveillance. The generation of escape variants is one of the most potent immune evasion strategies utilized by HCV (48). HCV-specific CD4⁺T-cell immune deficiency is the primary cause of CD8⁺T-cell functional exhaustion. Increased expression of checkpoint blockers, such as PD-1 and CTLA4, and an imbalance between Th17 and Treg cells impair HCV-specific CD4⁺T-cell help (41, 49, 50). Combined PD-1 and CTLA-4 blockade helps to rescue their *in vitro* function (51). Genetic factors, including KIR2DL3 gene combined with its ligand HLA-C1 allele determine the fate of acute HCV infection (52). HLA-B27, HLA-B57 and HLA-A3 allotypes associate with spontaneous HCV clearance (53). Together, these results implicated the important role of HCV-specific cellular immune responses in protection.

HCV associated metabolic disorders

Chronic HCV infection is associated with increased rates of insulin resistance, diabetes, and steatosis (54–56). Liver steatosis is most common among patients infected with HCV genotype 3, possibly due to direct effect of viral proteins, and partially related to the metabolic disorders. HCV mediated insulin resistance involve multiple processes, including upregulation of inflammatory cytokines, phosphorylation of IRS-1, up-regulation of gluconeogenic genes like G6P, PCK2, and accumulation of lipid droplets (57, 58).

Several mechanisms are proposed to account for the development of steatosis and fatty liver during HCV infection, and include expression of SREBP-1c and fatty acid synthase, controlled by the forkhead box transcription factor FoxO1 (58). Further, HCV infection significantly decreases both medium-chain acyl coenzyme A dehydrogenase and short-chain acyl coenzyme A dehydrogenase expression, controlled by FoxA2. HCV infection also increases lipid droplet accumulation, perilipin-2 expression, and decreases hormone-sensitive lipase activity. Knockdown of FoxO1 decreases lipogenesis and overexpression of FoxA2 increases β -oxidation. FoxO1 and FoxA2 might be useful therapeutic targets for HCV associated metabolic disorders. HCV infection resembles non-alcoholic steatohepatitis (NASH) from metabolic disorders by the presence of steatosis, serum dyslipidemia, and oxidative stress in the liver. Steatosis often disappear in the genotype 3 patients after treatment and recurs when HCV relapse. HCV infection also induces oxidative stress genes (59). HCV core protein alters mitochondrial function to increase the cellular abundance of ROS, and consequent increase in cellular lipid peroxidation (60). Chronic oxidative stress leads to mitochondrial and chromosomal DNA damage. HCV-associated metabolic disorders, oxidative stress and inflammation result in hepatic fibrogenesis and liver disease progression.

HCV associated inflammation and liver pathogenesis

HEPATIC STELLATE CELL ACTIVATION

Understanding the in-depth molecular mechanisms for HCV mediated pathogenesis are challenging due to absence of an appropriate animal model. Chronic HCV infected individuals often develop liver fibrosis/cirrhosis. The link among inflammation, fibrosis/cirrhosis and hepatocellular carcinoma associated with HCV infection is an emerging area of research. HCV associated HSC activation occurs by multiple mechanisms (Fig. 3). HCV infected hepatocytes secrete macrophage colony-stimulating factor and IL-34 which in turn induces PDGF and TGF- β from monocyte derived macrophages, and activates HSCs from resting or quiescent state (61). Macrophages/Kupffer cells exposed to HCV secret several cytokines and chemokines, including TNF- α , IL-1 β and CCL5 (32). Recognition of HCV proteins (Core, NS3, NS4, and NS5) by TLR4 in Kupffer cells during chronic infection increase TNF- α secretion. TNF- α enhances inflammasome markers in primary human HSCs, but does not activate fibrosis marker genes (32). On the other hand, CCL5, secreted from HCV exposed macrophages/Kupffer cells, enhances inflammation and activation of primary human HSCs. Clinically, HCV-infected individuals display upregulation of CCL5 in sera and liver (62), and correlates with HCV RNA load and histological activity index, with or without bridging necrosis and portal inflammation. CCL5 recently gained attention as a antifibrotic target and is in clinical trial.

HCV infection initiates an epithelial–mesenchymal transition state and tumor-initiating cancer stem–like cells (TISCs) in human hepatocytes (63). HCV-induced TISCs, when implanted into immunodeficient mice, activate stromal fibroblast (63, 64). TGF- β , a multifunctional cytokine, secreted in conditioned medium from HCV-infected human hepatocytes activates fibrosis-related markers in HSCs (32), further suggesting its role in fibrosis activation. Exosomes are extracellular vesicles and are considered important

mediators of cell-cell communication by delivering the information they carry to neighbouring cells (65, 66). In the liver, exosomes carrying miRNAs may take part in fibrosis by modulating epigenetic regulation of HSCs (67). Exosome-mediated intercellular transfer of connective tissue growth factor is also implicated in fine-tuning of liver fibrosis. Exosomes carrying miR-19a shuttles from HCV-infected hepatocytes to HSCs, and targets SOCS3 to induce STAT3-TGF- β 1 axis for fibrogenic activation (66).

POTENTIATION OF HEPATOCELLULAR CARCINOMA

Immune mediated inflammation during chronic HCV infection is implicated to trigger hepatocellular carcinoma (HCC), although the mechanism is not well understood. Several HCV proteins exhibit oncogenic potential. HCV Core protein promotes metabolic disorder, immortalizes primary human hepatocytes, enhances reactive oxygen species (ROS) formation, and cell growth (59, 68). Transgenic mice with liver specific core protein expression display HCC. HCV NS5A protein is involved with a variety of host signal transduction pathways such as anti-apoptosis, ROS production, immune evasion and cell proliferation. HCV NS5A binds to tumor suppressor p53, translocates into cytoplasm and disrupt p53 activity (5). Further, HCV core and NS5A proteins inhibit TNF- α mediated apoptosis. Other HCV proteins, such as E2, NS2, NS3 and NS5B, are also involved in cell growth regulation by interacting with host proteins. HCV-associated metabolic disorders, reactive oxidative stress and inflammation result in fibrogenesis, cirrhosis, and create genomic instability or pro-oncogenic microenvironment (Fig. 4). Therefore, long time interaction of HCV proteins with host cellular proteins and involvement in signaling cascade may promote malignant transformation.

Vaccine development

HCV exists as multi-genotypes, subtypes and quasispecies. Thus, an effective vaccine for prevention of HCV infection is a challenging task. An ongoing effort is underway for vaccine development against HCV. An earlier phase I vaccine trial with recombinant HCV E1/E2 envelope glycoproteins did not induce a strong immune response in the majority of the healthy volunteers (69). Only a small number of neutralization positive sera showed cross-protection with other HCV genotypes. In a different study, E1 therapeutic vaccination using recombinant envelope glycoprotein in chronically infected patients showed improved liver histopathology (70). However, therapeutic vaccination might be challenging for already established immune modulation by HCV in a chronically infected host. The importance of broadly neutralizing antibody response and breadth of cellular immune responses for protection against HCV are eminent from the already available information in the literature. A human monoclonal antibody to discontinuous epitope outside CD81 binding site of E1/E2 complex display broad neutralizing activity to diverse HCV genotypes and protects from heterologous challenge infection in a small animal model (71). The use of broadly neutralizing antibodies inhibiting HCV infection in human liver chimeric mouse model also suggested therapeutic potential (72), especially for preventing cell to cell spread of infection.

HCV E2 induces immune regulatory cytokine IL-10 and CD163 protein expression and enhances STAT3 and suppresses STAT1 activation in macrophages, suggesting the

possibility for macrophage polarization more like towards M2 phenotype (37). Further, E2 suppresses C3 complement expression, suggesting potential for impairment of immune cell priming. E2 associated macrophage polarization depends on the interaction with CD81 for EGFR activation. HCV E2 also inhibits the function of NK cells, B cells and T cells by binding with CD81 (73). Discrete domains within E2, rather than using the entire ectodomain or intact E2 in a vaccine preparation may prove to be more effective in eliciting a stronger protective immune response.

Predominant CD4⁺T-cell epitopes in HCV non-structural proteins are immunogenic in the context of class II molecules. Replication-defective adenoviral constructs, chimpanzee adenoviral vector, and modified vaccinia Ankara vector are in use as vehicle for vaccine development using HCV non-structural regions in prime-boost regimens of human trial (74, 75). Sustained and balanced memory and effector T-cell responses were noted using prime and boost vaccination strategy. Human efficacy study is underway in a large cohort of high-risk individuals. However, challenges remain for distinct T-cell specificity among different HCV genotypes (76) and the duration of protective immune response. Cocktail DNA vaccine elicits strong cellular immune response to the non-structural proteins in mice (77), suggesting potential for universal HCV vaccine development. Immunization with E1, selected regions of E2, and NS3-NS4a by appropriate delivery to immune system may help in generation of HCV cross-genotype specific broad neutralizing antibodies and T-cell responses.

Conclusions

HCV research made an extraordinary progress from discovery of genome to anti-viral agents in less than 25 years. Despite of major obstacles, including lack of virus growth in cell culture in the early years and absence of suitable small animal model for infection as the major tools for research, we have learned numerous biological functions of HCV RNA and proteins over the past several years. Majority of the research efforts were rationalized based on the use of molecular biology and immunology techniques. The use of HCV genomic regions for expression in cell culture, experiments from limited available chimpanzees and genetically modified small animal models, and clinical studies from HCV infected humans have significantly advanced HCV field. Obviously, the road to success was challenging. A major achievement of HCV research is the development of interferon-free DAA with a sustained virological response. Current DAA therapy is well tolerated and works on pan-genotypes in eradicating HCV RNA. However, one major hurdle for HCV infected individuals is early diagnosis for treatment. We do not have way to know who are infected, since HCV infection is asymptomatic and causes silent liver disease. Screening for HCV in high prevalence group and efforts to discover a potential biomarker for progressive liver disease would help in early treatment. miRNAs may act as a signature molecule for prediction of HCV chronicity and work is in progress for identifying predictive biomarkers. Further, DAA treatment cost, potential for reinfection, generation of mutant virus, reactivation of other viruses, and end stage liver disease progression even after virus clearance raise concerns.

Here, we have discussed numerous ways by which HCV manipulates humans as its favorite host for long term relationship and liver damage. We have learned that HCV induced miRNAs play a role in impairment of IFN signaling and induction of autophagy, which protect the virus from initial host cell insult. Exosomes are secreted from healthy, neoplastic and virus infected cells, and gaining attention as a cargo for transport of numerous biologicals by intercellular communications. Primary human HSCs, when exposed to exosomes carrying miRNA from HCV infected hepatocytes, display fibrogenic activation, implicating exosomes carry sensors. Further study of exosomes carrying stimuli will provide a rich area to enhance our understanding of the role of exosomes in HCV persistence and pathogenesis. Given that multiple mechanisms operating for impairment of innate immune system, HCV also triggers proinflammatory cytokine production for liver pathogenesis. It is still a mystery how HCV overcomes all the barriers of innate immunity pathways for establishment of chronic infection, and would require more precise understanding on virus and cell interactions for targeted novel therapy to inhibit liver disease progression. A global need for comprehensive strategy to control HCV infection requires effective and safe vaccine. The diversity of viral genome and the lack of an immunocompetent small animal model are the major obstacles for HCV vaccine development, and work is in progress to overcome these challenges. Thus, understanding host and viral factors will provide important information for successful vaccine-mediated protection and novel therapeutic approaches for HCV associated liver disease.

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Abbreviation:

DA	direct acting anti-viral
HCV	hepatitis C virus
HCC	hepatocellular carcinoma
HSC	hepatic stellate cell
IFN	interferon
ISG	interferon stimulating gene
IRES	internal ribosome entry site
KC	Kupffer cells
MAVS	mitochondrial antiviral protein
microRNA	miRNA

NK	natural killer
PAMP	pathogen-associated molecular patterns
PPR	pathogen recognition receptor
SOCS3	suppressor of cytokine signaling 3
STAT	signal transducer and activator of transcription
TISC	tumor-initiating cancer stem cell
UTR	untranslated region

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FIG. 1.

Hepatitis C virus genome and proteins. The single-strand HCV RNA of ~9.6 Kb genome is shown. The 5' untranslated region (UTR) contains an internal ribosome entry site (IRES). The 3' UTR contains a variable poly(U/UC) tract, followed by a conserved ~100 nucleotides that constitute the 3' X-tail sequence. The IRES-mediated translation of the open reading frame leads to a single polyprotein, which is processed to ten viral proteins by cellular proteases (shown by purple and pink arrows) and viral protease (shown by blue or green arrows). Three structural proteins (Core, E1 and E2) are processed from the amino terminus of the polyprotein. The nonstructural proteins p7 (viroporin - an ion channel) and NS2 (a cis-acting protease) are important for virus morphogenesis. Other nonstructural proteins: NS3 (protease and helicase), NS4A (protease cofactor of NS3), NS4B (integral membrane protein), NS5A (phosphoprotein) and NS5B (RNA-dependent RNA polymerase) are required for RNA replication. Direct acting antivirals targeting HCV proteins are shown at the bottom.

FIG. 2.

HCV viral proteins impairing interferon signaling pathways. HCV targets several pathways in interferon signaling. HCV NS3/4A protein blocks IFN- β induction through cleavage of the host proteins MAVS and TRIF. HCV core protein activates SOCS1/3, which in turn blocks STAT1 activation. PAMPs, present in the HCV RNA, are also sensed by RIG-I and MDA5 following virus infection. IRF-7 fails to translocate in nucleus and activate IFN- α signaling in HCV infected cells. HCV induced miR-130a inhibits IFITM1 expression, while miR-373 targets JAK1 and IRF9 resulting in inhibition of ISG activation.

FIG. 3.

Cross-talk among HCV infected hepatocytes with other liver cells. HCV released from infected hepatocytes when exposed to Kupffer cells (KC) secrete proinflammatory cytokines/chemokines, including TNF- α and CCL5. These mediators promote quiescent hepatic stellate cells (HSCs) to activated HSCs. On the other hand, HCV infected hepatocytes secrete TGF- β , which potentiates activation of quiescent HSCs. Exosomes carrying miRNAs, such as miR-19a, secreted from HCV infected hepatocytes also activate quiescent HSCs.

FIG. 4.

HCV persistence and liver disease progression. Chronic HCV infection induces multistep processes involving metabolic disorders, steatosis, cirrhosis and hepatocellular carcinoma (HCC). Key biochemical and immunological changes occurring during the processes are shown on the right.