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Molecular pathogenesis of hepatitis C virus-associated hepatocellular carcinoma

Dong-Yan Jin

Department of Biochemistry, The University of Hong Kong, Hong Kong

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1. ABSTRACT

Chronic infection with hepatitis C virus (HCV) is causally associated with the development of hepatocellular carcinoma (HCC). HCV is not cytolytic and replicates entirely in the cytoplasm. Viral interaction with the host leads to subversion of immune response and other defense mechanisms. The recent development of robust cell culture systems for HCV infection provides new opportunities for the study of virus-cell interaction and viral pathogenesis. HCV infection causes active inflammation and fibrosis, which ultimately progresses to cirrhosis. The onset of cirrhosis usually precedes the multistage process of tumor development, in which common themes of viral carcinogenesis can be identified. While chronic inflammation and cirrhosis are thought to play an important role in tumor initiation, the underlying mechanisms are incompletely understood. Recent studies have revealed that infection with HCV induces genome instability, leading to further genetic and epigenetic alterations which contribute to the full development of HCC tumor. The expression of viral oncoproteins such as C and NS5A is critically involved both in the induction of genome instability and in dysregulating cellular control of growth and signal transduction. A better understanding of the molecular pathogenesis of HCV will reveal novel strategies for the prevention and treatment of related diseases including HCC.

2. MOLECULAR BIOLOGY OF HCV

HCV, a member of the *Hepacivirus* genus in the *Flaviviridae* family, causes acute and chronic hepatitis, and HCC (1). Approximately 2% of the world's population (123 million people) are currently infected with HCV (2). The prevalence of HCV infection in different countries varies from ~3% in China, ~2% in USA, Italy and Japan, 0.6% in Germany to 0.01% in Hong Kong (2). Up to 85% of HCV infections become chronic and persistent, leading frequently to cirrhosis, end-stage liver disease and HCC (3). Thus, HCV is a significant infectious cause of morbidity and mortality worldwide. Particularly, HCV is the commonest chronic blood-borne infection as well as a leading cause of liver transplantation and HCC in many industrialized countries (3).

HCV is parenterally transmitted. Transfusion, hemodialysis and organ transplantation were major routes for transmission of HCV before blood screening was routinely performed (2). At this time, spread of HCV in developed countries occurs most commonly among users of injectable drugs, professional sex workers, and in some countries, professional blood donors (2, 4). However, in the developing world, blood contamination is still a major concern in the prevention of HCV infection (2).

The drugs approved for therapeutic treatment of HCV infection have only limited efficacy and no effective

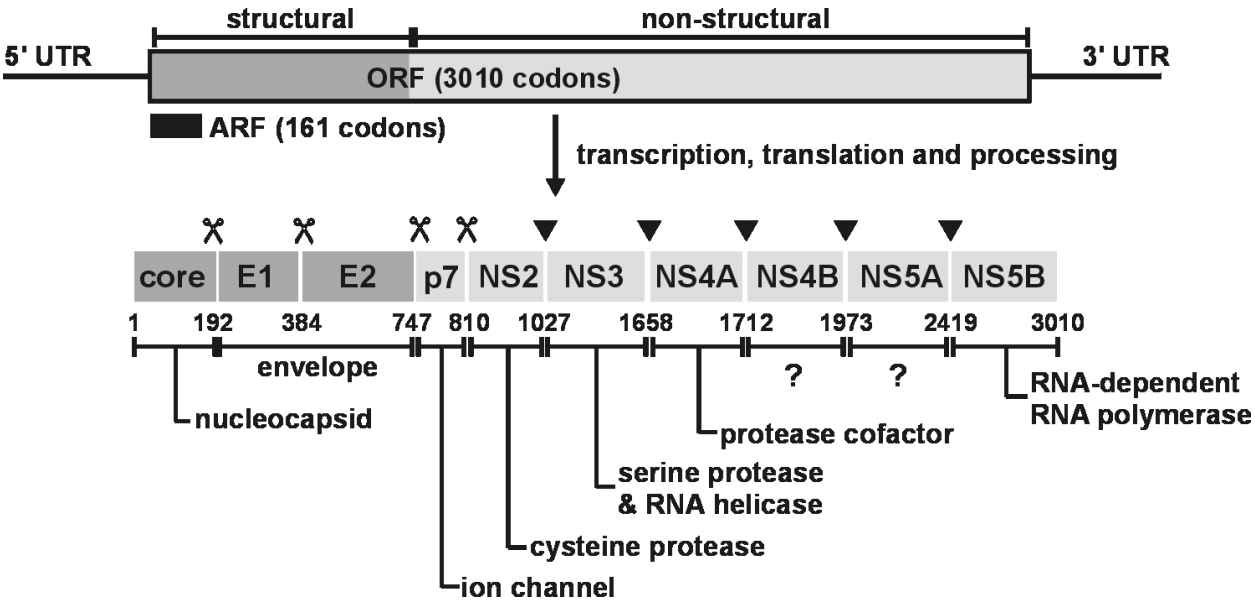

Figure 1. HCV genome and proteins. The numbers indicate the positions of the cleavage sites of cellular (✂) and viral (▼) proteases.

vaccine is available (5, 6). In addition, there are no non-primate animal models of HCV infection, and a cell culture system that efficiently produces HCV viral particles has not been established until very recently (7-11). Understanding the molecular biology of HCV and developing new model systems for the study of HCV infection are therefore a prerequisite for continued improvement of preventive measures and treatment modalities.

2.1. HCV and its life cycle

HCV has a positive stranded RNA genome of ~ 9600 nucleotides comprising a large open reading frame (ORF) flanked by highly structured untranslated regions (UTRs) located at both 5' and 3' termini (Figure 1). Translation of a polyprotein from the large ORF is driven by an internal ribosome entry site (IRES) in the highly conserved 5' UTR. The polyprotein is subsequently cleaved by cellular and viral proteases into three structural (core, E1, E2) and at least seven non-structural (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) proteins (12). Core (C) protein is well conserved among all HCV isolates. It is a highly basic protein with RNA-binding activity and forms the nucleocapsid of the virus. E1 and E2 are surface glycoproteins of the virion and they mediate virus-cell fusion and receptor binding (13). p7 is a small hydrophobic protein localized to mitochondria (14, 15). It is noteworthy that p7 has an ion channel activity (14) which is critical for the infectivity of HCV (16). The other non-structural proteins are thought to have enzyme activities required for viral replication. NS2 is a cysteine protease. NS3 has serine protease and RNA helicase activities, which are further enhanced by NS4A (17). Both NS4B and NS5A are membrane proteins, but their functions are not well understood (18). NS5B is an RNA-dependent RNA polymerase (19). In addition to the above proteins, another small HCV protein is also produced by ribosomal frameshift from the C region of the genome (20, 21). This protein known as F or ARFP (alternative reading frame protein) is an endoplasmic reticulum (ER)-associated short-lived protein that may have a function in viral morphogenesis or replication (22).

The life cycle of HCV starts with the interaction of viral glycoproteins with cellular receptors. After viral entry into the cell and uncoating, the HCV genome functions to translate viral proteins and replicate viral RNA. The newly formed viral RNA then interacts with structural proteins to generate virions, which bud into the ER and are subsequently released from the cell (12). Replication of HCV occurs entirely in membrane-associated structures within the cytoplasm (23). Membranes are critical for the maturation of all HCV proteins except for NS3 (24).

HCV life cycle has been well studied and comprehensive reviews can be found elsewhere (3, 12). Below I will only discuss new developments in two areas that are of particular interest: cellular receptor of HCV and IRES-dependent translation.

E2 glycoprotein binds tightly to a putative HCV receptor CD81, a tetraspanin expressed in hepatocytes and various other cells (25). More recent studies using retroviral particles pseudotyped with HCV E1/E2 glycoproteins demonstrate that CD81 is indeed critical for viral entry, but it likely acts at a post-attachment step (26, 27). The binding of HCV E2 protein to CD81 on the surface of hepatic stellate cells and lymphocytes has been shown to modulate intracellular signaling (28, 29) and more surprisingly, to induce genome instability (30). In addition to CD81, several other molecules including L-SIGN, DC-SIGN and scavenger receptor SR-BI have also been shown to have receptor or coreceptor function in HCV infection (31-34). Because forced expression of CD81 on

non-permissive human hepatic but not murine cells enables the entry of HCV pseudovirus (26), a primary receptor of HCV, which functions in concert with CD81 and probably other candidate receptors such as SR-BI (34), remains to be identified. The recent development of a robust cell culture system that supports HCV infection (7-11) will substantially facilitate the identification of this receptor.

HCV IRES-mediated translation uses a sequential pathway distinct from canonical cap-dependent initiation of protein synthesis (35). This process is initiated when IRES binds directly to 40S ribosomal subunit in the absence of canonical translation initiation factors such as eIF4F. The subsequent recruitment of eIF3 and eIF2 produces a unique 48S preinitiation complex. Finally, the formation of a translationally active 80S complex is rate-limiting and requires GTP-dependent association of the 60S ribosomal subunit (36). IRES-driven translation utilizes a specific subset of host factors, the inhibition of which may protect cells from viral infection (37). This IRES-dependent process is also regulated by viral proteins C and NS5A (38, 39).

2.2. Virus-host interaction

HCV is very successful in establishing persistent and life-long infection in a majority of individuals. To achieve this, HCV has developed highly effective viral strategies to combat and defeat immune response and other antiviral defenses of the host (40-44). During genome replication, HCV forms double stranded RNA (dsRNA) intermediates, which potently induce the cellular dsRNA-sensing machinery, leading to the expression and/or activation of various proteins involved in antiviral defense, including interferons (IFNs), interferon regulatory factors (IRFs), signal transducers and activators of transcription (STATs), interferon-stimulated genes (ISGs), and NFκB (45). For survival, HCV fights back with its core, E2, NS3 and NS5A proteins that counteract various cellular factors critically important in the mobilization of innate immune response (40, 43). Examples for this inhibition of cellular antiviral activities by HCV proteins are numerous and include the suppression of dsRNA-activated protein kinase PKR by NS5A and E2 (46, 47). In this review I will comment on several recent findings that provide new mechanistic insight into HCV inhibition of innate immunity.

On the other hand, HCV is also very effective in the subversion of adaptive immunity, in which T cells play a central role (41, 42). One central event in this scenario is an HCV-specific loss of $CD4^+$ T cell help (41). While the mechanisms for the subversion of HCV-specific T-cell immunity are poorly understood, existing evidence supports the notion of mutational escape of HCV epitopes (41). NS5B is an error-prone RNA-dependent RNA polymerase that has no proof-reading activity. In addition, two other factors may also facilitate the generation of HCV escape mutants. First, HCV quasispecies exist in the body before adaptive immunity is fully developed. Second, the replication rate of HCV is relatively high. Thus, immune evasion likely represents a major mechanism of HCV persistence (42). Other mechanisms such as hepatic expansion of HCV-specific regulatory T cell population (48) have also been proposed. However, further investigations are required to establish the contributory roles of all these mechanisms in HCV persistence.

In the following paragraphs I will summarize our current understanding in three areas relevant to the interaction between HCV and the host cell, with an emphasis on new developments: suppression of dsRNA-activated antiviral defense by HCV, modulation of ER stress and unfolded protein response (UPR) by HCV, and regulation of HCV replication by a cellular microRNA (miRNA).

Toll-like receptor 3 (TLR3) is a dsRNA receptor, the activation of which induces IFN-β production through the recruitment of two kinases, TANK-binding kinase 1 (TBK1) and IκB kinase ε (IKKε), that phosphorylate transcription factor IRF3 (45). In addition, DexD/H box RNA helicase RIG-I has also been shown to function as a cytoplasmic sensor of dsRNAs and it triggers antiviral response by activating IRF3 and NFκB (49). To deal with these dsRNA-induced antiviral defenses, HCV employs its NS3-NS4A protease to target several key factors in these signaling pathways (Figure 2). First, NS3-NA4A cleaves and inactivates TRIF, an adaptor protein that links TLR3 to TBK1 and IKKε (50). Second, NS3-NS4A physically interacts with TBK1, leading to its dissociation with IRF3 (51). Third, NS3-NS4A cleaves and inactivates Cardif, a CARD-domain-containing protein that adapts RIG-I signaling to IRF3 and NFκB activation (52). Thus, NS3-NS4A serves multiple functions in the inhibition of cellular antiviral responses through its protease activity and/or protein-protein interactions. This example illustrates how HCV empowers one of its nonstructural proteins to effectively disarm cellular antiviral defense.

HCV life cycle is closely associated with the ER. Viral activities including the synthesis and processing of viral proteins induce ER stress and have a profound impact on ER functions (53). ER stress activates several cell signaling pathways known as unfolded protein response (UPR), which enhances the biosynthetic capacity to maintain ER homeostasis. ATF6, PERK and IRE1 are three major regulators of UPR. Their target genes such as Grp78, Grp94 and CHOP are critically involved in protein folding or apoptosis (54). The activation of UPR by HCV has been shown with the subgenomic replicon system (55). In particular, the expression of E2, core and NS5A proteins activates the promoter of Grp78, Grp94 and CHOP (56-59). In contrast, the IRE1-XBP1 pathway of UPR, which governs protein refolding and degradation, is suppressed by HCV (60). Thus, HCV specifically modulates UPR to its own benefits: on one hand, it stimulates one arm of UPR to facilitate the folding of viral proteins; on the other hand, it inhibits another arm of UPR to prevent degradation of viral proteins. The functional consequence of UPR modulation by HCV includes ER sequestration and reduced cell-surface presentation of major histocompatibility complex class I (MHC-I) molecules (61), and the generation of reactive oxygen species (ROS; Ref. 53). Both down regulation of MHC-I and production of ROS have important implications in HCV pathogenesis.

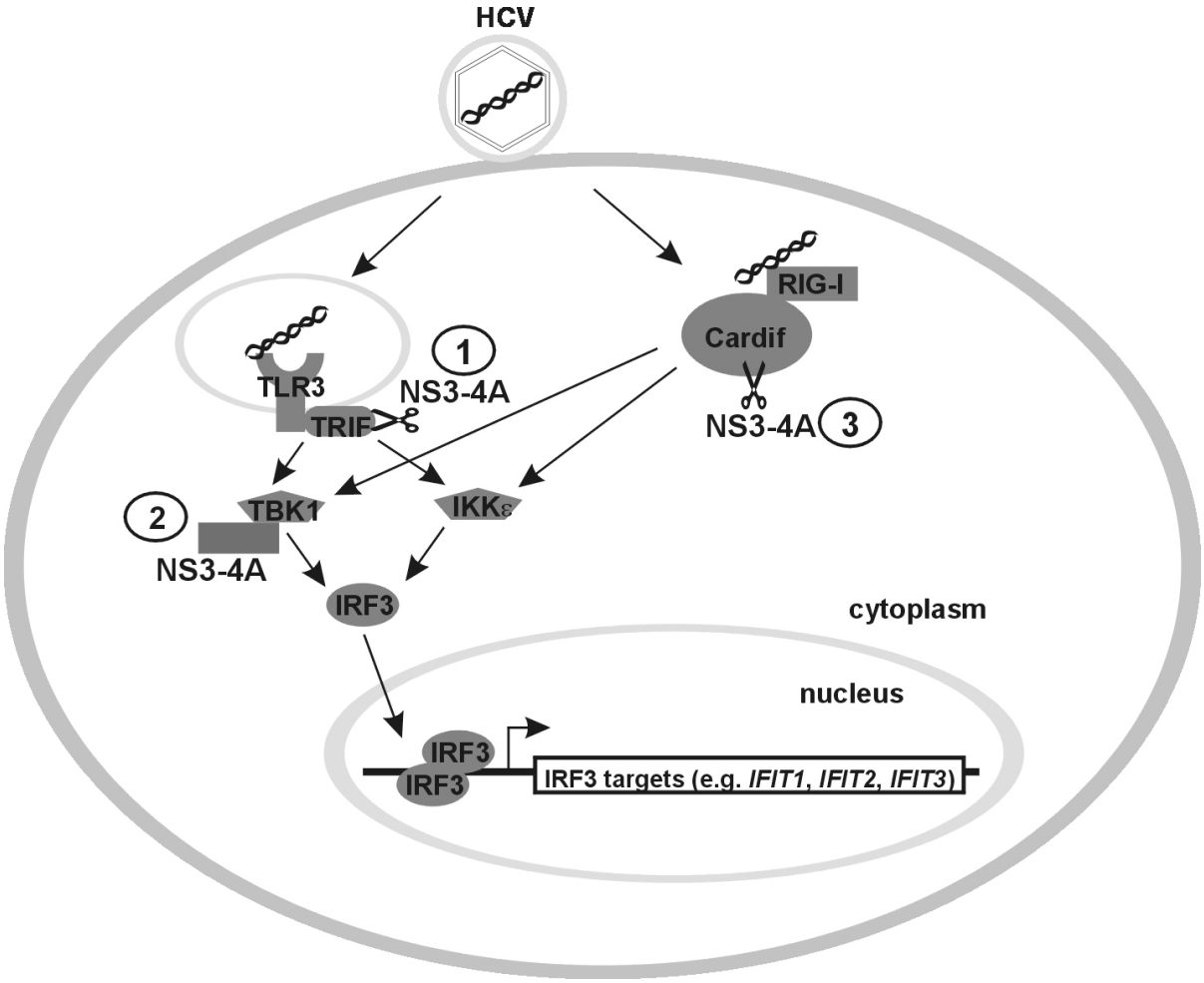

Figure 2. Inhibition of dsRNA-activated antiviral response by HCV NS3-NS4A protein. NS3-NS4A protease cleaves TRIF (1), associates and inhibits TBK1 (2), and cleaves Cardif (3).

Both Huh7 and HepG2 cells are derived from human HCC tissues. However, HCV replicates only in Huh7 cells, but not in HepG2 cells (9). Recent analysis of the expression pattern of a liver-specific microRNA miR-122 indicates that it is abundantly expressed in Huh-7 but absent in HepG2 cells (62). Interestingly, suppression of miR-122 with a 2'-O-methylated RNA oligonucleotide leads to inhibition of HCV replication. A miR-122 binding site has been identified in the 5' untranslated region of HCV and shown to interact directly with miR-122. In addition, this interaction is required for the modulation of HCV RNA abundance. These findings suggest that miR-122 facilitates HCV replication (62). Intriguingly, miR-122 does not affect stability of HCV RNA or translation of HCV proteins. In line with this, IRES-dependent translation has recently been shown to be resistant to inhibition by miRNA (63). Thus, new experiments are required to elucidate the mechanisms by which miR-122 modulates HCV replication.

2.3. HCV replication and infection in cultured cells

The development of HCV subgenomic replicon in cell culture is a major progress that has facilitated the study of HCV biology (64, 65). These replicon systems have incorporated a selective marker. The efficient replication of viral subgenome depends on the cell culture-adaptive mutations of the viral genes and higher permissiveness of the particular cell lines (66). However, HCV genomes with the culture-adaptive mutations have been found to be unable to produce viral particles in Huh-7 cells and highly attenuated for replication in chimpanzees (67). The isolation of a genotype 2a HCV genome called JFH-1, which replicates efficiently in cell culture in the absence of adaptive mutations, provides a good opportunity to circumvent the obstacle for efficient production of HCV virions caused by these mutations (7).

The combination of JFH-1 viral isolate with the highly permissive Huh-7.5 cell line leads to the establishment of HCV cell culture systems that efficiently produce infectious particles (9, 10). Similar systems have also been developed with other HCV genotypes or using stably transfected cDNAs (8, 11). The development of robust cell culture systems for HCV infection provides many new opportunities for HCV research and drug discovery. Ideally, all discoveries made with cloned viral

genes or with the subgenomic replicon system should be verified in the new systems that produce HCV particles. The use of these new systems represents a new era of HCV research in which all steps of viral life cycle can be studied by genetic analysis and manipulations in the context of HCV infection.

3. HCV-ASSOCIATED HCC: THEMES AND VARIATIONS

The association of HCV with HCC adds an RNA virus to the list of human oncogenic viruses, which include hepatitis B virus (HBV), Epstein-Barr virus (EBV), human T-cell leukemia virus type I (HTLV-I), human papillomavirus types 16, 18 and 45, and human herpesvirus 8 (68). Some common themes of viral oncogenesis are also identified in HCV-related HCC. Similar to other oncogenic viruses, HCV establishes persistent infection and it takes several decades (~30 years) to develop from initial exposure to virus to clinically recognizable HCC in a small percentage (1-2%) of infected individuals. Both viral and host factors are influential in the course of tumor development. For example, some evidence suggests that HCV of genotype 1b is more frequently associated with severe liver diseases and HCC (69). On the other hand, most but not all HCV-related HCC occurs in cirrhotic liver (70). Once cirrhosis is established, HCC develops at the rate of 3.7-7.1% per year in patients with HCV (71). The arising HCC tumor is monoclonal and contains additional genetic and epigenetic alterations.

The contribution of HCV to HCC varies in different geographic areas. In industrialized countries such as US, Japan, Italy, France and Spain, the HCC incidence attributable to HCV infection has increased significantly in recent years (2). In Japan, ~90% of HCC is thought to be associated with HCV (1). In US, 52% of patients with HCC are infected with HCV (72). In contrast, 7.3% and 9.4% of HCC in Hong Kong and China, respectively, are linked to HCV infection (73). Strong evidence supports that co-infection with HBV and HCV is associated with an increased risk of HCC (71, 74). Another important cofactor is alcohol, which is known to have synergistic effect on chronic hepatitis C in the development of HCC (71). Additionally, genetic factors have also been shown to influence the development of HCC in patients chronically infected with HCV (75).

HCV is the only positive-stranded RNA virus in the group of human oncogenic viruses. The NS5B polymerase of HCV does not have reverse transcriptase activity. The HCV RNA is not known to integrate into the cellular genome. In this regard, the course of HCV-associated hepatocarcinogenesis is distinct from that caused by HBV and HTLV-I, in which insertional mutagenesis might play an important role.

4. MOLECULAR MECHANISMS OF HCV CARCINOGENESIS

Due to the close association of cirrhosis with HCV-related HCC, studies on the molecular mechanisms of HCV carcinogenesis have been focused on the role of chronic inflammation, steatosis and fibrosis, all of which contribute to the development of cirrhosis. On the other hand, the multiple functions of the HCV proteins and their impacts on cell signaling have led to the concept that the interactions between viral and cellular proteins are instrumental in driving carcinogenesis. In addition, the induction of a mutator phenotype by the expression of HCV proteins provides the key evidence in support of a new model for the development of HCV-associated HCC, in which HCV creates genome instability. Below I will briefly review new progress in the above three aspects of research on HCV carcinogenesis.

4.1. Chronic inflammation, steatosis and fibrosis

In patients chronically infected with HCV, the level of viral replication and the severity of liver disease are associated with a higher risk of HCC (71). Particularly, cirrhosis is an important contributory factor in the development of HCC (70). Cirrhosis is the terminal stage of fibrosis. Prior to the occurrence of fibrosis, the patients have usually exposed to chronic inflammation for many years. Both inflammation and steatosis influence the initiation and progression of fibrosis.

HCV-related HCC is a prototype for inflammation-induced cancer. Persistence of HCV infection is thought to be ascribed at least in part to the selection of quasispecies that have escaped and taken control of host response (40). Viral evasion of innate and adaptive immune response results in the suppression of antiviral defense (40) and the HCV-specific loss of $CD4^+$ T cell help (41). However, HCV is a potent inducer of cellular dsRNA-sensing machinery that responds by activating NFκB and various pro-inflammatory cytokines (76). On the other hand, HCV-specific $CD8^+$ T cells can survive for many years in the chronically infected liver. The liver infiltrating lymphocytes and macrophages are important producers of pro-inflammatory cytokines and key mediators of tissue injury (41). These immune cells that are insufficient for viral clearance are thought to promote tumor progression.

NFκB is one molecular link between inflammation and cancer (76, 77). Particularly, the classical pathway of NFκB activation through IκB kinase β (IKK-β) is an important mediator of inflammation-induced tumor promotion and progression (78). Constitutive activation of NFκB has been well documented in various cancers (76). On one hand, many NFκB target genes encode pro-inflammatory cytokines, chemokines and anti-apoptotic proteins (Figure 3). On the other hand, NFκB activation can induce cellular transformation and promote cellular proliferation (79). Genetic evidence that links NFκB to inflammation-induced cancer is provided by *Mdr2*-knockout mice, a model of cholestatic hepatitis-induced HCC (80). In these mice, the disruption of *Mdr2* leads to bile duct inflammation caused by the bile acid and phospholipid accumulation. HCC usually appears in these mice within 8-10 months after birth. The inflammatory process triggers NFκB activation in hepatocytes through tumor necrosis factor α (TNFα) in adjacent endothelial and

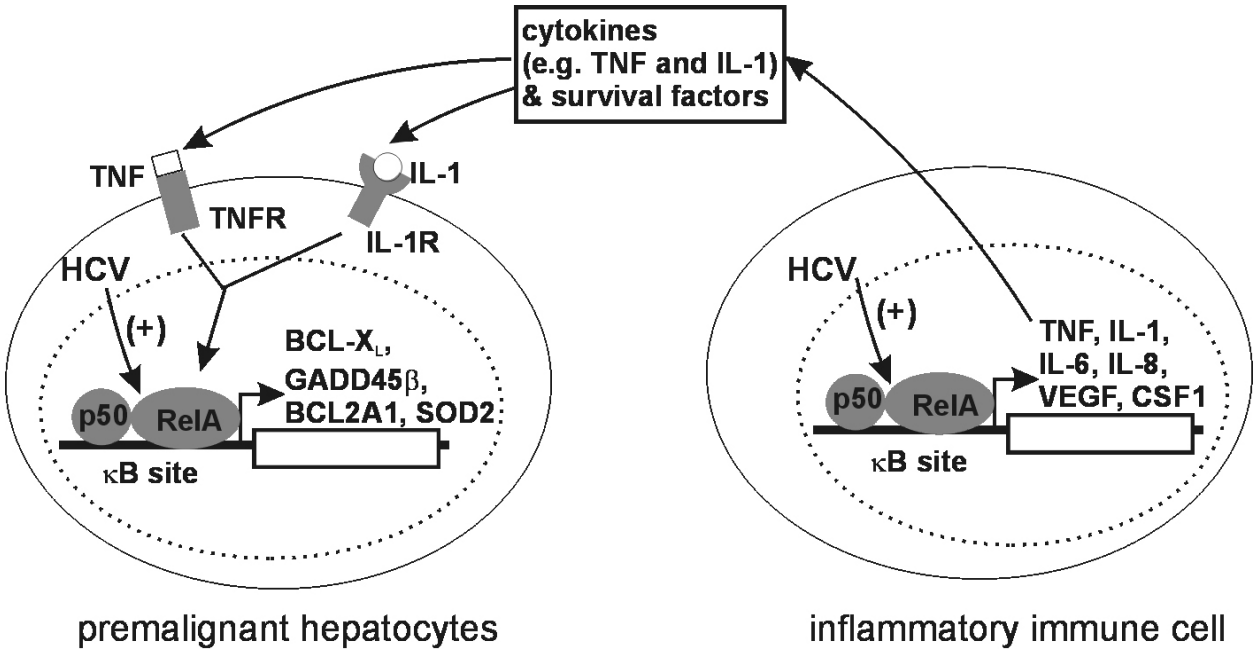

Figure 3. Role of NFκB in inflammation-promoted HCC.

inflammatory cells. Interestingly, suppression of NFκB using an IκB super-repressor in mice from birth to 7 months of age influences neither the course of hepatitis nor the initiation of HCC. However, inhibition of NFκB in later stages leads to apoptosis of transformed hepatocytes and prevention of HCC progression (80). Thus, NFκB is dispensable for early stages of HCC, but required for tumor promotion and progression. Additional genetic evidence of the involvement of IKKβ and NFκB in carcinogenesis comes from mice with a deletion of IKKβ in intestinal epithelial or myeloid cells. Attenuation of the formation of colitis-associated tumor in these mice lends further support to the notion that NFκB links inflammation to cancer (78).

Hepatic steatosis is a common histologic feature of chronic hepatitis C (81, 82). The prevalence of steatosis in patients with chronic hepatitis C is ~50%, ranging from 30% to 70%, which is 2.5-fold higher than expected to occur by chance alone (83). Thus, HCV has a causative role in the development of steatosis. Consistent with this, expression of HCV core protein alone has been shown to have steatogenic effect in transgenic mice and cultured cells (84, 85). On the other hand, steatosis influences disease progression in patients chronically infected with HCV. Indeed, steatosis has recently been identified as an independent risk factor for HCC in people with chronic hepatitis C (86). In addition, steatosis is associated with increased severity of fibrosis in chronic infection of HCV (81, 82). Mechanisms by which steatosis promotes fibrosis are not understood. Activated production of pro-inflammatory and profibrotic cytokines associated with steatosis could play a role in this process. In addition, the induction of oxidative stress has also been thought to be influential in the progression from steatosis to fibrosis (83). Steatosis provides substrates for HCV-related oxidative stress induced by HCV proteins such as core protein (87, 88). Oxidative stress leads to increased lipid peroxidation, the products of which have pro-inflammatory and profibrotic activities (81). These same factors including pro-inflammatory cytokines and oxidative stress are also critical in steatosis-related carcinogenesis (83). Thus, adjuvant therapy directed at steatosis might have beneficial effects in HCV-related cirrhosis and HCC.

Chronic infection of HCV progresses to cirrhosis in ~20 years in 20-30% of patients (70, 89). Cirrhosis is the most important risk factor for HCC in chronic hepatitis C (71). Hepatic cirrhosis is the terminal stage of fibrosis. Fibrosis is the excessive deposition of extracellular matrix (ECM), which reflects a net gain in the processes of fibrogenesis and fibrolysis. Hepatic fibrosis is a slow and multistage process in which several events have to take place. As discussed earlier, some HCV proteins such as core, NS3 and NS5A can induce steatosis and may have direct fibrogenic activity. The generation of oxidative stress and the secretion of profibrotic cytokines are thought to be crucial in the induction of fibrosis (89). One major profibrotic cytokine is transforming growth factor (TGF) β1 (90). Interestingly, certain mutations and polymorphisms in TGFβ1 gene as well as in other cytokine genes (e.g. interleukin 10 and TNFα) have been found to predispose patients to hepatic fibrosis (91). One consensus in the field is that a combination of several factors (i.e. the first and second hits) may determine the outcome of fibrosis. These contributory factors include viral proteins, genetic predisposition, oxidative stress, profibrotic cytokines and growth factors, and immune response (89). Because fibrosis and cirrhosis are causally associated with HCC, the development of antifibrotic therapeutics targeting

TGFβ1 and other steps of hepatic fibrosis provides the opportunity to prevent HCC in patients with chronic hepatitis C.

4.2. Genome instability

Genomic instability refers to an increase in mutability. This is a mutator phenotype in which genetic alterations occur at much higher rates (92). It is now generally accepted that genome instability, a hallmark of cancer, is also required for the initiation of tumorigenesis (92, 93). Two recent studies have suggested that HCV induces genome instability of infected cells (94, 95). Specifically, infection with HCV causes a 5- to 10-fold increase in mutation frequency in immunoglobulin heavy chain, p53, and β-catenin genes in several HCV-infected cells including HCC cells. This mutator phenotype is caused by an increase in DNA double strand breaks (DSBs) and an induction of error-prone DNA polymerases (94). Further analysis of the phenotype indicates that the increase in DSBs is attributed to elevated production of nitric oxide (NO) through activation of the gene encoding inducible NO synthase (iNOS) by HCV core and NS3 proteins. This sequence of events provides one mechanism for HCV-induced genome instability in HCC (95). In addition, an alternative mechanism for HCV-induced genome instability has also been proposed. In that scenario, the interaction of HCV E2 protein with CD81 on the surface of B cells induces the expression of activation-induced cytidine deaminase (AID) and hypermutation of immunoglobulin gene (30). With the development of cell culture systems for HCV infection (7-11), it will be of great interest to re-assess the influence of HCV infection on genome instability and to further investigate the underlying mechanisms in those systems that are more relevant to HCV biology.

The establishment of genome instability is a gateway for additional genetic and epigenetic alterations, which contribute to the promotion and progression of tumor. Above we have discussed mutations in TGFβ locus that confer susceptibility to hepatic fibrogenesis (91). Global search for additional susceptibility genes in Japanese patients with chronic hepatitis C has been performed using a large-scale candidate gene approach (75). Loss-of-heterozygosity (LOH) analysis, comparative genome hybridization (CGH), and promoter methylation analysis have also been carried out on HCV-related HCC samples (96-98). In one of these studies, hypermethylation of tumor suppressor genes SOCS-1, APC, and p15 has been found to be more frequently seen in HCV-related HCC (98). In general, genetic and epigenetic changes in HCV-related HCC are common and significantly different from those seen in HBV-associated HCC.

4.3. HCV oncoproteins

Above I have already mentioned the roles of HCV proteins, particularly core, E2, NS3 and NS5A, in the subversion of host immunity (2.2), stimulation of inflammation (4.1), induction of oxidative stress (4.1) and ER stress (2.2), promotion of steatosis and fibrosis (4.1), and creation of genome instability (4.2). Because HCV is an RNA virus that does not integrate into cellular genome, the oncogenic properties of the virus have been thought to be ascribed to its proteins. Hence, HCV proteins have been extensively studied in transfected cells in culture. Several recent and comprehensive reviews on HCV proteins can be found elsewhere (1, 3, 18, 99, 100). Below I will only summarize the major findings on HCV proteins in relation to their transforming activities.

Four structural and nonstructural proteins of HCV (core, NS3, NS4B and NS5A) have been shown to be transforming in murine fibroblasts (1). In addition, some lines of transgenic mice expressing HCV core protein develop HCC (101, 102). HCV proteins including core, NS3 and NS5A are truly multifunctional (1, 18). These viral proteins fulfill their functions in the cell mainly through interaction with cellular partners. For example, HCV core interacts with a long list of partner proteins found in the cytoplasm, mitochondria, ER, nucleus and other subcellular compartments (1, 88, 99, 100, 103). Moreover, proteomic analysis of partner proteins for HCV core using 2D PAGE followed by mass spectrometry (104) holds the promise to add new candidates to this list. In addition to direct protein-protein interactions, HCV NS3/NS4A protease is able to modulate cell signaling by digesting host signaling proteins (50, 52), as described above in Section 2.2.

Interactions between HCV proteins and their cellular partners are implicated in all major models that have been proposed to explain HCV carcinogenesis. Above I have discussed several of these mechanisms that emphasize the roles of inflammation and immunity (2.2 and 4.1), genome instability (4.2) and oxidative stress (4.1) in the initiation, promotion and progression of HCC tumor associated with HCV infection. HCV proteins are thought to play a central role in all the proposed mechanisms and many of their cellular interaction partners have been identified and characterized. However, additional partners or targets of HCV proteins remain to be understood. For example, HCV core protein has been shown to induce a mutator phenotype (95) likely through interactions with its cellular partners. These partners of HCV core are yet to be identified. On the other hand, further investigations are required to elucidate the functional implications of many HCV binding partners identified through yeast two-hybrid screening or proteomic profiling (104). Importantly, because most if not all of these interactions have been demonstrated only in transfected cells in culture, their relevance to HCV biology is uncertain and should be re-visited with the newly available culture systems for HCV infection (7-11).

In addition to an impact on genome stability and chronic inflammation, HCV proteins are thought to activate cellular oncoproteins and inactivate tumor suppressors through direct protein-protein interactions. Here I will give three examples for the inhibition of tumor suppressors by HCV. First, HCV NS5A has been demonstrated to interact directly with and inhibit p53 (18). Second,, HCV core targets bZIP transcription factor CREB3/LZIP (103), which is a candidate tumor suppressor closely related to liver-specific transactivator CREB-H (105). Third, HCV NS5B

forms a complex with Rb and targets it for degradation (106). On the other hand, the interaction of HCV NS5A with phosphoinositide 3 kinase leads to the stabilization of β-catenin, product of a cellular proto-oncogene (107). Thus, HCV proteins subvert cellular growth control through their stimulatory interactions with cellular oncoproteins and inhibitory interaction with tumor suppressors.

HCV proteins are also known to modulate apoptosis. However, existing data on this issue are very controversial. Both HCV core and NS5A proteins have been shown to be proapoptotic in some studies, and antiapoptotic in others (1, 18, 99). The use of cell culture systems in which HCV proteins are overexpressed could at least in part be accountable for the controversy. Another possibility is that some HCV proteins might have proapoptotic and antiapoptotic activities depending on the cell context and the stage of viral infection. Nevertheless, these discrepancies should be resolved using the new cell culture systems for HCV infection (7-11).

5. SUMMARY AND PERSPECTIVES

More than 15 years after the discovery of HCV, great progress has been made in understanding chronic HCV infection and its association with the development of HCC. It is widely accepted that host immunity plays an important role in HCV pathogenesis. On the other hand, HCV proteins can exert direct effects on cellular growth, metabolism, signal transduction and genome integrity. In this context, key factors that are influential in HCV-related HCC include chronic inflammation, steatosis, fibrosis/cirrhosis, oxidative stress, genome instability, and viral interference with cellular growth control. HCV-associated HCC serves as a good example for inflammation-induced cancer, in which the activation NFκB might adapt pro-inflammatory signals to tumor initiation and development. Concurrently, genome instability induced by HCV proteins is another driving force in the early stages of carcinogenesis. Most mechanistic insights on HCV-related HCC are derived from cultured cells in which HCV proteins are overexpressed. Robust cell culture systems that produce infectious HCV particles have not been available until very recently. It will be of great interest to revisit key issues and resolve major discrepancies in HCV pathogenesis and carcinogenesis using the new systems for HCV infection. One fundamental question to be addressed in these systems is whether chronic infection with HCV really leads to cellular transformation. In addition, global analysis of gene expression patterns in the new cell culture systems for HCV infection using microarray and proteomic approaches will shed significant light on HCV pathology and virus-cell interaction. Other important areas for future research include mechanisms of hepatic fibrosis and its progression to cirrhosis and HCC, mechanisms of HCV-induced genome instability, genetic and epigenetic alteration in HCV-related HCC, genetic susceptibility factors, as well as the role of host immunity in HCV pathogenesis and carcinogenesis. In addition, the development of non-primate small animal model for HCV and related diseases including HCC is still of a high priority. We have entered a new era of HCV research. With the robust cell culture systems for HCV infection, molecular studies on HCV pathogenesis and carcinogenesis will advance at an unprecedented and steady pace.

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Send correspondence to: Dr Dong-Yan Jin, 3/F Laboratory Block, 21 Sassoon Road, Pokfulam, Hong Kong, Tel: 852-28199491, Fax: 852-28551254, E-mail: dyjin@hkucc.hku.hk

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