**Name of Journal: *World Journal of Hepatology***

**Manuscript NO: 36565**

**Manuscript Type: Minireviews**

**Molecular basis of hepatocellular carcinoma induced by hepatitis C virus infection**

Irshad M *et al*. HCV induced HCC

**Mohammad Irshad, Priyanka Gupta, Khushboo Irshad**

**Mohammad Irshad, Priyanka Gupta, Khushboo Irshad,** Clinical Biochemistry Division,Department of Laboratory Medicine,All India Institute of Medical Sciences,New Delhi 110029, India

**ORCID number:** Mohammad Irshad (0000-0002-0674-3679); Priyanka Gupta (0000-0001-9813-2260); Khushboo Irshad (0000-0002-1552-3686).

**Author contributions:** All authors made equal contribution in the preparation of this manuscript and final approval of the version of it to be published.

**Conflict-of-interest statement:** The authors declare here that there is no conflict of interest related to this study among them.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Correspondence to:** **Mohammad Irshad, Professor,** Clinical Biochemistry Division, Department of Laboratory Medicine, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India. drirshad54@yahoo.com

**Telephone:** +91-11-26594981

**Fax:** +91-11-26588663

**Received:** October 9, 2017

**Peer-review started:** October 10, 2017

**First decision:** November 7, 2017

**Revised:** November 8, 2017

**Accepted:** December 5, 2017

**Article in press:**

**Published online:**

**Abstract**

Present study outlines a comprehensive view of published information about the underlying mechanisms operational for progression of chronic hepatitis C virus (HCV) infection to development of hepatocellular carcinoma (HCC). These reports are based on the results of animal experiments and human based studies. Although, the exact delineated mechanism is not yet established, there are evidences available to emphasize the involvement of HCV induced chronic inflammation, oxidative stress, insulin resistance, ER stress, hepatosteatosis and liver fibrosis in the progression of HCV chronic disease to HCC. Persistent infection with replicating HCV not only initiates several liver alterations but also creates an environment for development of liver cancer. Various studies have reported that HCV acts both directly as well as indirectly in promoting this process. Whereas HCV related proteins, like HCV core, E1, E2, NS3 and NS5A, modulate signal pathways, dysregulating cell cycle and cell metabolism, the chronic infection produces similar changes in an indirect way. HCV is an RNA virus and does not integrate with host genome and therefore, HCV induced hepatocarcinogenesis pursues a totally different mechanism causing imbalance between suppressor and proto-oncogenes and genomic integrity. However, the exact mechanism of HCC inducement still needs a full understanding of various steps involved in this process.

**Key words:** Hepatitis C virus; Hepatocellular carcinoma; Fibrosis; Core; NS5A; Inflammation

**© The Author(s) 2017.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Hepatocellular carcinoma (HCC) is one of the most common cancer occurring in human population all over the world. Chronic hepatitis C virus (HCV) infection is considered as a major cause of producing HCC in developed countries. HCV infection induces chronic inflammation in liver, which initiates several changes including production of oxidative stress, steatosis, progressive fibrosis, cirrhosis and finally HCC. HCV related proteins also interact directly with cellular proteins at various steps of cell signaling disturbing cell cycle and regeneration process. HCC is supposed, now days, to be the foremost indication for liver transplant.

Irshad M, Gupta P, Irshad K. Molecular basis of hepatocellular carcinoma induced by hepatitis C virus infection. *World J Hepatol* 2017; In press

**INTRODUCTION**

Hepatitis C virus (HCV) infection is a global health problem reported from all parts of the world. HCV was characterised by Choo *et al* and Kuo *et al*[1,2] in 1989. As per WHO report, about 3% world population is having HCV infection with 170 million people becoming as chronic carriers of HCV[3]. These people always remain at high risk of developing cirrhosis of liver and hepatocellular carcinoma (HCC) in later years. There is an increase in the cases of HCC with 1%-7% chronic HCV infected patients developing HCC after establishment of cirrhosis[4,5]. HCC caused by HCV infection is a prominent indication for liver transplant[6].

HCV is an enveloped RNA virus included under Flaviviridae family[7]. It has 9.6 kb single standard RNA with positive polarity. HCV genome encodes a long protein of 3000 amino acids which undergoes proteolysis to yield structural proteins (Envelop E1, E2 and Core) and nonstructural proteins (P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B)[8]. Whereas structural proteins play important role in its morphological features and entry into the host cell, nonstructural proteins are involved mainly in viral replication, assembly and pathogenesis of diseases caused. HCV genome is highly heterogeneous with 32%-35% variations in different HCV genotypes[9]. Based on current reports at least seven genotypes and several subtypes of HCV have been reported till date[10]. Although, variability of genomic sequence has been reported throughout the viral genome, the E1 and E2 regions have been reported to be maximally variable [10].

HCC develops more frequently in cirrhotic patients in comparison to those having mild fibrosis[11]. In addition, hepatitis B virus infection, insulin resistance, obesity and steatohepatitis also promote HCV related HCC[12]. HCC may result from a combined effect of host, environment and viral factors[13]. Immune mediated chronic inflammation during HCV infection is supposed to facilitate the development of HCC. Simultaneously, It may induce HCC by altering many cell pathways involved in cell proliferation, energy metabolism, and apoptosis[14].

As such, HCV is a non-cytopathic virus and initiates hepatic injury by immune mediated reaction-cascade. Although, it is not fully established, however, on the basis of animal experiments and human studies, it is assumed that HCV plays both direct as well as indirect role in inducing HCC[15,16]. Current literature demonstrates that cell death, regeneration, inflammation, oxidative stress and steatosis noted during chronic HCV infection are some of the main reasons responsible for hepatocarcinogenesis[13,17]. Similarly, dysregulation of cell cycle by altered intracellular signaling cascade arising during chronic HCV infection is an important phenomenon in the direction of HCC development. In fact, mechanism of hepatocarcinogenesis during chronic HCV infection is slightly distinct from those responsible for causing other types of cancers. HCV core protein was found to induce HCC in absence of genetic aberrations and so, this was named as “non- Vogelstein- type” carcinogenesis in some reports[18]. This may explain a high incidence and multicentric nature of HCC developed during HCV infection. Present review describes a compilation of informations on the mechanisms of HCC development during chronic hepatitis C virus infection.

***Mechanisms of HCV induced HCC***

HCV is a hepatotropic virus and enters host cell *via* a complex sets of molecules present on cell surface including CD81 (receptor molecule), SRB-1 (scavenger receptor) and Occluding-1 and Claudin (tight junction proteins)[19-21]. After its entry, HCV replicates in hepatocytes and leads to different types of cellular and immune mediated changes. A majority of patients infected with HCV fail to clear the virus. In these patients HCV persists for longer duration causing chronic HCV infection and a high risk for progressive hepatic fibrosis, cirrhosis and HCC[22]. Simultaneously, the ensuing chronic inflammation associated with oxidative stress and emerging cellular DNA damage, also contribute to development of HCV associated HCC. The question whether cancer develops in infected hepatocytes or in uninfected hepatocytes still needs to be answered. Based on some experimental studies it was reported that Ki67 proliferation marker is raised in advanced HCV infected hepatocytes pointing towards HCV infected cells at higher risk for HCC as compared to uninfected cells[23,24]. Several studies suggest that liver cancer develops by an interplay of host, viral and environmental factors. All these finally bring some epigenetic changes in HCV infected hepatocytes leading to development of HCC [13,25].

Chronic HCV infection is often accompanied by several disturbances including inflammation, steatosis and progressive fibrosis in the liver[25]. All these changes ultimately progress to cirrhosis and hepatocarcinogenesis. Therefore, it is suggested that HCC is caused by an interplay of chronic inflammation, insulin resistance (IR), hepatosteotasis, oxidative stress, fibrosis, and the resulting liver damages by chronic HCV infection. This interplay produces a pro-oncogenic microenvironment which promotes fibrogenesis and genetic instability[26]. Simultaneous with a direct transforming role of HCV, the liver microenvironment is supposed to have a modulating effect on cell transforming process during HCC development. Several HCV proteins have direct oncogenic effects and use liver changes in upregulating mitogenic process[27]. At the same time, increasing cell proliferation in this environment also results in DNA damage causing genomic disturbances. This becomes another basis for malignant transformation of hepatocytes. In view of all these available reports[25-27], the mechanism of HCV induced HCC may be illustrated by a direct and indirect role of HCV in relation to the microenvironment produced by chronic HCV infection (Figure 1).

**HOST FACTORS**

***Inflammation and oxidative stress in HCV induced HCC***

Immune mediated inflammation caused during chronic HCV infection indirectly triggers hepatocarcinogensis. Simultaneous with a direct role of HCV in inducement of HCC by altering several cellular pathways involved in metabolism, DNA repair and apoptosis[14], chronic HCV infection enhances the reactive oxygen species (ROS) which damages the liver cells. At the same time, HCV also induces inflammation by activating hepatic stellate cells (HSCs)[28]. These HSCs get activated by ROS, growth factors, cytokines, adipokines and chemokines secreted by hepatocytes, Kuffer cells and inflammatory cells[29]. The progress of disease is increased by cumulative effect of inflammation, ROS, steatosis and IR caused during chronic HCV infection. The activated HSCs, under the effect of fibrogenic cytokines undergo epithelial to mesenchymal trans-differentiation (EMT) into myofibroblast like cells which cause liver fibrosis[14]. Transforming growth factor beta (TGF- β) cytokine regulates EMT demonstrating its pro-oncogenic functions[30]. Hepatic fibrosis is closely associated with HCC development. EMT pathway plays a major role in transition of hepatocyte to cancerous cell and process of metastasis know with expression of E-cadherin and Vimentin[31]. The IR stimulates HSCs and links fibrosis with steatosis. The process of fibrogenesis is regulated by a number of signaling pathways including SMADs, phosphatidylinositide 3-kinases (PI3K), Akt, Mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinases (JNKs) pathways. JNK activation by IL1- β cytokine increases fibrogenesis, oncogenesis and cell motility[32,33]. Thus all these liver alterations finally produce a suitable environment for development of HCC in chronic HCV infection.

***Insulin resistance and hepatosteatosis in HCV induced HCC***

It has been observed that HCV *genotype-3* induces steatosis in patients with chronic HCV infection[34]. HCV induces steatosis by increasing lipid synthesis and reducing its secretion and degradation. The structural and nonstructural proteins of HCV directly interferes in lipid synthesis[35] and Very-low-density lipoprotein (VLDL) secretion[36,37]. These HCV related proteins also inhibit fatty acid oxidation[38,39] and enhance fatty acid release from adipocytes[34]. All this finally results in hepatic steatosis. The HCV related proteins are also involved in producing ROS[40] and glucose homeostasis. HCV interferes with insulin signaling by proteosomal degradation of Insulin receptor substrate 1 (IRS-1) and Insulin receptor substrate 2 (IRS-2) by Suppressor of cytokine signaling (SOCS) protein or PI3K/Akt/mTOR pathway. IRS-1 is reported to be inactivated by TGF-αand PI3K/Akt also[41]. In this manner, the early stage of chronic HCV infection with increasing steatosis and IR creates an environment to help in hepatcarcinogensis leading to development of HCC.

***Immune mediated liver alteration in HCV induced HCC***

HCV influences both innate and adaptive immunity. This virus inhibits type 1 Interferon production and CD4+ T-cell transformation to Th2, Th17 and regulatory T-cell. This disturbs the function of cytotoxic CD8+ T-cells and natural killer (NK) cells[42-48]. It results in chronic liver inflammation which disturbs tissue homeostasis and promotes pro-carcinogenic environment. Simultaneously, there is an increase in the release of ROS, NO (Nitric oxide), cytotoxic cytokines and lipid peroxidation. It also helps in immune escape of neoplastic transformed cells facilitating the development of HCC[49]. During chronic HCV infection, the inflammatory cytokine like TNF-α, IL-1, IL-23, IL-6 and Lymphotoxins-alpha and beta (LT-α and β) are also increased causing chronic liver inflammation and HCC progression[49-51]. There is already a report demonstrating an important role of LT-α and LT-β in the development of HCC[51]. In fact, activation of NF-κB pathway by LTs triggers the hepatocarcinogenesis by increasing production of chemokines and cytokines. In patients with chronic HCV infection, the liver infiltrating T and B-cells not only fail viral clearances but also increase chronic inflammation[51,52]. Also, an increased number of CD8+ is accompanied by reduction in NK and NKT cells which are involved in cancer immune surveillance[52]. These informations indicate that during chronic HCV infection there is a regular tumor promoting inflammation and impaired anticancer immune scanning, which ultimately facilitates towards HCC.

***Hepatic fibrosis in HCV induced HCC***

As described earlier there is high occurrence of steatohepatitis in patients with chronic HCV infection. The accumulation of free fatty acid induces production of ROS and mitochondrial dysfunction and Endoplasmic reticulum (ER) stress. In turn, oxidative stress stimulates lipid peroxidation and increases inflammation in liver tissue. Increased ROS levels have direct effect on fibrosis by increasing collagen 1 expression[50]. The HCV induced steatosis changes the liver T-cell function. HCV related proteins in the liver develop extensive steatosis which is accompanied by an infiltrate of CD8+ T-cell secreting Th2 type cytokine[53]. A massive liver infiltration by CD8+ and NKT cells induces steatosis, inflammation and carcinogenesis[54]. In HCV infected patients, the risk of HCC development may also be linked with the severity of liver fibrosis. TGF-β is an important cytokine involved in fibrogenesis. Its expression is directly affected by HCV related proteins or oxidative/ ER stress and NF-κB pathway activation[55-58]. This concludes that hepatic fibrosis caused by various mechanisms is a big inducer promoting hepatocarcinogenesis.

***Genetic factors in HCV induced HCC***

There are a number of genes associated with HCV induced HCC. The tumor suppressor gene P53 was the first one noted for its association with development of HCC. Recent studies have shown a subset of genes frequently mutated in HCV patients[59,60]. Oncogene CTNNB1 which encodes β-catenin protein of WNT-pathway shows a mutation of 30%. WNT ligands activate signal transduction cascade resulting in inhibition of beta-catenin degradation complex. It has been observed that WNT pathway get mutated in HCC, which stabilizes beta-catenin, this beta catenin translocates to the nucleus and regulate genes responsible for cell survival and proliferation. NS5A indirectly regulate the WNT pathway through PI3K and activate Akt. Increased beta-catenin has been observed in HCV infected cells. The significance of beta-catenin with is HCV infected cells is still uncertain[61]. However, Its level is increased mostly in HCC patients. Similarly, reduction in the size of telomere triggers cellular senescence. Activation mutation in the telomerase reverse transcriptase (TERT) promoter gene has been detected in HCC induced by HCV infection in addition to other etiologies[62-64]. HCV core protein downregulates CDKN2A expression to overcome hepatocyte senescence. Increased telomerase activity a characteristic of transforming or transformation prone cells was observed in HCV core- transfected primary human hepatocytes that acquired an immortalized phenotype. In line with this observation, somatic mutation in the TERT promoter that enhance TERT expression were shown to be among the earliest and most prevalent neoplastic events associated with all major etiologies including HCV. Host genetic variants are also associated with a high risk of HCC[65]. PNPLA3 gene (Patatin-like phospholipase domain-containing protein-2) shows a significant association with fatty liver disease in HCV patients having a higher risk of HCC[66-68]. On a similar pattern, polymorphisms in several other cytokines/receptors genes have been found to be associated with HCC. These are cytokines TNF-α, IL-10, IL-23R and VEGF *etc.* genes. Host respond differently to variation in viral genome for example HCV genotype 1a and 1b reported to be associated with HCC[69].

***Epigenetic alterations in HCV induced HCC***

Various studies have demonstrated a dysregulation of epigenetic regulatory genes in HCC[70]. Histone- lysine N- Methyltransferase enzyme (EZH2) is one such an example which is aberrantly expressed in HCC[71] and this also targets expression of tumor suppressor miRNAs[72]. The changes in gene methylation were also related with virus induced tumors[73]. Various tumor suppressor genes including CDKN2A, GSTP1, RUNX3, APC, SOCS-1 and RASSF1Aare highly methylated in HCC caused by HBV and HCV infection[74]. Epigenetic alterations in HCC may be mediated by changes in miRNAs and long noncoding RNAs. There are several miRNAs which modulate HCV replication in a positive and negative manner[75].

***Neoangiogenesis in HCV induced HCC***

Structural and nonstructural HCV proteins have a direct role in inducing neoangiogenesis. HCV core promotes angiogenesis by upregulating hypoxia inducible factor 1-alpha which regulates vascular endothelial growth factor (VEGF) and cyclooxygenase 2. VEGF is an important endothelium specific growth factor in HCC and for this reason, VEGF level in serum isused as a prognostic factor in HCC[76]. Angiopoietin-2 is also upregulated by HCV infection[77].

**VIRAL FACTORS**

HCV replicates and releases its protein component in cytosol, HCV related proteins which have a major role in regulating viral replication and HCV particle assembly, have been demonstrated to influence several cell signal pathways and metabolic mechanisms indicating their role in cell cycle and cell transformation. Both structural and nonstructural proteins interact with different host cellular proteins to promote malignant transformation of hepatocytes. Based on these studies we describe here the role of each individual HCV protein in the process of cell transformation to malignant liver cell.

***Core protein***

HCV core protein, which regulates HCV RNA translation and its replication, interacts with component proteins of various cell-signaling pathways. In addition, this protein modulates host immune response, oxidative stress, lipid metabolism and also apoptosis[78]. In some recent studies on HCV infected patients, core gene has been found to undergo frequent mutations[79]. The role of core protein in the development of HCC was studied in transgenic mouse model. The information collected from these studies indicate that core gene overexpression results in steatosis in early life with development of adenoma and HCC in later years[80]. In few other studies, the presence of steatosis in liver induced by core protein could not be related to HCC development[29,81]. According to recent reports, Core protein shows interference with cellular proteins and it is considered as a major risk factor for the progression of HCC[82]. Of course, the presence of core protein has been associated with its activation of lipogenic pathway in HCC cases[83]. Core protein often remains associated with lipid droplets in CHC cases and possibly causes steatosis through several mechanisms including peroxisome proliferator-activated receptor alpha (PPAR-α) and sterol-regulatory element binding protein-1 pathways[46,81,84].

Similarly, core protein also interacts with ER or mitochondria and induces ER stress by accumulation of ROS[85]. ROS causes DNA damage and accelerates hepatocarcinogenesis. The effect of HCV core have also been demonstrated on signaling pathways responsible for cell cycle like stimulation of G1/S transition by increasing the levels of cyclin E/Cdk2[86] and apoptosis. Core protein interacts with tumor suppressor including P53, P73 and P21[87] as well as regulator of apoptosis like TNF-α signaling or Bcl-2 members. Core proteins also effects growth and proliferation of cells through activation of signaling pathways like RAF/MAPK (Mitogen activated protein kinase)[88], WNT/β-cateninand Transforming growth factor-β (TGF-β)[39,89]. All these pathways have been reported to be active in HCC. Therefore, these findings about HCV core indicate that this protein has a potential role in cell proliferation and reduction of apoptosis during development of HCC.

***E1/E2 protein***

The effect of structural proteins E1/E2 was also studied on malignant transformation of hepatocytes. The results indicated these proteins to interfere with Interferon actions by inhibiting dsRNA protein kinase (PKR)[90,91]. In addition, E2 protein also inhibits activation of T and NK cells[91] and MAPK/ERK (Extracellular signal regulated protein kinase) pathway including the transcription factor ATF-2 and promotes cell proliferation and cell survival[92].

***NS2 protein***

NS2 activates cyclin D/ CDK4 and induces expression of Cyclin E[93]. Some studies also supported its role in the inhibition of apoptosis by interference with p53 pathway.

***NS3 protein***

The NS3 transforms mammalian cells but its role in HCC is less clear[94,95]. This protein interacts with tumor suppressor p53. NS3 protein modulates various signal transduction pathways having transformation potential. NS3 interacts with Protein Kinase A (PKA) and inhibits its translocation to nucleus. NS3 also inhibit Interferon response factor (IRF-3) mediated induction of type-1 interferon, necessary to escape immune surveillance. NS3/4A interacts with ATM, Check point kinase, preventing DNA repair. This also disturbs endoplasmic reticulum leading to cell death[96]. Similarly, NS3/4A target adaptor molecules in TLR3 and RIG 1 signal pathway, thereby interfering with activation of IRF3 transcription factor and promoting proliferation[97-99]. All these reactions contribute to cancer promoting effect of HCV.

***NS5A protein***

This protein is needed for replication of HCV genome. It forms part of viral replicates complex. Inside the nucleus, NS5A acts as transcription factor activator[100] and interacts with various signaling pathways including cell cycle/ apoptosis, lipid metabolism[46,101] and also shares some signaling targets with core. It has been reported to interfere with PKR-p38 signaling pathway and inducing aberrant mitosis and chromosomal instability leading to HCC[102]. NS5A inhibits TGF-β signaling by preventing nuclear translocation of SMAD proteins down regulating tumor suppressor CDKN1A[103]. On a similar pattern, NS5A inhibits TNF- α (Tumor necrosis factor-α) mediated apoptosis[104]. NS5A acts a transcriptional activator for many genes including p53. NS5A also interacts with pathways like Bcl-2, PI3K, WNT/β-catenin signal and mTOR for proliferation of cells and inhibition of apoptosis. It has been found that HCV NS5A influences EMT pathway and helps in transition process of epithelial cells to mesenchymal stem cells. NS5A work in cooperation to TGF-beta to activate stellate cell causing fibrosis. Also HCV core protein was found to induce EMT in primary hepatocyte by suppressing cytostatic effect via SMAD3[105,106]. Thus NS5A and core produce cells in tumor mass that are not differentiated and mobile via EMT pathway EMT contributes to liver fibrosis on the line as in lungs, kidney and intestine.

***NS5B protein***

NS5B binds with Rb and promotes its cytoplasmic relocalization and proteasomal degradation[107,108]. This finally activates E2F responsive genes, which in turn stimulates cell cycle progression[108].

Above reports demonstrate a clear effect of HCV-related proteins on various pathways engaged in progression of infected cells to malignant cells. These proteins enhance the level of underlying inflammation, oxidative stress, ER stress, steatosis, fibrogenesis and finally cell proliferation. Although it is not possible to emphasis their direct effect in exclusion either on initiation or progression, but there is no doubt that involvement of these proteins at various steps of complex mechanism, helps in progression of carcinogenesis resulting in development of HCC.

**CONCLUSION**

This update on the development of hepatocellular carcinoma following chronic hepatitis C virus infection demonstrates that HCV infection is a serious health problem recorded globally. A majority of patients progress to end stage liver diseases including liver cirrhosis and hepatocellular carcinoma (HCC). Once established, the chronic HCV infection produces several changes in the liver including chronic inflammation, insulin resistance, oxidative stress, steatosis and continuing liver fibrosis. These changes are caused by the mechanism influenced either directly or indirectly by HCV particles. HCV related proteins interact with several cellular proteins thereby modulating cell signaling. Similarly, chronic inflammation caused by HCV inflammation also promotes all above liver changes. During this interplay of various reaction cascade there is possibility of genomic imbalance disturbing the normal reactions leading to abnormal cell cycle and apoptosis. The cumulative effect of all these finally facilitates the tumorigenesis in liver causing HCC. Although several lines of information are available, however, much more still needs to be answered to extricate this mystery.

**ACKNOWLEDGEMENTS**

We appreciate the infrastructure provided by All India Institute of Medical Sciences, New Delhi, India, for conduct of this study.

**REFERENCES**

1 **Choo QL**, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; **244**: 359-362 [PMID: 2523562]

2 **Kuo G**, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevens CE. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science* 1989; **244**: 362-364 [PMID: 2496467]

3 **Global surveillance and control of hepatitis C.** Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. *J Viral Hepat* 1999; **6**: 35-47 [PMID: 10847128]

4 **Yoshida H**, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, Nishiguchi S, Kuroki T, Imazeki F, Yokosuka O, Kinoyama S, Yamada G, Omata M. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med* 1999; **131**: 174-181 [PMID: 10428733]

5 **Bandiera S**, Billie Bian C, Hoshida Y, Baumert TF, Zeisel MB. Chronic hepatitis C virus infection and pathogenesis of hepatocellular carcinoma. *Curr Opin Virol* 2016; **20**: 99-105 [PMID: 27741441 DOI: 10.1016/j.coviro.2016.09.010]

6 **Testino G**, Sumberaz A, Leone S, Borro P. Recurrent hepatitis C and non-alcoholic fatty liver disease in transplanted patients: a review. *Minerva Med* 2013; **104**: 225-232 [PMID: 23514999]

7 **Lauer GM**, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41-52 [PMID: 11439948]

8 **Penin F,** Dubuisson J, Rey FA, Moradpour D, Pawlotsky JM. Structural biology of hepatitis C virus. Hepatology 2004; 39: 5–19

9 **Irshad M**, Ansari MA, Singh A, Nag P, Raghvendra L, Singh S, Badhal SS. HCV-genotypes: a review on their origin, global status, assay system, pathogenecity and response to treatment. *Hepatogastroenterology* 2010; **57**: 1529-1538 [PMID: 21443116]

10 **Smith DB**, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, Simmonds P. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. *Hepatology* 2014; **59**: 318-327 [PMID: 24115039 DOI: 10.1002/hep.26744]

11 **Llovet JM**, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, Gores G. Hepatocellular carcinoma. *Nat Rev Dis Primers* 2016; **2**: 16018 [PMID: 27158749 DOI: 10.1038/nrdp.2016.18]

12 **El-Serag HB**. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273.e1 [PMID: 22537432 DOI: 10.1053/j.gastro.2011.12.061]

13 **Vescovo T**, Refolo G, Vitagliano G, Fimia GM, Piacentini M. Molecular mechanisms of hepatitis C virus-induced hepatocellular carcinoma. *Clin Microbiol Infect* 2016; **22**: 853-861 [PMID: 27476823 DOI: 10.1016/j.cmi.2016.07.019]

14 **Bartosch B**, Thimme R, Blum HE, Zoulim F. Hepatitis C virus-induced hepatocarcinogenesis. *J Hepatol* 2009; **51**: 810-820 [PMID: 19545926 DOI: 10.1016/j.jhep.2009.05.008]

15 **Mailly L**, Robinet E, Meuleman P, Baumert TF, Zeisel MB. Hepatitis C virus infection and related liver disease: the quest for the best animal model. *Front Microbiol* 2013; **4**: 213 [PMID: 23898329 DOI: 10.3389/fmicb.2013.00212]

16 **Billerbeck E**, de Jong Y, Dorner M, de la Fuente C, Ploss A. Animal models for hepatitis C. *Curr Top Microbiol Immunol* 2013; **369**: 49-86 [PMID: 23463197 DOI: 10.1007/978-3-642-27340-7\_3]

17 **Waller LP,** Deshpande V, Pyrsopoulos N. Hepatocellular carcinoma: A comprehensive review. World J Hepatol 2015; 7: 2648-2663 [doi:10.4254/wjh.v7.i26.2648]

18 **Koike K**. Hepatitis C virus contributes to hepatocarcinogenesis by modulating metabolic and intracellular signaling pathways. *J Gastroenterol Hepatol* 2007; **22 Suppl 1**: S108-S111 [PMID: 17567457]

19 **Pileri P**, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa D, Grandi G, Abrignani S. Binding of hepatitis C virus to CD81. *Science* 1998; **282**: 938-941 [PMID: 9794763]

20 **Sasaki M**, Yamauchi K, Nakanishi T, Kamogawa Y, Hayashi N. In vitro binding of hepatitis C virus to CD81-positive and -negative human cell lines. *J Gastroenterol Hepatol* 2003; **18**: 74-79 [PMID: 12519228]

21 **Meredith LW**, Wilson GK, Fletcher NF, McKeating JA. Hepatitis C virus entry: beyond receptors. *Rev Med Virol* 2012; **22**: 182-193 [PMID: 22392805 DOI: 10.1002/rmv.723]

22 **Sebastiani G**, Gkouvatsos K, Pantopoulos K. Chronic hepatitis C and liver fibrosis. *World J Gastroenterol* 2014; **20**: 11033-11053 [PMID: 25170193 DOI: 10.3748/wjg.v20.i32.11033]

23 **Koskinas J**, Petraki K, Kavantzas N, Rapti I, Kountouras D, Hadziyannis S. Hepatic expression of the proliferative marker Ki-67 and p53 protein in HBV or HCV cirrhosis in relation to dysplastic liver cell changes and hepatocellular carcinoma. *J Viral Hepat* 2005; **12**: 635-641 [PMID: 16255765]

24 **Dutta U**, Kench J, Byth K, Khan MH, Lin R, Liddle C, Farrell GC. Hepatocellular proliferation and development of hepatocellular carcinoma: a case-control study in chronic hepatitis C. *Hum Pathol* 1998; **29**: 1279-1284 [PMID: 9824107]

25 **Goossens N**, Hoshida Y. Hepatitis C virus-induced hepatocellular carcinoma. *Clin Mol Hepatol* 2015; **21**: 105-114 [PMID: 26157746 DOI: 10.3350/cmh.2015.21.2.105]

26 **Okuda M**, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, Weinman SA. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology* 2002; **122**: 366-375 [PMID: 11832451]

27 **He QQ**, Cheng RX, Sun Y, Feng DY, Chen ZC, Zheng H. Hepatocyte transformation and tumor development induced by hepatitis C virus NS3 c-terminal deleted protein. *World J Gastroenterol* 2003; **9**: 474-478 [PMID: 12632500]

28 **Sun B**, Karin M. NF-kappaB signaling, liver disease and hepatoprotective agents. *Oncogene* 2008; **27**: 6228-6244 [PMID: 18931690]

29 **McGivern DR**, Lemon SM. Virus-specific mechanisms of carcinogenesis in hepatitis C virus associated liver cancer. *Oncogene* 2011; **30**: 1969-1983 [PMID: 21258404 DOI: 10.1038/onc.2010.594]

30 **Hoshida Y**, Fuchs BC, Bardeesy N, Baumert TF, Chung RT. Pathogenesis and prevention of hepatitis C virus-induced hepatocellular carcinoma. *J Hepatol* 2014; **61**: S79-S90 [PMID: 25443348 DOI: 10.1016/j.jhep.2014.07.010]

31 **Nalluri SM**, O'Connor JW, Gomez EW. Cytoskeletal signaling in TGFβ-induced epithelial-mesenchymal transition. *Cytoskeleton (Hoboken)* 2015; **72**: 557-569 [PMID: 26543012 DOI: 10.1002/cm.21263]

32 **Matsuzaki K**, Murata M, Yoshida K, Sekimoto G, Uemura Y, Sakaida N, Kaibori M, Kamiyama Y, Nishizawa M, Fujisawa J, Okazaki K, Seki T. Chronic inflammation associated with hepatitis C virus infection perturbs hepatic transforming growth factor beta signaling, promoting cirrhosis and hepatocellular carcinoma. *Hepatology* 2007; **46**: 48-57 [PMID: 17596875]

33 **Giannelli G**, Bergamini C, Fransvea E, Sgarra C, Antonaci S. Laminin-5 with transforming growth factor-beta1 induces epithelial to mesenchymal transition in hepatocellular carcinoma. *Gastroenterology* 2005; **129**: 1375-1383 [PMID: 16285938 DOI: 10.1053/j.gastro.2005.09.055]

34 **Negro F**. Mechanisms and significance of liver steatosis in hepatitis C virus infection. *World J Gastroenterol* 2006; **12**: 6756-6765 [PMID: 17106922]

35 **Waris G**, Felmlee DJ, Negro F, Siddiqui A. Hepatitis C virus induces proteolytic cleavage of sterol regulatory element binding proteins and stimulates their phosphorylation via oxidative stress. *J Virol* 2007; **81**: 8122-8130 [PMID: 17507484 DOI: 10.1128/JVI.00125-07]

36 **Wetterau JR**, Lin MC, Jamil H. Microsomal triglyceride transfer protein. *Biochim Biophys Acta* 1997; **1345**: 136-150 [PMID: 9106493]

37 **Domitrovich AM**, Felmlee DJ, Siddiqui A. Hepatitis C virus nonstructural proteins inhibit apolipoprotein B100 secretion. *J Biol Chem* 2005; **280**: 39802-39808 [PMID: 16203724 DOI: 10.1074/jbc.M510391200]

38 **Cheng Y**, Dharancy S, Malapel M, Desreumaux P. Hepatitis C virus infection down-regulates the expression of peroxisome proliferator-activated receptor alpha and carnitine palmitoyl acyl-CoA transferase 1A. *World J Gastroenterol* 2005; **11**: 7591-7596 [PMID: 16437683]

39 **Dharancy S**, Malapel M, Perlemuter G, Roskams T, Cheng Y, Dubuquoy L, Podevin P, Conti F, Canva V, Philippe D, Gambiez L, Mathurin P, Paris JC, Schoonjans K, Calmus Y, Pol S, Auwerx J, Desreumaux P. Impaired expression of the peroxisome proliferator-activated receptor alpha during hepatitis C virus infection. *Gastroenterology* 2005; **128**: 334-342 [PMID: 15685545]

40 **Tardif KD**, Waris G, Siddiqui A. Hepatitis C virus, ER stress, and oxidative stress. *Trends Microbiol* 2005; **13**: 159-163 [PMID: 15817385 DOI: 10.1016/j.tim.2005.02.004]

41 **Aytug S**, Reich D, Sapiro LE, Bernstein D, Begum N. Impaired IRS-1/PI3-kinase signaling in patients with HCV: a mechanism for increased prevalence of type 2 diabetes. *Hepatology* 2003; **38**: 1384-1392 [PMID: 14647049 DOI: 10.1016/j.hep.2003.09.012]

42 **Gong G**, Waris G, Tanveer R, Siddiqui A. Human hepatitis C virus NS5A protein alters intracellular calcium levels, induces oxidative stress, and activates STAT-3 and NF-kappa B. *Proc Natl Acad Sci U S A* 2001; **98**: 9599-9604 [PMID: 11481452 DOI: 10.1073/pnas.171311298]

43 **Piccoli C**, Quarato G, Ripoli M, D'Aprile A, Scrima R, Cela O, Boffoli D, Moradpour D, Capitanio N. HCV infection induces mitochondrial bioenergetic unbalance: causes and effects. *Biochim Biophys Acta* 2009; **1787**: 539-546 [PMID: 19094961 DOI: 10.1016/j.bbabio.2008.11.008]

44 **Tsukiyama-Kohara K**. Role of oxidative stress in hepatocarcinogenesis induced by hepatitis C virus. *Int J Mol Sci* 2012; **13**: 15271-15278 [PMID: 23203124 DOI: 10.3390/ijms131115271]

45 **Park SH**, Rehermann B. Immune responses to HCV and other hepatitis viruses. *Immunity* 2014; **40**: 13-24 [PMID: 24439265 DOI: 10.1016/j.immuni.2013.12.010]

46 **Lee HC**, Sung SS, Krueger PD, Jo YA, Rosen HR, Ziegler SF, Hahn YS. Hepatitis C virus promotes T-helper (Th)17 responses through thymic stromal lymphopoietin production by infected hepatocytes. *Hepatology* 2013; **57**: 1314-1324 [PMID: 23150092 DOI: 10.1002/hep.26128]

47 **Urbani S**, Amadei B, Fisicaro P, Tola D, Orlandini A, Sacchelli L, Mori C, Missale G, Ferrari C. Outcome of acute hepatitis C is related to virus-specific CD4 function and maturation of antiviral memory CD8 responses. *Hepatology* 2006; **44**: 126-139 [PMID: 16799989 DOI: 10.1002/hep.21242]

48 **Rehermann B**. Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells. *Nat Med* 2013; **19**: 859-868 [PMID: 23836236 DOI: 10.1038/nm.3251]

49 **Grivennikov SI**, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010; **140**: 883-899 [PMID: 20303878 DOI: 10.1016/j.cell.2010.01.025]

50 **Nakagawa H**, Maeda S, Yoshida H, Tateishi R, Masuzaki R, Ohki T, Hayakawa Y, Kinoshita H, Yamakado M, Kato N, Shiina S, Omata M. Serum IL-6 levels and the risk for hepatocarcinogenesis in chronic hepatitis C patients: an analysis based on gender differences. *Int J Cancer* 2009; **125**: 2264-2269 [PMID: 19585572 DOI: 10.1002/ijc.24720]

51 **Haybaeck J**, Zeller N, Wolf MJ, Weber A, Wagner U, Kurrer MO, Bremer J, Iezzi G, Graf R, Clavien PA, Thimme R, Blum H, Nedospasov SA, Zatloukal K, Ramzan M, Ciesek S, Pietschmann T, Marche PN, Karin M, Kopf M, Browning JL, Aguzzi A, Heikenwalder M. A lymphotoxin-driven pathway to hepatocellular carcinoma. *Cancer Cell* 2009; **16**: 295-308 [PMID: 19800575 DOI: 10.1016/j.ccr.2009.08.021]

52 **Ramzan M**, Sturm N, Decaens T, Bioulac-Sage P, Bancel B, Merle P, Tran Van Nhieu J, Slama R, Letoublon C, Zarski JP, Jouvin-Marche E, Marche PN, Leroy V. Liver-infiltrating CD8(+) lymphocytes as prognostic factor for tumour recurrence in hepatitis C virus-related hepatocellular carcinoma. *Liver Int* 2016; **36**: 434-444 [PMID: 26215124 DOI: 10.1111/liv.12927]

53 **Alonzi T**, Agrati C, Costabile B, Cicchini C, Amicone L, Cavallari C, Rocca CD, Folgori A, Fipaldini C, Poccia F, Monica NL, Tripodi M. Steatosis and intrahepatic lymphocyte recruitment in hepatitis C virus transgenic mice. *J Gen Virol* 2004; **85**: 1509-1520 [PMID: 15166435 DOI: 10.1099/vir.0.19724-0]

54 **Wolf MJ**, Adili A, Piotrowitz K, Abdullah Z, Boege Y, Stemmer K, Ringelhan M, Simonavicius N, Egger M, Wohlleber D, Lorentzen A, Einer C, Schulz S, Clavel T, Protzer U, Thiele C, Zischka H, Moch H, Tschöp M, Tumanov AV, Haller D, Unger K, Karin M, Kopf M, Knolle P, Weber A, Heikenwalder M. Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. *Cancer Cell* 2014; **26**: 549-564 [PMID: 25314080 DOI: 10.1016/j.ccell.2014.09.003]

55 **Shin JY**, Hur W, Wang JS, Jang JW, Kim CW, Bae SH, Jang SK, Yang SH, Sung YC, Kwon OJ, Yoon SK. HCV core protein promotes liver fibrogenesis via up-regulation of CTGF with TGF-beta1. *Exp Mol Med* 2005; **37**: 138-145 [PMID: 15886528 DOI: 10.1038/emm.2005.19]

56 **Taniguchi H**, Kato N, Otsuka M, Goto T, Yoshida H, Shiratori Y, Omata M. Hepatitis C virus core protein upregulates transforming growth factor-beta 1 transcription. *J Med Virol* 2004; **72**: 52-59 [PMID: 14635011 DOI: 10.1002/jmv.10545]

57 **Lin W**, Tsai WL, Shao RX, Wu G, Peng LF, Barlow LL, Chung WJ, Zhang L, Zhao H, Jang JY, Chung RT. Hepatitis C virus regulates transforming growth factor beta1 production through the generation of reactive oxygen species in a nuclear factor kappaB-dependent manner. *Gastroenterology* 2010; **138**: 2509-2518, 2518.e1 [PMID: 20230822 DOI: 10.1053/j.gastro.2010.03.008]

58 **Chusri P**, Kumthip K, Hong J, Zhu C, Duan X, Jilg N, Fusco DN, Brisac C, Schaefer EA, Cai D, Peng LF, Maneekarn N, Lin W, Chung RT. HCV induces transforming growth factor β1 through activation of endoplasmic reticulum stress and the unfolded protein response. *Sci Rep* 2016; **6**: 22487 [PMID: 26927933 DOI: 10.1038/srep22487]

59 **Tornesello ML**, Buonaguro L, Izzo F, Buonaguro FM. Molecular alterations in hepatocellular carcinoma associated with hepatitis B and hepatitis C infections. *Oncotarget* 2016; **7**: 25087-25102 [PMID: 26943571 DOI: 10.18632/oncotarget.7837]

60 **Sghaier I**, Mouelhi L, Rabia NA, Alsaleh BR, Ghazoueni E, Almawi WY, Loueslati BY. Genetic variants in IL-6 and IL-10 genes and susceptibility to hepatocellular carcinoma in HCV infected patients. *Cytokine* 2017; **89**: 62-67 [PMID: 28340949 DOI: 10.1016/j.cyto.2016.10.004]

61 **Wang W**, Pan Q, Fuhler GM, Smits R, Peppelenbosch MP. Action and function of Wnt/β-catenin signaling in the progression from chronic hepatitis C to hepatocellular carcinoma. *J Gastroenterol* 2017; **52**: 419-431 [PMID: 28035485 DOI: 10.1007/s00535-016-1299-5]

62 **Chen YL**, Jeng YM, Chang CN, Lee HJ, Hsu HC, Lai PL, Yuan RH. TERT promoter mutation in resectable hepatocellular carcinomas: a strong association with hepatitis C infection and absence of hepatitis B infection. *Int J Surg* 2014; **12**: 659-665 [PMID: 24866078 DOI: 10.1016/j.ijsu.2014.05.066]

63 **Nault JC**, Calderaro J, Di Tommaso L, Balabaud C, Zafrani ES, Bioulac-Sage P, Roncalli M, Zucman-Rossi J. Telomerase reverse transcriptase promoter mutation is an early somatic genetic alteration in the transformation of premalignant nodules in hepatocellular carcinoma on cirrhosis. *Hepatology* 2014; **60**: 1983-1992 [PMID: 25123086 DOI: 10.1002/hep.27372]

64 **Nault JC**, Mallet M, Pilati C, Calderaro J, Bioulac-Sage P, Laurent C, Laurent A, Cherqui D, Balabaud C, Zucman-Rossi J. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat Commun* 2013; **4**: 2218 [PMID: 23887712 DOI: 10.1038/ncomms3218]

65 **Dragani TA**. Risk of HCC: genetic heterogeneity and complex genetics. *J Hepatol* 2010; **52**: 252-257 [PMID: 20022654 DOI: 10.1016/j.jhep.2009.11.015]

66 **Miura M**, Maekawa S, Kadokura M, Sueki R, Komase K, Shindo H, Ohmori T, Kanayama A, Shindo K, Amemiya F, Nakayama Y, Kitamura T, Uetake T, Inoue T, Sakamoto M, Okada S, Enomoto N. Analysis of viral amino acids sequences and the IL28B SNP influencing the development of hepatocellular carcinoma in chronic hepatitis C. *Hepatol Int* 2012; **6**: 386-396 [PMID: 22020823 DOI: 10.1007/s12072-011-9307-6]

67 **Valenti L**, Al-Serri A, Daly AK, Galmozzi E, Rametta R, Dongiovanni P, Nobili V, Mozzi E, Roviaro G, Vanni E, Bugianesi E, Maggioni M, Fracanzani AL, Fargion S, Day CP. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 1209-1217 [PMID: 20373368 DOI: 10.1002/hep.23622]

68 **Sato M**, Kato N, Tateishi R, Muroyama R, Kowatari N, Li W, Goto K, Otsuka M, Shiina S, Yoshida H, Omata M, Koike K. Impact of PNPLA3 polymorphisms on the development of hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *Hepatol Res* 2014; **44**: E137-E144 [PMID: 24125181 DOI: 10.1111/hepr.12258]

69 **El-Shamy A**, Shindo M, Shoji I, Deng L, Okuno T, Hotta H. Polymorphisms of the core, NS3, and NS5A proteins of hepatitis C virus genotype 1b associate with development of hepatocellular carcinoma. *Hepatology* 2013; **58**: 555-563 [PMID: 23281009 DOI: 10.1002/hep.26205]

70 **Ma L**, Chua MS, Andrisani O, So S. Epigenetics in hepatocellular carcinoma: an update and future therapy perspectives. *World J Gastroenterol* 2014; **20**: 333-345 [PMID: 24574704 DOI: 10.3748/wjg.v20.i2.333]

71 **Sudo T**, Utsunomiya T, Mimori K, Nagahara H, Ogawa K, Inoue H, Wakiyama S, Fujita H, Shirouzu K, Mori M. Clinicopathological significance of EZH2 mRNA expression in patients with hepatocellular carcinoma. *Br J Cancer* 2005; **92**: 1754-1758 [PMID: 15856046 DOI: 10.1038/sj.bjc.6602531]

72 **Au SL**, Wong CC, Lee JM, Fan DN, Tsang FH, Ng IO, Wong CM. Enhancer of zeste homolog 2 epigenetically silences multiple tumor suppressor microRNAs to promote liver cancer metastasis. *Hepatology* 2012; **56**: 622-631 [PMID: 22370893 DOI: 10.1002/hep.25679]

73 **Li HP**, Leu YW, Chang YS. Epigenetic changes in virus-associated human cancers. *Cell Res* 2005; **15**: 262-271 [PMID: 15857581 DOI: 10.1038/sj.cr.7290295]

74 **Feng Q**, Stern JE, Hawes SE, Lu H, Jiang M, Kiviat NB. DNA methylation changes in normal liver tissues and hepatocellular carcinoma with different viral infection. *Exp Mol Pathol* 2010; **88**: 287-292 [PMID: 20079733 DOI: 10.1016/j.yexmp.2010.01.002]

75 **Shrivastava S**, Steele R, Ray R, Ray RB. MicroRNAs: Role in Hepatitis C Virus pathogenesis. *Genes Dis* 2015; **2**: 35-45 [PMID: 25984557 DOI: 10.1016/j.gendis.2015.01.001]

76 **Yvamoto EY**, Ferreira RF, Nogueira V, Pinhe MA, Tenani GD, Andrade JG, Baitello ME, Gregório ML, Fucuta PS, Silva RF, Souza DR, Silva RC. Influence of vascular endothelial growth factor and alpha-fetoprotein on hepatocellular carcinoma. *Genet Mol Res* 2015; **14**: 17453-17462 [PMID: 26782388 DOI: 10.4238/2015.December.21.16]

77 **Li Y**, Chen J, Wu C, Wang L, Lu M, Chen X. Hepatitis B virus/hepatitis C virus upregulate angiopoietin-2 expression through mitogen-activated protein kinase pathway. *Hepatol Res* 2010; **40**: 1022-1033 [PMID: 20887338 DOI: 10.1111/j.1872-034X.2010.00712.x]

78 **Liang TJ**, Heller T. Pathogenesis of hepatitis C-associated hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S62-S71 [PMID: 15508105]

79 **Nguyen LT**, Dunford L, Freitas I, Holder P, Nguyen LA, O'Gorman J, Connell J, Carr M, Hall W, De Gascun C. Hepatitis C Virus Core Mutations Associated with False-Negative Serological Results for Genotype 3a Core Antigen. *J Clin Microbiol* 2015; **53**: 2697-2700 [PMID: 25994168 DOI: 10.1128/JCM.01062-15]

80 **Moriya K**, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, Matsuura Y, Kimura S, Miyamura T, Koike K. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. *Nat Med* 1998; **4**: 1065-1067 [PMID: 9734402 DOI: 10.1038/2053]

81 **Perlemuter G**, Sabile A, Letteron P, Vona G, Topilco A, Chrétien Y, Koike K, Pessayre D, Chapman J, Barba G, Bréchot C. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. *FASEB J* 2002; **16**: 185-194 [PMID: 11818366 DOI: 10.1096/fj.01-0396com]

82 **Selimovic D**, El-Khattouti A, Ghozlan H, Haikel Y, Abdelkader O, Hassan M. Hepatitis C virus-related hepatocellular carcinoma: An insight into molecular mechanisms and therapeutic strategies. *World J Hepatol* 2012; **4**: 342-355 [PMID: 23355912 DOI: 10.4254/wjh.v4.i12.342]

83 **Yamashita T**, Honda M, Takatori H, Nishino R, Minato H, Takamura H, Ohta T, Kaneko S. Activation of lipogenic pathway correlates with cell proliferation and poor prognosis in hepatocellular carcinoma. *J Hepatol* 2009; **50**: 100-110 [PMID: 19008011 DOI: 10.1016/j.jhep.2008.07.036]

84 **Schmoldt A**, Benthe HF, Haberland G. Digitoxin metabolism by rat liver microsomes. *Biochem Pharmacol* 1975; **24**: 1639-1641 [PMID: 10 DOI: 18188449]

85 **Li Y**, Boehning DF, Qian T, Popov VL, Weinman SA. Hepatitis C virus core protein increases mitochondrial ROS production by stimulation of Ca2+ uniporter activity. *FASEB J* 2007; **21**: 2474-2485 [PMID: 17392480 DOI: 10.1096/fj.06-7345com]

86 **Cho JW**, Baek WK, Suh SI, Yang SH, Chang J, Sung YC, Suh MH. Hepatitis C virus core protein promotes cell proliferation through the upregulation of cyclin E expression levels. *Liver* 2001; **21**: 137-142 [PMID: 11318983]

87 **Kao CF**, Chen SY, Chen JY, Wu Lee YH. Modulation of p53 transcription regulatory activity and post-translational modification by hepatitis C virus core protein. *Oncogene* 2004; **23**: 2472-2483 [PMID: 14968111 DOI: 10.1038/sj.onc.1207368]

88 **Tsutsumi T**, Suzuki T, Moriya K, Shintani Y, Fujie H, Miyoshi H, Matsuura Y, Koike K, Miyamura T. Hepatitis C virus core protein activates ERK and p38 MAPK in cooperation with ethanol in transgenic mice. *Hepatology* 2003; **38**: 820-828 [PMID: 14512869 DOI: 10.1053/jhep.2003.50399]

89 **Tsai WL**, Chung RT. Viral hepatocarcinogenesis. *Oncogene* 2010; **29**: 2309-2324 [PMID: 20228847 DOI: 10.1038/onc.2010.36]

90 **Taylor DR**, Shi ST, Romano PR, Barber GN, Lai MM. Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. *Science* 1999; **285**: 107-110 [PMID: 10390359]

91 **Crotta S**, Stilla A, Wack A, D'Andrea A, Nuti S, D'Oro U, Mosca M, Filliponi F, Brunetto RM, Bonino F, Abrignani S, Valiante NM. Inhibition of natural killer cells through engagement of CD81 by the major hepatitis C virus envelope protein. *J Exp Med* 2002; **195**: 35-41 [PMID: 11781363]

92 **Zhao LJ**, Wang L, Ren H, Cao J, Li L, Ke JS, Qi ZT. Hepatitis C virus E2 protein promotes human hepatoma cell proliferation through the MAPK/ERK signaling pathway via cellular receptors. *Exp Cell Res* 2005; **305**: 23-32 [PMID: 15777784 DOI: 10.1016/j.yexcr.2004.12.024]

93 **Bittar C**, Shrivastava S, Bhanja Chowdhury J, Rahal P, Ray RB. Hepatitis C virus NS2 protein inhibits DNA damage pathway by sequestering p53 to the cytoplasm. *PLoS One* 2013; **8**: e62581 [PMID: 23638118 DOI: 10.1371/journal.pone.0062581]

94 **Hassan M**, Ghozlan H, Abdel-Kader O. Activation of c-Jun NH2-terminal kinase (JNK) signaling pathway is essential for the stimulation of hepatitis C virus (HCV) non-structural protein 3 (NS3)-mediated cell growth. *Virology* 2005; **333**: 324-336 [PMID: 15721365 DOI: 10.1016/j.virol.2005.01.008]

95 **Kasprzak A**, Adamek A, Przybyszewska W, Olejniczak K, Biczysko W, Mozer-Lisewska I, Zabel M. p21/Wafl/Cipl cellular expression in chronic long-lasting hepatitis C: correlation with HCV proteins (C, NS3, NS5A), other cell-cycle related proteins and selected clinical data. *Folia Histochem Cytobiol* 2009; **47**: 385-394 [PMID: 20164022 DOI: 10.2478/v10042-009-0096-x]

96 **Feng DY**, Sun Y, Cheng RX, Ouyang XM, Zheng H. Effect of hepatitis C virus nonstructural protein NS3 on proliferation and MAPK phosphorylation of normal hepatocyte line. *World J Gastroenterol* 2005; **11**: 2157-2161 [PMID: 15810084]

97 **Li K**, Foy E, Ferreon JC, Nakamura M, Ferreon AC, Ikeda M, Ray SC, Gale M Jr, Lemon SM. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. *Proc Natl Acad Sci U S A* 2005; **102**: 2992-2997 [PMID: 15710891 DOI: 10.1073/pnas.0408824102]

98 **Li XD**, Sun L, Seth RB, Pineda G, Chen ZJ. Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. *Proc Natl Acad Sci U S A* 2005; **102**: 17717-17722 [PMID: 16301520 DOI: 10.1073/pnas.0508531102]

99 **Wang N**, Liang Y, Devaraj S, Wang J, Lemon SM, Li K. Toll-like receptor 3 mediates establishment of an antiviral state against hepatitis C virus in hepatoma cells. *J Virol* 2009; **83**: 9824-9834 [PMID: 19625408 DOI: 10.1128/JVI.01125-09]

100 **Yamashita T**, Honda M, Kaneko S. Molecular mechanisms of hepatocarcinogenesis in chronic hepatitis C virus infection. *J Gastroenterol Hepatol* 2011; **26**: 960-964 [PMID: 21443660 DOI: 10.1111/j.1440-1746.2011.06723.x]

101 **Benga WJ**, Krieger SE, Dimitrova M, Zeisel MB, Parnot M, Lupberger J, Hildt E, Luo G, McLauchlan J, Baumert TF, Schuster C. Apolipoprotein E interacts with hepatitis C virus nonstructural protein 5A and determines assembly of infectious particles. *Hepatology* 2010; **51**: 43-53 [PMID: 20014138 DOI: 10.1002/hep.23278]

102 **Wu SC**, Chang SC, Wu HY, Liao PJ, Chang MF. Hepatitis C virus NS5A protein down-regulates the expression of spindle gene Aspm through PKR-p38 signaling pathway. *J Biol Chem* 2008; **283**: 29396-29404 [PMID: 18728014 DOI: 10.1074/jbc.M802821200]

103 **Choi SH**, Hwang SB. Modulation of the transforming growth factor-beta signal transduction pathway by hepatitis C virus nonstructural 5A protein. *J Biol Chem* 2006; **281**: 7468-7478 [PMID: 16407286 DOI: 10.1074/jbc.M512438200]

104 **Ghosh AK**, Majumder M, Steele R, Meyer K, Ray R, Ray RB. Hepatitis C virus NS5A protein protects against TNF-alpha mediated apoptotic cell death. *Virus Res* 2000; **67**: 173-178 [PMID: 10867196]

105 **Battaglia S**, Benzoubir N, Nobilet S, Charneau P, Samuel D, Zignego AL, Atfi A, Bréchot C, Bourgeade MF. Liver cancer-derived hepatitis C virus core proteins shift TGF-beta responses from tumor suppression to epithelial-mesenchymal transition. *PLoS One* 2009; **4**: e4355 [PMID: 19190755 DOI: 10.1371/journal.pone.0004355]

106 **Bose SK**, Meyer K, Di Bisceglie AM, Ray RB, Ray R. Hepatitis C virus induces epithelial-mesenchymal transition in primary human hepatocytes. *J Virol* 2012; **86**: 13621-13628 [PMID: 23035229 DOI: 10.1128/JVI.02016-12]

107 **Munakata T**, Liang Y, Kim S, McGivern DR, Huibregtse J, Nomoto A, Lemon SM. Hepatitis C virus induces E6AP-dependent degradation of the retinoblastoma protein. *PLoS Pathog* 2007; **3**: 1335-1347 [PMID: 17907805 DOI: 10.1371/journal.ppat.0030139]

108 **Munakata T**, Nakamura M, Liang Y, Li K, Lemon SM. Down-regulation of the retinoblastoma tumor suppressor by the hepatitis C virus NS5B RNA-dependent RNA polymerase. *Proc Natl Acad Sci USA* 2005; **102**: 18159-18164 [PMID: 16332962 DOI: 10.1073/pnas.0505605102]

**P-Reviewer:** Kocazeybek B, Pekgoz M **S-Editor:** Cui LJ **L-Editor: E-Editor:**

**Specialty type:** Gastroenterology and Hepatology

**Country of origin:** India

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0



**Figure 1 Direct and indirect role of hepatitis C virus In Causing hepatocellular carcinoma.** Role of hepatitis C virus (HCV) and its structural and non-structural proteins in inducement of hepatocellular carcinoma (HCC) during chronic HCV infection. Viral onset causes various cellular alterations leading to activation of hepatic stellate cells which in turn, produces progressive fibrosis leading to cirrhosis of liver. Simultaneously, HCV also dysregulate cell cycle causing cell proliferation. Both cirrhosis and cell proliferation induce development of HCC. In this figure, the top half portion shows an indirect role of HCV via cellular alterations and causing cirrhosis by inter-related mechanisms and cell dysregulation leading to cell proliferation. The lower half shows a direct role of HCV by interaction of its proteins with various cellular pathways producing different effects as preconditions for inducement of HCC. The link bars show the underlying pathways and the bottom boxes show the end effects.