Infectious Agents and Cancer

REVIEW

Open Access

From viruses to cancer: exploring the role of the hepatitis C virus NS3 protein in carcinogenesis

Carole-Anne Martineau[1], Nathalie Rivard[2] and Martin Bisaillon[1*]

Abstract

Hepatitis C virus (HCV) chronically infects approximately 170 million people worldwide and is a known etiological agent of hepatocellular carcinoma (HCC). The molecular mechanisms of HCV-mediated carcinogenesis are not fully understood. This review article focuses on the oncogenic potential of NS3, a viral protein with transformative effects on cells, although the precise mechanisms remain elusive. Unlike the more extensively studied Core and NS5A proteins, NS3's roles in cancer development are less defined but critical. Research indicates that NS3 is implicated in several carcinogenic processes such as proliferative signaling, cell death resistance, genomic instability and mutations, invasion and metastasis, tumor-related inflammation, immune evasion, and replicative immortality. Understanding the direct impact of viral proteins such as NS3 on cellular transformation is crucial for elucidating HCV's role in HCC development. Overall, this review sheds light on the molecular mechanisms used by NS3 to contribute to hepatocarcinogenesis, and highlights its significance in the context of HCV-associated HCC, underscoring the need for further investigation into its specific molecular and cellular actions.

HCV infection

Hepatitis C virus (HCV) is a member of the *Flaviviridae* family harboring an approximately 9.6 kbp positive single-stranded RNA genome [1]. Upon infection of hepatocytes, the genome is released in the cytoplasm where the single open reading frame (ORF) is transcribed in a polyprotein using an internal ribosome entry site (IRES) sequence [1]. Viral and cellular proteases are responsible for the cleavage and production of 10 viral proteins: structural proteins Core, E1, and E2, and non-structural proteins P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B [1]. Genomic variations within studied HCV genomes have led to the classification of six different genotypes, further divided into subtypes [1]. After acute infection, 75–85% of patients progress to chronic hepatitis, which significantly increases the risk of cirrhosis, fibrosis, and hepatocellular carcinoma (HCC) [2]. The prevalence of HCV infection is high; 170 million people worldwide live with chronic HCV infection [2]. HCV-associated HCC usually develops after the establishment of cirrhosis, and carcinogenesis is tightly connected to immune-induced inflammation [3]. However, recent studies have demonstrated the direct effect of HCV on carcinogenesis, mainly through the Core, NS3 and NS5A viral proteins [4]. This review will focus on the implication of the viral protein NS3 in carcinogenesis, as multiple studies have shown the transformative effects of this protein on cells, although the precise mechanism is still unclear.

*Correspondence:
Martin Bisaillon
Martin.Bisaillon@USherbrooke.ca
[1] Département de Biochimie et de Génomique Fonctionnelle, Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke, 3201 Rue Jean-Mignault, Sherbrooke, QC J1E 4K8, Canada
[2] Département d'Immunologie et Biologie Cellulaire, Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke, 3201 Rue Jean-Mignault, Sherbrooke, QC J1E 4K8, Canada

HCV NS3 protein

NS3 is a 631-amino acid protein that harbors two distinct enzymatic activities [5]. The first 180 amino acids in the N-terminal region of the protein act as a serine protease which is required for the cleavage of the viral polyprotein at four specific junctions (Fig. 1A) [5]. This NS3 protease domain adopts a β-barrel structure (Fig. 1B) [5]. To achieve its functionality, NS3 needs both a zinc ion and the viral NS4A cofactor, which allows the formation of an eight-stranded β-barrel structure required for the correct orientation of NS3 catalytic triad [5]. The interaction between the two viral proteins is essential for the processing of the polyprotein. NS4A is tethered to the endoplasmic reticulum (ER), and its interaction with NS3 anchors it to the ER [6, 7]. Numerous studies have investigated the co-expression of NS3 and NS4A to unravel their intricate interplay.

The C-terminal region of NS3 houses an essential NTPase/helicase domain. NS3 interacts with nucleotides and utilizes energy from hydrolysis to move along RNA or DNA molecules. This region of the protein is further divided into distinct domains. Within domains 1 and 2, NS3 exhibits the seven canonical SF2 helicase motifs (I, Ia, and II through VI) [8]. Additionally, NS3 also possesses other conserved domains that vary across different HCV genotypes [9]. Two noteworthy features are the Arg-clamp motif, which connects domains IV and V, and the Phe-loop, situated between motifs V and VI. Residues responsible for binding nucleotides include Thr269 in domain 1 and Thr411 in domain 2 [10]. The ATP binding site lies between these two domains, forming two RecA-like domains, and comparative studies suggest that Lys210 may contact ATP [8]. Overall, the helicase activity of NS3 is critical for HCV replication, although its precise function is not fully resolved but likely involves unwinding RNA duplexes or secondary structures within the viral RNA [8].

Since NS3 possesses two enzymatic activities, it has been a target for inhibitor development. The inhibitors targeting NS3 protease are divided into two main categories: first-generation protease inhibitors, which operate through reversible covalent interactions, and second-generation protease inhibitors, characterized by noncovalent binding mechanisms [11]. Treatment strategies vary depending on the HCV genotypes, with protease inhibitors such as Telaprevir and Simeprevir showing efficacy [11]. Despite the presence of helicase inhibitors, their advancement is hindered by the conserved nature of

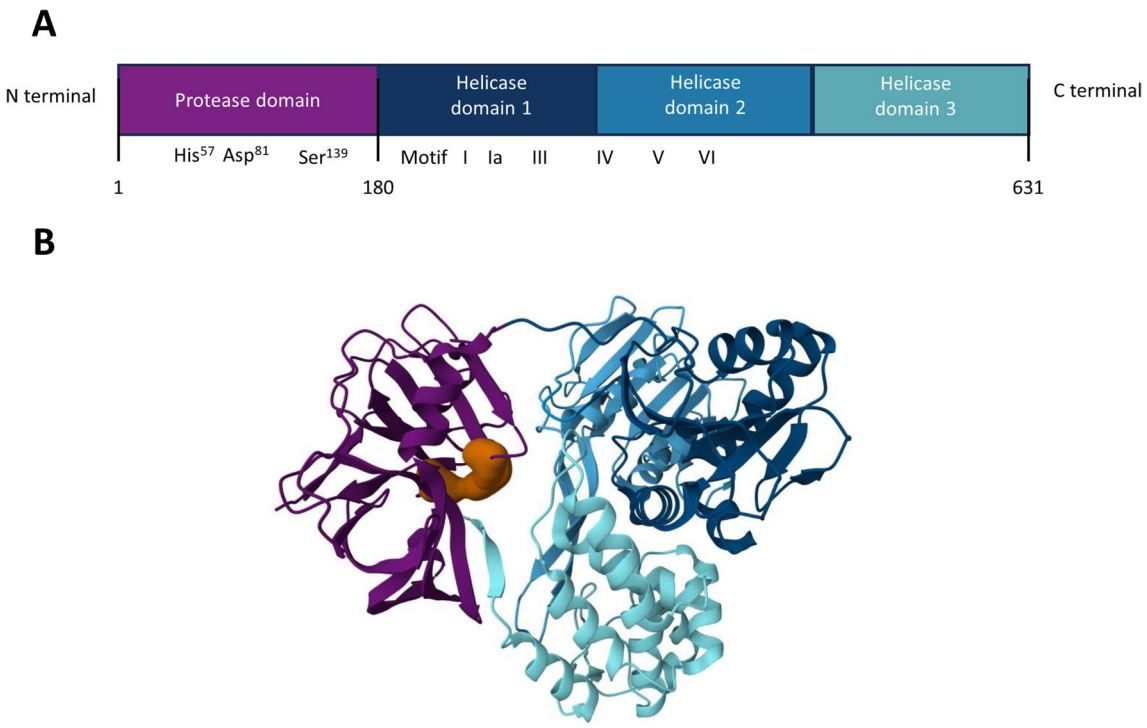

Fig. 1 Representation of HCV-NS3 domains. (**A**) N-terminal contains the protease domain from residues 1–180 represented in purple. Residues His^{57}, Asp^{81} and Ser^{139} compose the catalytic triad. C-terminal contains a NTPase/helicase domain from 180 to 631, itself composed of three domains represented in different shades of blue. Helicase domain I, Ia and III to VI are shown in domains 1 and 2. (**B**) 3D representation of NS3 from genotype 1b obtained by X-ray diffraction (PDB: 3O8B). The same color code was used as in (**A**), residues in the catalytic triad are in orange and represented in their gaussian surface form

the NS3 helicase domain across various cellular helicases, posing significant challenges in their development [11]. Approaches employing small molecules or nucleic acid-based inhibitors to target RNA or DNA binding sites and disrupt helicase function have been explored, yet inhibitors specifically targeting the helicase activity are still not available for clinical use [11].

NS3 in hepatocellular carcinoma

The correlation between NS3 and cellular transformation was initially highlighted in a seminal study dating back to 1995. In this study, researchers expressed either the 5′ or the 3′ genome segment of NS3 in NIH3T3 fibroblasts and meticulously monitored transformation-associated characteristics such as contact inhibition, doubling time, cloning efficiency, and tumor induction upon injection into mice [12]. Notably, the transforming capacity was unequivocally demonstrated by the NS3 5′ genome segment, corresponding to the N-terminal protease domain [13–15]. The helicase domain was also associated with HCC as levels of antibodies that recognize peptides in this region (1381–1403) are lower in HCV patients in chronic stage compared to HCV patients in cirrhotic or HCC stage [16]. Different groups have studied the immunological impact of different peptides spanning NS3 sequence, without studying the association with the stage of the disease [17–19]. However, there is an association between viral clearance and immunological response to the NS3 epitope 1248–1261 [18]. Multiple studies have also associated NS3 mutants and polymorphisms with HCC development. For instance, within HCV genotype 1b, the presence of the Tyr1082/Gln1112 polymorphism in NS3 is associated with a heightened risk of HCC [20]. NS3 from genotype 1b is also less immunological, as sera from patients infected with 1b are less reactive than genotypes 1a and 3a [21, 22]. Those observations could indicate that immunological suppression by NS3 is an important feature in HCC development. Moreover, viruses within genotype 1b can be stratified into distinct subgroups based on NS3 secondary structure, with certain subgroups, particularly group B, exhibiting a heightened incidence of HCC [23, 24]. Collectively, these findings underscore the intricate involvement of NS3 in carcinogenesis.

The following paragraphs provide an overview of the mechanisms potentially involving NS3 in carcinogenesis. Cancer emerges when cells divide uncontrollably, seizing control of tissues and organs. It is characterized by various cellular alterations, as reviewed in *The Hallmarks of Cancer* [25]. For cells to become cancerous, they must acquire the ability to achieve replicative immortality, resist cell death, circumvent growth suppression, instigate angiogenesis, and facilitate invasion and metastasis. Recently, researchers have added two more traits to this list: altering cellular energy processing and avoiding detection by the immune system. Additionally, factors like inflammation and genomic instability also play significant roles in cancer development.

NS3 molecular mechanisms of carcinogenesis

Sustaining proliferative signaling

Cell division is intricately regulated by multiple extracellular signals. These signals culminate in proliferation through the mitogen-activated protein kinases (MAPK) signaling pathway, among others. Extensive research has explored the role of NS3 in MAPK signaling. Across various studies, NS3 has been implicated in the activation of multiple MAPK pathways, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 [26–31]. This activation, as elucidated by different research groups, triggers distinct patterns of MAPK phosphorylation, consequently modulating the activity of activator Protein-1 (AP-1) transcription factor involved in cell growth regulation [26–29]. Some of those studies have shown that NS3-mediated MAPK activation is required for the viral protein's effect on cell growth [26, 29]. In HepG2 and HeLa, NS3 expression mediates cell growth through activation of JNK, leading to AP-1, activating transcription factor-2 (ATF-2), and c-jun expression [26]. One of AP-1 transcriptional targets is cyclin D1, which is involved in the progression of G1/S phase transition of cell cycle. Moreover, NS3 expression in QSG7701 cells induces an increase in cyclin D1 levels through the activation of ERK and nuclear factor-κB (NF-κB) pathways [29]. However, it's crucial to note the inconsistency among studies regarding which specific MAPK pathway NS3 activates, highlighting the lack of consensus in the literature on this subject.

Other cell-growth pathways, such as NOTCH and epidermal growth factor receptor (EGFR), are also modulated by NS3. However, direct involvement of NS3 in cell proliferation via these pathways remains unconfirmed. In HEK 293 cells, NS3 was shown to interact with Snf2-related CBP activator protein (SRCAP), a mediator of NOTCH activation [32]. Interaction between SRCAP and NS3 activates NOTCH-dependent transcriptional activity in Hep3B cells [32]. NS3-mediated NOTCH activation also requires the presence of another protein possibly interacting with NS3, namely p400 [32]. Despite reports of NOTCH upregulation in HCC, a pathway known for its dual roles as both an oncogene and tumor suppressor, the exact impact of NS3-mediated NOTCH activation on proliferation necessitates further investigation [33, 34]. NS3/4A is also responsible for the cleavage and inhibition of T-cell protein tyrosine phosphatase (TC-PTP), a phosphatase involved in regulation of EGFR signaling

[35]. A negative correlation between NS3 and TC-PTP was confirmed in liver biopsies from patients infected with HCV, supporting the evidence of cleavage by NS3 [36]. This cleavage results in the activation of EGFR and protein kinase B (Akt), with Akt activation proving pivotal for both viral replication and resistance to apoptosis in HCV-infected cells [35, 37]. However, the involvement of this pathway's activation on NS3-mediated cell growth needs further studies. Furthermore, NS3 may activate EGFR through an alternate mechanism involving neuregulin 1 (NRG1). NS3 was shown to cleave NRG1, which could explain the upregulation of EGFR pathway in cells infected with HCV (Fig. 2, Table 1) [38]. However, the physiological implications of this mechanism remain largely uncharacterized, warranting further investigation into its role in cellular physiology.

The co-expression of NS3 with its cofactor NS4A appears to modulate NS3's ability to enhance cell growth. While various studies have demonstrated NS4A's inhibitory effect on NS3-mediated growth promotion, this inhibition does not extend to the activation of MAPK pathways, as detailed previously [26, 39]. Intriguingly, certain research groups have also noted the antiproliferative properties of NS3 [39, 40]. In hepatic cell lines, NS3 expression inhibits cell cycle progression either in G0/G1 or G2/M phases, depending on the study [39, 40]. However, the absence of p21 cell cycle inhibitor expression in the cell lines was noted, potentially explaining the disparate findings [40]. Moreover, in one particular report, despite the inhibitory effect of NS3 on cell growth in vitro, mice inoculated with HepG2 cells expressing NS3 exhibited augmented proliferative capacities [39]. Further investigation is warranted to elucidate the precise mechanisms underlying these observations.

Resisting cell death

NS3 interacts with p53, a transcription factor involved in the control of cell cycle progression, via specific amino acids (Leu106 and Phe43), effectively inhibiting p53's function [41–44]. In cells expressing NS3, both p53 and its target gene p21 exhibit reduced mRNA levels [39]. Although another study found that NS3 does not affect p53 stability or transcriptional activity, NS3 inhibits p21 transcription in a manner depending on the number of p53 binding domains in the p21 promoter [45]. The authors hypothesized that p21 expression could be repressed by the interaction between NS3 and p53, which could explain the increased cell growth observed in NIH 3T3 cells. Additionally, another group has linked the NS3-p53 interaction to the upregulation of ubiquitin D (UBD), which correlates with proliferative effects [46].

Beyond its pivotal role in cell cycle regulation, p53 is intricately linked to apoptosis. NIH 3T3 cells expressing the N-terminal region of NS3 are more resistant to

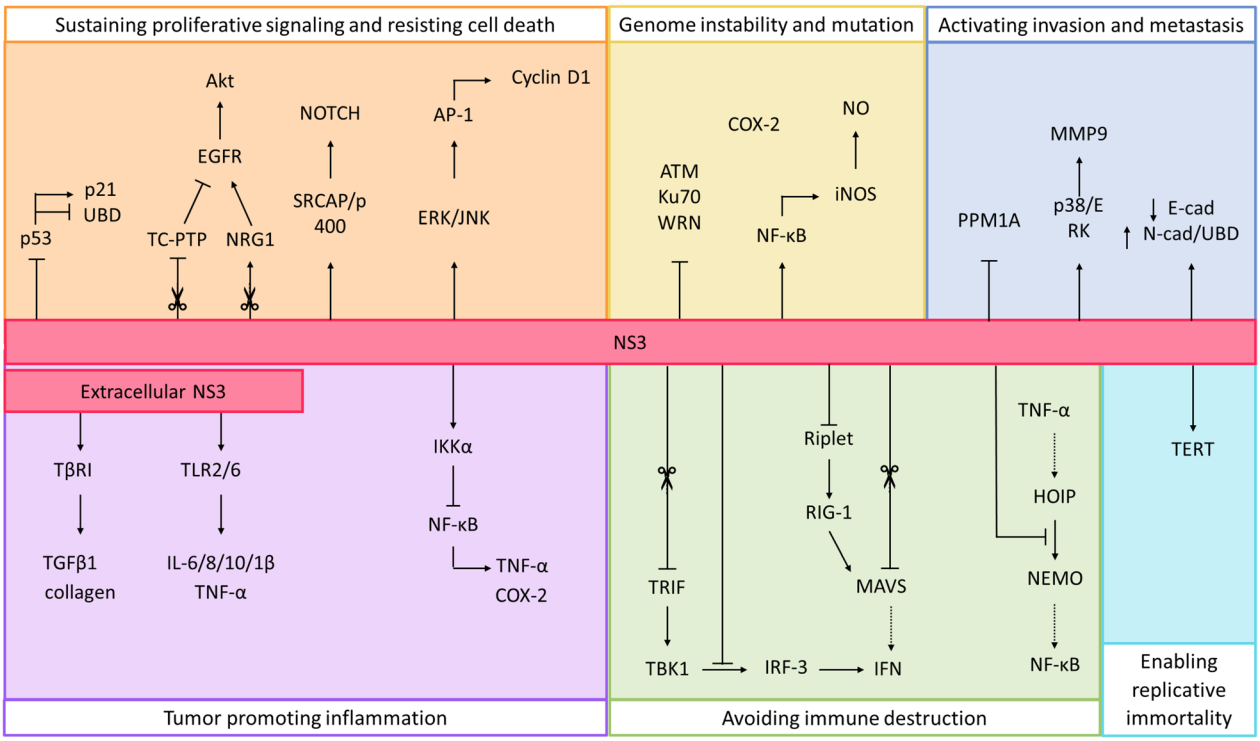

Fig. 2 Visual summary of the different mechanisms by which NS3 could contribute to carcinogenesis

Table 1 Summary of the effects of NS3 on different pathways involved in carcinogenesis

Cancer hallmark	Cellular protein/pathway	Effect of NS3	Impact on cellular pathway	References
Sustaining proliferative signaling	JNK	Activation	Activation of AP-1 and ATF-2 leading to higher cell growth	Hassan et al. [26]
	ERK	Activation	Activation of AP-1 and NF-κB leading to the expression of cyclin D1 and higher cell growth	Li et al. [29]
	TC-PTP	Cleavage	Activation of EGFR and Akt	Brenndörfer et al. [35]
	NRG1	Cleavage	Increase in EGF-like domain	Schwartz et al. [38]
	SCRAP	Interaction	Activation of NOTCH receptor	Iwai et al. [32]
	p53	Inhibition	Downregulation of p21 expression leading to higher growth rate	Kwun et al. [45]
		Interaction	Inhibition of p53 transcriptional activity	Deng et al. [42]
		Interaction	Resistance to apoptosis	Tanaka et al. [43]
		Downregulation	Resistance to apoptosis	Fujita et al. [47]
		Downregulation	Upregulation of UBD	Li et al. [46]
	iNOS	Upregulation of expression	NO production leading to dsDNA breaks	Machida et al. [53, 54]
	ATM	Interaction and relocalization to the cytoplasm	Impaired DNA repair	Lai et al. [58]
	WRN	Degradation	Impaired NHEJ DNA repair	Chen et al. [59]
	Ku70	Reduction of the capacity to bind DSB	Impaired DNA repair	
Activating invasion and metastasis	ERK and p38	Activation	Activation of NF-kB and expression of MMP9	Lu et al. [61]
	PPM1A	Proteasome-mediated degradation	Enhanced cell invasion	Zhou et al. [60]
	TC-PTP	Cleavage	Activation of EGFR leading to formation of invadopodium and cell invasion	Ninio et al. [63]
	UBD	Upregulation	Increased migration capacities	Li et al. [46]
	TLR2	Activation	Activation of inflammatory pathways and cytokine expression	Dolganiuc et al. [59]
Tumor promoting inflammation	IKKα	Activation	IkB-α degradation leading to activation of NF-kB and expression of TNF-α	Hassan et al. [27]
	JNK, ERK, and PKD2	Activation	Activation of NF-κB leading to the expression of COX-2	Lu et al. [30]
	TLR2, TLR1, and TLR6	Activation	Impaired cytokine expression	Chang et al. [70]
	TLR2and TLR6	Activation	Activation of NF-kB leading to pro-inflammatory cytokine expression	Rajalakshmy et al. [71]
	TβRI	Interaction and activation	Expression of collagen and TGFβ1 contributing to fibrosis	Sakata et al. [73]
Avoiding immune destruction	MAVS	Cleavage	Inhibition IFN-β promoter	Meylan et al. [86]
				Li et al. [89]
			Inhibition IFN-β promoter and RIG-I mediated IRF3/7 activation	Lin et al. [92]
			Disruption of MAVS and IKKe localization at the mitochondria	Lin et al. [92]

Table 1 (continued)

Cancer hallmark	Cellular protein/pathway	Effect of NS3	Impact on cellular pathway	References
			Inhibition of RIG-1 interaction and IRF-3 signaling	Loo et al. [87]
			Inhibition of MAVS oligomerization	Baril et al. [88]
			Inhibition of IFN response	Bender et al. [93]
			Inhibition of ISG response	Ferreira et al. [91]
	Riplet	Cleavage	Inhibition of RIG-I polyubiquitination and activation	Oshiumi et al. [94]
	TRIF	Cleavage	Inhibition of TLR3 signaling	Li et al. [89]
	HOIP	Interaction	Inhibition of NEMO polyubiquitination, blocking TNF-α-mediated activation of NF-κB	Chen et al. [83]
	TBK1	Interaction	Inhibition of TBK1 interaction with IRF-3	Otsuka et al. [98]
Enabling replicative immortality	TERT	Interaction	Increased TERT activity	Zhu et al. [102]

actinomycin D-induced apoptosis and present reduced protein levels of p53 [47]. However, the impact of NS3 on apoptosis remains a subject of debate. While some studies find no discernible effect of this viral protein on apoptosis, others reveal a pro-apoptotic influence of NS3 [48]. First, a study demonstrated the apoptotic effect of NS3 on mature dendritic cells, which correlated with a decrease in p21 expression [49]. Subsequently, NS3 was found to interact with caspase-8, an apoptosis effector, thus promoting this form of cell death [50, 51]. Intriguingly, NS3-mediated apoptosis in phagocytes may contribute to carcinogenesis. Exposure of phagocytes to NS3 activates NADPH oxidase, resulting in heightened oxidative stress and subsequent apoptosis [52]. Consequently, NS3-induced apoptosis impairs phagocyte response, a process crucial for eliminating infected cells.

Genome instability and mutation

Genomic instability is a hallmark of cancer cells [25]. Mutations in DNA are usually repaired by different mechanisms. However, when those processes fail, mutations accumulate, leading to genomic instability. Therefore, genes involved in DNA repair play a critical role as tumor suppressors [25]. The expression of NS3 has been shown to induce cellular DNA damage which could mediate carcinogenesis. In Raji cells expressing NS3, inducible nitric oxide synthase (iNOS) expression was upregulated [53, 54]. Remarkably, this upregulation correlates with the number of NF-κB copies in the iNOS promoter, suggesting that NF-κB acts as a mediator in this process [53]. Notably, iNOS generates nitric oxide (NO), a genotoxic molecule that inflicts various types of DNA damage [55]. Furthermore, in NS3-expressing cells, double-stranded breaks occur, further emphasizing the intricate interplay between NS3, DNA damage, and potential carcinogenic pathways [54].

The HCV NS3 protein plays a significant role in promoting DNA damage and disrupting DNA repair through various mechanisms. Notably, the NS3/4A complex interacts with key DNA repair proteins such as ataxia telangiectasia mutated (ATM), Ku70, and Werner syndrome helicase (WRN), thereby affecting the cellular response to DNA lesions. ATM is essential for recognizing double-stranded DNA breaks and for the phosphorylation of histone H2AX on serine 139 (γ-H2AX), a marker of DNA damage [56]. The interactions of NS3 and NS3/4A with ATM do not lead to cleavage of ATM but result in its relocation to the cytoplasm, impairing the phosphorylation of γ-H2AX [57, 58]. This mislocalization correlates with ineffective DNA damage repair, as observed in U2OS cells expressing NS3 [58]. Additionally, the NS3/4A complex promotes the degradation of WRN through proteasomal pathways, thus interfering with WRN's role in DNA repair. NS3 also binds to Ku70, a protein crucial for non-homologous end joining (NHEJ), diminishing its ability to bind to DNA breaks [59]. This interaction significantly reduces the effectiveness of NHEJ, as demonstrated in studies involving NS3 and NS3/4A [59]. These findings collectively highlight the complex role of NS3 in hindering DNA repair processes.

Activating invasion and metastasis

Migration and invasion are key aspects of cancer cell transformation [25]. The NS3 protein significantly influences these processes through multiple mechanisms. Firstly, NS3 interacts with protein phosphatase 1A

(PPM1A) and promotes its ubiquitination and subsequent degradation. PPM1A is a phosphatase that plays a role in several cellular pathways, and its degradation has been associated with increased cell migration and invasion in Huh-7 cells [60]. Additionally, NS3 expression is linked to changes in key molecules involved in epithelial-mesenchymal transition (EMT). This includes increased levels of vimentin and N-cadherin, along with decreased expression of E-cadherin, further facilitating the migration and invasion capabilities of cancer cells [60].

NS3 enhances cell migration through several mechanisms. One such mechanism involves increasing the expression of gelatinase-B (MMP-9), an enzyme that degrades collagen-IV and promotes tumor invasion. In various cell lines, NS3 activates the transcription of MMP-9 by triggering the ERK1/2 and p38 MAPK pathways [61]. This activation leads to the translocation of NF-κB to the nucleus, where it binds to the MMP-9 promoter [61]. Additionally, NS3 further boosts MMP-9 levels through expression of cyclooxygenase-2 (COX-2), an enzyme involved in inflammation and other pathways important for carcinogenesis [61, 62].

Moreover, NS3/4A increases invasion by promoting invadopodia formation, cell protrusions involved in matrix degradation and invasion [63]. As previously described, NS3 achieves this by cleaving TC-PTP, which activates the EGFR pathway, a critical process in invadopodium assembly [63]. Furthermore, a protein network analysis revealed that proteins associated with focal adhesion interact with NS3, influencing cellular dynamics [64]. Cells expressing NS3 or NS3/4A exhibit a significantly reduced ability to bind to fibronectin in vitro [64], a key component of the cellular matrix. Additionally, upregulation of UBD in HepG2 cells expressing NS3 also leads to increased cell migration, reinforcing NS3's role in enhancing invasive properties [46].

Tumor-related inflammation

Chronic inflammation is tightly linked to cancer as it creates a niche favorable for the malignant transformation of cells [65]. Multiple processes induced by chronic inflammation are associated with cancer, such as proliferation, invasion, and fibrosis [65, 66]. Since viruses such as HCV create a hepatic cancer niche from chronic inflammation, it was suspected that viral proteins participated in inflammation induction [65]. NF-κB is involved in inflammation by regulating the expression of different genes, including tumor necrosis factor α (TNF-α), an inflammatory cytokine [27, 67–69]. It was demonstrated that NS3 promotes the activation of IκB Kinase α (IKKα) in HepG2 and Huh7 [27]. IKKα is responsible for the degradation of NFKB inhibitor α (IκB-α), a negative regulator of NF-κB [27]. NS3 activation of IKKα is mediated through IKKα/NF-κB but also requires JNK/AP-1 signalling [27]. Others have reported that activation of NF-κB, and other signalling pathways such as JNK and ERK, in HepG2 leads to an upregulation in COX-2 expression [30].

NS3 has also extracellular inflammatory effects: a study showed that monocytes are activated in response to NS3 exposition, which leads to the expression of TNF-α and interleukin-10 (IL-10) [68]. A downregulation of IκB-α was also observed in the same study, suggesting the involvement of NF-κB [68]. Furthermore, exposing myeloid dendritic cells to NS3 reduces their allostimulatory capacity by inhibiting their differentiation resulting in irregular cytokine expression [68]. The same group demonstrated the capacity of NS3 exposition on HEK 293 cells, mouse macrophages, and human monocytes to activate toll-like receptor 2 (TLR2) [69]. In this case, TLR2, ERK, and JNK/AP-1 pathways were necessary to induce TNF-α [69]. Further studies highlighted the importance of TLR2, TLR1, and TLR6 in cytokine expression mediated by NS3 exposition in human monocytes [70]. Similar results were obtained in microglia cell line CHME3 exposed to NS3 in which inflammatory cytokine expression, such as IL-8, IL-6, and IL-1β, was induced through TLR2 or TLR6, but not TLR1 [71].

Furthermore, co-expression of HCV nonstructural proteins in hepatic stellate cells (HSC) promotes the expression of pro-inflammatory molecules such as RANTES, monocyte chemoattractant protein-1 (MCP-1), IL-8, and intercellular Adhesion Molecule 1 (ICAM-1) through a mechanism involving upregulation of intracellular Ca^{2+} and reactive oxygen species (ROS) formation [28]. Those results were obtained in HSC expressing NS3-NS5 with an adenovirus system. While it was shown that HSC express receptors necessary for HCV infection, the capacity of the virus to infect this cell type was not characterized [28]. Nevertheless, NS3 does have extracellular effects on HSC by activating pro-inflammatory signaling pathways such as c-Jun, p38, AP-1, and NF-κB [28].

Fibrosis is an inflammatory response where cells deposit extracellular matrix to repair the tissue [72]. It is a mechanism correlated with cancer as they both arise from chronic inflammation. However, fibrosis can lead to cytokine production, creating a positive feedback of inflammation response [72]. Non structural proteins (NS3–NS5) expression in HSC leads to fibrosis induction by secretion of transforming growth factor β1 (TGFβ1) and increased procollagen α1 expression [28]. Another study found that NS3 binds the type I subunit of the transforming growth factor receptor (TβRI) by mimicking its ligand TGFB2 transforming growth factor beta 2 (TGF-β2) [73]. In LX-2 HSC cells treated with NS3 but not normal hepatic cells, this interaction results in the expression of collagen and TGFβ1, a cytokine promoting

fibrosis [73]. Those results suggest once again that NS3 has extracellular effects on HSC. Dysregulation of microRNA (miRNA) expression by NS3 is also involved in fibrosis. Human monocytes stimulated with NS3 show an increase of miR-155, responsible for activating inflammatory signaling pathways [74, 75]. Furthermore, NS3 expressing cells show a downregulation of miR-335, miR-150, and miR-122, with anti-fibrotic effects, and an upregulation of miR-27a, with pro-fibrotic effect [76–78].

Avoiding immune destruction

The progression of HCV infection into chronic infection is a risk factor for HCC development. Indeed, most HCV-induced HCC occur after the onset of inflammation and cirrhosis, although cases that defy that statement have been reported [79–81]. Immune system evasion contributes to chronic infection, which can lead to carcinogenesis. Avoiding immune destruction is also an important cancer hallmark. As mentioned in the last section, many studies have demonstrated the activation of NF-κB and the upregulation of TNF-α expression in the presence of NS3. However, it was shown that NS3/4A expressed in mice hepatocytes in vivo confers resistance to TNF-α treatment [82]. Another study showed a downregulation of NF-κB activation following TNF-α stimulation in HEK 293T and Huh-7.5.1. This effect was attributed to NS3 and its interaction with HOIL-1-interacting protein (HOIP), a member of the linear ubiquitin chain assembly complex (LUBAC), competing for NF-κB essential modulator (NEMO), both involved in NF-κB signalling [83]. In Huh-7.5.1, perturbation of this interaction by NS3 inhibits polyubiquitination of NEMO, which is important in the activation of NF-κB [83].

HCV can be recognized by different pathogen recognition receptors such as retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and TLRs [84]. After viral RNA sensing by RLRs, the caspase recruitment domain (CARD) of RLRs interacts with the same domain on the mitochondrial antiviral-signaling protein (MAVS) present in the mitochondrion and peroxisome membrane [85]. MAVS then activates signalization, leading to interferon (IFN) expression [85]. NS3 impairs this pathway through cleavage of MAVS at Cys-508, inhibiting IFN production [86–93]. Another cleavage target of NS3/4A in this pathway is Riplet, which is necessary for the ubiquitination and activation of RIG-1 [94]. Another study revealed that residue Y16 of NS4A is essential for NS3/4A Riplet inhibition in Huh7 cells [95].

In addition to RLR dysregulation, NS3 controls TLR signaling. Indeed, in vitro assays demonstrated the capacity of NS3/4A to cleave TIR domain-containing adapter inducing IFN-β (TRIF), a downstream effector of TLR3, involved in the recognition of dsRNA [96]. TRIF cleavage was also shown in osteosarcoma cells, impairing IFN response [97]. However, another team observed no cleavage of TRIF in HEK 293T expressing NS3 or NS3/4A [98]. Still in the TLR pathway, TANK-binding kinase 1 (TBK1) was demonstrated to be an interactor of NS3 [98]. In HEK 293T, NS3 competitively binds TBK1, inhibiting its interaction with interferon regulatory factor 3 (IRF-3), a transcription factor responsible for IFN expression [98]. Concordantly, another study observed lower levels of phosphorylated IRF-3 in UHCV11 cells expressing NS3/4A following IRF-3 activation by Sendai virus [99]. Upon IFN secretion, the molecule binds to its receptor, activating a signaling cascade involving signal transducer and activator of transcription 1 (STAT1). In Huh-T7 cells expressing NS3/4A, lower levels of STAT1 are observed, although the two proteins don't interact [100].

Enabling replicative immortality

Cells have a limited division potential according to the length of their telomeres framing chromosome ends, after which, apoptosis or senescence occurs. To evade this unfortunate destiny, cancer cells reactivate telomerase (TERT), an enzyme catalyzing the lengthening of telomeres. In normal cells, telomere shortening limits cellular replication by inducing senescence or apoptosis. Many different proteins from oncogenic viruses control TERT activity [101]. This prevents telomere shortening and allows continuous cell division, effectively enabling cells to bypass replicative limits. However, very few studies have focused on the role of NS3 in replicative immortality. Still, NS3/4A was found to interact with C-terminal region of TERT and increase its activity in HEK 293 expressing NS3/4A [102]. Considering the crucial role of TERT in cancer development, further studies on the relationship between NS3 and replicative immortality are warranted.

The *Flaviviridae* NS3 protein

The *Flaviviridae* family contains four genera: *Flavivirus*, *Hepacivirus*, *Pestivirus*, and *Pegivirus* [103]. Members of the first two genera cause disease in humans [103]. Viruses in the *Flaviviridae* family have a similar genome organization and possess a conserved NS3 protease-helicase protein that requires a viral co-factor (NS2B for *Flavivirus* and NS4A for *Hepacivirus*) [103]. However, HCV, the only human virus in the *Hepacivirus* genus, is one of the only *Flaviviridae* associated with cancer. Furthermore, attenuated forms of *Flavivirus* have been proposed as oncolytic therapies [104]. Reasonably, HCV-NS3 is far more studied for cell transformation than other *Flaviviridae*. Nonetheless,

some studies have shown similar roles of NS3 from other *Flaviviridae* to those presented in this review.

First, *Flaviviridae* NS3 has a role in intrinsic immunity and inflammation, as reviewed in Latanova et al. [105] and Chen et al. [106]. It was observed that Kunjin virus (KUNV) and Dengue virus (DENV) NS2B/NS3 inhibit IFN response [107–109]. DENV-NS2B/NS3 targets STING, involved in pathogen-associated molecular pattern recognition, for cleavage and impairs IFN expression [109]. DENV-NS3 also targets mediator of IRF3 activation (MITA) for cleavage [110]. Moreover, DENV-NS3 interacts with 14-3-3ε, leading to inhibition of RIG-1 translocation to MAVS [111]. Zika virus (ZK) NS3 also interacts with 14-3-3ε and 14-3-3η, inhibiting RIG-1 and melanoma differentiation-associated protein 5 (MDA5) translocation to mitochondria [112]. Furthermore, ZK-NS3 induces MAVS and MITA degradation leading IFN-β expression inhibition [113]. ZK-NS3 was also associated with the degradation of nucleotide-binding oligomerization domain-like receptor 3 (NLRP3), involved in activation of the inflammasomes [114].

However, contrary to some reports on HCV-NS3, multiple studies reported the apoptotic effect of NS3 from different *Flaviviridae*. First, DENV-NS2B/NS3 was shown to induce apoptosis [115, 116]. DENV-NS2B/NS3 cleaves IKKα/β, leading to activation of NF-κB and promotion of apoptosis through extrinsic pathways [116]. West Nile Virus (WNV) NS3 cleaves procaspase-8 triggering apoptosis [117]. Japanese encephalitis virus (JEV) was also shown to induce apoptosis through the caspase and mitochondrial pathways [118, 119]. Finally, Langat virus NS3 triggers apoptosis through caspase-8 binding [120].

The *Pegivirus* genus, to which HCV bears more resemblance than the *Flavivirus* genus, contains viruses that infect humans (human *pegivirus*: HPgV) [121]. Besides, HCV-NS3 shares more similarities with HPgV-1-NS3 than other *Flaviviruses* NS3, such as WNV, KUNV, ZK, and DENV (Fig. 3). Considering this resemblance, it could be hypothesized that HPgV-NS3 has oncogenic functions, as does HCV-NS3. HPgV is a lymphotropic virus, but its pathogenicity is unclear; it is not considered a causal agent of any disease but is associated, among others, with the development of non-Hodgkin lymphoma (NHL) [122]. The association between HPgV infection and NHL is not understood mechanistically, but it has been hypothesized that reduced immune surveillance in chronic HPgV infection could be in cause [122, 123]. Nevertheless, HPgV-1-NS3/4A/B was shown to cleave MAVS, as HCV-NS3, leading to impaired IFN response [124, 125].

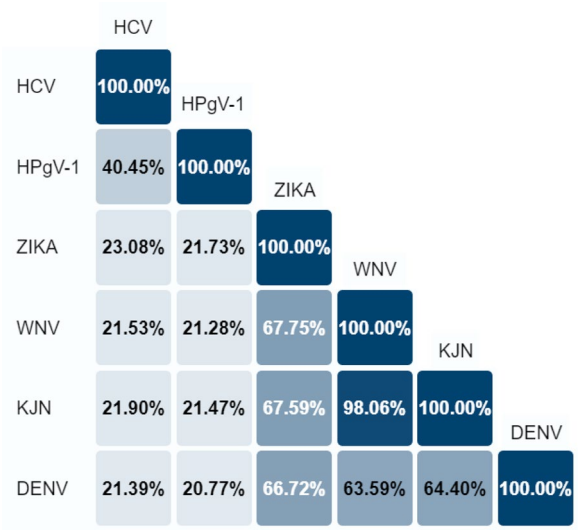

Fig. 3 Percent identity matrix of NS3 alignment from different *Flaviviridae*. Alignment and analysis were done with Uniprot Align tool with the Clustal Omega program. HCV genotype 1b isolate BK (P26663); WNV strain NY99 (Q9Q6P4); KUNJIN strain MRM61C (P14335); ZIKA strain MR 766 (Q32ZE1); DENV type 2 strain IQT2913 (Q9WDA6); HPgV-1 (Q69422)

Conclusion

The HCV NS3 protein disrupts a variety of cellular pathways, potentially contributing to the development of HCC. Additionally, NS3 may influence other cancer-related characteristics that are not discussed in this review. Indeed, other oncogenic viral proteins are known to promote angiogenesis, such as the human papillomavirus E6 protein, which increases the expression of vascular endothelial growth factor (VEGF) [126]. Furthermore, oncogenic viruses can disrupt cellular metabolism; for example, HCV infection alters glucose metabolism through several mechanisms, including increased glycolysis [127, 128]. Moreover, oncogenic viruses like Epstein-Barr virus and Hepatitis B virus are known to modify alternative splicing (AS) [129–131]. Interestingly, the interaction between NS3 and PKC-δ leads to its activation. PKC could then phosphorylate human antigen R (HuR), allowing its relocalization to the cytoplasm, where it plays a role in viral replication. HuR is also involved in pre-mRNA splicing through its binding of AU-rich sequences [132]. HuR is involved in many viral infections; for example, in Sindbis virus infection, HuR interacts with viral RNA 3'-untranslated regions, which mediates its translocation to the cytoplasm [133, 134]. HuR sequestration in the cytoplasm leads to changes in AS [133]. AS alterations are frequent in cancer, and some even consider it as a hallmark [135, 136]. Given these observations, it is plausible to hypothesize that oncogenic

viral proteins, such as HCV-NS3, may contribute to cancer development through modulations in AS.

Despite the numerous pathways altered by the HCV NS3 protein, there is no consensus on the exact mechanism by which it contributes to cell transformation, as findings from various studies often contradict each other. These discrepancies could stem from several factors, including differences in the expression systems used, the types of cells studied, and whether other co-factors are co-expressed. Additionally, the protease activity and localization of NS3 are influenced by its interaction with NS4A, and co-expression of these two proteins can alter the outcomes when protease activity is a factor. The validity of results derived from ectopic expression of viral proteins is also debatable. Issues such as the level of protein expression and the absence of other viral proteins create conditions that do not fully mimic natural viral infections. For instance, other HCV proteins like E2 and Core have been shown to inhibit NF-κB activity, which contradicts many studies focused solely on NS3 [137, 138].

Understanding the interaction between the NS3 protein and its host cell is crucial for effective HCV diagnosis and treatment [139, 140]. NS3 participates in various cellular signaling pathways, which may influence the success of therapies. For instance, cancer cells often upregulate COX-2 expression in response to chemotherapy drugs, a behavior also observed in cells expressing NS3 [141]. Consequently, inhibiting COX-2 has been shown to enhance the effectiveness of chemotherapy [141]. Additionally, evidence suggests that COX-2 inhibitors can slow the progression of hepatocellular carcinoma (HCC) in various models [142].

In conclusion, while NS3 has the potential to transform cells and various mechanisms have been proposed to explain its effects, further research is necessary to clarify these mechanisms and reconcile the conflicting results.

Abbreviations

HCV	Hepatitis C virus
IRES	Internal ribosome entry site
HCC	Hepatocellular carcinoma
ER	Endoplasmic reticulum
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
MAPK	Mitogen-activated protein kinases
ERK	Extracellular signal-regulated kinase
JNK	C-Jun N-terminal kinase
AP-1	Activator protein-1
ATF-2	Activating transcription factor-2
NF-κB	Nuclear factor-κB
EGFR	Epidermal growth factor receptor
SRCAP	Snf2 related CREBBP activator protein
TC-PTP	T cell protein tyrosine phosphatase
NRG1	Neuregulin 1
UBD	Ubiquitin D
iNOS	Inducible nitric oxide synthase
NO	Nitric oxide
ATM	Ataxia telangiectasia mutated
WRN	Werner syndrome helicase
NHEJ	Non-homologous end joining
PPM1A	Protein phosphatase 1A
EMT	Epithelial-mesenchymal transition
MMP-9	Matrix metalloproteinase-9
COX-2	Cyclooxygenase-2
TNF-α	Tumour necrosis factor alpha
IKKα	IκB Kinase α
IκB-α	NFKB inhibitor α
IL	Interleukin
TLR	Toll-like receptors
MCP-1	Monocyte chemoattractant protein-1
ICAM-1	Intercellular Adhesion Molecule 1
ROS	Reactive oxygen species
HSC	Hepatic stellate cells
TGFβ1	Transforming growth factor beta 1
TβRI	Transforming growth factor receptor
TGF-β2	TGFB2 transforming growth factor beta 2
HOIP	HOIL-1-interacting protein
LUBAC	Linear ubiquitin chain assembly complex
NEMO	NF-κB essential modulator
miRNA	MicroRNA
RIG-I	Retinoic acid-inducible gene I
RLR	RIG-I-like receptors
CARD	Caspase recruitment domain
MAVS	Mitochondrial antiviral-signaling protein
IFN	Interferon
TRIF	TIR domain-containing adapter inducing IFN-β
TBK1	TANK-binding kinase 1
IRF-3	Interferon regulatory factor 3
STAT1	Signal transducer and activator of transcription 1
dsRNA	Double-stranded RNA
TERT	Telomerase
KUNV	Kunjin virus
DENV	Dengue virus
MITA	Mediator of IRF3 activation
ZK	Zika virus
MDA5	Melanoma differentiation-associated protein 5
NLRP3	Nucleotide-binding oligomerization domain-like receptor 3
WNV	West Nile virus
JEV	Japanese encephalitis virus
HPgV	Human *pegivirus*
NHL	Non-Hodgkin lymphoma
VEGF	Vascular endothelial growth factor
AS	Alternative splicing
HuR	Human antigen R

Acknowledgements
Not applicable.

Author contributions
C.A.M. drafted the manuscript and generated the figures. N.R. and M.B. substantively revised the manuscript. All authors read and approved the final manuscript.

Funding
This work was supported by a Fonds de recherche du Québec – Santé scholarship to C.A.M., and a grant from the Natural Sciences and Engineering Research Council of Canada (RGPIN-2022-04026) to M.B.

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 5 July 2024 Accepted: 21 August 2024
Published online: 27 August 2024

References

1. Chevaliez S, Pawlotsky JM. HCV genome and life cycle. In: Tan SL, editor. Hepatitis C viruses: genomes and molecular biology. Norfolk: Horizon Bioscience; 2006.
2. Chen SL, Morgan TR. The natural history of hepatitis C virus (HCV) infection. Int J Med Sci. 2006;3(2):47–52.
3. Hoshida Y, Fuchs BC, Bardeesy N, Baumert TF, Chung RT. Pathogenesis and prevention of hepatitis C virus-induced hepatocellular carcinoma. J Hepatol. 2014;61(1):S79-90.
4. Kasprzak A, Adamek A. Role of hepatitis C virus proteins (C, NS3, NS5A) in hepatic oncogenesis. Hepatol Res. 2008;38(1):1–26.
5. Lin C. HCV NS3–4A serine protease. In: Tan SL, editor. Hepatitis C viruses: genomes and molecular biology. Norfolk: Horizon Bioscience; 2006.
6. Tanji Y, Hijikata M, Satoh S, Kaneko T, Shimotohno K. Hepatitis C virus-encoded nonstructural protein NS4A has versatile functions in viral protein processing. J Virol. 1995;69(3):1575–81.
7. Wölk B, Sansonno D, Kräusslich HG, Dammacco F, Rice CM, Blum HE, et al. Subcellular localization, stability, and trans-cleavage competence of the hepatitis C virus NS3-NS4A complex expressed in tetracycline-regulated cell lines. J Virol. 2000;74(5):2293–304.
8. Frick DN. The hepatitis C virus NS3 protein: a model RNA helicase and potential drug target. Curr Issues Mol Biol. 2007;9(1):1–20.
9. Lam AMI, Keeney D, Frick DN. Two novel conserved motifs in the hepatitis C virus NS3 protein critical for helicase action. J Biol Chem. 2003;278(45):44514–24.
10. Lin C, Kim JL. Structure-based mutagenesis study of hepatitis C virus NS3 helicase. J Virol. 1999;73(10):8798–807.
11. Salam KA, Akimitsu N. Hepatitis C virus NS3 inhibitors: current and future perspectives. Biomed Res Int. 2013;2013: 467869.
12. Sakamuro D, Furukawa T, Takegami T. Hepatitis C virus nonstructural protein NS3 transforms NIH 3T3 cells. J Virol. 1995;69(6):3893–6.
13. Zemel R, Gerechet S, Greif H, Bachmatove L, Birk Y, Golan-Goldhirsh A, et al. Cell transformation induced by hepatitis C virus NS3 serine protease. J Viral Hepatitis. 2001;8(2):96–102.
14. 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(3):474–8.
15. Smirnova IS, Aksenov ND, Vonsky MS, Isaguliants MG. Different transformation pathways of murine fibroblast NIH 3T3 cells by hepatitis C virus core and NS3 proteins. Cell Biol Int. 2006;30(11):915–9.
16. Brakha C, Arvers P, Villiers F, Marlu A, Buhot A, Livache T, et al. Relationship between humoral response against hepatitis C virus and disease overcome. Springerplus. 2014;3:56.
17. Lamonaca V, Missale G, Urbani S, Pilli M, Boni C, Mori C, et al. Conserved hepatitis C virus sequences are highly immunogenic for CD4+ T cells: implications for vaccine development. Hepatology. 1999;30(4):1088–98.
18. Diepolder HM, Gerlach JT, Zachoval R, Hoffmann RM, Jung MC, Wierenga EA, et al. Immunodominant CD4+ T-cell epitope within nonstructural protein 3 in acute hepatitis C virus infection. J Virol. 1997;71(8):6011–9.
19. Wertheimer AM, Miner C, Lewinsohn DM, Sasaki AW, Kaufman E, Rosen HR. Novel CD4+ and CD8+ T-cell determinants within the NS3 protein in subjects with spontaneously resolved HCV infection. Hepatology. 2003;37(3):577–89.
20. 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(2):555–63.
21. Alaee M, Rajabi P, Sharifi Z, Farajollahi MM. Immunoreactivity assessment of hepatitis C virus NS3 protease and NS5A proteins expressed in TOPO cloning system. J Microbiol Immunol Infect. 2014;47(4):282–91.
22. Carlos MP, Yamamura Y, Vu Q, Conzen K, Anderson DE, Torres JV. Humoral immunity to immunodominant epitopes of Hepatitis C virus in individuals infected with genotypes 1a or 1b. Clin Immunol. 2004;111(1):22–7.
23. Ogata S, Huab Florese R, Nagano-Fujii M, Hidajat R, Deng L, Ku Y, et al. Identification of hepatitis C virus (HCV) subtype 1b strains that are highly, or only weakly, associated with hepatocellular carcinoma on the basis of the secondary structure of an amino-terminal portion of the HCV NS3 protein. J Clin Microbiol. 2003;41(7):2835–41.
24. Nishise Y, Saito T, Sugahara K, Ito JI, Saito K, Togashi H, et al. Risk of hepatocellular carcinoma and secondary structure of hepatitis C virus (HCV) NS3 protein amino-terminus, in patients infected with HCV subtype 1b. J Infect Dis. 2007;196(7):1006–9.
25. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57–70.
26. 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(2):324–36.
27. Hassan M, Selimovic D, Ghozlan H, Abdel-Kader O. Induction of high-molecular-weight (HMW) tumor necrosis factor(TNF) alpha by hepatitis C virus (HCV) non-structural protein 3 (NS3) in liver cells is AP-1 and NF-κB-dependent activation. Cell Signal. 2007;19(2):301–11.
28. Bataller R, Paik Y, Lindquist JN, Lemasters JJ, Brenner DA. Hepatitis C virus core and nonstructural proteins induce fibrogenic effects in hepatic stellate cells. Gastroenterology. 2004;126(2):529–40.
29. Li B, Li X, Li Y, Guo H, Sun SY, He QQ, et al. The effects of hepatitis C virus non-structural protein 3 on cell growth mediated by extracellular signal-related kinase cascades in human hepatocytes in vitro. Int J Mol Med. 2010;26(2):273–9.
30. Lu L, Wei L, Peng G, Mu Y, Wu K, Kang L, et al. NS3 protein of hepatitis C virus regulates cyclooxygenase-2 expression through multiple signaling pathways. Virology. 2008;371(1):61–70.
31. 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(14):2157–61.
32. Iwai A, Takegami T, Shiozaki T, Miyazaki T. Hepatitis C virus NS3 protein can activate the notch-signaling pathway through binding to a transcription factor, SRCAP. PLoS ONE. 2011;6(6): e20718.
33. Gramantieri L, Giovannini C, Lanzi A, Chieco P, Ravaioli M, Venturi A, et al. Aberrant Notch3 and Notch4 expression in human hepatocellular carcinoma. Liver Int. 2007;27(7):997–1007.
34. Radtke F, Raj K. The role of Notch in tumorigenesis: Oncogene or tumour suppressor? Nat Rev Cancer. 2003;3(10):756–67.
35. Brenndörfer ED, Karthe J, Frelin L, Cebula P, Erhardt A, Schulte am Esch J, et al. Nonstructural 3/4A protease of hepatitis C virus activates epithelial growth factor-induced signal transduction by cleavage of the T-cell protein tyrosine phosphatase. Hepatology. 2009;49(6):1810–20.
36. Rahbin N, Frelin L, Aleman S, Hultcrantz R, Sällberg M, Brenndörfer ED. Non-structural 3 protein expression is associated with T cell protein tyrosine phosphatase and viral RNA levels in chronic hepatitis C patients. Biochem Biophys Res Commun. 2013;433(1):31–5.
37. Mannová P, Beretta L. Activation of the N-Ras-PI3K-Akt-mTOR pathway by hepatitis C virus: control of cell survival and viral replication. J Virol. 2005;79(14):8742–9.
38. Schwartz N, Pellach M, Glick Y, Gil R, Levy G, Avrahami D, et al. Neuregulin 1 discovered as a cleavage target for the HCV NS3/4A protease by a microfluidic membrane protein array. New Biotechnol. 2018;45:113–22.
39. Zhang J, Ishigaki Y, Takegami T. Hepatitis C virus NS3 protein modulates the biological behaviors of malignant hepatocytes by altering the expression of host cell microRNA. Mol Med Rep. 2015;12(4):5109–15.
40. Siavoshian S, Abraham JD, Kieny MP, Schuster C. HCV core, NS3, NS5A and NS5B proteins modulate cell proliferation independently from p53 expression in hepatocarcinoma cell lines. Arch Virol. 2004;149(2):323–36.
41. Ishido S, Hotta H. Complex formation of the nonstructural protein 3 of hepatitis C virus with the p53 tumor suppressor. FEBS Lett. 1998;438(3):258–62.
42. Deng L, Nagano-Fujii M, Tanaka M, Nomura-Takigawa Y, Ikeda M, Kato N, et al. NS3 protein of Hepatitis C virus associates with the tumour

suppressor p53 and inhibits its function in an NS3 sequence-dependent manner. J Gen Virol. 2006;87(6):1703–13.
43. Tanaka M, Nagano-Fujii M, Deng L, Ishido S, Sada K, Hotta H. Single-point mutations of hepatitis C virus NS3 that impair p53 interaction and anti-apoptotic activity of NS3. Biochem Biophys Res Commun. 2006;340(3):792–9.
44. Sabri S, Idrees M, Rafique S, Ali A, Iqbal M. Studies on the role of NS3 and NS5A non-structural genes of hepatitis C virus genotype 3a local isolates in apoptosis. Int J Infect Dis. 2014;25:38–44.
45. Kwun HJ, Jung EY, Ahn JY, Lee MN, Jang KL. p53-dependent transcriptional repression of p21waf1 by hepatitis C virus NS3. J Gen Virol. 2001;82(9):2235–41.
46. Li H, Liu S, Lin Y, Shi X, Du N, Yao J, et al. Identification and verification of ubiquitin D as a gene associated with hepatitis C virus-induced hepatocellular carcinoma. Intervirology. 2022;65(4):195–205.
47. Fujita T, Ishido S, Muramatsu S, Itoh M, Hotta H. Suppression of actinomycin D-induced apoptosis by the NS3 protein of hepatitis C virus. Biochem Biophys Res Commun. 1996;229(3):825–31.
48. Berg CP, Schlosser SF, Neukirchen DK, Papadakis C, Gregor M, Wesselborg S, et al. Hepatitis C virus core protein induces apoptosis-like caspase independent cell death. Virol J. 2009;6(1):213.
49. Siavoshian S, Abraham JD, Thumann C, Paule Kieny M, Schuster C. Hepatitis C virus core, NS3, NS5A, NS5B proteins induce apoptosis in mature dendritic cells. J Med Virol. 2005;75(3):402–11.
50. Han Y, Niu J, Wang D, Li Y. Hepatitis C virus protein interaction network analysis based on hepatocellular carcinoma. PLoS ONE. 2016;11(4): e0153882.
51. Prikhod'ko EA, Prikhod'ko GG, Siegel RM, Thompson P, Major ME, Cohen JI. The NS3 protein of hepatitis C virus induces caspase-8-mediated apoptosis independent of its protease or helicase activities. Virology. 2004;329(1):53–67.
52. Thorén F, Romero A, Lindh M, Dahlgren C, Hellstrand K. A hepatitis C virus-encoded, nonstructural protein (NS3) triggers dysfunction and apoptosis in lymphocytes: role of NADPH oxidase-derived oxygen radicals. J Leukoc Biol. 2004;76(6):1180–6.
53. Machida K, Cheng KTH, Sung VMH, Lee KJ, Levine AM, Lai MMC. Hepatitis C virus infection activates the immunologic (Type II) isoform of nitric oxide synthase and thereby enhances DNA damage and mutations of cellular genes. J Virol. 2004;78(16):8835–43.
54. Machida K, McNamara G, Cheng KTH, Huang J, Wang CH, Comai L, et al. Hepatitis C virus inhibits DNA damage repair through reactive oxygen and nitrogen species and by interfering with the ATM-NBS1/Mre11/Rad50 DNA repair pathway in monocytes and hepatocytes. J Immunol. 2010;185(11):6985–98.
55. Burney S, Caulfield JL, Niles JC, Wishnok JS, Tannenbaum SR. The chemistry of DNA damage from nitric oxide and peroxynitrite. Mutat Res Fundam Mol Mech Mutagen. 1999;424(1):37–49.
56. Podhorecka M, Skladanowski A, Bozko P. H2AX phosphorylation: its role in DNA damage response and cancer therapy. J Nucleic Acids. 2010;2010: 920161.
57. Ariumi Y, Kuroki M, Dansako H, Abe KI, Ikeda M, Wakita T, et al. The DNA damage sensors ataxia-telangiectasia mutated kinase and checkpoint kinase 2 are required for hepatitis C virus RNA replication. J Virol. 2008;82(19):9639–46.
58. Lai CK, Jeng KS, Machida K, Cheng YS, Lai MMC. Hepatitis C virus NS3/4A protein interacts with ATM, impairs DNA repair and enhances sensitivity to ionizing radiation. Virology. 2008;370(2):295–309.
59. Chen TI, Hsu YK, Chou CY, Chen YH, Hsu ST, Liou YS, et al. Hepatitis C virus NS3 protein plays a dual role in WRN-mediated repair of nonhomologous end joining. J Virol. 2019;93(22):e01273. https://doi.org/10.1128/jvi.01273-19.
60. Zhou Y, Zhao Y, Gao Y, Hu W, Qu Y, Lou N, et al. Hepatitis C virus NS3 protein enhances hepatocellular carcinoma cell invasion by promoting PPM1A ubiquitination and degradation. J Exp Clin Cancer Res. 2017;36(1):42.
61. Lu L, Zhang Q, Wu K, Chen X, Zheng Y, Zhu C, et al. Hepatitis C virus NS3 protein enhances cancer cell invasion by activating matrix metalloproteinase-9 and cyclooxygenase-2 through ERK/p38/NF-κB signal cascade. Cancer Lett. 2015;356(2, Part B):470–8.
62. Hashemi Goradel N, Najafi M, Salehi E, Farhood B, Mortezaee K. Cyclooxygenase-2 in cancer: a review. J Cell Physiol. 2019;234(5):5683–99.
63. Ninio L, Nissani A, Meirson T, Domovitz T, Genna A, Twafra S, et al. Hepatitis C virus enhances the invasiveness of hepatocellular carcinoma via EGFR-mediated invadopodia formation and activation. Cells. 2019;8(11):1395.
64. de Chassey B, Navratil V, Tafforeau L, Hiet MS, Aublin-Gex A, Agaugué S, et al. Hepatitis C virus infection protein network. Mol Syst Biol. 2008;4:230.
65. Afify SM, Hassan G, Seno A, Seno M. Cancer-inducing niche: the force of chronic inflammation. Br J Cancer. 2022;127(2):193–201.
66. Elinav E, Nowarski R, Thaiss CA, Hu B, Jin C, Flavell RA. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. Nat Rev Cancer. 2013;13(11):759–71.
67. van Loo G, Bertrand MJM. Death by TNF: a road to inflammation. Nat Rev Immunol. 2023;23(5):289–303.
68. Dolganiuc A, Kodys K, Kopasz A, Marshall C, Do T, Romics L Jr, et al. Hepatitis C virus core and nonstructural protein 3 proteins induce pro- and anti-inflammatory cytokines and inhibit dendritic cell differentiation1. J Immunol. 2003;170(11):5615–24.
69. Dolganiuc A, Oak S, Kodys K, Golenbock DT, Finberg RW, Kurt-Jones E, et al. Hepatitis C core and nonstructural 3 proteins trigger toll-like receptor 2-mediated pathways and inflammatory activation. Gastroenterology. 2004;127(5):1513–24.
70. Chang S, Dolganiuc A, Szabo G. Toll-like receptors 1 and 6 are involved in TLR2-mediated macrophage activation by hepatitis C virus core and NS3 proteins. J Leukoc Biol. 2007;82(3):479–87.
71. Rajalakshmy AR, Malathi J, Madhavan HN. Hepatitis C virus NS3 mediated microglial inflammation via TLR2/TLR6 MyD88/NF-κB pathway and toll like receptor ligand treatment furnished immune tolerance. PLoS ONE. 2015;10(5): e0125419.
72. Wu B, Sodji QH, Oyelere AK. Inflammation, fibrosis and cancer: mechanisms, therapeutic options and challenges. Cancers. 2022;14(3):552.
73. Sakata K, Hara M, Terada T, Watanabe N, Takaya D, Yaguchi S, et al. HCV NS3 protease enhances liver fibrosis via binding to and activating TGF-β type I receptor. Sci Rep. 2013;3(1):3243.
74. Bala S, Tilahun Y, Taha O, Alao H, Kodys K, Catalano D, et al. Increased microRNA-155 expression in the serum and peripheral monocytes in chronic HCV infection. J Transl Med. 2012;10:151.
75. O'Connell RM, Rao DS, Baltimore D. microRNA regulation of inflammatory responses. Annu Rev Immunol. 2012;30(1):295–312.
76. Khanizadeh S, Ravanshad M, Hosseini SY, Davoodian P, Almasian M, Khanlari Z. The effect of the hepatitis C virus (HCV) NS3 protein on the expression of miR-150, miR-199a, miR-335, miR-194 and miR-27a. Microb Pathog. 2017;110:688–93.
77. Khanizadeh S, Ravanshad M, Hosseini SY, Davoodian P, Zadeh AN, Sabahi F, et al. The possible role of NS3 protease activity of hepatitis C virus on fibrogenesis and miR-122 expression in hepatic stellate cells. Acta Virol. 2016;60(03):242–8.
78. Vettori S, Gay S, Distler O. Role of MicroRNAs in fibrosis. Open Rheumatol J. 2012;6:130–9.
79. De Mitri MS, Pisi E, Poussin K, Paterlini P, Bréchot C, Baccarini P, et al. HCV-associated liver cancer without cirrhosis. Lancet. 1995;345(8947):413–5.
80. Nash KL, Woodall T, Brown AS, Davies SE, Alexander GJ. Hepatocellular carcinoma in patients with chronic hepatitis C virus infection without cirrhosis. World J Gastroenterol. 2010;16(32):4061–5.
81. Yeh MM, Daniel HDJ, Torbenson M. Hepatitis C associated hepatocellular carcinomas in non-cirrhotic livers. Mod Pathol. 2010;23(2):276–83.
82. Frelin L, Brenndörfer ED, Ahlén G, Weiland M, Hultgren C, Alheim M, et al. The hepatitis C virus and immune evasion: non-structural 3/4A transgenic mice are resistant to lethal tumour necrosis factor α mediated liver disease. Gut. 2006;55(10):1475–83.
83. Chen Y, He L, Peng Y, Shi X, Chen J, Zhong J, et al. The hepatitis C virus protein NS3 suppresses TNF-α–stimulated activation of NF-κB by targeting LUBAC. Sci Signal. 2015;8(403):ra118.
84. Chan ST, Ou J. Hepatitis C virus-induced autophagy and host innate immune response. Viruses. 2017;9(8):224.
85. Rehwinkel J, Gack MU. RIG-I-like receptors: their regulation and roles in RNA sensing. Nat Rev Immunol. 2020;20(9):537–51.
86. Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, Bartenschlager R, et al. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature. 2005;437(7062):1167–72.

87. Loo YM, Owen DM, Li K, Erickson AK, Johnson CL, Fish PM, et al. Viral and therapeutic control of IFN-β promoter stimulator 1 during hepatitis C virus infection. Proc Natl Acad Sci USA. 2006;103(15):6001–6.
88. Baril M, Racine ME, Penin F, Lamarre D. MAVS dimer is a crucial signaling component of innate immunity and the target of hepatitis C virus NS3/4A protease. J Virol. 2009;83(3):1299–311.
89. 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 USA. 2005;102(49):17717–22.
90. Horner SM, Liu HM, Park HS, Briley J, Gale M. Mitochondrial-associated endoplasmic reticulum membranes (MAM) form innate immune synapses and are targeted by hepatitis C virus. Proc Natl Acad Sci USA. 2011;108(35):14590–5.
91. Ferreira AR, Magalhães AC, Camões F, Gouveia A, Vieira M, Kagan JC, et al. Hepatitis C virus NS3-4A inhibits the peroxisomal MAVS-dependent antiviral signalling response. J Cell Mol Med. 2016;20(4):750–7.
92. Lin R, Lacoste J, Nakhaei P, Sun Q, Yang L, Paz S, et al. Dissociation of a MAVS/IPS-1/VISA/Cardif-IKKε molecular complex from the mitochondrial outer membrane by hepatitis C virus NS3-4A proteolytic cleavage. J Virol. 2006;80(12):6072–83.
93. Bender S, Reuter A, Eberle F, Einhorn E, Binder M, Bartenschlager R. Activation of type I and III interferon response by mitochondrial and peroxisomal MAVS and inhibition by hepatitis C virus. PLoS Pathog. 2015;11(11): e1005264.
94. Oshiumi H, Miyashita M, Matsumoto M, Seya T. A distinct role of riplet-mediated K63-linked polyubiquitination of the RIG-I repressor domain in human antiviral innate immune responses. PLoS Pathog. 2013;9(8): e1003533.
95. Vazquez C, Tan CY, Horner SM. Hepatitis C virus infection is inhibited by a noncanonical antiviral signaling pathway targeted by NS3-NS4A. J Virol. 2019;93(23):e00725. https://doi.org/10.1128/jvi.00725-19.
96. Ferreon JC, Ferreon ACM, Li K, Lemon SM. Molecular determinants of TRIF proteolysis mediated by the hepatitis C virus NS3/4A protease. J Biol Chem. 2005;280(21):20483–92.
97. Li K, Foy E, Ferreon JC, Nakamura M, Ferreon ACM, Ikeda M, et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. Proc Natl Acad Sci. 2005;102(8):2992–7.
98. Otsuka M, Kato N, Moriyama M, Taniguchi H, Wang Y, Dharel N, et al. Interaction between the HCV NS3 protein and the host TBK1 protein leads to inhibition of cellular antiviral responses. Hepatology. 2005;41(5):1004–12.
99. Foy E, Li K, Wang C, Sumpter R, Ikeda M, Lemon SM, et al. Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease. Science. 2003;300(5622):1145–8.
100. Lin W, Choe WH, Hiasa Y, Kamegaya Y, Blackard JT, Schmidt EV, et al. Hepatitis C virus expression suppresses interferon signaling by degrading STAT1. Gastroenterology. 2005;128(4):1034–41.
101. Tornesello ML, Cerasuolo A, Starita N, Tornesello AL, Bonelli P, Tuccillo FM, et al. The molecular interplay between human oncoviruses and telomerase in cancer development. Cancers. 2022;14(21):5257.
102. Zhu Z, Tran H, Mathahs MM, Moninger TO, Schmidt WN. HCV induces telomerase reverse transcriptase, increases its catalytic activity, and promotes caspase degradation in infected human hepatocytes. PLoS ONE. 2017;12(1): e0166853.
103. Neufeldt CJ, Cortese M, Acosta EG, Bartenschlager R. Rewiring cellular networks by members of the Flaviviridae family. Nat Rev Microbiol. 2018;16(3):125–42.
104. Nazarenko AS, Vorovitch MF, Biryukova YK, Pestov NB, Orlova EA, Barlev NA, et al. Flaviviruses in antitumor therapy. Viruses. 2023;15(10):1973.
105. Latanova A, Starodubova E, Karpov V. Flaviviridae nonstructural proteins: the role in molecular mechanisms of triggering inflammation. Viruses. 2022;14(8):1808.
106. Chen S, Wu Z, Wang M, Cheng A. Innate immune evasion mediated by flaviviridae non-structural proteins. Viruses. 2017;9(10):291.
107. Liu WJ, Wang XJ, Mokhonov VV, Shi PY, Randall R, Khromykh AA. Inhibition of interferon signaling by the New York 99 strain and Kunjin subtype of west Nile virus involves blockage of STAT1 and STAT2 activation by nonstructural proteins. J Virol. 2005;79(3):1934–42.
108. Rodriguez-Madoz JR, Belicha-Villanueva A, Bernal-Rubio D, Ashour J, Ayllon J, Fernandez-Sesma A. Inhibition of the type I interferon response in human dendritic cells by dengue virus infection requires a catalytically active NS2B3 complex. J Virol. 2010;84(19):9760–74.
109. Aguirre S, Maestre AM, Pagni S, Patel JR, Savage T, Gutman D, et al. DENV inhibits type I IFN production in infected cells by cleaving human STING. PLoS Pathog. 2012;8(10): e1002934.
110. Yu CY, Chang TH, Liang JJ, Chiang RL, Lee YL, Liao CL, et al. Dengue virus targets the adaptor protein MITA to subvert host innate immunity. PLoS Pathog. 2012;8(6): e1002780.
111. Chan YK, Gack MU. A phosphomimetic-based mechanism of dengue virus to antagonize innate immunity. Nat Immunol. 2016;17(5):523–30.
112. Riedl W, Acharya D, Lee JH, Liu G, Serman T, Chiang C, et al. Zika virus NS3 mimics a cellular 14-3-3-binding motif to antagonize RIG-I- and MDA5-mediated innate immunity. Cell Host Microbe. 2019;26(4):493-503.e6.
113. Li W, Li N, Dai S, Hou G, Guo K, Chen X, et al. Zika virus circumvents host innate immunity by targeting the adaptor proteins MAVS and MITA. FASEB J. 2019;33(9):9929–44.
114. Gim E, Shim DW, Hwang I, Shin OS, Yu JW. Zika virus impairs host NLRP3-mediated inflammasome activation in an NS3-dependent manner. Immune Netw. 2019;19(6): e40.
115. Shafee N, AbuBakar S. Dengue virus type 2 NS3 protease and NS2B-NS3 protease precursor induce apoptosis. J Gen Virol. 2003;84(8):2191–5.
116. Lin JC, Lin SC, Chen WY, Yen YT, Lai CW, Tao MH, et al. Dengue viral protease interaction with NF-κB inhibitor α/β results in endothelial cell apoptosis and hemorrhage development. J Immunol. 2014;193(3):1258–67.
117. Ramanathan MP, Chambers JA, Pankhong P, Chattergoon M, Attatippaholkun W, Dang K, et al. Host cell killing by the West Nile virus NS2B–NS3 proteolytic complex: NS3 alone is sufficient to recruit caspase-8-based apoptotic pathway. Virology. 2006;345(1):56–72.
118. Yiang GT, Chen YH, Chou PL, Chang WJ, Wei CW, Yu YL. The NS3 protease and helicase domains of Japanese encephalitis virus trigger cell death via caspase-dependent and -independent pathways. Mol Med Rep. 2013;7(3):826–30.
119. Yang TC, Shiu SL, Chuang PH, Lin YJ, Wan L, Lan YC, et al. Japanese encephalitis virus NS2B-NS3 protease induces caspase 3 activation and mitochondria-mediated apoptosis in human medulloblastoma cells. Virus Res. 2009;143(1):77–85.
120. Prikhod'ko GG, Prikhod'ko EA, Pletnev AG, Cohen JI. Langat flavivirus protease NS3 binds caspase-8 and induces apoptosis. J Virol. 2002;76(11):5701–10.
121. Hartlage AS, Cullen JM, Kapoor A. The strange, expanding world of animal hepaciviruses. Annu Rev Virol. 2016;3(1):53–75.
122. Stapleton JT. Human Pegivirus type 1: A common human virus that is beneficial in immune-mediated disease? Front Immunol. 2022;13: 887760.
123. Fama A, Xiang J, Link BK, Allmer C, Klinzman D, Feldman AL, et al. Human Pegivirus infection and lymphoma risk and prognosis: a North American study. Br J Haematol. 2018;182(5):644–53.
124. Chowdhury AY, Tavis JE, George SL. Human pegivirus (GB virus C) NS3 protease activity inhibits induction of the type I interferon response and is not inhibited by HCV NS3 protease inhibitors. Virology. 2014;456–457:300–9.
125. Chen Z, Benureau Y, Rijnbrand R, Yi J, Wang T, Warter L, et al. GB virus B disrupts RIG-I signaling by NS3/4A-mediated cleavage of the adaptor protein MAVS. J Virol. 2007;81(2):964–76.
126. López-Ocejo O, Viloria-Petit A, Bequet-Romero M, Mukhopadhyay D, Rak J, Kerbel RS. Oncogenes and tumor angiogenesis: the HPV-16 E6 oncoprotein activates the vascular endothelial growth factor (VEGF) gene promoter in a p53 independent manner. Oncogene. 2000;19(40):4611–20.
127. Ripoli M, D'Aprile A, Quarato G, Sarasin-Filipowicz M, Gouttenoire J, Scrima R, et al. Hepatitis C virus-linked mitochondrial dysfunction promotes hypoxia-inducible factor 1α-mediated glycolytic adaptation. J Virol. 2010;84(1):647–60.
128. Noch E, Khalili K. Oncogenic viruses and tumor glucose metabolism: like kids in a candy store. Mol Cancer Ther. 2012;11(1):14–23.
129. Francies FZ, Dlamini Z. Aberrant splicing events and epigenetics in viral oncogenomics: current therapeutic strategies. Cells. 2021;10(2):239.

130. Armero VES, Tremblay MP, Allaire A, Boudreault S, Martenon-Brodeur C, Duval C, et al. Transcriptome-wide analysis of alternative RNA splicing events in Epstein–Barr virus-associated gastric carcinomas. PLoS ONE. 2017;12(5): e0176880.
131. Tremblay MP, Armero VES, Allaire A, Boudreault S, Martenon-Brodeur C, Durand M, et al. Global profiling of alternative RNA splicing events provides insights into molecular differences between various types of hepatocellular carcinoma. BMC Genomics. 2016;17:683.
132. Lebedeva S, Jens M, Theil K, Schwanhäusser B, Selbach M, Landthaler M, et al. Transcriptome-wide analysis of regulatory interactions of the RNA-binding protein HuR. Mol Cell. 2011;43(3):340–52.
133. Barnhart MD, Moon SL, Emch AW, Wilusz CJ, Wilusz J. Changes in cellular mRNA stability, splicing, and polyadenylation through HuR protein sequestration by a cytoplasmic RNA virus. Cell Rep. 2013;5(4):909–17.
134. Boudreault S, Roy P, Lemay G, Bisaillon M. Viral modulation of cellular RNA alternative splicing: A new key player in virus–host interactions? WIREs RNA. 2019;10(5): e1543.
135. Bonnal SC, López-Oreja I, Valcárcel J. Roles and mechanisms of alternative splicing in cancer—implications for care. Nat Rev Clin Oncol. 2020;17(8):457–74.
136. Ladomery M. Aberrant alternative splicing is another hallmark of cancer. Int J Cell Biol. 2013;2013: 463786.
137. McKay LGA, Thomas J, Albalawi W, Fattaccioli A, Dieu M, Ruggiero A, et al. The HCV envelope glycoprotein down-modulates NF-κB signalling and associates with stimulation of the host endoplasmic reticulum stress pathway. Front Immunol. 2022;13: 831695.
138. Joo M, Hahn YS, Kwon M, Sadikot RT, Blackwell TS, Christman JW. Hepatitis C virus core protein suppresses NF-κB activation and cyclooxygenase-2 expression by direct interaction with IκB kinase β. J Virol. 2005;79(12):7648–57.
139. Warkad SD, Song KS, Pal D, Nimse SB. Developments in the HCV screening technologies based on the detection of antigens and antibodies. Sensors. 2019;19(19):4257.
140. Hepatitis C. Guidance 2018 update: AASLD-IDSA recommendations for testing, managing, and treating hepatitis C virus infection. Clin Infect Dis. 2018;67(10):1477–92.
141. Bell CR, Pelly VS, Moeini A, Chiang SC, Flanagan E, Bromley CP, et al. Chemotherapy-induced COX-2 upregulation by cancer cells defines their inflammatory properties and limits the efficacy of chemoimmunotherapy combinations. Nat Commun. 2022;13(1):2063.
142. Cervello M, Montalto G. Cyclooxygenases in hepatocellular carcinoma. World J Gastroenterol. 2006;12(32):5113–21.

Publisher's Note