Molecular mechanisms of hepatocarcinogenesis in chronic hepatitis C virus infection

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Abstract

Hepatitis C virus (HCV) infection is a major cause of hepatocellular carcinoma (HCC) and chronic liver disease worldwide. Recent developments and advances in HCV replication systems *in vitro* and *in vivo*, transgenic animal models, and gene expression profiling approaches have provided novel insights into the mechanisms of HCV replication. They have also helped elucidate host cellular responses, including activated/inactivated signaling pathways, and the relationship between innate immune responses by HCV infection and host genetic traits. However, the mechanisms of hepatocyte malignant transformation induced by HCV infection are still largely unclear, most likely due to the heterogeneity of molecular paths leading to HCC development in each individual. In this review, we summarize recent advances in knowledge about the mechanisms of hepatocarcinogenesis induced by HCV infection.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the third leading cause of cancer death worldwide [1]. The majority of HCCs arise from a background of chronic liver diseases caused by infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) [2]. Although both viruses are hepatotropic and regarded as causative agents of HCC, the underlying mechanisms of hepatocarcinogenesis are considered to be largely different, partly due to differences in the nature of DNA viruses (with an integration capacity for the host genome) and RNA viruses (with no genome integration capacity).

HCV is an RNA virus that is unable to integrate into the host genome but, instead, its proteins interact with various host proteins and induce host responses that potentially contribute to the malignant transformation of cells. In addition, HCC usually develops in the setting of liver cirrhosis after long-term continuous inflammation/regeneration processes; these accelerate the turnover of hepatocytes with increased risk of replication errors and DNA damage. Furthermore, recent genome-wide association studies have suggested that the natural course of HCV infection might be modified by the genetic background of the host [3, 4]. Thus, both host and virus factors are considered to affect the process of hepatocarcinogenesis in a complex manner.

In this review, we summarize the current knowledge of the mechanisms of hepatocarcinogenesis induced by HCV infection. We also focus on recent findings of transcriptomic characteristics of HCV-related HCC and summarize the potential signaling pathways that are altered in this condition.

Epidemiology

Chronic HCV infection is a major risk factor for the development of HCC worldwide. According to the World Health Organization (WHO), approximately 170 million people are chronically infected with HCV. Although epidemiological evidence has suggested a clear, close relationship between HCV infection and HCC, [5, 6] the prevalence of HCV infection in HCC patients differs noticeably between geographical regions. Thus, HCV infection is found in 70–80% of HCC patients in Japan, 70% in Egypt, 40–50% in Italy and Spain, about 20% in the United States (US), and less than 10% in China [7-9]. In industrialized countries including the US, a recent increase in HCC incidence and mortality has been observed, potentially due to the rising incidence of HCV infection transmitted through contaminated blood [10].

HCV increases the risk of HCC by promoting inflammation and fibrosis of the infected liver that eventually results in liver cirrhosis. Once HCV-related cirrhosis is established, HCC develops at an annual rate of about 4–7% [11]. Other factors including alcohol intake, diabetes, and obesity have also been reported to increase the risk of HCC development by about 2-4 fold, indicating a strong life-style effect on the process of hepatocarcinogenesis [12, 13]. Age and male gender are also contributing risk factors for HCV-related HCC, although the detailed mechanisms are still debatable.

Virus proteins and host responses

HCV belongs to the Flaviviridae family. It has a positive-stranded linear RNA genome of about 9.6-kb containing a single large open reading frame encoding three structural (core, E1, and E2) and seven non-structural (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins [14]. The structural proteins form the HCV virions whereas non-structural proteins are involved in the processes of viral replication, assembly, and maturation. HCV proteins are known to be processed by host and viral proteases. Both structural and non-structural proteins can interact with various host cellular proteins to potentially promote the malignant transformation of hepatocytes (see recent reviews [7, 15, 16]). In this review, because of space limitations, we focus on the findings of core and NS5A proteins in terms of host responses potentially evoked during the process of HCV-related hepatocarcinogenesis.

Core protein

HCV core is a 21 kDa nucleocapsid protein with an RNA-binding capacity. In addition to its function in regulating HCV-RNA translation and HCV particle assembly, core protein is known to be involved in mediating the alteration of various host cell signaling pathways, transcriptional activation, modulation of immune responses, apoptosis, oxidative stress, and lipid metabolism [7]. Several recent studies have indicated the statistically significant high frequency of mutations in the *core* gene in HCV-infected patients who developed HCC [17, 18]. However, the functional relevance of mutant core proteins on the malignant transformation of hepatocytes or the HCV life cycle has yet to be clarified.

Evidence of core protein as a causative agent of HCC was initially obtained from the transgenic mice model in which *core* gene overexpression, under the regulation of the HBV regulatory element used as a promoter, resulted in steatosis of mouse livers in early life, with subsequent development of adenoma and HCC [19]. However, another mouse model using a different promoter and of a different strain background resulted only in steatosis or different phenotypes without HCC development [20, 21]. Similar controversial findings were reported in transgenic mice expressing HCV polyprotein or structural protein with regards to the development of HCC [22, 23]. Thus, the role of core protein alone in the development of HCC remains unclear in transgenic mouse models.

Although the direct role of core protein in the malignant transformation of hepatocytes is still under investigation, it seems to be related to the development of hepatic steatosis [19, 24]. Indeed, steatosis is one of the risk factors for the development of HCV-related HCC [25, 26], and activation of the lipogenic pathway has been reported in a subset of HCC cases [27]. Core protein is associated with the surface of lipid droplets in infected cells and might be directly related to steatosis through several factors responsible for lipid biogenesis and degradation, including peroxisome proliferator-activated receptor alpha and sterol-regulatory element binding protein-1 [21, 28-30]. Furthermore, core protein is reported to interact with endoplasmic reticulum (ER) or mitochondrial outer membranes and induce ER stress by perturbation of protein folding or by the accumulation of reactive oxygen species (ROS) through mitochondrial dysfunction [31, 32]. ROS produced in this way might result in DNA damage to the host genome and accelerate the process of hepatocarcinogenesis. Increased hepatic iron deposition may also induce oxidative stress

and lipid peroxidation, thus increasing the risk of HCC development in HCV polyprotein transgenic mice [33].

Since the discovery of HCV, various studies have investigated the role of core on host cells. Its effects have been demonstrated on signaling pathways responsible for the cell cycle, and apoptosis through interaction with several tumor suppressors including p53, p73, and p21 [34-39] as well as apoptosis regulators such as TNF-α signaling or Bcl-2 members [40-42]. However, the data obtained from these studies are relatively inconsistent with each other and have varied across experimental models. Core protein may influence the growth and proliferation of host cells through activation of signaling pathways such as Raf/mitogen activated protein kinase (MAPK) [43], Wnt/beta catenin [16], and transforming growth factor beta (TGF-β) [15, 44]. These pathways are known to be activated in HCC [45]. The findings therefore indicate a potential role for core in cell proliferation or suppression of apoptosis during malignant transformation of hepatocytes in the liver of chronic hepatitis C, where chronic inflammation and regeneration of hepatocytes continuously occurs.

NS5A protein

NS5A is a 56–58 kDa protein phosphorylated at serine residues by serine-threonine kinase [46] and is essential for replication of the HCV genome. NS5A protein forms part of the viral replicase complex and is localized mainly in the cytoplasm of infected cells in association with the ER. NS5A can become a lower molecular weight protein through post-translational modification, after which it can undergo translocation to the nucleus where it acts as a transcriptional activator. High frequencies of wild-type *NS5A* genes were reported

to be dominant in liver cirrhosis patients who finally developed HCC compared with those who did not [47], but the mechanistic significance of the *NS5A* wild/mutant genotypes in the process of HCV-related hepatocarcinogenesis remains uncertain.

NS5A protein has been suggested to interact with various signaling pathways including cell cycle/apoptosis [48] and lipid metabolism [28, 49, 50] in host cells and shares some signaling targets with core protein. NS5A is recognized as a transcriptional activator for many target genes [51] including p53 and its binding protein, TATA binding protein (TBP). Transcription factor IID activities were reported to be modified by NS5A in the suppression of p53-dependent transcriptional transactivation and apoptosis [52, 53]. NS5A may also interact with pathways such as Bcl2 [54], phosphatidylinositol 3-kinase (PI3-K) [55], Wnt/beta catenin signaling [56], and mTOR [57] to activate cell proliferation signaling and inhibit apoptosis.

Taken together, intriguing data concerning the function of core and NS5A proteins on host cell signaling pathways, transcriptional activation, apoptosis, oxidative stress, and lipid metabolism described above suggest a diverse role for HCV proteins in the pathophysiology of chronic hepatitis C that leads to malignant transformation in infected hepatocytes. Key findings and present concepts are summarized in Figure 1).

Transcriptomic characteristics of HCV-related HCC

As described above, HCV proteins can evoke various host responses in infected cells at transcriptional/translational/post-translational levels. Furthermore, enhanced cell death/regeneration processes are considered to induce DNA damage and accelerate replication errors that cause frequent mutations and genomic alteration in the host genome. The central dogma is defined as the flow of genetic information from DNA to mRNA and then to protein, so genetic/genomic alterations and transcriptional/translational modifications are ultimately considered to affect the cellular signaling pathway at the transcriptional level.

Over the past decade, several methods (including differential display, serial analysis of gene expression (SAGE), and microarray) have been developed to allow comparative studies of gene expression between normal and cancer cells on a genome-wide scale [58], and the analysis of a set of all RNA molecules (mainly indicating mRNAs) is termed as whole transcriptome analysis. Extensive transcriptome analysis of HCC and corresponding non-cancerous livers has been performed, and the results have greatly increased our knowledge about the transcriptome characteristics of HCV-related HCC.

Early microarray and SAGE studies investigating the gene expression patterns of chronic hepatitis B and C tissues indicated that these two chronic hepatitis tissues had distinct gene expression profiles; the genes activated in chronic hepatitis C were correlated with signaling pathways associated with apoptosis, oxidative stress responses, and Th1 cytokine signaling [59, 60]. An early study comparing genes activated in HCV-related and HBV-related HCCs showed that the genes associated with xenobiotic metabolism were

more abundantly expressed in HCV-related HCC [61], suggesting a detoxification role which is potentially induced by chronic inflammation and generation of ROS resulting from HCV infection. In contrast, HBV-related HCC might closely correlate with the activation of imprint genes, including IGF-II as investigated by oligo-DNA microarray [62], suggesting a role of de-differentiation or epigenetic alteration of the host genome in HBV-related HCC. Activation of genes associated with interferon, oxidative stress, apoptosis, and lipid metabolism signaling was detected in HCV-related HCC and chronic hepatitis C specimens [27, 60, 63], consistent with numerous functional studies that have investigated the host response evoked by HCV structural and non-structural proteins [48].

Transcriptome analysis has also recently shed new light on the transcriptional alteration events occurring in early stages of HCV-related hepatocarcinogenesis. *GPC3* (encoding Glypican 3) was identified as one of the most activated transcripts in the early stage of hepatocarcinogenesis [60, 64], while several recent studies showed that gene signatures including *GPC3* can successfully discriminate HCCs from pre-malignant dysplastic nodules and cirrhosis nodules [65, 66]. Close examination of genes differentially expressed among cirrhotic nodules, dysplastic nodules, and early and advanced HCV-related HCC tissues has also suggested roles for Toll-like receptor signaling, Wnt signaling, BMP/TGF-β signaling, JAK-STAT signaling, and DNA repair/cell cycle responses in each step of the malignant transformation processes [67]. These processes might therefore provide candidate molecular targets for the chemoprevention of HCV-related HCC.

Recent advances in transcriptome analysis have also provided detailed information on the status of small noncoding RNAs, microRNAs, that can regulate the expression of target genes and viral replication in normal and cancer tissues. Expression of microRNAs including miR-122 and -199a has been reported to modulate HCV replication [68-70], and miR-122 expression can be regulated by host interferon signaling and responses [71]. HCV protein expression in turn could induce miRNAs and might affect the tumor suppressor DLC1 and the chemosensitivity of malignantly transformed cells [72, 73]. Several microRNAs were also differentially expressed between HCV-related and HBV-related HCCs as well as their corresponding non-cancerous liver tissues. The candidate signaling pathways potentially altered by microRNAs in HCV-related tissues were those associated with antigen presentation, cell cycle, and lipid metabolism [74], consistent with the mRNA microarray data described above. MicroRNAs have also recently been reported to successfully discriminate between HCC and cirrhotic liver tissues [75], implicating their role in the early stages of malignant transformation. These data suggest that microRNAs may be good targets for the eradication of HCC as well as hepatocytes infected with HCV.

Conclusion

The heterogeneity of genetic/transcriptomic/proteomic events observed in hepatocytes or cell lines expressing HCV proteins and HCV-related HCCs reported thus far has suggested that complex mechanisms underlie malignant transformation induced by HCV infection. These potentially act through convoluted virus-host interactions including HCV replication

with host cell cycles, apoptosis, proliferation, quality control of protein synthesis, lipid metabolism, and DNA damage responses. Indeed, HCC is a heterogeneous disease in terms of drug sensitivity, metastatic capacity, and clinical outcome. The heterogeneity of HCV-related HCC may closely correlate with the origin of malignantly transformed cells where multifaceted cellular reactions including apoptosis and cell proliferation are induced by HCV infection. An in-depth understanding of these molecular complexities associated with HCV-related HCC may provide the opportunity for effective chemoprevention of HCC among those with HCV-cirrhosis, and to design tailor-made treatment options for HCV-related HCC patients in the future.

References

Figure Legend

Figure 1. Signaling pathways potentially affected by HCV proteins. EGF, epidermal growth factor; IGF, insulin-like growth factor; MAPK, mitogen activated protein kinase; PI3-K, phosphatidylinositol 3-kinase; ER, endoplasmic reticulum; TBP, TATA binding protein; PPAR, peroxisome proliferator-activated receptor; SREBP, sterol-regulatory element binding protein; mTOR, mammalian target of rapamycin; ROS, reactive oxygen species.

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Figure 1