Review

Oncogenic Potential of Hepatitis C Virus Proteins

Arup Banerjee [1], Ratna B. Ray [2] and Ranjit Ray [1,3,]*

[1] Department of Internal Medicine, Edward A. Doisy Research Center, 1100 S. Grand Blvd.,
8th Floor, St. Louis, MO 63104, USA; E-Mail: abanerj1@slu.edu

[2] Department of Pathology, Edward A. Doisy Research Center, 1100 S. Grand Blvd., 2nd Floor,
St. Louis, MO 63104, USA; E-Mail: rayrb@slu.edu

[3] Molecular Microbiology & Immunology, Edward A. Doisy Research Center, 1100 S. Grand Blvd.,
8th Floor, St. Louis, MO 63104, USA

* Author to whom correspondence should be addressed; E-Mail: rayr@slu.edu;
Tel.: 1-314- 977-9034; Fax: 1-314-771-3816.

Abstract: Chronic hepatitis C virus (HCV) infection is a major risk factor for liver disease progression, and may lead to cirrhosis and hepatocellular carcinoma (HCC). The HCV genome contains a single-stranded positive sense RNA with a cytoplasmic lifecycle. HCV proteins interact with many host-cell factors and are involved in a wide range of activities, including cell cycle regulation, transcriptional regulation, cell proliferation, apoptosis, lipid metabolism, and cell growth promotion. Increasing experimental evidences suggest that HCV contributes to HCC by modulating pathways that may promote malignant transformation of hepatocytes. At least four of the 10 HCV gene products, namely core, NS3, NS5A and NS5B play roles in several potentially oncogenic pathways. Induction of both endoplasmic reticulum (ER) stress and oxidative stress by HCV proteins may also contribute to hepatocyte growth promotion. The current review identifies important functions of the viral proteins connecting HCV infections and potential for development of HCC. However, most of the putative transforming potentials of the HCV proteins have been defined in artificial cellular systems, and need to be established relevant to infection and disease models. The new insight into the mechanisms for HCV mediated disease progression may offer novel therapeutic targets for one of the most devastating human malignancies in the world today.

1. Introduction

Over 200 million people are estimated to be infected with hepatitis C virus (HCV) worldwide, reflecting the unique capacity of this virus to establish long-standing, persistent infection. Within the United States, HCV infection is the leading cause of chronic hepatitis and cirrhosis, and is an increasingly important factor in the etiology of hepatocellular carcinoma (HCC) [1]. Increased incidence of HCC observed over the past several decades is due to an expansion in the number of individuals chronically infected with HCV [2,3]. HCC typically develops often in the setting of cirrhosis, although the underlying mechanisms for cancer progression remain poorly defined.

While genetic alterations are the predominant mechanisms of oncogenesis, viruses have evolved additional methods to affect the same critical pathways in an attempt to promote viral replication. Viruses often encode proteins that modulate normal cellular processes favoring viral replication [4]. Genomic expression profiling studies have identified varying gene expression for HCC associated with HCV infection [5-7]. HCV mediated HCC may reflect distinct molecular mechanisms, including alteration of normal cellular signaling pathways to stimulate host cell growth, and cellular transformation. Indirect mechanisms, including long-standing hepatic inflammation with associated oxidative stress and the potential for DNA damage, are also likely to contribute to the development of HCC [8-10]. There are strong evidences that the HCV proteins (including core, NS3, NS5A and NS5B) potentiate oncogenic transformation. Expression of these HCV proteins, alone or together, promotes growth, when stably expressed in cells or in transgenic mice [11-16]. The consequences of the host immune response to HCV infection, including immune mediated destruction of infected hepatocytes that induces repeated liver regeneration cycles, may as well be involved in disease progression to HCC. In this review, we discuss how multiple interactions of HCV proteins, especially the core protein, with host-cells contribute to the development of liver cancer in chronically infected patients.

2. HCV genome organization, protein synthesis and life cycle

HCV is classified within the genus *Hepacivirus*, and belongs to the family *Flaviviridae*. The genome of the virus is ~9.6 kb long and contains a long open reading frame, flanked by untranslated 5' and 3' sequences (Figure 1). The untranslated 5' and 3' sequences are important for translation and replication of the viral RNA [17,18]. Six genotypes and more than 50 subtypes have been reported based on HCV genomic sequence variations [19,20]. The positive-strand RNA genome of the virus encodes a single large polyprotein that is co- and post-translationally processed by cellular and viral proteases into at least 10 structural and nonstructural (Core, E1, E2/p7, NS2, NS3, NS4A, NS4B,

NS5A and NS5B) viral proteins. Proteins derived from the amino-terminal third of the viral polyprotein include the three structural proteins: core and two envelope glycoproteins, E1 and E2.
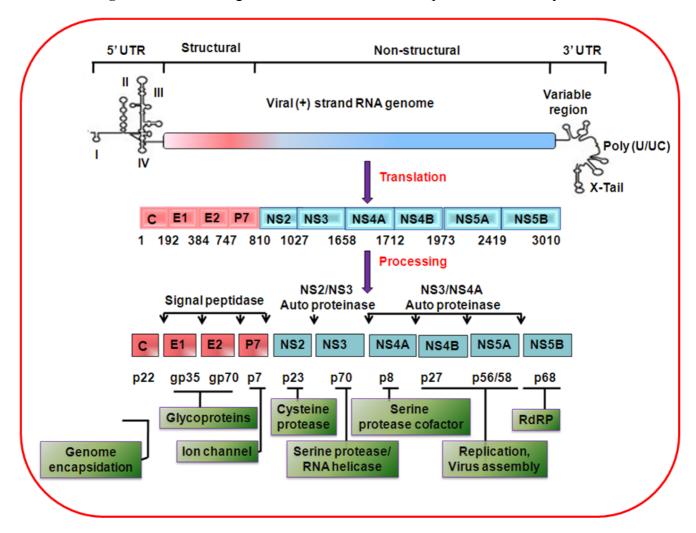
Figure 1. Genomic organization and function of the proteins encoded by HCV.

HCV core is a basic protein with RNA-binding activity that is thought to comprise the nucleocapsid of the virus. Several forms of the core protein of variable molecular weights (17–23 kDa) have been identified [21-24]. Synthesis of proteins encoded from alternative open reading frames from the core genomic region has also been shown [25-28]. However, we did not observe sharing of the major properties of core protein with the translated protein from its alternate open reading frame [29]. HCV core protein has been detected in various subcellular compartments, including cytosol, lipid droplets, endoplasmic reticulum/golgi apparatus, mitochondria, and nuclei. The broad intracellular distribution raises the possibility that HCV core protein may modulate multiple cellular processes [30]. HCV envelope glycoproteins interact with multiple cell surface molecules and LDL-R for orchestration of virus entry into mammalian cells [31,32]. Downstream of these proteins is p7, a small transmembrane protein with ion channel activity. NS2, a non-structural protein, plays a critical role in polyprotein processing and virus assembly. The remaining non-structural proteins (NS3, NS4A, NS4B, NS5A, and NS5B) are required for viral RNA replication [33]. NS3 is a serine protease that is responsible for *cis* or *trans* cleavage at four sites within the HCV polyprotein, thereby generating the amino termini of

NS4A, NS4B, NS5A, and NS5B [34]. NS3 also functions as an RNA helicase and NTPase, and is an essential component of the RNA replicase complex [35,36]. NS4A, a small 54-amino-acid protein, forms a stable complex with the amino-terminal third of NS3, protease domain, and is required for complete serine protease activity [37]. NS4B, an integral membrane protein, is mostly localized on the cytoplasmic side of the ER membrane and is implicated in assembly of the replicase complex on lipid rafts [38,39]. NS5A, a phosphoprotein, plays a role in viral resistance to interferon [40,41]. NS5A also plays a role in RNA replication, and virus assembly [42]. NS5B is the RNA-dependent RNA polymerase, and acts as the catalytic core of the macromolecular replicase complex essential for HCV RNA replication [43,44].

Experimental findings using cloned HCV gene expression in mammalian cells, the development of subgenomic or full-length replicon derived from HCV, and the generation of infectious HCV genotypes 1a and 2a in human hepatocyte derived cell lines [45-52] have significantly contributed to the advancement of HCV research. Recently, autophagy has gained importance as it plays an important role in HCV life cycle. We and others have shown that HCV induces autophagy in hepatocytes [53-56]. HCV may induce accumulation of autophagosomes via the induction of ER stress and the unfolded protein response [54,57]. Similar to poliovirus and coxsackieviruses, the induction of autophagosomes may play an important role in HCV replication, as siRNA-knockdown of autophagy related cellular genes, including Atg7, LC3, Atg4B, Atg12 and Beclin-1, altered HCV RNA replication levels [54-56].

3. Transcriptional modulation and oncogene regulation by HCV

HCV core protein may directly and indirectly interact with numerous transcription factors, including heterogeneous nuclear ribonucleoprotein K [58], leucine zipper transcription factor (LZIP) [59], 14-3-3 protein [60], RNA helicase CAP-Rf [61], p53 [62], p21 [63,64], and NF-kB [65], and RNA helicase DEAD box DDX3 protein [66,67]. HCV core protein aberrantly sequesters LZIP in the cytoplasm to inactivate its function, and potentiates cellular transformation [59]. Development of HCC might be associated with activation of the Ras/Raf/MAP kinase pathway [68]. The 14-3-3 protein family is known to associate with components of several signal transduction pathways, including the Raf-1 kinase cascade [60]. HCV core protein activates Raf-1 kinase through interaction with 14-3-3 protein family. Thus, HCV core protein may play an important role in regulating hepatocyte growth, senescence, and differentiation through its interaction with 14-3-3 protein. Constitutive expression of HCV core protein results in a high basal activity of MAP kinase kinase, as determined by immunodetection of hyperphosphorylated ERK-1 and ERK-2 [69]. HCV core protein also represses p21 promoter activity [63]. Interaction between HCV core protein and DEAD Box protein DDX3 may be involved in HCV replication [70] although recent studies suggest that the requirement of DDX3 for replication is unrelated to its interaction with the viral core protein [71]. HCV core can also modulate the expression of the cyclin-dependent inhibitor p21, which is a major target of p53 and regulates the activities of cyclin/cyclin-dependent kinase complexes involved in cell-cycle control and tumor formation [63,72,73]. HCV core protein suppresses NF-кB activity in TNF-α, PMA, OA, and H_2O_2 treated cells; while upregulates AP1 [69]. Whether activation of AP-1 and suppression of NF-кB by the HCV core are linked to activation of the MAPK pathway is not clear. HCV core protein also

selectively reduces phosphorylated STAT1 accumulation in the nucleus in a proteasome-dependent manner, and impairs IFN-α induced signal transduction via expression of suppressor of cytokine signaling (SOCS)-3 [74-76].

Wnt/β-catenin pathway plays a major role in HCC carcinogenesis [77]. The transcriptional upregulation of both wnt-1 and its downstream target WISP-2 by HCV core protein suggested possible activation of the wnt-1 signaling pathway in the promotion of cell growth. NS5A expression in the context of HCV polyprotein inhibits the Akt substrate Forkhead transcription factor and stimulates the phosphorylation of glycogen synthase kinase-3β, leading to stabilization of cellular β-catenin and stimulation of β-catenin-responsive transcription [78,79]. NS5A protein thus may directly be involved in Wnt/β-catenin-mediated liver pathogenesis

We and others have reported transcriptional regulation of cellular genes, such as p53, c-myc and hTERT by HCV core protein [8,30,62,63,69,80-84]. Expression of wild type p53 significantly diminishes STAT3 phosphorylation, STAT3 DNA binding activity, and inhibits STAT3-dependent transcriptional activity [85]. HCV NS5A activates STAT3 in Huh-7 cells [86] and in transgenic mouse liver [87]. STAT3 activation may not only provide a growth advantage [80,88], but also confer resistance to conventional therapies that rely on the apoptotic machinery to eliminate tumor cells.

4. Hepatocyte growth regulation by HCV proteins

HCV proteins promote cell proliferation by interfering with cellular proteins involved in different phases of the cell cycle. Normal progression through the cell cycle is regulated by sequential activation of cyclin and cyclin-dependent kinase (CDK) complexes. Active cyclin-CDK complexes in G1 phosphorylate the retinoblastoma family of proteins (pRb, p130, and p107) allowing the release of E2F transcription factors and upregulation of cellular genes to positively reinforce progression through this phase of the cell cycle [89]. Importantly, these checkpoints require active p53 and Rb pathways [90,91].

During the G1/S transition, p53 activates transcription of p21 which, in turn, binds to and inhibits CDK2, causing cell cycle arrest while the cell attempts to repair the DNA damage. Anti-growth signals such as checkpoint activation can limit the replication of oncogenic viruses, particularly if the checkpoint is activated in response to viral infection. We and others have shown that HCV NS5A physically associates with p53 and downregulates the cell cycle regulatory gene p21 [92-94]. Another recent study found that the NS5A protein downregulates the expression of the mitotic spindle protein ASPM through the PKR-p38 signaling pathway and induces aberrant mitoses, chromosome instability and HCC [95].

HCV proteins can bind to p53 [96], p73 [97], and pRb [98,99], but the functional consequences of these interactions have not fully been elucidated. HCV core interacts with p73, causes nuclear translocation of core protein and prevents p73-α-dependent cell growth arrest in a p53-dependent manner [97]. Conditional expression of the core protein results in a decreased abundance of Rb in immortalized rat embryo fibroblasts, leading to enhanced E2F transcription-factor activity [98].

NS5B has been shown to form a cytoplasmic complex with Rb in infected cells [99]. NS5B dependent downregulation of Rb leads both to activation of E2F-dependent transcription and to increased cellular proliferation. Another cell-cycle checkpoint, the mitotic spindle checkpoint (MSC),

is also a target for HCV proteins. Significantly, the integrity of Rb appears to be particularly important in the normally quiescent hepatocyte, as liver-specific loss of Rb has been shown to promote ectopic cell-cycle entry and aberrant ploidy [100], which likely contributes to neoplastic transformation. The interaction of the HCV polymerase NS5B with Rb results in the degradation of Rb and activates the *MAD2* promoter [15]. Thus, infection with HCV may lead to a loss of host-cell genomic stability due to deregulation of Rb pathway.

Cross-talk between cellular protein and HCV core protein may be a major risk factor for potentiating HCC. HCV core protein expression alone in a transgenic mouse model was sufficient to induce tumor formation in liver [16]. HCV core can induce spontaneous, persistent, age dependent and heterogeneous activation of PPARα, which may contribute to HCC [101-103]. We also observed that introduction of HCV core protein stimulates primary human hepatocytes to escape from replicative senescence and promotes an immortalized phenotype [104]. Cells retaining an immortalized phenotype display a weak level of core protein expression and exhibit continuous growth. Reactivation of telomerase was observed in the immortalized hepatocytes. HCV core protein introduction resulted in an increase in expression of IL-6, gp130, leptin receptor, and STAT3 [105]. Upregulation of these genes in turn may regulate c-myc and cyclin D1, downstream of the STAT3 signaling pathway, promoting cellular transformation. Repression of the core gene expression in immortalized hepatocytes by a construct of the antisense orientation of the core gene under the control of an inducible metallothionine promoter resulted in apoptosis and characteristic changes in p53, c-myc, and hTERT expression [84]. However, immortalized hepatocytes passaged for a longer time did not display apoptosis from expression of antisense core, likely due to anchorage dependent growth on soft agar, thus proceeding to a transformed phenotype.

A direct role of NS3 was reported in the neoplastic transformation of hepatocytes *in vivo* and *in vitro* [106,107]. Transformation and tumorigenicity occurs upon transfection with HCV NS3 DNA in the non-tumorigenic mouse fibroblast cell line NIH 3T3 into nude mice. HCV NS3 C-terminal-deleted protein also showed transforming and oncogenic potential [108]. Stable expression of the NS3 protein in human hepatocytes induced transformed characters with reduced population doubling time, anchorage-independent growth and tumor development with increase expression of phospho-p44/42 and phospho-p38 proteins. The NS3 protein also forms complexes with p53 [109], and inhibits p21 promoter activity. The NS3 domain of protease and helicase/NTP-ase activity was responsible for the inhibition of p21.

5. Role of HCV proteins in cytokine modulation

Various components of the host immune system are involved in the pathogenesis and outcome of HCV infection. There has been an increasing recognition of the roles played by the cell mediated response, especially the cytokine systems; in the immunopathogenesis of chronic hepatitis C. Disease progression due to persistent HCV infection is usually associated with an imbalance between pro-inflammatory and anti-inflammatory cytokines. The development and resolution of an inflammatory process are regulated by a complex interplay between cytokines that have pro- and anti-inflammatory effects [110]. Conflicting data exists concerning the cytokine profile associated with the development of HCC in chronic HCV infection. Some investigators report that the development of HCC in the

cirrhotic liver is associated with a predominant Th-2 cytokine profile with increased IL-10 expression [110]. HCV also subverts cellular immunity by inducing IL-10, which in turn inhibits the activation of dendritic cells (DC) and development of Th-1 cells [111,112]. Similarly, there are reports of increased Th-1 cytokine in the setting of HCC [113].

A recent study has highlighted the relationship between the activation of genes involved in the IL-6 signaling pathway and the development of HCC [114]. An increase in the β-2 microglobulin serum level as well as IL-6 level was observed among HCV infected HCC patients. Weakening of the immune system, due to IL-6, may be responsible for a more severe progression of HCC and the hyperexpression of β-2 microglobulin [115]. We have recently shown that HCV core protein attenuates IL-6 stimulated acute-phase response, and may contribute to impaired innate immunity for viral persistence [116]. TNF-α plays a diverse role in HCV infection. Activation of TNF-α has a pivotal role in the inflammatory process of chronic hepatitis C, and TNF-α levels correlate with the degree of inflammation [117,118].

HCV core also upregulates the expression of TGF-β [119,120], and NS5A modulates TGF-β signaling through interaction with TGF-β receptor I [121]. As HCV-infected livers progress from chronic hepatitis to cirrhosis and/or HCC, hepatocytic pSmad3L/PAI-1 increases with fibrotic stage and necroinflammatory grade, and pSmad3C/p21 decreases [122]. Therefore, it is possible that chronic inflammation associated with HCV infection shifts hepatocytic TGF-β signaling from tumor suppression to fibrogenesis, accelerating liver fibrosis and increasing the risk of HCC. Another study showed that different thresholds of Smad3 activation control TGF-β responses in hepatocytes and that liver cancer-derived HCV core protein, by decreasing Smad3 activation, switches TGF-β growth inhibitory effects to tumor-promoting responses [123]. A recent study found that HCV core triggers the production of both TGF-β2 and VEGF proteins through multiple pathways, including PKC, RB/E2F1, ASK1-JNK/p38 and ERK [124]. HCV core protein also behaves as a positive regulator in androgen receptor signaling and enhances the expression of VEGF in hepatocytes [125].

6. Role of HCV in oxidative stress and apoptosis

Oxidative stress induced by HCV infection plays a role in the pathogenesis of liver disease. HCV core protein induces oxidative DNA damage, while it inhibits apoptosis accompanied by enhanced ROS production [126,127], indicating two independent functional aspects. Inducible nitric oxide synthase (iNOS) is upregulated in HCV core introduced hepatocytes [105]. iNOS induces the production of total nitric oxide (NO) from L-arginine in inflamed tissues. NO plays an important role in many physiological and pathological conditions, serving as an intercellular and intracellular messenger and antimicrobial agent [128]. NO induces DNA cleavage, and enhances the chance of mutation. This sequence of events may contribute to HCV mediated pathogenesis and oncogenesis [129]. Oxidative stress leads indirectly from DNA damage to p53 induction, which can lead to activation of BAX and apoptosis [130,131].

Apoptosis is a key element in a host organism's defense against viral infections, inhibiting viral spread and persistence. Alterations in cell survival contribute to the pathogenesis of a number of human diseases, including viral oncogenesis [132]. During HCV infection, hepatocyte apoptosis could be induced by immune attack on infected cells or directly by viral infection. Hepatocyte damage plays

a role in the recruitment and activation of stellate cells and macrophages and the subsequent development of fibrosis [133,134]. HCV infected patients have higher levels of immune related death ligands; TRAIL, TNF-α, FAS, and FASL [135-137]. The expression of HCV proteins may inhibit Fas mediated apoptosis and death in mice by repressing the release of Cyt-C from mitochondria, thereby suppressing caspases-9 and -3/7 activation [138]. At least two HCV viral proteins (Core and NS5A) play an important role in modulation of apoptosis [139,140]. Our observations suggest that HCV NS5A protein impairs TNF mediated apoptosis, but not by Fas antibody, in a transgenic mouse model [141]. HCV nonstructural proteins are the key mediators of sensitization to TRAIL. Sensitization to TRAIL was shown to be caspase-9 dependent and mediated in part via the mitochondrial pathway [142], and may contribute to the elimination of virus infected hepatocytes. Earlier study by our group suggested that HCV core mediates a novel mechanism of apoptosis, in which hepatocytes death correlates with an increase in Apaf-1 [143]. The subsequent activation of caspase-9, leading to the initiation of the intrinsic cell death pathway, occurs in the absence of cytochrome c translocation to the cytosol. HCV core protein suppresses apoptosis mediated by TNF-α [144]. A sustained expression of c-FLIP, an endogenous caspase-8 inhibitor, inhibits TNF-α induced apoptotic pathway in HCV core expressing hepatocytes [145]. Apoptotic activity of common chemotherapeutic drugs (5-fluorouracil, doxorubicin or cisplatin) or chemotherapeutic cytokine are highly dependent on the status of p53 [146,147]. HCV core protein mediated modulation of p53 may protect cells from chemotherapeutic drug induced apoptosis, allowing cancer cells to proliferate or survive inappropriately. Cytokine or drug induced apoptosis is modulated by HCV core protein in different cells [63,69,144,148]. We have recently identified an association between HCV core and cellular HAX-1 proteins, which may promote 5-FU mediated p53-dependent caspase-7 activation and hepatocyte growth inhibition [149]. p53 is a critical component for apoptosis. HCV NS5A has also been shown to inhibit p53 induced apoptosis [92,94,150,151]. NS5A interacts with and partially sequesters p53 and hTAF (II), a component of TFIID and an essential coactivator of p53, and suppresses p53-mediated transcriptional activation and apoptosis [152]. NS5A also forms complexes with the TBP and p53 and inhibits the binding of both p53 and TBP to their DNA consensus binding sequences *in vitro*. Further, this may inhibit p53-TBP and p53-excision repair cross complementing factor 3 protein–protein complex formations [94]. NS5A interacts also with Bax as a Bcl-2 homolog and prevents apoptosis in a p53-independent manner [153].

Expression of either HCV genome or individual HCV structural protein (core or E1) induces endoplasmic reticulum (ER) stress [154,155] and the unfolded protein response (UPR), which can lead to apoptosis. Recently, HCV infection in chimeric *SCID/Alb-uPA* mice correlated with increased levels of the ER chaperone GPR78/BiP, a key regulator of the unfolded protein response. In addition, levels of pro-apoptotic BAX were increased, while anti-apoptotic NF-κB and BCL-xL were decreased in HCV infected cells [156]. Therefore, ER stress induced by HCV combined with lower NF-κB and BCL-xL levels may sensitize hepatocytes to apoptosis.

7. HCV associated metabolic disorders and liver disease progression

The metabolic syndrome is a constellation of problems that includes insulin resistance, obesity, hypertension, and hyperlipidemia. Increasingly, components of the metabolic syndrome are being linked to various forms of cancer with respect to both increased risk of disease and worsened outcome.

Experimental studies indicate that insulin resistance occurring in HCV core-transgenic mice is due at least partly to an increase in TNF-α secretion [157]. TNF-α has also systemic effects that result in insulin resistance and type 2 diabetes (T2D). Marked increases in both sTNFR1 and sTNFR2 were demonstrated in HCV-diabetic patients [158]. Possible explanations for the unique association between insulin resistance and HCV infection may be related to differences in the clinical course of liver inflammation and fibrosis, or in the mode of TNF-receptor activation or cleavage. Thus, in the correlation between liver disease and insulin resistance, a link among chronic HCV infection, TNF-α, and T2D possibly exists [159,160].

We have observed that HCV core protein alone or together with other viral proteins upregulates serine phosphorylation of insulin receptor substrate-1 and impair the downstream Akt/protein kinase B signaling pathway for insulin resistance [161]. Insulin resistance is paradoxically associated with a reduced ability of insulin signaling to inhibit glucose production, whereas insulin-stimulated lipogenesis is enhanced in the liver and two transcription factors, FoxO1 and FoxA2 play an important role in this process. A recent study on 165 consecutive patients with newly diagnosed HCC suggested that insulin resistance is associated with HCC in chronic hepatitis C infection [162]. We have shown that HCV can differentially modulate activation of forkhead transcription factors and insulin induced metabolic gene expression [163].

Insulin resistance and subsequent hyperinsulinemia are highly associated with fatty liver disease and is an important risk factor for the progression of fibrosis in chronic hepatitis C [160,164,165]. From the metabolic aspect, hepatitis C resembles non-alcoholic steatohepatitis (NASH) in numerous features, such as the presence of steatosis, serum dyslipidemia, and oxidative stress in the liver [166]. In contrast, there are noticeable differences between hepatitis C and NASH, in that HCV modulates cellular gene expression and intracellular signal transduction, while such details have not been noted for NASH. A recent report suggests that HCV may actively contribute to the fibrogenic process via the paracrine effect of IL-8 secreted by infected hepatocytes. [167].

HCV core protein expression leads to the development of progressive hepatic steatosis (fatty change) and HCC in transgenic mice [168]. Persistent activation of PPARα has also been suggested for the pathogenesis of hepatic steatosis and hepatocellular carcinoma in HCV core expressing transgenic mice [102]. Hepatic steatosis occurs at a high rate (40–86%) in chronic HCV patients, and a close relationship between steatosis and intrahepatic core protein expression has been noted [169]. Insulin resistance is a prominent mechanism linking steatosis and fibrogenesis although this link is complex and poorly understood.

Hepatic stellate cells (HSCs) are one of the sinusoid constituent cells that play multiple roles in liver pathophysiology and, in particular, in liver fibrosis [170]. Liver fibrosis is one of the major complications associated with HCV infection, but the mechanism underlying the molecular basis of HCV-related fibrosis is unclear. Progressive liver fibrosis may eventually lead to cirrhosis and HCC. Insulin resistance is a significant risk factor for hepatic fibrosis in patients with chronic HCV, either directly or by favoring hepatic steatosis. HCV infection generates oxidative stress, TNF-α, and IL-6 production in the liver. Oxidative stress and these cytokines are well known profibrogenic mediators [171]. HCV may induce fibrosis directly either by stimulating secretion of profibrogenic cytokines by hepatocytes, by interacting with sinusoidal endothelium, or by directly provoking fibrogenesis by HSCs.

8. Induction of miRNAs by HCV

miRNAs affect gene silencing via both translational inhibition and mRNA degradation [172]. The expression of host cell miRNAs can be modulated by HCV. Several studies have shown that the expression of miRNAs is altered in human HCC, implicating them in hepatocarcinogenesis. [173]. Abnormally expressed miRNAs may work as functional actors in HCC initiation and progression. miRNAs that are unique to certain virus-related HCC have been identified. By comparing HCV-HCC tissues and adjacent non HCC tissues, 29 differentially expressed miRNAs were identified [174]. Nineteen of these miRNAs are differentially expressed between HBV-HCC and HCV-HCC [175]. Since most of these miRNAs are HCC-associated, these results suggest dual roles of miRNAs in viral replication and HCC development. Among several mRNAs modulated in HCV infected liver tissues, the function of miR122 was extensively studied. MiR-122 enhances HCV RNA translation [176-180]. Studies of miRNA expression in liver tissues of HCV-infected patients showed increased expression of several miRNAs, including miR-122 in HCC tissues when compared with normal adjacent tissues, suggesting that the underlying HCV infection can modulate the expression of miRNAs in cancer [174]. Contrary to this finding of miR-122 upregulation in HCV associated HCC; other investigators have reported a down regulation in hepatoma cell lines with etiologies other than HCV infection [174,181]. Because miR122 closely interacts with the HCV genome and miR-122 expression pattern in HCV associated HCC is directly opposed to non-HCV infected HCC, we speculate that HCV infected transformed hepatocytes are able to circumvent tumorigenic repression of miR-122. HCV dependent modulation of miRNAs expression including miR-122 was also studied in HCV expressing hepatoma cell lines [179,182]. Interestingly, cell culture study reveals that miR-122 is downregulated (~3 fold) during acute HCV infection [182].

Recently, Peng *et al.* [183] carried out a computational study of HCV associated miRNAs-mRNA regulatory modules in human livers. They found differential profile of cellular miRNAs that target the genes involved in chemokine, B cell receptor, PTEN, IL-6, ERK/MAPK and JAK/STAT signaling pathways, suggesting a critical role of miRNAs in the replication, propagation, and latency of virus in the host cell. Upregulation of miR-155 was correlated with the growth promotion of HCC cells [184], and HCV replication associates with an increase in expression of cholesterol biosynthesis genes that are regulated by miR-122 [185]. Together, these findings suggest that miRNAs have the potential to become novel drug targets in virally induced infectious or malignant diseases.

9. Cooperative interactions of HCV and other agents in promoting liver disease

HCV increases the risk for HCC by promoting the development of liver fibrosis and cirrhosis. The question remains whether HCV causes HCC directly or promotes as a cooperative oncogene for end stage liver disease progression. HCC arising from a noncirrhotic liver vary according to geographic location (0% to 68.4%), and represents an uncommon and poorly defined subgroup of HCC [186]. Several studies suggested that patients infected with HCV genotype 1b have more rapid progression of associated liver dysfunction and a 2-6 fold increased risk for HCC [187].Viral proteins, including HCV core, play important role in liver disease associated with infection [188-190]. The variables affecting the range of pathology induced by HCV and the widely differing rates of disease progression are

poorly understood and are likely to be multi-factorial, including aspects of host genetics, immune responses, diet, and alcohol consumption. Viral factors such as viral load, genotype, and variation within individual viral genes may as well affect the range of pathology. Several studies suggest that HCC might be a hormone-responsive neoplasm, and the role of sex hormone receptors in primary liver tumors have been implicated [191]. Androgen receptor (AR) expression is detected with more intense expression in HCC than in non-tumoral liver tissue. Our recent study demonstrated that HCV core protein alone or in context with other HCV proteins enhances AR-mediated transcriptional activity and further augments in the presence of androgen [125]. Subsequent study suggested that HCV core protein acts as a positive regulator in AR signaling, providing further insight into oncogenic potential in the development of HCC in HCV infected individuals.

Dual infection with HCV and HBV in cirrhotic patients has been linked to an increased risk of HCC. A meta-analysis of case-control studies found a synergism between the two viruses with regard to carcinogenesis, the risk being more additive than multiplicative [192,193]. In cohort studies among Italian or Chinese patients with cirrhosis, those with HCV/HBV coinfection had a two- to six-fold higher risk of developing HCC compared with those with single infection [194]. HCC occurs at a younger age and after a shorter period of HCV infection in subjects coinfected with human immunodeficiency virus (HIV) compared with patients with HCV related HCC but without HIV infection [195]. Since newer therapies are decreasing mortality from HIV infection, it is anticipated that an increase in the incidence of HCC will appear in the future among HCV/HIV coinfected persons. Case-control studies have shown that there is more than additive interaction between alcohol and HCV infection in the development of HCC [196-198]. Together, these reports suggest that cooperative interactions of other agents with HCV have prolonged effect on HCV induced liver pathogenesis.
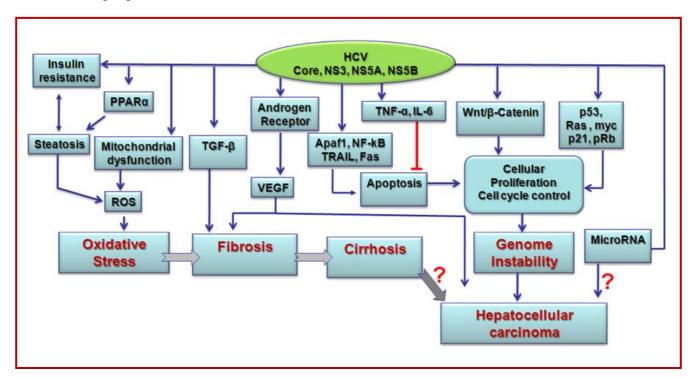
10. Summary

Chronic HCV infection is a major risk factor for the development of end stage liver disease, including HCC. Immune mediated liver damage may occur from HCV infected hepatocyte death and rapid turnover of hepatocytes with altered genetic changes for development of HCC. However, hepatocyte death does not appear to occur at a high rate as the liver transaminase upregulation is modest and intermittent during HCV chronicity. We have highlighted some of the major effects of HCV proteins promoting cell growth with the potential for oncogenesis (Figure 2). While the transcriptional and cellular effects from HCV are well studied, there are still gaps in our understanding of how HCV influences oncogenesis. Many intriguing functions related to HCV core protein, which may significantly contribute to disease progression have been reported.

Alterations in cell cycle proteins and their regulation are clearly involved in cancer progression and cellular transformation pathways. Activities of the HCV proteins are thought to contribute to the development of HCV associated promotion of hepatocyte growth, which may develop into HCC. Further understanding of the cellular factors targeted by HCV proteins and their effects on viral replication and cellular components of the liver could provide new insight and provide a better understanding of the development of liver cancer in chronically HCV infected patients. HCV appears to program hepatocyte cell machinery for viral replication and growth promotion towards the

development of HCC. Most of the putative transforming potentials of the HCV proteins have been defined in artificial cellular systems, which may not be applicable to HCV infection *in vivo*, and still need to be established relevant to infection and disease models. Unfortunately, we are yet to develop a suitable small animal disease model from HCV infection. Thus, the true biologic relevance of these observations remains still to be established in a relevant infection model scenario.

Figure 2. Schematic view of the molecular mechanisms for HCV mediated end stage liver disease progression.

HCC arising from a noncirrhotic liver, although uncommon, suggests that this disease process may follow a distinct pathway, independent of cirrhosis. Genetic and environmental factors and other cooperative agents may be involved with HCC. HCV proteins interact with a number of host factors and signaling pathways, and thus contribute to the progression from chronic hepatitis C to liver cirrhosis and HCC. However, it is difficult to demonstrate specific roles of HCV proteins *in vivo*, and in the microenvironment due to the lack of a suitable animal model. Role of miRNAs in viral life cycle is an emerging field, and future studies will elude their specific role in HCV mediated pathogenesis. As HCV mediated liver disease progression is slow and often takes more than a decade, there is longtime for treatment opportunity. Thus, we hope understanding the mechanism for liver disease progression from chronic HCV infection would offer opportunity for optimum treatment and intervention strategies.

Acknowledgments

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