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Review

Molecular mechanisms of hepatitis C virus–induced hepatocellular carcinoma

T. Vescovo [1], G. Refolo [1], G. Vitagliano [1,2], G.M. Fimia [1,3,*], M. Piacentini [1,2]

[1] 'L. Spallanzani' National Institute for Infectious Diseases, IRCCS, Italy
[2] Department of Biology, 'Tor Vergata' University of Rome, Rome, Italy
[3] Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Lecce, Italy

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ABSTRACT

Hepatitis C virus (HCV) is a major leading cause of hepatocellular carcinoma (HCC). HCV-induced hepatocarcinogenesis is a multistep process resulting from a combination of pathway alterations that are either caused directly by viral factors or immune mediated as a consequence of a chronic state of inflammation. Host genetic variation is now emerging as an additional element that contribute to increase the risk of developing HCC. The advent of direct-acting antiviral agents foresees a rapid decline of HCC rate in HCV patients. However, a full understanding of the HCV-mediated tumourigenic process is required to elucidate if pro-oncogenic signatures may persist after virus clearance, and to identify novel tools for HCC prevention and therapy. In this review, we summarize the current knowledge of the molecular mechanisms responsible for HCV-induced hepatocarcinogenesis. **T. Vescovo, CMI 2016;22:853**

Introduction

Hepatocellular carcinoma (HCC), the fifth most common cancer in men and the ninth in women, represents an urgent clinical problem, being the second leading cause of cancer-related death worldwide (GLOBOCAN 2012, http://globocan.iarc.fr).

Hepatitis C virus (HCV) is a major risk factor for chronic liver disease and for the increasing HCC incidence in most Western countries [1]. Approximately 3% of the world population is infected with HCV, and the severe consequences of virus infection makes HCV one of the most pressing emergencies worldwide (World Health Organization, http://www.who.int/).

The majority of infected patients are unable to clear the infection and develop a chronic hepatitis C (CHC) infection [2]. CHC results in inflammation-induced lesions in the liver frequently associated with hepatic fat accumulation (steatohepatitis) and progressive fibrosis, which over 20 to 40 years may evolve in cirrhosis (10–20% of patients) or HCC (1–5%) (Fig. 1) [3,4]. It is estimated that 27% of cases of cirrhosis and 25% of HCC cases worldwide can be attributed to HCV infection [5].

In chronically infected people, the risk of developing HCC is strictly correlated to fibrosis stage, with the incidence of HCC more frequent in patients with cirrhotic liver than in those with mild fibrosis [6]. Moreover, several risk factors, such as hepatitis B virus or HIV coinfection, obesity, insulin resistance or nonalcoholic steatohepatitis, actively enhance HCV-related HCC progression [7].

Sustained viral response to interferon (IFN)-based therapies decreases HCC incidence in a large portion of HCV patients, indicating the importance of eradicating the virus to prevents tumourigenesis [8,9]. However, despite a successful virus clearance, the risk of HCC is not fully abrogated in individuals with severe fibrosis, and continuous HCC surveillance is recommended [10].

The introduction of direct-acting antiviral (DAA) drugs as anti-HCV therapy, which allows a high rate of virus eradication, will provide important indications about the effectiveness of virus clearance in HCC prevention with respect to the disease stage of HCV patients [11,12].

Hepatocarcinogenesis in HCV-Infected Patients

HCV-related carcinogenesis results from a complex combination of host, environmental and viral factors. The induction of

* Corresponding author. G.M. Fimia, 'L. Spallanzani' National Institute for Infectious Diseases, IRCCS, Via Portuense 292, 00149 Rome, Italy.
E-mail address: gianmaria.fimia@inmi.it (G.M. Fimia).

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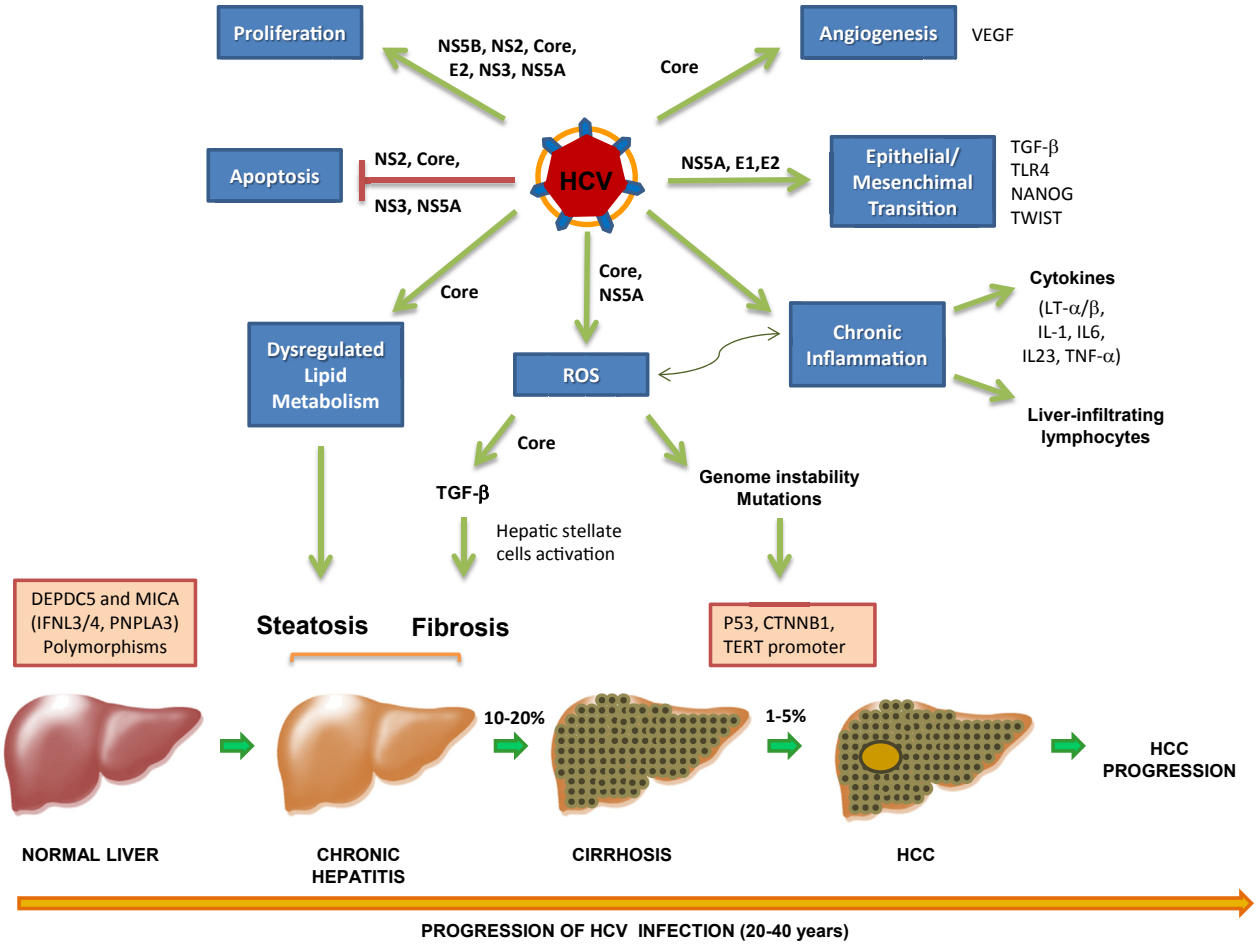

Fig. 1. HCV-related mechanisms of carcinogenesis: from HCV infection to HCC. Chronic HCV and associated liver cirrhosis represent major risk factors for HCC development. Hepatocarcinogenesis is a multistep process that may last for years; it involves progressive accumulation of different genetic alterations which lead to malignant transformation. Malignant transformation of hepatocytes occurs through increased liver cell turnover, induced by chronic liver injury and regeneration, in the context of inflammation and oxidative stress. HCV proteins may directly up-regulate mitogenic pathways, block cell death and induce ROS production. Moreover, HCV triggers persistent inflammation with accumulation of liver-infiltrating lymphocytes and production of several cytokines, such as LTα and LTβ, which are tightly linked to HCC development. Chronic inflammation exacerbates ROS production, which is considered a main source of genetic mutations. ROS are also associated with TGF-β pathway induction, leading to hepatic stellate cell activation and fibrogenesis. TGF-β, together with TLR4, plays an important role in the epithelial–mesenchymal transition. HCV dysregulates host lipid metabolism, causing liver fat accumulation which in many patients is associated with HCC. HCV is also able to induce angiogenic and metastatic pathways. Polymorphisms, mainly in *DEPDC5* and *MICA* genes, have been recently shown to increase the risk of developing HCC. HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ROS, reactive oxygen species; TGF, transforming growth factor.

hepatocarcinogenesis is assumed to be mainly triggered via the indirect effect of immune-mediated chronic inflammation. However, HCV may also directly induce HCC by altering several host regulatory pathways involved in proliferation, energy metabolism, angiogenesis, epithelial–mesenchymal transition (EMT), DNA repair, apoptosis and oxidative/endoplasmic reticulum (ER) stress (Fig. 1) [13].

Chronic inflammation leads to increased levels of reactive oxygen species (ROS), which damage hepatocytes both at metabolic and genetic levels, eventually causing cell death. Compensative liver regeneration, which occurs in a HCV-altered environment, favours chromosomal instability and irreversible genetic/epigenetic changes, which promote neoplastic transformation of hepatocytes and the progression of malignant clones [14].

HCV Directly Interferes With Pro- and Antioncogenic Pathways

Host tumour suppressors and proto-oncogenes are direct targets of HCV proteins (Fig. 1) [15]. An important example is represented by the Retinoblastoma (Rb) protein, which is known to control cell proliferation mainly by repressing the activation of E2F, a transcription factor needed for S-phase entry in the cell cycle [16]. HCV infection in cell culture has been shown to negatively regulate the levels of Rb [17]. This negative effect is mediated by NS5B, which binds Rb and promotes its cytoplasmic relocalization and proteasomal degradation [18,19]. The final outcome is the activation of E2F-responsive genes that stimulate cell cycle progression [19].

Other HCV proteins are able to stimulate G1/S transition by activating cyclin/Cdk complexes. NS2 is able to activate cyclin D/Cdk4 and induce expression of cyclin E [20], while Core increases the levels of cyclin E and Cdk2 [21], suggesting that HCV has evolved multiple ways to ensure cell cycle progression [15].

Constitutive activation of growth factor signaling pathways plays an important role in the initiation and the maintenance of HCC [22]. HCV Core, E2, NS3 and NS5A stimulate cellular proliferation by interfering with RAF/MAPK/ERK-regulated pathways, which are known to be associated with a more aggressive HCC phenotype [23–26]. The Wnt/β-catenin pathway is also altered by HCV. This pathway is activated upon binding of Wnt ligands to the Frizzled receptor and results in the inhibition of β-catenin degradation complex and consequent up-regulation of wide range of

protumoural genes, including the proliferation regulators c-myc and cyclin D [27]. Both NS5A and Core have been reported to activate β-catenin when overexpressed in cell culture through an indirect mechanism that involves phosphorylation and inactivation of GSK-3β, and subsequent stabilization of β-catenin [28,29]. More recent data suggest that β-catenin may also be activated through a direct interaction with NS5A [30,31]. The functional significance of the activation of β-catenin within HCV-infected cells is still unclear. Although dysregulation of Wnt signaling seems not to be sufficient of causing malignant transformation of hepatocytes, β-catenin levels are increased in most HCC and may sustain tumour growth [22].

An important event in the multistep process of tumourigenesis is the inhibition of apoptosis, which allows transformed cells to escape crucial checkpoints during cell cycle. HCV infection has a complex and incompletely understood interplay with apoptosis.

In cell culture experiments, apoptosis changes as a function of time, with apoptotic activity being higher in the early stage of infection and decreasing over time [32]. In patients, excessive apoptosis is observed in acute and fulminant hepatitis, as well as with chronic hepatitis. Sustained apoptosis is associated with persistent inflammation and fibrogenesis, while deficient apoptosis contributes to the development of HCC [33].

The p53 protein is a critical tumour suppressor that coordinates cell-cycle arrest, senescence and apoptosis [34]. Numerous studies have indicated that the HCV proteins Core, NS2, NS3 and NS5A interact with p53 when overexpressed in cell culture [35]. Core protein directly deregulates the p53 pathway, although controversial results on the consequence on this interaction have been reported. These discrepancies could be in part dependent on virus protein concentration, since it was shown that low levels of HCV Core protein stimulates the transcriptional activity of p53, while high levels of Core expression repress it [36]. HCV NS2, NS3/4A and NS5A interfere with the p53 pathway by inducing its relocalization from nucleus to cytoplasmic/perinuclear regions [20,37,38]. In particular, the anti-p53 activity of NS5A is mediated by its direct interaction, which results in the inhibition of p53-induced apoptosis [39–41].

HCV has evolved mechanisms that antagonize cell death induced by tumour necrosis factor (TNF)-α, a major apoptotic cytokine. HCV core protein blocks TNF-α-mediated apoptosis signaling by inducing the inhibitor FLICE [42]. HCV NS5A protects against TNF-α mediated apoptotic cell death by blocking the activation of caspase-3 and inhibiting the proteolytic cleavage of the death substrate poly (ADP-ribose) polymerase (PARP1) [43,44]. Moreover, NS5A can direct interfere with the apoptotic machinery, promoting a calpain-mediated proteolysis of Bid, a proapoptotic member of the Bcl2 family [45].

Transforming growth factor β (TGF-β) signaling is another important pathway targeted by HCV. TGF-β exerts antiproliferative and proapoptotic functions, but also have protumoural activities due to its role in EMT, a process involved in epithelial degeneration, fibrogenesis, metastatic process and invasiveness [46]. HCV NS5A has been shown to block TGF-β signaling through direct interaction with TGF-β receptor I (TGFBR1). NS5A inhibits TGF-β-mediated phosphorylation and nuclear translocation of Smad2, as well as Smad3/Smad4 heterodimerization [47]. Moreover, TGF-β pathway is also a target of HCV core. Interestingly, Pavio *et al.* [48], by isolating natural core variants from HCC and their nontumoural counterparts, showed that tumour-derived core variants inhibit the TGF-β pathway, while moderate or no effects were observed in the nontumoural ones. The authors also reported that the inhibition of TGF-β signaling occurs through a direct interaction with Smad3, which results in the inhibition of the DNA-binding activity of the Smad3/4 complex. These data suggest that during chronic infection, virus variants are selected to favour cell transformation by providing resistance to TGF-β antiproliferative effects. Relevant to this regard is the observation of Battaglia *et al.* [49] showing that HCV may interfere with the antitumoural effect of TGF-β, without a significant impact on EMT. They found that the expression of HCC-derived HCV core proteins renders primary human or mouse hepatocytes less responsive to tumour suppressive effects of TGF-β by reducing Smad3 signaling. However, the residual activity of Smad3 is sufficient to sustain the pro-EMT activity of TGF-β, which may contribute to HCC progression.

Related to the dual role of TGF-β in cancer, it was observed that, in HCV-infected patients, chronic inflammation shifts the TGF-β activity from tumour-suppressor to fibrogenic, thus increasing the risk for HCC. In particular, a phosphorylation of Smad3 mediated by c-Jun N-terminal kinase (JNK), different from that performed by classical TGFBRI pathway, promotes extracellular matrix deposition by up-regulating plasminogen activator inhibitor 1 (PAI-1), and is expressed during disease progression from chronic hepatitis through cirrhosis and HCC [50].

Furthermore, HCV may indirectly inhibit apoptosis by stimulating the PI3K-Akt survival pathway. In particular, this effect appears to be mediated by NS5A, which is able to down-regulate PTEN and relieves its inhibitory effect on PI3K-Akt pathway [51].

Another important survival pathway modulated by HCV is autophagy, a process that may play either anti- or protumour functions in the early and late phases of carcinogenesis [52,53]. Defective autophagy in mice results in spontaneous development of HCC. This is mainly due to accumulation of the autophagy cargo p62 and the consequent persistent activation of the transcription factor Nrf2, which regulates the expression a variety of cytoprotective genes [54,55]. Moreover, autophagy deficiency in hepatocytes has been also shown to favour EMT [56]. On the other hand, autophagy stimulation may promote the growth of early preneoplastic liver nodules in a Resistant-Hepatocyte rat model [57]. Autophagy is induced by HCV infection, playing important roles in the control of virus replication/secretion, inflammation and lipid homeostasis [58–63]. Interestingly, recent studies indicate that p62 levels are increased in HCV-induced HCC, suggesting that the impairment of autophagy may play a role in tumour development [64,65].

Other alterations caused by HCV infection that may affect the development of HCC are related to the dysregulated production of ROS. There are many reports describing elevated ROS levels in HCV replicating cells and in liver tissue from HCV-infected patients [66,67]. Mitochondria are considered the major site for the production of ROS in HCV-infected cells [68]. HCV core protein affects the release of ROS from mitochondria because of its association with mitochondrial membranes, where it alters the electron transport chain and the antioxidant enzyme systems [69–71]. NS5A plays also a role in the production of ROS, since its overexpression induces calcium release from ER, which is quickly absorbed by mitochondria, resulting in an elevation of ROS [72,73].

The presence of high levels of ROS due to unbalanced oxidative stress are known to favour the development of HCC by directly modifying mitochondrial and nuclear DNA, which during the repairing process may result in gene mutation [74].

Role of Immune-Mediated Liver Alterations in HCV-Induced HCC

To establish a chronic infection, HCV deregulates both innate and adaptive immunity through multiple mechanisms, including inhibition of type I IFN production and alteration of CD4 T cell differentiation towards unfavourable Th2, Th17 and regulatory T cells (Treg) subsets, which impairs the function of cytotoxic

lymphocytes CD8 T and natural killer (NK) cells [75–78]. These alterations result in a low-grade chronic liver inflammation that persistently perturbs tissue homeostasis and promotes a procarcinogenic environment inducing the release of ROS, nitric oxide species, lipid peroxidation and aberrant expression of cytotoxic cytokines [79]. At the same time, this condition may favour the impairment of immune surveillance and consequently the immune escape of neoplastic transformed cells, thus contributing to HCC development [80].

Many inflammatory cytokines, including TNF-α, interleukin (IL)-1, IL-23, IL-6 and lymphotoxins (LT) α and β have been implicated in chronic liver inflammation and HCC progression (Fig. 1) [80–82]. In particular, HCV-related hepatocarcinogenesis appears to be dependent on a delicate balance between pro- and anti-inflammatory stimuli. A high ratio of TNF-α/IL-10 levels has been observed in sera from patients with severe liver damage and HCC [83]. Moreover, the protumoural role of inflammatory cytokines, such as IL-6, seems to be dependent on the presence of estrogen. In fact, serum levels of IL-6 are higher in CHC patients than in healthy controls, but only female patients display an increased risk of HCC development [81].

Among inflammatory cytokines, evidence points at an important role of LTα and LTβ in the development HCC [82]. Notably, liver-specific overexpression of LTα and LTβ in mice leads to high inflammation levels accompanied by severe hepatotoxicity and spontaneous HCC development [82]. Experimental data point to the activation of NF-κB pathway by LTs as the triggering event of hepatocarcinogenesis by causing an amplification in the production of chemokines and cytokines, which trigger hepatic cell death, tissue remodelling and cell malignant transformation [82]. Severe liver damages induced by high expression of LTs correlate with an increased numbers of liver-infiltrating lymphocytes [82].

During HCV infection, liver-infiltrating lymphocytes fail virus clearance and accumulate in the liver, contributing to establish chronic inflammation [82,84]. Notably, Ramzan *et al.* [84] have observed the presence of higher levels of liver-infiltrating T and B cells in cirrhotic parenchyma of HCV infected patients with HCC compared to HCV patients without HCC. In particular, a high number of $CD8^+$ T cells in cirrhotic areas, but not in tumour of HCV-HCC patients, correlates with HCC occurrence and is a prognostic factor for recurrence after surgical resection [84]. Moreover, in HCV patients with HCC, an increased number of inflammatory $CD8^+$ cells is accompanied by a decrease of NK and NKT cells, which are known to participate in cancer immunosurveillance [84]. In contrast, an accumulation of T-reg cells, key suppressors of antitumour immunity, has been described in both HCC and cirrhotic tissue of HCV-infected patients [85]. Altogether, these data indicate that the synergistic effect of tumour-promoting inflammation and impaired anticancer immunity may be crucial in the progression from HCV-related cirrhosis to HCC.

Steatohepatitis and Hepatic Fibrosis in HCC

HCV-related HCC is frequently accompanied by steatohepatitis, suggesting that alteration of lipid metabolism, associated with inflammation, may take part to the multistep process of carcinogenesis [86]. It is well established that HCV proteins interfere with host lipid metabolism, mainly resulting in lipogenic pathways activation and decreased lipid catabolism, and consequently may trigger lipotoxicity in infected cells [62,87]. Indeed, an accumulation of free fatty acids is responsible for the production of ROS as a consequence of mitochondrial dysfunctions and ER stress. Oxidative stress can in turn stimulate lipid peroxidation and activate, over a certain threshold, an inflammatory cascade culminating in the release of several inflammatory cytokines, such as TNF-α and IL-1, which are intimately implicated in the development of steatohepatitis and insulin resistance [88]. Increased ROS levels have also a direct impact on fibrosis, by stimulating collagen I expression [89], and on genome abnormalities, by favouring gene mutations and chromosomal instability [90–92].

Accordingly, high expression levels of lipogenic genes have been reported in clinical HCC samples [93]. Moreover, in patients with chronic HCV, the administration of statins has been associated with a dose-dependent reduction in incident cirrhosis and HCC [94], confirming an intimate relationship between steatosis and HCC. In line with this idea, obesity and diabetes represent dangerous risk factors for HCC progression in chronically HCV-infected patients [95].

A novel mechanism by which HCV and obesity may synergize in inducing HCC has been recently proposed. Mice expressing HCV NS5A in the liver develop HCC when fed with alcohol and/or a high-fat diet in a TLR4-dependent manner [96,97]. Notably, NS5A, by increasing the levels of TLR4, amplifies the induction of the stem cells transcription factor NANOG stimulated by circulating endotoxins, thus generating tumour-initiating stemlike cells (TIC). They also found that NANOG promotes tumourigenesis by inducing Twist1, a master regulator of EMT, in cooperation with the adipocyte-derived leptin-pSTAT3 pathway, as well as by activating fatty acid oxidation to support TIC self-renewal and drug resistance [97,98].

An important aspect in the pro-HCC characteristics of HCV-induced steatosis is the alteration of liver T cell function. Transgenic mice, constitutively expressing HCV proteins in the liver, develop, with age, extensive steatosis which is accompanied by a consistent T cell infiltrate made up predominately of $CD8^+$ T cells secreting Th2-type cytokines [99]. More recently, in a mouse model of combined steatohepatitis and obesity, it has been observed that the massive liver infiltration and the activation of $CD8^+$ and NKT cells are required to induce steatosis, inflammation and carcinogenesis [100]. Moreover, a direct link was found between increased lipid production, in particular linoleic acid, and selective $CD4^+$ T cells loss in obesity-induced mice, which enhances HCC development by impairing antitumour immunity [101]. Altogether, these data emphasize the controversial and crucial role of immune system in HCV-related HCC development, indicating the importance of developing a targeted immune-based treatment approach for curing HCC [102].

In HCV-infected patients, an increasing risk of HCC onset is also tightly correlated with the severity of liver fibrosis. Specifically, profibrogenic cytokines have been also identified as key modulators of HCC progression [50]. TGF-β is a crucial cytokine involved in fibrogenesis, whose expression is induced directly by HCV core protein or through oxidative/ER stress and NF-κB pathway activation (Fig. 1) [103–106]. High levels of TGF-β have been observed both in serum and liver tissue of chronic HCV infected patients [107,108], as well as in patients with HCC [109]. Although Kupffer cells and liver-infiltrating lymphocytes are in large part responsible for TGF-β release [92], hepatocytes have been also identified as a source [103–105]. Recently Jee *et al.* [110] have shown that hepatocytes of HCV-infected patients express high levels of TGF-β and, *in vitro,* the amount of secreted TGF-β is sufficient to activate hepatic stellate cells. Upon stimulation, quiescent hepatic stellate cell transdifferentiate in myofibroblast and initiate to release profibrogenic mediators, inflammatory cytokines and chemokines, exacerbating liver inflammation and fibrogenesis [111]. In addition to its fibrogenic property, TGF-β also acts as immunosuppressor, thus favouring the immune escape of neoplastic transformed cells [112].

Neoangiogenesis and Metastasis in HCV-Induced HCC

Neoangiogenesis is an essential step for the growth and the survival of cancer cells. Many studies have shown that among all cases of HCC, microvessel density is higher in HCV-positive patients [113,114].

Experimental data suggest that structural and nonstructural HCV proteins could play a direct role in the induction of this process (Fig. 1). HCV core protein promotes angiogenesis by up-regulating hypoxia inducible factor Iα [115], which transcriptionally up-regulates vascular endothelial growth factor (VEGF) [116] and cyclooxygenase 2, as well as by activating matrix metalloproteinases, such as MMP-2 and MMP-9 [117]. VEGF has been reported to be an important endothelium-specific growth factor in HCC [118,119]. For this reason, serum levels of VEGF are used as prognostic factor of HCC [120–122]. Angiopoietin-2 (Ang2) was also shown to be up-regulated by HCV infection [123]. Ang2, with the receptor Tie-1, controls vessel quiescence. Under VEGF stimulation, Ang2 could be released from the endothelial cells and promote the proangiogenic action of VEGF.

Neoangiogenesis is not only required for tumour cell survival but also allows malignant cells to spread through the neovascularization and lead to intrahepatic and extrahepatic metastasis. The portal tract of HCC lesions is the first site of intrahepatic metastasis because cancer cells enter the portal vein via efferent flow [124].

A central role in the metastatic process is played by EMT, a process through which epithelial cells acquire mesenchymal characteristics, displace the tight and adherens junction components and increase cellular motility and invasion [125]. Notably, NS5A has been reported to trigger EMT through activating Twist2, a key transcriptional regulator of this process [126]. An alternative EMT induction pathway is triggered by E1/E2 proteins and uses TGF-β and VEGF signaling. TGF-β and VEGF are also regulated by HIF-1α, which, as previously described, is up-regulated by HCV core protein and increases hepatoma migration and permeability [127].

Role of Host and Virus Genetic Factors in HCV-Related HCC

Several gene mutations have been associated with hepatocarcinogenesis [128]. The first identified gene was the tumour suppressor gene *p53*, the mutation of which may lead to either gene inactivation or the production of dominant-negative forms [129]. The use of whole-genome sequencing has now provided a more detailed picture of genetic alteration in HCC, identifying a subset of genes more frequently mutated in HCV patients [130]. In particular, a high rate of mutations (>30%) was found in the oncogene *CTNNB1*, which encodes the β-catenin protein of the Wnt signaling pathway [131–133]. Another frequent genetic alteration detected in HCC regards the telomerase reverse transcriptase (*TERT*) gene, often involving its promoter region, which results in an increased expression of this pro-oncogenic factor (Fig. 1) [134–136].

Besides mutations in tumour suppressor genes, recent evidences indicate that host genetic variants are associated with a higher risk of HCC development [137]. Next-generation sequencing technology has been extensively applied to in-depth analysis of genetic variations in HCV patients.

An interesting example is represented by the *IFNL3/4* genes, two members of the IFN-λ cytokine family in which variants are known to be strongly associated with HCV patient response to therapy and natural clearance of infection [138]. Of note, one of these polymorphisms, which is located in the 3′ untranslated region of *IFNL3* mRNA, dictates transcript stability by influencing the binding of microRNAs (miRNAs) that are induced by HCV infection [139]. The *IFNL3* unfavourable polymorphisms have been also associated with an increased risk for HCC, especially in patients without a sustained viral response, although a series of validation studies failed to detect this association [140–144].

Similar incongruous observations has been reported for the *PNPLA3* gene (patatin-like phospholipase domain-containing protein 3), which shows a significant association with the presence of nonalcoholic fatty liver disease in HCV patients, while either positive or negative results were obtained concerning the relation to higher HCC risk [144–148].

Polymorphisms in other cytokines and/or related receptors have been found to be associated with HCC. Variants were identified in two main actors of the inflammatory response against HCV: the proinflammatory cytokine TNF-α and the anti-inflammatory cytokine IL-10. Patients carrying low-production haplotypes of *IL-10* and *TNF-α* 308 GG genotype have a higher risk of developing HCC [149]. Moreover, a rare variant of *IL-23R* was observed to correlate with reduced risk of HCV-related HCC in Egyptian patients [150]. Also, a HCC-associated polymorphism was found in the *VEGF* gene, which correlates with higher VEGF expression levels [122].

Recent genomewide association studies in large Japanese cohorts identified genetic variants in *DEPDC5* and *MICA* (MHC class I polypeptide-related sequence A) genes that are associated with HCV-related HCC [151,152]. While *DEPDC5* function is still unknown, *MICA* is a plasma membrane protein that is required for the binding to NK group 2D (NKG2D) cells and mediates their cytotoxic activity towards target cells in tumour immune surveillance. Unexpectedly, a validation study on a Caucasian cohort found that the same polymorphism has a protective impact on HCC development, while no association was observed for *DEPDC5*. Moreover, the study identified a novel variant in the *HCP5* gene located upstream of *MICA* as a stronger predictor of HCC [153]. Further validation analyses in different ethnic populations will help to define the predictive value of these markers.

Variations at the level of HCV sequence could also influence the risk of developing HCC. Polymorphisms of Core, NS3 and NS5A in circulating HCV genotype 1b and Core in genotype 1a have been reported to be associated with the development of HCC [154,155]. HCV sequences were recently compared between tumoural and nontumoural tissues; this analysis revealed a significant decrease of HCV levels in transformed cells and the presence of different quasispecies in the two districts, suggesting segregation of specific virus variants in HCC [156].

Epigenetic Alterations in HCV-Related HCC

Epigenetic regulatory genes are also dysregulated in HCC, which can result in a profound and permanent modification of gene expression [157]. An example is represented by the histone–lysine *N*-methyltransferase enzyme EZH2, which is aberrantly expressed in HCC [158] and also targets, among many genes, the expression of tumour suppressor miRNAs [159,160].

Another epigenetic-related modification observed during HCV infection is the inhibition of histone H4 arginine methyltransferase 1 (PRMT1), which is associated with the virus-mediated up-regulation of protein phosphatase 2A (PP2Ac) [161].

Changes in gene methylation were also correlated with virus-induced tumours [162]. In particular, a variety of tumour suppressor genes, including *CDKN2A, GSTP1, RUNX3, APC, SOCS-1* and *RASSF1A* are highly methylated in HCC from hepatitis B virus and HCV [163–166]. From a mechanistic point of view, a major role in DNA methylation changes is played by the HCV core protein. In fact, HCV core protein has been reported to inhibit the expression of the *CDKN2A* gene, which encodes for p16INK and p14Arf, two inhibitors of cell proliferation, by up-regulating the methyltransferases

DNMT1 and DNMT3b [167]. Moreover, HCV core protein also increases the methylation of *RASSF1A* promoter, a negative regulator of the Ras pathway, by inducing the histone methyltransferase SMYD3 (SET and MYND domain containing 3) [168].

Epigenetic alterations relevant for HCC may be mediated by changes in miRNA and long noncoding RNAs. A variety of miRNAs have been reported to modulate HCV replication in a positive or negative manner [169]. One of the most characterized HCV-related miRNA is miR-122, a liver-specific human miRNA that is highly expressed, that could pair to the 5′ untranslated region of HCV genomic RNA and enhance the virus replication within liver cells [170]. Less characterized is the role of miRNA in hepatocarcinogenesis. Some of miRNAs (miR-122, miR-27a and miR-181c) involved in the inhibition of proliferation, lipid metabolism and hepatocyte growth are down-regulated in HCV-related HCC, while others (miR-21, mi-R-221, miR-130α, Let7b, miR-155 and miR-200c) that regulate metabolism and immune response have been found to be up-regulated [60,171,172]. Recently up-regulation of miR-200c and miR-21 have been linked to hepatic fibrosis in HCV patients [169]. Because a relation between serum miR-122 levels and clinical features in HCV patients has been observed, it will be interesting to analyse whether miR-122 or other miRNAs may also serve as circulating biomarkers of HCC [173].

Conclusion

Our understanding of how HCV triggers HCC by direct and indirect mechanisms has significantly improved. In particular, new insights on the interrelation between HCV-induced alterations of immune cell activity and the development of HCC are promising to provide, in the near future, novel tools to monitor the progression of liver disease to HCC and to stimulate an effective anticancer immunity. Moreover, it is foreseeable that the ongoing identification of genetic and epigenetic factors associated with HCC will help to identify HCV patients at higher risk.

In the next years, current DAA therapeutic strategies, which have high sustained viral response rates in both patients with compensated and decompensated cirrhosis, are expected to prevent the progression of HCV-related pathologies, including HCC [174]. A first study on HCV-infected patients with advanced cirrhosis confirmed this expectation, showing a significant improvement of liver function (Model for End Stage Liver Disease (MELD) score) when DAA therapies are successful [175]. More recently, a study on HCV-infected patients treated with sofosbuvir reported a significant reduction in liver fibrosis when monitored by serologic and ultrasound-based tests [176]. A further important result observed with DAA is the reduction in recurrence of HCV infection in patients with HCC after liver transplantation [177]. However, in apparent contrast with this observation, a recent study on HCV patients with HCC undergoing chemotherapy and successful IFN-free DAA treatment revealed a high rate of early HCC recurrence, suggesting a negative effect of DAA on tumour immunosurveillance, possibly due to decreased levels of inflammation observed after HCV clearance [178]. If the negative impact of DAA on antitumoural immunity is confirmed, it will be important to evaluate if DAA-mediated HCV clearance also influences the progression of preneoplastic lesions and if immunotherapies may compensate for the decreased ‘beneficial’ inflammatory state.

Transparency Declaration

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