

Review Article

Interaction of Hepatitis C Viral Proteins with Cellular Oncoproteins in the Induction of Liver Cancer

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Hepatitis C virus infection is a major health problem all over the world. A large proportion of patients infected by HCV develop liver cirrhosis or cancer. However, the mechanism(s) remain to be elucidated. Since HCV does not carry any known oncogene, it is thought that interaction between virally encoded proteins and host proteins is responsible for carcinogenesis. Many crucial interactions between HCV-encoded proteins and host proteins have been reported. In this review we focus on the interaction of viral proteins with important regulators of cell cycle—oncoproteins YB-1, p53, and cyclin D1—which play a major role in cell proliferation, apoptosis, DNA repair, and genomic stability. Genetic variants of HCV accumulate in patients and alter these interactions of host cell proteins. It is a battle between the virus and host and the final outcome depends on the winner; if the host succeeds in clearing the virus the patient may not develop serious liver diseases. On the other hand, if the virus dominates by evolving quasispecies which code for altered proteins that interact differently with host proteins, or induce mutations in host protooncogenes, then the patient may develop liver cirrhosis and/or liver cancer.

1. Introduction

Hepatitis C virus (HCV) infection is a major health problem and it appears that about 170 million people worldwide (about 2.0% of world population) are chronically infected and more than 350,000 people die every year from hepatitis C-related liver diseases [1]. It is estimated that about 3 to 4 million new cases are added each year. In Africa the prevalence is the highest and in some countries like Egypt about 15% of the people are infected [2, 3]. Initially HCV does not cause any serious disease but in about 80% of the infected people the virus establishes a chronic infection that leads to the more severe form of liver diseases—primarily cirrhosis and hepatocellular carcinoma (HCC). A large proportion of these chronic carriers (10 to 20%) may end up with cirrhosis and/or HCC over a period of 10 to 20 years after infection. In the USA alone, more than 165,000 HCV-infected people will die of liver diseases or HCC in this decade [4]. Thus, both disease burden and economic burden worldwide are extremely high. Although direct acting drugs are effective in controlling HCV infections, already globally more than 170 million people

were infected; a large number of these high risk patients will develop cirrhosis and HCC. Therefore, understanding the interactions between viral proteins and host cell proteins is very important to develop drugs for these liver diseases and HCC. A large number of publications appeared in the last 3 to 4 decades but in this review only the recent ones that are directly related to this topic are cited.

2. Hepatitis C Virus

HCV belongs to the *Hepacivirus* genus within the family Flaviviridae. It contains a positive sense single-stranded RNA genome of about 9500 nucleotides that is composed of 5' - and 3' noncoding regions (NCR) serving important regulatory functions during replication. Within the 5' NCR, an internal ribosome entry site is present to facilitate CAP-independent translation initiation of genomic RNA [5]. After receptor-mediated endocytosis involving multiple HCV receptors including CD81, scavenger receptor B1, tight junction proteins claudin1 and occludin, and the LDL receptor [6], its

genomic RNA is translated into a single polyprotein of about 3050 amino acids, which is subsequently cleaved by host and viral proteases into structural proteins—nucleocapsid Core and envelope proteins E1, E2, and p7 and nonstructural proteins—NS2, NS3, NS4A, NS4B, NS5A, and NS5B [7–9]. The viral RNA replicates in the cytoplasm of infected hepatocytes in a specialized convoluted structure called “membranous web” derived from the membrane of endoplasmic reticulum [10–12]. Host cell lipid metabolism plays a major role in the infectious life cycle of HCV and several of the viral proteins are shown to be involved in the recruitment of viral complexes to the lipid droplets, LDs [6, 12]. The Core protein, NS3/4A, and NS5A play a central role in the recruitment and transfer of viral RNA to LDs and assembly of viral particles [13–15].

Among RNA viruses, excluding retroviruses, HCV is the only RNA virus whose infection is associated with liver cancer, either by directly activating or indirectly interacting with cellular macromolecules. How HCV infection results in cirrhosis and HCC remains an enigma. In a large proportion of infected people HCV establishes persistent infection. In most cases, the cell-mediated immune system eventually clears the virus within a few weeks. However, in the case of HCV, interaction of viral proteins with key host proteins inhibits or diverts innate immunity thus evading immediate viral clearance to establish a slow persistent infection [16]. Viral proteins Core, NS2, NS3, and NS5A subvert the normal functioning of many crucial cellular proteins such as p53 and YB-1 and oncogenic products such as Myc and Ras, transforming a normal cell into a cancer cell.

Several studies on the interaction of HCV proteins with host cellular proteins by proteomic analysis in infected cells indicated that at least 420 host proteins interact with viral proteins [17], including components of the Jak/STAT, Insulin, TGF β , and focal adhesion molecules pathways with a majority of these interacting with Core protein, NS3, and NS5A [17]. Interestingly NS5A interacts with many proteins implicated in signal transduction, cell growth and death, and in cancer [18]. How many of these interactions remain in chronic patients several years after infections remains to be determined. Nevertheless, it is clear from the vast literature on this subject that viral proteins play a central role in regulating metabolic processes, cell-to-cell adhesion, and cytoskeletal organization, leading to HCV pathogenesis.

Some of these interactions play a pivotal role in viral replication, in eliciting strong cellular and humoral response, in developing strategies to avoid immune recognition, and in the induction of chronic hepatitis leading to cirrhosis and HCC [16, 19]. HCV has evolved strategies to hijack several critical host proteins including the Y-box binding protein-1 and its associated proteins, DEAD box helicases DDX3 and DDX6, and heteronuclear RNA protein A1, all of which play a major role in virus replication, assembly, and virus egress [15, 20]. CSNK2A1, the catalytic subunit of Casein kinase II (CKII) phosphorylates HCV NS5A which is required for the production of infectious virus particles [21]. NS5A activates a component of cell-adhesion complex, CTNNB1, which is positively regulated by CKII and Akt and has also been implicated in HCC [22]. Incidentally YB-1 is

also phosphorylated by CKII and Akt and this modification is required for its function in cell proliferation [23, 24]. Genome-wide analysis of host mRNA translation indicated that the strongest translation regulation was observed for mRNAs encoding proteins involved in pre-mRNA splicing, mRNA translation, and protein folding, all of which appear to be directly due to HCV replication rather than to HCV entry [25].

Upon establishing persistent infection, some of the viral proteins interact with host cellular proteins and change their properties and functions. As a result the cells and extracellular matrix components change over a period of several years and can end up in remodeling the tissue and also result in loss of control of regulation of cell proliferation, the hallmark of cancer. Marked induction of reactive oxygen species (ROS) in infected cells leading to oxidative stress [26] and suppression of host immunity by viral proteins have been observed in infected patients and in tissue culture systems expressing viral proteins [16, 26].

It is extremely interesting to note that virus evolved ways of cannibalizing some host proteins for its own replication and some other proteins to ensure host cell proliferation by inducing DNA synthesis and preventing apoptosis and others to overcome immune recognition by the host [16, 27]. Viral proteins Core, NS2, NS3/4A, and NS5A have been shown to be involved in interacting with key host oncoproteins and contribute to the development of HCC in HCV-infected patients. However, the mechanism of these interactions remains elusive. Some of these issues have been reviewed recently [27, 28]. However, in this review we focus on the interactions of HCV proteins with the cellular master regulator genes YB-1, p53, and cyclin D1 in the induction of liver cancer.

3. Y-Box-Binding Protein YB-1 and p53

The tumor suppressor gene p53, which is frequently mutated in human cancers, is a gate keeper of cell cycle arrest and apoptosis [29]. It is a transcription factor induced by stress and ensures completion of DNA repair and integrity of the genome and depending on the stage of the cell it can promote cell cycle arrest, apoptosis, and senescence of the cell [29]. The Y-box-binding protein 1 (YB-1), which belongs to the cold shock domain superfamily of proteins, is a DNA/RNA binding protein [30]. YB-1, like p53, is a multifunctional protein and appears to be involved in many cellular processes (see Table 1). It plays a major role in DNA proliferation, transcription and translation of RNA, DNA repair, and drug resistance [30–33]. It activates many genes involved in cell proliferation, directly activates genes involved in ribosomal RNA synthesis, binds to several mRNAs, and represses translation initiation [30, 33]. It also physically interacts with several key regulators of cell survival including p53. YB-1 can disable the p53 pathway by directly interacting with p53 and regulating its expression and activity [34, 35]. A comparison of the functions of YB-1 and p53 (Table 1) indicated that both of them are involved in many cellular functions. However, some notable differences between YB-1 and p53 are also evident. For example, YB-1 is unique

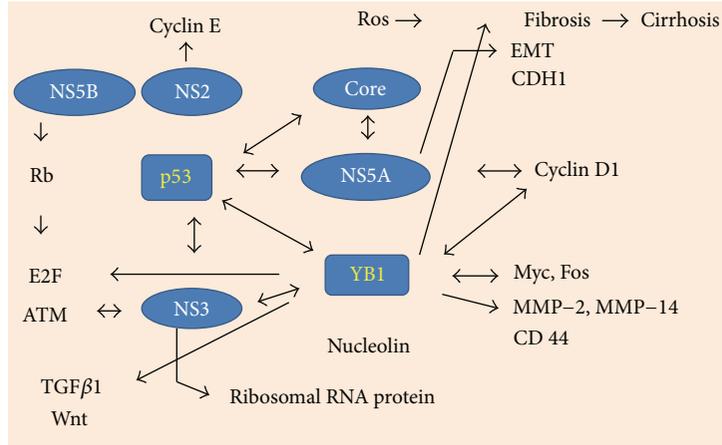


FIGURE 1: Interactions between HCV proteins and oncoproteins, YB-1 and p53 and other important host cells proteins implicated in hepatocellular carcinogenesis: the tumor suppressor gene p53 is more frequently inactivated in a variety of cancers. YB-1, a master regulator of several genes involved in cell proliferation and apoptosis, has also been shown to be activated in practically all types of cancer. The Core protein of HCV has been shown to bind to p53 and change its properties. NS5A interacts with p53 and cyclin D1 and this interaction prevents phosphorylation of RB and stimulates transcription of various cell cycle genes induced by the transcription factor E2F. YB-1 also activates E2F transcription factors by sequestering cyclin D1 and modulating RB gene expression. NS3/4 interacts with YB-1 and many host cell proteins involved in ribosomal RNA synthesis, and nucleolin, which binds to various RNAs and regulates their translation. NS2 has been shown to upregulate cyclin E. Additionally, YB-1 was shown to regulate major signaling pathways implicated in several tumors. YB-1 also is implicated in EMT through activation of cadherin genes and this phenotypic change is important for cancer. Also it has been shown that depending on the concentration of YB-1 and its cellular localization, it can directly activate or repress transcription of extracellular matrix components, which play a major role in fibrosis leading to cirrhosis. YB-1 also activates transcription of matrix metalloproteinases genes which are involved in tumor metastasis. YB-1 also regulates expression of Myc and Fos oncogenes. HCV proteins are shown in white with blue background.

TABLE 1: Comparison of the roles played by p53 and YB-1 in various cellular processes. Note that both proteins are actively involved in all the events that lead to cell proliferation and apoptosis, DNA damage and repair, genomic stability, and transcriptional regulation. Unlike p53, YB-1 plays an important role in translational regulation of various mRNAs, in inducing multidrug resistance especially in cancers. Another major difference between p53 and YB-1 is that the YB-1 knockout mice are embryonic lethal due to defects in translation of mRNAs and cell proliferation, whereas p53 knockout mice are prone to cancer without affecting normal embryonic development.

	Role in cellular processes	
	p53	YB-1
Oncogene activation	+	+
DNA damage	+	+
DNA Repair	+	+
Genomic stability	+	+
Apoptosis	+	+
Hypoxia	+	+
Cell cycle arrest	+	+
Transcriptional regulation	+	+
Translational regulation	-	+
Ribosomal stress	+	-
Drug resistance	-	+
Redox signaling	-	+
Knockout	Prone to cancer	Embryonic lethal

in the sense that it regulates translation of several mRNAs and activates genes involved in drug resistance and redox signaling that is important for generation of reactive oxygen species. However, the most important difference between YB-1 and p53 was observed with knockout mice. Knockout of both alleles of p53 did not affect the development of mice but made them prone to cancer [29, 36], whereas YB-1 knockout causes an embryonic lethal phenotype, which appears to be related to cell proliferation, defects in translation of mRNAs, and neural tube defects [37–39]. Consistent with this evidence we found very little expression of YB-1 in adult liver; however, it is over-expressed in embryonic liver, regenerating liver and in all cancers implying a role for YB-1 in cell proliferation [40]. Activation of YB-1 and suppression of p53 could be one mechanism by which viral proteins immortalize hepatocytes and establish persistent infection in infected individuals.

4. Interaction of HCV Core Protein with p53

At least 4 of the 10 HCV encoded proteins may participate in inducing HCC by interacting with different cellular target proteins [41] (Figure 1). Core is the most conserved protein and intensely studied with respect to its function [42]. Besides interacting with other HCV proteins, Core interacts with several host proteins, the most important for HCC being p53 and retinoblastoma protein RB [27], and indirectly with YB-1 [20]. The Core protein enhances the DNA binding

activity of p53 due to hyperacetylation of *lys273* and *lys382* and phosphorylation of *ser15*. Core protein also relieves the suppressive effect of p53 on RNA polymerases I and III, which is required for cell growth and proliferation [43].

Studies on the interaction of Core with p53 by pull down assays indicated that the N-terminal 50 amino acids (residues 319–393) of p53 are required [43]. Interestingly the same domain of p53 also interacts with YB-1 [30]. Further proof for interaction between Core and p53 was also obtained by colocalization studies using confocal immunofluorescence analysis [43]. Normally HCV proteins are present mainly in the cytoplasm of infected cells, whereas exogenous p53 is diffusely present in nucleus. However, when coexpressed, Core and p53 are predominantly localized at the perinuclear region [43]. They have further shown that this interaction results in the modulation of transcriptional activity of p53 [44]. An opposite effect of Core on p53 and p21 has also been suggested implying that depending on the interaction, Core can activate or repress transcriptional regulation of p53 and its downstream effectors [45]. HCV Core has also been implicated in various signaling pathways including Erk, Wnt/ β catenin, NF- κ B, and PI3K/Akt/mTOR [46]. These results suggest that HCV Core modulates many transcriptional and posttranslational events which make target cells more prone to cancerous phenotype. However, Core alone may not be sufficient to induce HCC because transgenic mice carrying Core gene, driven to expression with albumin or HBV promoter, induced HCC in about 14 to 30% of transgenic C57BL/6 mice, whereas the same gene expressed under MUP (major urinary protein) promoter did not produce any tumors [47–49]. Core in other combinations like Core-E1-E2 failed to induce HCC in significant numbers [50]. Other species of mice are totally refractory to HCC, suggesting that both the levels of expression of the viral protein and the host are important for developing cirrhosis and HCC [51]. The effect of Core appears to depend on the levels of its expression as it has been shown to activate p53 and repress p21^{WAF1/Cip1/Sid1} [44, 45, 52]. Thus, Core appears to play a dual role in pro- and antiapoptotic effects depending on the cell system. Even in individual patients, these pro- and antiapoptotic effects have been described suggesting that differences in the quantity of Core proteins made by the quasispecies of HCV and their interactions may be exerting different effects [53].

5. Interaction of HCV Proteins NS2, NS3/4A, and NS5A with p53

It has been shown that several HCV proteins are involved in p53 and DNA damage sensor pathways. Recently it has been shown that HCV NS2 protein inhibits DNA damage pathway by sequestering p53 in the cytoplasm, through ATM checkpoint pathway by inducing phosphor-Chk2. In addition, NS2 appears to interact with cyclin E and induce cell proliferation [54]. Cyclin E is known to bind to cdk2 and this kinase activity phosphorylates p27 and leads to its degradation. As a result the cell cycle arrest caused by p27 is relieved and the cells progress from G1 to S [55]. HCV

Core protein also upregulates cyclin E, [56] suggesting that HCV has evolved multiple ways to ensure host cell cycle progression.

HCV NS3/4 forms a complex with p53 and inhibits its function; it also interacts with ATM and impairs repair of DNA making the cells more sensitive to radiation [57, 58]. HCV NS5A binds directly to p53 and colocalizes p53 in the perinuclear region. NS5A physically interacts with p53 and regulates p21/waf gene expression and transcriptional transactivation by p53 in a dose-dependent manner [59]. The p53-induced apoptosis was abrogated by NS5A and the inhibitory effect correlated well with the binding ability of NS5A to p53. In addition, HCV NS5A protein interacts with and colocalizes hTAFII32, a component of TFIID and an essential coactivator of p53, *in vivo*. These results suggest that HCV NS5A interacts with and partially sequesters p53 and hTAFII32 in the cytoplasm and suppresses p53-mediated transcriptional transactivation and apoptosis during HCV infection, which may contribute to the hepatocarcinogenesis of HCV infection [60]. The 118 amino acid carboxyl domain of NS5A interacts directly with p53, causing p53 to relocate to perinuclear region from its normal nuclear localization and inhibits transcriptional activity of p53, which downregulates the downstream effector molecules like p21/waf1 [60]. These results suggest that HCV has evolved a mechanism to ensure host cell proliferation, which is probably required for virus survival and persistence. HCV NS2 and NS3/4A have been shown to be involved in p53 and DNA damage sensor pathways by sequestering p53 and ATM (ataxia telangiectasia mutated) and rendering them, relocalizing to the perinuclear region instead of their normal nuclear localization [51, 54, 58, 61]. p53 mislocalization is mediated via phosphorylation of checkpoint kinase 2, Chk2, which causes inhibition of apoptosis leading to cell proliferation.

NS5A is a phosphoprotein and serves an important function in the replication of HCV. It has three important functional domains separated by two low complexity sequences (LCS1, amino acids 214–250 and LCSII, amino acids 343–355). An amphipathic domain is located at the amino end of the molecule (amino acids 5–25) in Domain I (amino acids 1–213), Domain II (amino acids 250–342), and Domain III (amino acids 356–448). Isolate-specific virus replication and production appear to depend on the proline-rich LCSII [62]. Expression of NS5A in tissue culture cells results in significant stimulation of ribosomal RNA (rRNA) transcription [63] and it appears that the proline-rich regions in Domain I and in LCSII may be involved in promoter-dependent transcription of rRNA. Since the polyproline-rich motifs bind to SH3 domains of a number of cellular proteins, the effect of NS5A on enhanced rRNA synthesis may be exerted through these proteins. Although the exact mechanism is not elucidated, it is likely that NS5A activates expression of cyclin D1-CDK4, which in turn activates the latent transcription factor UBF1 by phosphorylating serine residue 484. Phosphorylated UBF-1 activates transcription of rRNA from rDNA templates [63].

6. Interaction of NS5A and YB-1 with Cyclin D1 and Other Host Cell Proteins

Cyclin D1 (CCND1) gene, which encodes the cell cycle protein cyclin D1, is one of the most frequently amplified genes in human cancers [64, 65]. The cyclin D1-CDK4/6 complexes phosphorylate pRB, p107, and p130 and release E2F transcription factors, which in turn activate a number of cellular genes implicated in cell cycle progression [66]. When the DNA in the cells gets damaged, proliferating cells respond by arresting their cell cycle progression by downregulating cyclin D1 [64, 65]. YB-1 also has been implicated at several steps in the DNA repair pathways [64]. It also promotes the expression of E2F1-dependent proliferative genes and prevents apoptosis in a number of ways by interacting at several nodal points in the PI3K/Akt/mTOR pathway [33, 67]. NS2 also induces cyclin E expression and promotes cell proliferation through phosphorylation of p27, an inhibitor of Cdk4/cyclin D complex activity, allowing cells to transit from G1 to S phase [54]. NS3 interacts with cyclin D1, which is upregulated in most of the tumors [68]. NS5B also interacts with RB and downregulates production of RB thus affecting cell cycle progression [51]. In this connection it is important to note that YB-1 interacts with cyclin D1 via its amino end A/P domain (proline-rich domain) [32].

7. Interaction of NS3/4A Protein with YB-1

The viral NS3/4A is a multifunctional protease-RNA helicase which plays a major role in evading innate immunity [16]. It is absolutely required for HCV replication. Proteomic analysis of NS3/4A interactome coupled with mass spectrometry has led to the identification of a cellular protein YB-1 and the DEAD box RNA helicases DDX3 and DDX6, as interacting partners with viral NS3/4A [15, 20]. Lamarre's group also showed that YB-1 interacts with many RNA binding proteins and this interaction is vital to control the equilibrium between viral RNA replication and NS3/4 dependent late steps in particle production [20]. HCV Core protein also interacts with DDX3 helicase but this interaction is not required for viral replication; rather it is important for controlling IFN- β induction, which in turn is involved in antiviral activity of host [69, 70]. Silencing YB-1 expression decreased viral RNA replication and severely impaired the propagation of the infectious HCV molecular clone JFH-I [15, 20]. Immunofluorescence studies further revealed a drastic HCV-dependent redistribution of YB-1 to the surface of the lipid droplets, an important platform for HCV assembly. Core and NS3 protein-dependent polyprotein maturation was shown to be required for YB-1 relocalization [15]. In addition, NS3/4A interacts with hnRNPs, several ribosomal proteins, and nucleolin [15]. Nucleolin is a multifunctional protein mainly localized in nucleolus and participates in RNA regulatory mechanisms including transcription and ribosome assembly (Figure 1). Nucleolin binds to target RNAs via its four RNA binding domains and alterations in its binding to target RNAs have been observed in several diseases particularly cancer [71]. NS3/4A and YB-1 also associate with tubulin and

promote *in vitro* nucleation; this can control the dynamics of cytoskeleton around the LDs by forming complexes with microtubules and actin filaments [72, 73].

8. Expression of YB-1 in Hepatocellular Carcinoma

Hepatocellular carcinoma, like other types of cancer, results from interactions of multiple factors in different signaling pathways leading to genetic changes in hepatocytes or precursor stem cells which cause uncontrollable cell proliferation and cell death. YB-1 has been reported to be a prognostic marker for a number of cancers including breast, ovarian, lung, thyroid, prostate, and liver cancers and its overexpression in various tumors is closely correlated with unfavorable patient prognosis [33, 74, 75]. It has been shown that about 89% of HCC cases were positive for YB-1 and in all of the positive cases and that YB-1 is predominantly localized to cytoplasm [76]. Intracellular pathways involved in hepatocarcinogenesis in HCV-infected patients include Wnt/ β -catenin, MAPK, Jak/STAT, and p53, all of which either directly or indirectly interact with YB-1 [33, 77]. In fact it is extremely interesting that a 18 kDa cleaved fragment of YB-1 containing the cold shock domains (CSD) 1-3 of the full length protein was detected by an immunoblot assay in the plasma of HCC and advanced carcinomas [78]. Strong YB-1 expression has also been shown to be associated with liver metastasis progression and can be used to predict shorter disease-free survival in advanced gastric cancer [79]. Oncogenes Met, Myc, Fos, and Jun and the cell surface death receptor appear to play a major role in liver cancer cell proliferation and apoptosis, and YB-1 regulates these genes at transcription and translation levels [80-84]. In addition, YB-1 has been shown to inhibit both Fas-mediated apoptosis pathway and expression of the proapoptotic protein BAX [81, 82, 85]. Several of YB-1 interacting partners such as ClQBP and hnRNP are overexpressed in HCC and have been shown to correlate with cancer aggressiveness and progression with poor survival prognosis [76, 79, 86]. Thus, YB-1 controls many cellular processes involved in cell proliferation and cell death, which are very critical for carcinogenesis.

YB-1 also appears to play a key role in the PI3K/Akt/mTOR pathway, which in turn is involved in the activation of E2F pathway. Indeed YB-1 depends on Akt for its nuclear translocation from cytoplasm following phosphorylation at Ser¹⁰² in a number of tumors [33, 85]. Thus, YB-1 has been shown to protect cells from p53-driven apoptosis; in other words, YB-1 regulates both apoptosis and cell proliferation pathways by interacting directly or indirectly with a number of cellular genes (p53, p21, cyclin D1, E2F, etc.) and HCV gene products (Core, NS2, NS3/4A, and NS5A). Overexpression of YB-1 appears to promote genomic instability, loss of regulation of cell cycle, and centrosomal amplification (Table 1) [87, 88]. Additional roles for YB-1 include regulation of energy metabolism in cancer cells via PI3K/Akt1/mTOR pathway, induction of angiogenesis, invasion and metastasis of various cancer cells, and evading immune destruction of cancer cells [33].

9. Role of YB-1 in Epithelial Mesenchymal Transition

EMT is very important for transition of normal hepatocytes to a cancer phenotype. It is generally thought that EMT is critical in the process of metastasis which is correlated with the expression of E-cadherin and vimentin [89]. NS5A appears to have a major role in this transition as expression of this protein in primary hepatic progenitor cells gave rise to modifications of cell polarity leading to EMT [90]. YB-1 also promotes transition of differentiated epithelial cells into mesenchymal stem cells with an ability to migrate to distant sites [31]. Further it has been shown that elevated levels of YB-1 were correlated with augmented tumor dissemination, thus serving as an early marker of metastasis [80, 91]. TGF- β , a key cytokine in the activation of stellate cells and fibrosis, also cooperates with NS5A in polarity changes of hepatic precursor cells leading to the EMT [90]. EMT is also driven by many pathways and molecules such as TGF- β 1, the translation of which is regulated by YB-1 [92] and Wnt/Notch pathways, which were also shown to be involved in EMT [93]. Interestingly, YB-1 has been shown to bind to the promoters of a number of Wnt pathway genes; a fragment of YB-1 has been identified as a ligand for Notch 3 receptors [94, 95]. HCV can induce EMT of primary hepatocytes and it is also interesting to note that HCV Core protein, which is implicated in oncogenesis, is capable of provoking EMT induction in primary hepatocytes by suppressing TGF- β -mediated cytostatic effect via Smad3 [96, 97]. YB-1 also promotes EMT by translational activation of snail and other developmentally regulated transcription factors [31]. Thus, EMT is required to produce cells in the tumor mass that are not differentiated and motile and we propose that the Core and NS5A of HCV and YB-1 of host play an important role in these cellular processes.

10. Evolution of Quasispecies and Changes in Virus-Host Interactions

The results discussed so far clearly indicate that HCV encoded proteins interact with cell cycle regulated oncoproteins such as YB-1, p53, cyclin D1, and RB. If these interactions are critical for the development of cirrhosis and HCC, then we expect to see induction of liver cancer in all the patients when the virus robustly replicates in the early stages of infection. Clearly this is not the case. This means that other genetic and phenotypic changes are required in the host and virus as well. Since a large proportion of infected patients establish persistent infections genetic variants in virus as well as in host target cells accumulate over a long period, alter the interactions, and cause permanent genetic and epigenetic changes leading to HCC. It is estimated that only about 40% of the HCV genotype 1 infected people respond to antiviral therapy and altogether about 80% of the infected people develop chronic infection and are at high risk for cirrhosis and HCC [98, 99]. Initially virus infection results in suppression of host innate immunity and activation of oxidative stress leading to production of

reactive oxygen species (ROS) followed by hepatitis [26]. In due course of time host immunity (humoral and cellular) and antiviral therapy reduce virus load by clearing the virus from most hepatocytes. Since the viral RNA polymerase lacks the proofreading function, mutants accumulate at a rapid rate leading to the establishment of persistent infections [100–103]. Within weeks to months, the original monoclonal virus stock could be replaced by emerging mutants called “quasispecies” [102–105]. In a typical example, it was observed that as many as 12 different variants appeared in 6 months in a single infected patient in the hypervariable region 1 of E2 [106]. In 3 years these 12 quasispecies were replaced with one dominant (75% of the virus) and 3 or 4 minor variants. Similar changes, albeit at a reduced frequency, were observed in other regions of the genome. Treatment with antiviral drugs including protease inhibitors induces resistant mutants [107, 108]. To summarize, HCV constantly evolves new variants during persistent infection and the host is constantly subjected to episodes of hepatitis and these variants evade immune recognition, alter interactions with the host cell proteins, and also induce chromosomal abnormalities [109] culminating in liver cancer.

Based on the enormous amount of information accumulated over 4 to 5 decades, Hanahan and Weinberg proposed “Hallmarks of Cancer” which include “sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis”; recently they added two more to this list—reprogramming of energy metabolism and evading immune destruction [110]. Evidence documented so far clearly indicates that YB-1 meets all these hallmarks [33]. This is accomplished by the HCV encoded proteins, Core, NS3/4, and NS5A. p53 and cyclin D1 also fulfill these criteria, as reviewed above. Another important feature of cancers is the development of multidrug resistance which has also been attributed to the YB-1 in a variety of cancers [30, 74, 111]. The proteins that are encoded by cancer genes normally regulate cell proliferation, differentiation and cell death, and mutations in these genes as well as mutations in genes involved in DNA repair also result in oncogenesis. Futreal et al. conducted a “census” of cancer genes and found that mutations in 1% of genes (~290 genes) contribute to human cancer [112]. The most common domains that are encoded by oncogenes are the protein kinases, DNA binding, and transcriptional regulation domains [112]. Most of these are due to somatic mutations in cancer genes. In a similar analysis on the pattern of somatic mutations in over 210 diverse human cancers, more than 1000 somatic mutations were recorded in about 274 megabases of DNA, corresponding to the coding exons of 518 protein kinase genes [113]. They further suggested that a high frequency of these somatic mutations may not contribute to oncogenesis; about 120 driver mutations may be involved in the development of different cancers. It is possible that some of the HCV-infected patients develop liver cancer because of these predispositions and HCV encoded mutated gene products interact differently than the originally infected genotype. For example, it has been shown that the NS5A quasispecies variants induce different levels of transcription according to the charge

of the residue [114]. Similarly it has been reported that subcellular localization of NS3 and its interaction with p53 vary with different NS3 sequences [57]. Many examples like this clearly indicate that evolution of quasispecies and their interactions with host proteins determine the final outcome in the patients.

The evolution of quasispecies of HCV in each patient fulfills the requirement of evading immune destruction as well as the participation of viral proteins in inducing sustained proliferative signaling for immortalization and spreading of cancer cells, as has been elegantly shown in recent patient studies [103, 106, 115]. In some of the best examples where they studied liver diseases (fibrosis, cirrhosis) induced by HCV, a monoclonal viral population was replaced in weeks to months by “quasispecies” in individual patients [103]. Further it was shown that the disease manifestation as evidenced by alanine aminotransferase levels (indicative of liver damage) was different in slow progressors compared to rapid progressors; HCV-specific RNA levels were also fluctuated at various times. For example, in slow progressors, there is a dramatic drop in the amount of virus released into the serum of the patient between 6 and 12 months; then new quasispecies (mutations detected in hypervariable region 1) emerged and persisted for almost 20 years [103]. In contrast, in the rapid progressors, viral RNA levels were maintained and the patients died within 7 years with end stage liver disease. Analysis indicated that rapid progressors displayed greater viral diversity and higher rate of synonymous mutations and also produced profibrogenic chemokines [106]. Recent studies by laser capture microdissection technique indicated that the proportion of HCV-infected hepatocytes in human liver ranged from 21 to 45% and that the level of viral RNA ranged from 1 to 50 infectious units per hepatocyte (115). More interestingly they observed that HCV infects in nonrandom clusters, whereas expression of antiviral molecules (interferon-induced transmembrane protein 3) is scattered among hepatocytes (115). These results indicate the heterogeneity in the infected hepatocytes and suggest that some of these cells in the clusters may behave differently. The dynamics of evolution of quasispecies of HCV and their interaction with host cell proteins as well as the development of mutations in host cell genes over a period of several years selects the cancerous cells which then outgrow normal cells producing liver cancer.

11. Conclusions

Evidence presented in this review clearly indicates the importance of host proteins, particularly YB-1, p53, and cyclin D1 in the dysregulation of cell cycle and DNA repair. A consequence of this dysregulation is the phenotypic changes in the quality and quantities of key proteins responsible for cell growth and proliferation. For instance, YB-1 is directly involved in HCV replication and assembly of the virus along with several other host proteins; when the rate of viral replication reduces or mutations in NS3/4A or helicases impair their interaction with YB1, then YB-1 can be redistributed from lipid droplets to other compartments including nucleus where it triggers cell proliferation. Also it appears that HCV

has developed a strategy to inhibit apoptosis by blocking p53 via Core and NS5A and at the same time ensured target cell proliferation by interacting with YB-1 via NS3/4A for its own survival. YB-1 can disable the p53 pathway by directly interacting with p53 and regulating its expression and activity. Other proteins like NS2 can inhibit p53 mediated repair and activate other cyclins involved in cell cycle. NS5A and YB-1 also suppress RB phosphorylation by cyclin D1/CDK4 which then turns on E2F transcription factors leading to activation of genes involved in DNA synthesis and cell proliferation. These are all very interesting observations and strongly suggest that the interaction between the viral proteins and host proteins can play a dominant role in inducing liver cancer. However, we have to consider the length of time in patients it takes to induce first cirrhosis and then HCC. How many of these qualitative and quantitative interactions occur with the same candidate protein 10 to 20 years later, when the originally infected virus is replaced by several variants, remains to be studied. This is a probability event and it takes a long time during which virus-host interactions are perturbed; mutations accumulate in viral genomes as well as in the host resulting in the transformation of some hepatocytes or cancer stem cells to a cancerous phenotype. This could lead to genetic instability and clonal expansion of the cancer cells to produce liver cancer. For cancer cells in tumor mass to survive and proliferate, they have to evade immune destruction, maintain energy metabolism, induce angiogenesis, and invade and metastasize. The tumor suppressor genes p53 and RB and the oncoprotein YB-1 and cell cycle regulators like cyclins all are likely to be involved. The HCV genome has evolved in such a way that its mutant proteins can alter the host cell for its own survival. In order to precisely understand the mechanism of virus-host interactions, the pattern of evolution of mutants in both the hepatitis C virus and host “driver mutations” in the 120 genes, the somatic mutations that have been found in human cancers, can be determined by systematic sequencing of genomes at various stages after infection, that is, initially when the infection is diagnosed and then at various stages of liver disease (cirrhosis and HCC) in humans.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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