
Review article:**HEPATITIS C VIRUS INFECTION: ESTABLISHMENT OF CHRONICITY AND LIVER DISEASE PROGRESSION**Young-Chan Kwon¹, Ratna B. Ray², Ranjit Ray^{1,3*}Departments of Internal Medicine¹, Pathology², and Molecular Microbiology & Immunology³, Saint Louis University, Missouri

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ABSTRACT

Hepatitis C virus (HCV) often causes persistent infection, and is an important factor in the etiology of fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). There are no preventive or therapeutic vaccines available against HCV. Treatment strategies of HCV infection are likely to improve with recently discovered direct antiviral agents (DAAs). However, a proportion of patients still progress to liver failure and/or HCC despite having been cured of the infection. Thus, there is a need for early diagnosis and therapeutic modalities for HCV related end stage liver disease prevention. HCV genome does not integrate into its host genome, and has a predominantly cytoplasmic life cycle. Therefore, HCV mediated liver disease progression appears to involve indirect mechanisms from persistent infection of hepatocytes. Studying the underlying mechanisms of HCV mediated evasion of immune responses and liver disease progression is challenging due to the lack of a naturally susceptible small animal model. We and other investigators have used a number of experimental systems to investigate the mechanisms for establishment of chronic HCV infection and liver disease progression. HCV infection modulates immune systems. Further, HCV infection of primary human hepatocytes promotes growth, induces phenotypic changes, modulates epithelial mesenchymal transition (EMT) related genes, and generates tumor initiating stem-like cells (TISCs). HCV infection also modulates microRNAs (miRNAs), and influences growth by overriding normal death progression of primary human hepatocytes for disease pathogenesis. Understanding these observations at the molecular level should aid in developing strategies for additional effective therapies against HCV mediated liver disease progression.

Keywords: HCV, DAAs, miRNAs, EMT, fibrosis, TISCs

INTRODUCTION

Hepatitis C virus (HCV) is an enveloped virus with a ~ 9.6 kb single-stranded RNA genome (Choo et al., 1989), a member of the Flaviviridae family and genus Hepacivirus. HCV genome encodes a single polyprotein

which is processed co-translationally into three structural and seven nonstructural (NS) polypeptides (Grakoui et al., 1993; Tanji et al., 1994; Ali et al., 2011). HCV core protein forms the capsid, which is surrounded by a lipid bilayer containing the envelope glycoproteins, E1 and E2 on the external surface.

These envelope glycoproteins are responsible for initiation of infection in a host cell. The nonstructural (NS) proteins coordinate the intracellular processes of the virus life cycle.

HCV is a major cause of chronic liver disease, with an estimated 180 million people infected worldwide. An important therapeutic advancement was achieved with the recent discovery of potent direct acting antiviral agents (DAAs) against HCV (Casey and Lee, 2013; Au and Pockros, 2014). Several clinical trials have shown various combinations of agents, including interferon-free regimens, to be highly effective in the clearance or sustained viral response (SVR) of chronic hepatitis C infection. However, significant challenges remain in deploying modern antivirals for patients with asymptomatic HCV infection and must be sought through screening programs. HCV infection particularly affects persons of low socioeconomic status who have less access to health care. The very high cost of HCV treatment may also contribute to delays in patients being treated.

Majority of the infected patients (approximately 80 %) develop chronic infection and are at high risk for end stage liver disease progression to cirrhosis and hepatocellular carcinoma (HCC). HCC is a common cancer worldwide and accounts for ~5.6 % of all cancers. It is the fifth common cancer in the world and the third common cause of cancer death (Bosch et al., 2004; Sherman, 2010). The incidence of HCC is rising precipitously, primarily as a result of the increasing prevalence of chronic HCV infection (Kanwal et al., 2011) and fatty liver disease in the United States (Nordenstedt et al., 2010; Zhang and Friedman, 2012). Liver fibrosis is strongly associated with HCC, since approximately 80-90 % of HCC cases are arising in cirrhotic livers (Seitz and Stickel, 2006; Lok et al., 2009). HCC development is also linked to alcoholic cirrhosis (Fattovich et al., 2004), nonalcoholic steatohepatitis (NASH) (Ascha et al., 2010). HCV does not integrate into its host genome and has a cytoplasmic

life cycle (Moradpour et al., 2007). HCC, therefore, must involve several indirect mechanisms including the interplay between HCV and host cell genes/proteins for pathological consequences. In addition, HCV induces epithelial to mesenchymal transition (EMT) state that is known as important element in cancer progression (Bose et al., 2012b). This review will discuss recent advances in HCV research with a focus on establishment of chronicity and liver disease progression.

EVASION OF INNATE/ADAPTIVE IMMUNE RESPONSES BY HCV

IFN response

HCV infection is sensed by multiple innate immune pathways, but often not cleared by immune responses, resulting in a chronic infection. HCV blocks the IFN response pathway by several mechanisms. HCV NS3/4A utilizes its protease domain to cleave key innate immune signaling adaptor proteins, effectively inactivating viral RNA detection program (Horner, 2014). HCV NS3/4A protein cleaves MAVS and TRIF (Baril et al., 2009; Li et al., 2005b; Lin et al., 2006; Loo et al., 2006; Meylan et al., 2005; Li et al., 2005a), and can alter RIG-I and TLR signaling pathway. Hepatocytes persistently infected with HCV and treated with IFN- α , PKR kinase is activated (Kang et al., 2009a) for translational suppression of host mRNAs, including ISGs, and antiviral functions of IFN (Garaigota and Chisari, 2009). Several HCV proteins have been implicated as regulators of the IFN response pathway. Expression of HCV proteins blocks IFN signaling at the level of the JAK/STAT pathway (Heim et al., 1999; Raychoudhuri et al., 2011), and impairs IRF-7 nuclear localization through its NS5A protein (Raychoudhuri et al., 2010; Chowdhury et al., 2014).

IFI6 is a type I ISG and plays a critical role in the regulation of apoptosis. IFI6 is strongly associated with the immune system, but its antiviral effects are not well known. Our recent (unpublished) experimental findings suggest co-localization of HCV co-

receptors during HCV entry are compromised by IFI6 mediated disruption of kinase function, thereby inhibiting HCV at the point of entry.

Cytokine response

A relationship between the activation of genes involved in the IL-6 signaling pathway and the development of HCC has been observed (Zekri et al., 2009). An increase of the β -2 microglobulin in serum level as well as IL-6 level was observed among HCV infected HCC patients. Weakening of the immune system, due to IL-6, may be responsible for a more severe progression of HCC and the hyperexpression of β -2 microglobulin (Tang et al., 2008). HCV core protein attenuates IL-6 stimulated acute-phase response, and contributes to impaired innate immunity for viral persistence (Malaguarnera et al., 2000; Ait-Goughoulte et al., 2010). TNF- α plays diverse roles, including in the inflammatory processes, in HCV infection (Saito et al., 2006). HCV may actively contribute to the fibrogenic process via the paracrine effect of IL-8 secreted by infected hepatocytes (Koike and Moriya, 2005).

Autophagy

Autophagy is a process of degradation of cytoplasmic materials, including damaged organelles and long-lived proteins, in the cells for the maintenance of cellular homeostasis. During autophagy, the double-membrane vesicles, called autophagosome, engulf the cytoplasmic materials and fuse with the lysosome for degradation. Autophagy has been identified as a component of the innate immune system against viral infection. We were the first to demonstrate that HCV induces autophagy in immortalized human hepatocyte (Ait-Goughoulte et al., 2008). Subsequently, HCV subgenomic replicon and infection were shown to induce autophagy in hepatoma cells (Sir et al., 2008; Dreux et al., 2009; Mizui et al., 2010). Autophagy proteins (Beclin-1, Atg4B, Atg5 and Atg12) are required for initiation of HCV replication (Dreux et al., 2009) and contrib-

ute to the effective production of virus particles (Tanida et al., 2009). Recently, we have shown that knockdown of autophagy proteins in HCV infected hepatocytes enhance interferon signaling pathway and induces apoptosis (Shrivastava et al., 2011). HCV mediated autophagy may promote infectious virus particle production and evade innate immune response for establishment of persistent infection (Shrivastava et al., 2011; Shrivastava and Ray, 2014).

Complement

The complement system is one of the vital effectors in the innate immune system for targeting and eliminating infected cells and invading microorganisms, including free virus particles (Mollnes et al., 2002; Gasque, 2004; Kim and Song, 2006). HCV escapes the complement response by regulating complement components. HCV proteins suppress C3/C4 complement expression (Mazumdar et al., 2012; Banerjee et al., 2011), and attenuates membrane attack complex (MAC)-mediated microbicidal activity by suppressing C9 expression (Kim et al., 2013). To avert damage from excessive complement activation and MAC formation, host cells express membrane-bound regulators of complement activation (RCA) proteins, including CD46, CD55 and CD59, to limit these processes (Hourcade et al., 2000; Williams et al., 2003; Pangburn et al., 2008). HCV core protein enhances transcription and surface expression of DAF/CD55 in infected hepatocytes and promotes incorporation onto mature HCV particles (Mazumdar et al., 2013). HCV also incorporates CD59 and protects against complement mediated lysis (Amet et al., 2012; Ejaz et al., 2012). DAF/CD55 expression has been associated with complement dependent cytolysis (CDC), antibody dependent cell cytolysis (ADCC), and NK cell function (Finberg et al., 1992; Bellone et al., 2012; Kim et al., 2014). The strategies adopted by HCV to modulate complement pathways imply a significant advantage for survival of chronically infected hepatocytes,

enhancing viral fitness in establishing chronicity and liver disease promotion.

Dendritic cell, NK cell, and T cell functions

HCV can have an inhibitory effect on antigen presenting cells, resulting in reduction of antigen-specific T-cell activation. These effects may contribute to the overall low level of immunogenicity of HCV observed in chronically infected patients (Saito et al., 2008). HCV has an inhibitory role on cathepsin S-mediated major histocompatibility complex (MHC) class II maturation, which may contribute to weak immunogenicity of viral antigens in chronically infected humans (Kim et al., 2012). Further, HCV has been shown to attenuate interferon induced MHC class I expression and decreases CD8+ T cell effector functions (Kang et al., 2014). HCV disables a key receptor ligand (MICA/B) in infected hepatocytes, inhibiting the ability of infected cells to respond to stimuli from NK cells to positively regulate complement synthesis (Kim et al., 2014). Reduced NK cell function may also contribute to the emergence of HCC in chronic liver disease. NK cells induce apoptosis in cells that have either down-regulated MHC class I expression or up-regulated stress-induced ligands (Kim et al., 2014). Broad and potent T cell responses (Neumann-Haefelin and Thimme 2013), and a rapid induction of neutralizing antibody responses help in virus clearance (Osburn, 2014).

Antibody response

In contrast to CD8+T cells, viral escape is likely not a major determinant of HCV specific CD4+ T cell failure (Fleming et al., 2010; Fuller et al., 2010). This is in agreement with the observation that HCV specific CD4+ T cell responses are very weak and dysfunctional in chronic infection and also in agreement with the concept that HCV specific CD4+ T cells primarily have a helper function rather than strong direct antiviral activity. Although, HCV specific neutralizing antibodies exist in infected patients, the virus often escapes from humoral immune

response by multiple mechanisms inhibiting evolution of viral quasispecies and display mutations within targeted epitopes (Farci et al., 1996; von Hahn et al., 2007; Dowd et al., 2009; Di Lorenzo et al., 2011). Most neutralizing antibodies show little cross-neutralization of heterologous viral strains; thus, identification of neutralizing antibodies with broad cross-neutralizing activity is an important prerequisite for the use of neutralizing antibodies in prophylactic or therapeutic vaccination strategies. In addition, HCV particles are protected through interaction of envelope glycoproteins with lipoproteins (Mazumdar et al., 2011) and scavenger receptor B1 (Scarselli et al., 2002; Bartosch et al., 2003) in facilitating virus entry into mammalian cells.

The important roles of viral escape in evasion from the neutralizing antibody response have been supported from study performed in patients who underwent liver transplantation (Fafi-Kremer et al., 2010). Reinfection of the liver graft included only few viral quasispecies that were present in the explanted liver. The quasispecies that established reinfection were resistant to homologous neutralizing antibodies, indicating viral escape, while the viral quasispecies that were lost after transplantation were sensible to neutralization by homologous antibodies. A HCV candidate vaccine phase I clinical trial was conducted at the Saint Louis University Vaccine and Treatment Evaluation Unit with the recombinant E1 and E2 glycoproteins in human volunteers, and suggested modest immunogenicity (Frey et al., 2010; Ray et al., 2010; Meyer et al., 2011; Ray, 2011). A different study aimed to elicit HCV specific T cells using a recombinant adenoviral vector strategy in a phase I study in human healthy volunteers (Barnes et al., 2012). The results suggested generation of broad, long-lasting, and functional T cell responses. The protective nature of these responses against HCV exposure remains to be understood.

HCV INFECTION AND METABOLIC DISORDERS

The metabolic syndrome is a constellation of problems that includes insulin resistance, obesity, hypertension, and hyperlipidemia. Increasingly, components of the metabolic syndrome are being linked to various forms of cancer with respect to both increased risk of disease and worsened outcome. HCV induced insulin resistance impairs antiviral effect of interferon (El-Zayadi and Anis, 2012). Possible explanations for the unique association between insulin resistance and HCV infection may be related to differences in the clinical course of liver inflammation and fibrosis, or in the mode of TNF-receptor activation or cleavage (Joyce et al., 2009). Marked increases in both sTNFR1 and sTNFR2 were demonstrated in HCV-diabetic patients (Shintani et al., 2004). Insulin resistance, a link among chronic HCV infection, TNF- α , and type 2 diabetes (T2D) possibly exists in the correlation with liver disease (Knobler et al., 2003; Sheikh et al., 2008; Ray et al., 1998; Ghosh et al., 2000; Saito et al., 2006; Bose and Ray, 2014).

We have reported that HCV infection up-regulates serine phosphorylation of insulin receptor substrate-1 and impairs the downstream Akt/protein kinase B signaling pathway for insulin resistance (Banerjee et al., 2008) via mTOR/S6K1 pathway (Bose et al., 2012a). Insulin resistance is paradoxically associated within a reduced ability of insulin signaling to inhibit glucose production, whereas insulin-stimulated lipogenesis is enhanced in the liver and two forkhead transcription factors, FoxO1 and FoxA2 to play important roles in this process. HCV can differentially modulate activation of forkhead transcription factors and insulin induced metabolic gene expression (Banerjee et al., 2010b; Bose et al., 2014).

Insulin resistance and subsequent hyperinsulinemia are highly associated with fatty liver disease and are important risk factors for the progression of fibrosis in chronic hepatitis C (Sheikh et al., 2008; Banerjee et

al., 2010a; Ortiz et al., 2002). Hepatitis C resembles non-alcoholic steatohepatitis (NASH) in numerous features from metabolic aspect, such as the presence of steatosis, serum dyslipidemia, and oxidative stress in the liver (Bugianesi et al., 2004). Steatosis is prevalent with HCV genotype 3 infection and correlates with the level of HCV replication (Adinolfi et al., 2013; Roingeard, 2013). HCV related steatosis predicts an advanced liver disease and a more rapid progression of fibrosis, as well as an increased risk of development of HCC. Viral fatty liver may not impact on response to therapy, while metabolic steatosis does (Negro, 2012). Similarly, viral insulin resistance may not reduce the rate of response to therapy to the same extent that metabolic insulin resistance does.

Micro RNAs

Micro RNAs (miRNAs) constitute a class of ~18-22 nucleotides long non-coding RNAs and play a crucial role in the regulation of gene expression. Deregulation of miRNA occurs frequently in a variety of diseases, including liver (Roberts et al., 2011). The major targets and functions of specific miRNAs vary under different physiological or pathological conditions and in different cell types. Several RNA viruses interact directly with cellular miRNAs in modulating viral genome replication (Cullen, 2013a; Conrad and Niepmann, 2014). The liver specific miR-122 is required for efficient HCV replication. HCV binds two molecules of this liver-specific miR-122 resulting in a novel, unprecedented up-regulation of the viral genome (Jopling et al., 2005). Sequestration of miR-122 in HCV-infected cultured cells or in livers of infected chimpanzees leads to a dramatic loss of infectious virus without emergence of resistant virus (Lanford et al., 2010). A phase 2 study using miR-122 antagonist (miravirsin) indicated effective anti-HCV activity (Janssen et al., 2013). Other miRNAs, such as miR-199a*, let-7b, miR-196 and miR-448, physically interact with HCV RNA and suppress the viral replication. HCV infection can alter miRNA ex-

pression profile of the host cell in facilitating escape from immune system (Shrivastava et al., 2013b; Cullen, 2013b). We have previously shown that miR-130a expression is up-regulated in HCV infected cells, and facilitates virus replication by inhibiting interferon-induced transmembrane protein IFITM1 (Bhanja Chowdhury et al., 2012). HCV infection suppresses miR-181c expression by down-regulating transcription factor C/EBP- β and promotes HOXA1 expression, which subsequently upregulates STAT3 and STAT5 expression (Mukherjee et al., 2014). In addition, exogenous expression of miR-181c restricts HCV replication by binding with E1 and NS5A.

INFLAMMATION, FIBROSIS/ CIRRHOSIS, HEPATOCELLULAR CARCINOMA

Inflammation

An inflammatory process resulting from infection and/or tissue damage is an early defense mechanism during which striking changes in protein synthesis occur mainly in the liver. Inflammatory cells and mediators are found frequently in the local environment of tumors, and inflammation is considered a hallmark of cancer (Hanahan and Weinberg, 2011). Kupffer cells are resident macrophages in the liver and play a pivotal role in triggering inflammation during liver diseases (Zimmermann et al., 2012). HCV infection is sensed by pattern recognition receptors (PRRs) on Kupffer cells and modulates inflammatory responses (Liaskou et al., 2012). Our recent results demonstrated that monocyte-derived human macrophages (THP-1) incubated with cell culture grown HCV enhance the secretion of IL-1 β /IL-18 into culture supernatants (Shrivastava et al., 2013a). A similar cytokine release was observed from peripheral blood mononuclear cells (PBMCs) derived primary human macrophages and Kupffer cells upon incubation with HCV. Macrophage cell line (THP-1) incubated with HCV led to caspase-1 activation and release of proinflammatory cytokines. HCV induces pro-IL-1 β and pro-IL-18

synthesis via the NF- κ B signaling pathway in macrophages, although consequence of these proinflammatory cytokine syntheses in liver pathogenesis remains to be elucidated.

Fibrosis

Hepatocellular injury followed by inflammation and activation of the innate immune system may lead to early stage liver fibrosis, resulting in hepatic stellate cell (HSC) activation (Hernandez-Gea and Friedman, 2011). Activated HSCs are both signaling and target cells for a great variety of stimulatory and inhibitory fibrogenic cytokines and growth factors. Hepatic fibrosis affects a large number of people worldwide, and contributes to the processes and pathways involved in malignant transformation. In fibrotic tissues, myofibroblasts accumulate and secrete an excessive amount of collagen that is deposited as fibers, thereby compromising organ function and leading to its failure. Quiescent stellate cells undergo activation to adopt myofibroblast morphology and secrete type I collagen, the principal matrix protein responsible for the development of liver fibrosis, cirrhosis and cancer progression (Kang et al., 2011; Zhang and Friedman, 2012).

Stellate cells produce growth factors, including interleukin 6, hepatocyte growth factor, and Wnt ligands, fostering an environment for hepatocyte proliferation (Friedman, 2008a, b). Similarly, hepatic myofibroblasts can enhance growth and migration of malignant hepatocytes, at least partially through platelet-derived growth factor (PDGF) and transforming growth factor- β (TGF- β) mediated mechanisms (van Zijl et al., 2009). TGF- β signaling are highly dependent on extracellular matrix (ECM) interactions. TGF- β is directly recruited to the ECM by latent TGF- β binding protein (LTBPs), which have affinity for both TGF- β and ECM fibrils. When bound to LTBP, TGF- β are unable to signal. This suggests that accumulation of ECM would lead to increased proliferation and decreased apoptosis, because TGF- β signaling would be suppressed.

However, LTBP1s contain multiple proteinase sensitive sites, and cleavage of those sites by MMPs leads to the release of TGF- β (Todorovic and Rifkin, 2012). In the setting of inflammation or increased migratory potential, elevated MMP activity can liberate sequestered TGF- β . Fibrotic ECM, containing more sequestered TGF- β , would release greater amounts of the cytokine. This could antagonize oncogenesis by inhibiting proliferation and promoting apoptosis. HCV core upregulates the expression of TGF- β (Torre et al., 1994; Taniguchi et al., 2004), and NS5A modulates TGF- β signaling through interaction with TGF- β receptor I (Shin et al., 2005). Another study showed that different thresholds of Smad3 activation control TGF- β responses in hepatocytes and that liver cancer-derived HCV core protein, by decreasing Smad3 activation, switches TGF- β growth inhibitory effects to tumor-promoting responses (Matsuzaki et al., 2007). HCV core also triggers the production of both TGF- β 2 and VEGF proteins through multiple pathways (Battaglia et al., 2009). As HCV infected livers progress from chronic hepatitis to cirrhosis and/or HCC, pSmad3L/PAI-1 increases with fibrotic stage and necroinflammatory grade; pSmad3C/p21 decreases (Choi and Hwang, 2006). HCV infected hepatocytes release TGF- β 1 and other profibrogenic factors that differentially modulate expression of several key genes that can activate HSCs in liver fibrosis (Schulze-Krebs et al., 2005). CD81 protein, a key entry coreceptor for HCV, is highly expressed in HSCs (Mazzocca et al., 2002) and HCV E2 protein can directly interact with CD81 on HSC surface, inducing fibrogenic effects on HSCs (Mazzocca et al., 2005). Therefore, it is possible that chronic inflammation associated with HCV infection shifts hepatocytic TGF- β signaling from tumor suppression to fibrogenesis, accelerating liver fibrosis and increasing the risk of HCC.

Fibrosis is defined by changing the amount and composition of ECM component, which contribute to tumorigenesis. Integrin family of transmembrane receptors

contributes to increase deposition of fibrillar collagen type I and III, as well as fibronectin in hepatic fibrosis. In addition to the fibrillar collagens, other ECM molecules including laminin, fibronectin, and several nonfibrillar collagens may also amplify carcinogenic signaling. Although these proteins are in relatively low abundance compared to the fibrillar collagens, their potential function as growth factor receptor ligands could amplify their carcinogenic impact. Increased ECM may stimulate integrin signaling in hepatocyte. Integrins promote growth and survival by activating phosphoinositide 3 kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling cascade (Cox et al., 2010), thereby enhancing the growth and survival of precancerous cells. The correlation of collagen expression, integrin expression and tumorigenicity is studied in human and animal HCC specimens (Lee et al., 2009; Lai et al., 2011). Other mechanisms for integrin-mediated tumorigenesis are increased migration (Fransvea et al., 2009; Fu et al., 2010) and survival through anti-apoptotic signaling (Zhang et al., 2002). In tumor cell lines, overexpression of integrin B1 actually leads to growth arrest, attributed to up-regulation of the cyclin-dependent kinase inhibitor p21 and p27. In addition, human HCC samples have decreased expression of integrin B3, and its overexpression in a human HCC cell line leads to apoptosis (Wu et al., 2009).

Interestingly, cell tracing studies have shown that a significant portion of these myofibroblasts arise from the conversion of epithelial cells through an EMT process (Iwano et al., 2002). Hepatocytes can undergo EMT and contribute significantly to liver fibrosis (Figure 1). Indeed, lineage-tracing in transgenic mice also indicates that hepatocytes undergo EMT during CCl₄ induced liver fibrosis (Zeisberg et al., 2007). Interestingly, hepatocytes derived from cirrhotic livers also display characteristics of EMT, which has implications for the progression to HCC (Nitta et al., 2008). We have shown that pri-

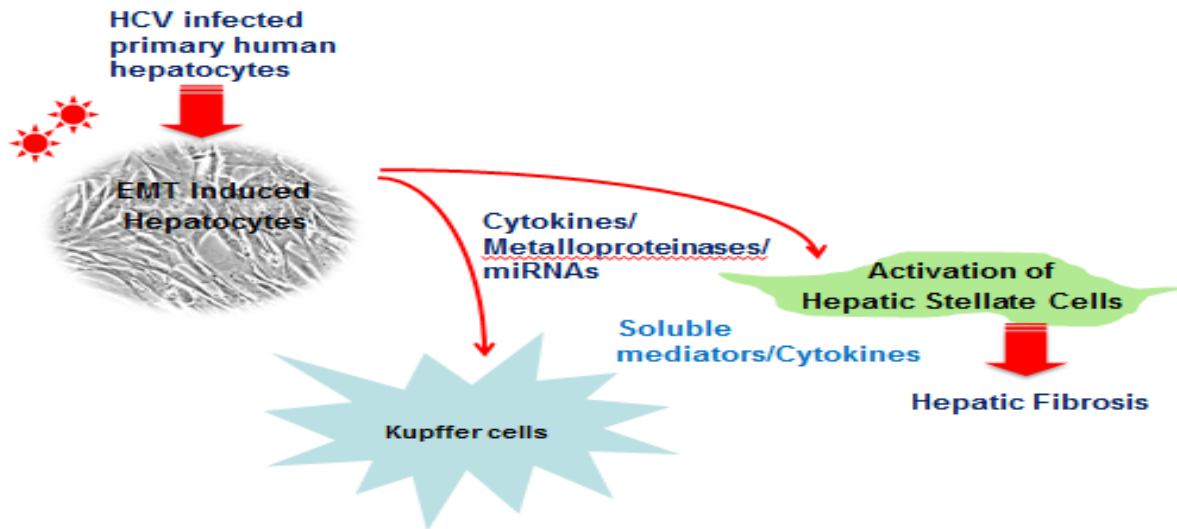


Figure 1: Pathways associated with fibrogenic potential of hepatic stellate cells

mary human hepatocytes infected in vitro with cell culture grown HCV display morphological and molecular alterations suggestive of EMT, and display an extended life span (Bose et al., 2012b). Similar observations have been noted in continuous cell types (Akkari et al., 2012; Wilson et al., 2012; Conti et al., 2013; Iqbal et al., 2014). EMT type II has been linked to escape from senescence and apoptosis, which suggest a role in epithelial cell growth promotion. Among HCV proteins, core and NS5A are suggested to induce EMT (Akkari et al., 2012; Quan et al., 2014). EMT is likely to play a major mechanism in tumor progression, local invasion, metastasis, and therapeutic resistance; and is linked to the development of stem-like properties by cancer cells (Mani et al., 2008; Thiery et al., 2009).

Hepatocellular carcinoma

Activated HSCs and myofibroblasts may directly support hepatic tumorigenesis and invasion of primary tumors (Kalluri and Zeisberg, 2006). Desmoplasia or cancer associated fibrosis is the growth of fibrous or connective tissue that usually occurs around a malignant neoplasm, causing dense fibrosis around the tumor (Kang et al., 2011; Zhang and Friedman, 2012; Yaqoob et al., 2012; Liu et al., 2013). Several studies have identi-

fied cells resembling activated stellate cells associated with the liver progenitor cell niche, suggesting that these cells may provide paracrine signals that promote stem cell expansion (Greenbaum and Wells, 2011). The nature of these paracrine signals, and the mechanisms underlying the supportive role of HSCs in stem cell expansion, are currently unknown and of intense interest. Intercellular signaling networks exist between tumors and tumor-associated fibroblasts. Tumor secretion of PDGF and TGF- β causes to changes in ECM composition and organization through stimulating myofibroblast activation. In addition, hepatic stellate cells secrete more angiopoietin 1 when activated (Taura et al., 2008), facilitating an angiogenic milieu that is supportive of tumor growth.

Tumors may signal to surrounding stroma. For example, elevated hedgehog signaling has been associated with liver injury in mice and humans (Jung et al., 2008; Lees et al., 2005), and promotes liver regeneration (Ochoa et al., 2010). Hedgehog signaling from tumors to the stromal microenvironment may be responsible for promoting tumor progression (Yauch et al., 2008). Since hedgehog signaling may induce EMT, the tumorigenic effect of hedgehog could be mediated by increased myofibroblast activa-

tion and fibrosis (Omenetti et al., 2008; Syn et al., 2009; Philips et al., 2011).

Increased stromal stiffness precedes and accompanies fibrosis in chronic liver disease (Georges et al., 2007; Yin et al., 2007), and elevated liver stiffness is associated with enhanced risk of HCC (Masuzaki et al., 2008). Stromal stiffness increases activation of stellate cells (Wells, 2008) and portal fibroblasts (Li et al., 2007), creating a positive feedback loop that continues to promote fibrosis. Stromal stiffness is regulated in part by matrix metalloproteinases (MMPs) and their inhibitors, but MMPs can regulate cell proliferation independently of their effects on stromal stiffness. Although MMPs degrade the stroma, they paradoxically increase HSC proliferation, liver growth, and tumor progression (Theret et al., 2001; Nishio et al., 2003; Zhou et al., 2006).

HCC diagnosed in cirrhotic and non-cirrhotic livers may display different imaging and pathological attributes such as size, differentiation, and encapsulation (Branca et al., 2002). When associated with NAFLD, HCC is often moderately or well differentiated and occurs as solitary large mass (Regimbeau et al., 2004; Bugianesi et al., 2002). HCC with mild or no fibrosis may share these characteristics (Yasui et al., 2011; Kawada et al., 2009; Iannaccone et al., 2007). Similarly, HCC complicating the metabolic syndrome and arising in non-fibrotic livers often remains well differentiated despite a larger size (Paradis et al., 2009). Deregulation of the Wnt/ β -catenin pathway has little role in the development of HCC associated with the metabolic syndrome in the absence of significant liver fibrosis (Paradis et al., 2009).

Alcoholic liver disease (ALD) is the most common cause of HCC, accounting for approximately one-third of all HCC cases (Morgan et al., 2004). Alcohol abuse has synergistic effects with other risk factors for the development of HCC, such as infection with HBV or HCV, diabetes and obesity (Hassan et al., 2002; Loomba et al., 2010).

Oncogenic potential of HCV proteins

Highly conserved HCV core protein is related to the induction of liver steatosis in transgenic mice and in HCV infected patients (Moradpour et al., 1996; Rouille et al., 2006; Barba et al., 1997). HCV core protein is involved in apoptotic changes, glucose and lipid metabolism, and malignant transformation. Among many interactions with cellular factors, HCV core has been shown to induce ROS production via interaction with heat shock protein Hsp60 (Kang et al., 2009), and modulates expression of the tumor suppressor protein p53 (Ray et al., 1997; Lu et al., 1999), p73 (Alisi et al., 2003) and pRb (Cho et al., 2001). Core also inhibits the expression of the cyclin-dependent kinase (CDK) inhibitor p21/Waf (Wang et al., 2002). p21 is a transcriptional target of p53 and blocks the cyclin/CDK complexes involved in cell cycle control and tumor formation. Core induces activation of the Raf1/MAPK pathway (Hayashi et al., 2000), protects cells from serum starvation and growth arrest and drives cells into proliferation. HCV core also activates the Wnt/ β -catenin pathway, which controls cell proliferation, DNA synthesis and cell-cycle progression (Fukutomi et al., 2005). We have shown that HCV core protein acts as a positive regulator in AR signaling, providing further insight into oncogenic potential in the development of HCC in HCV infected individuals (Kanda et al., 2008). HCV core protein behaves as a positive regulator in androgen receptor signaling and enhances the expression of VEGF in hepatocytes (Hassan et al., 2009). HCV NS2 also retains p53 into the cytoplasm, although the mechanism is not well understood (Bittar et al., 2013). A direct role of HCV NS3 was reported in the neoplastic transformation of hepatocytes *in vivo* and *in vitro* (Sakamuro et al., 1995). The NS3 protein also forms complexes with p53, and inhibits p21 promoter activity (He et al., 2003). HCV NS5A protein interacts with various signaling pathways including cell cycle/apoptosis (Kasprzak and Adamek, 2008) in host cells and shares some signaling

targets with core protein. NS5A is recognized as a transcriptional activator for many target genes (Kato et al., 1997). Transcription factor IID activities are modified by NS5A in the suppression of p53-dependent transcriptional transactivation and apoptosis (Lan et al., 2002; Majumder et al., 2001). NS5A also interacts with pathways, such as Bcl2 (Chung et al., 2003), phosphatidylinositol 3-kinase (PI3-K) (He et al., 2002), Wnt/beta catenin signaling (Park et al., 2009), and mTOR (Peng et al., 2010) to activate cell proliferation signaling and inhibits apoptosis. HCV polymerase NS5B forms a cytoplasmic complex with Rb in infected cells (Munakata et al., 2007). NS5B dependent down-regulation of Rb leads to activation of E2F-dependent transcription and increases cell proliferation. The interaction of the NS5B with Rb results in the degradation of Rb and activates the MAD2 promoter (Munakata et al., 2005). Thus, infection with HCV may lead to a loss of host-cell genomic stability due to deregulation of Rb pathway. The integrity of Rb appears to be important in the normally quiescent hepatocytes, as liver-specific loss of Rb may promote ectopic cell-cycle entry, aberrant ploidy and neoplastic transformation (Machida et al., 2009).

CONCLUSIONS

HCV remains a major cause of cirrhosis, liver failure and HCC despite recent dramatic advances in antiviral treatment. Some patients may experience progression of liver disease or HCC despite viral clearance. Trace amounts of HCV RNA from successfully treated patients can be infectious (Veerapu et al., 2014). We do not know the long-term efficacy of treatment with the new generation of DAAs, particularly with interferon-free regimens; and generation of potential resistant virus (Di Bisceglie et al., 2014; Poveda et al., 2014). Thus, it is important to understand the underlying mechanisms of interferon mediated and interferon free DAA mediated clearance of chronic HCV infection. Safety profile of DAAs with

side effects, especially in patients with advanced liver fibrosis is also an important point for consideration (D'Ambrosio and Colombo, 2013). The treatment may not work well upon repeated reinfection, especially among the drug addicts. An effective vaccine against multiple genotypes along with DAAs will be most appropriate to combat HCV infection. While there is evidence of a strong link between chronic HCV infection, fibrosis/cirrhosis, and HCC, how HCV promotes the disease processes is under intense investigation. HCV causes persistent infection, although the viral genome does not integrate into the host cell genome. Somatic cells have the ability to become pluripotent cells when transiently exposed to strong stimuli that they would not normally experience in their living environments (Obokata et al., 2014a). This reprogramming does not require nuclear transfer or genetic manipulation (Obokata et al., 2014b). Primary human hepatocytes, when infected in vitro with cell culture grown HCV, display an extended life span, and morphological and molecular alterations suggestive of epithelial-mesenchymal transition (EMT) state and tumor initiating stem cell (TISC) generation (Figure 2). This may promote to fibrosis/cirrhosis and HCC, and needs investigations to unveil the underlying mechanisms and overlaps in developing appropriate therapeutic modalities.

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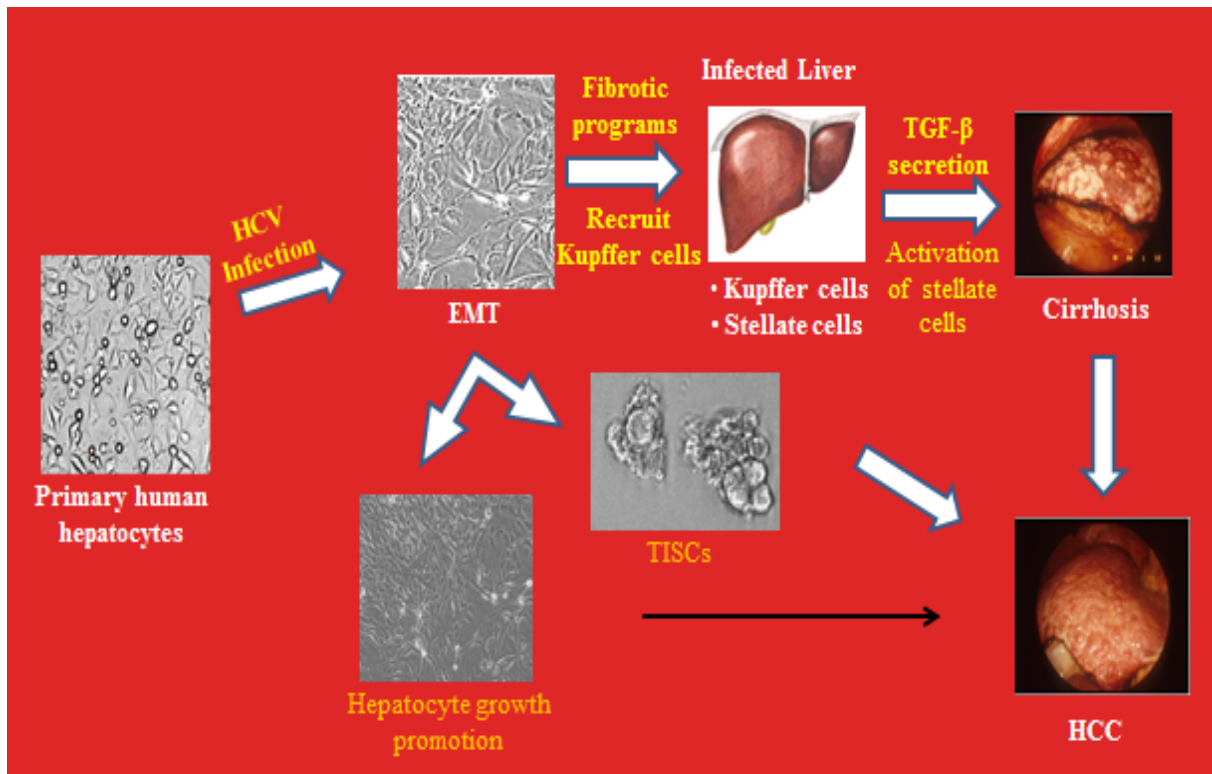


Figure 2: Hypothesis for HCV induced fibrosis/cirrhosis and HCC in chronically infected liver

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