



Metabolic dysfunction and cancer in HCV: Shared pathways and mutual interactions

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Summary

HCV hijacks many host metabolic processes in an effort to aid viral replication. The resulting hepatic metabolic dysfunction underpins many of the hepatic and extrahepatic manifestations of chronic hepatitis C (CHC). However, the natural history of CHC is also substantially influenced by the host metabolic status: obesity, insulin resistance and hepatic steatosis are major determinants of CHC progression toward hepatocellular carcinoma (HCC). Direct-acting antivirals (DAAs) have transformed the treatment and natural history of CHC. While DAA therapy effectively eradicates the virus, the long-lasting overlapping metabolic disease can persist, especially in the presence of obesity, increasing the risk of liver disease progression. This review covers the mechanisms by which HCV tunes hepatic and systemic metabolism, highlighting how systemic metabolic disturbance, lipotoxicity and chronic inflammation favour disease progression and a precancerous niche. We also highlight the therapeutic implications of sustained metabolic dysfunction following sustained virologic response as well as considerations for patients who develop HCC on the background of metabolic dysfunction.

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Introduction

HCV is a leading cause of end-stage liver disease and hepatocellular carcinoma (HCC). It is estimated that 71.1 million people worldwide have chronic HCV infection,^{1,2} resulting in 400,000 predicted liver-related deaths per year. Whilst the hepatic manifestations are well known, chronic hepatitis C (CHC) is a multisystem disease with several extrahepatic manifestations. These include: mixed cryoglobulinaemic vasculitis, autoimmune disease, lymphoma, and cardiometabolic disease.³ The biological pathways underlying this multisystem disease are not fully understood; however, host metabolic dysfunction overlapping with and/or in response to HCV infection is a unifying process for some of these manifestations. Specifically, CHC is often associated with obesity and/or an impairment of glucose and lipid metabolism, as well as with a higher prevalence of the metabolic syndrome (MetS) and its components/complications, such as insulin resistance and type 2 diabetes mellitus (T2DM), hepatic steatosis, dyslipidaemia, and cardiovascular disease (CVD).^{4–7}

The recent development of direct-acting antivirals (DAAs) has revolutionised the treatment of CHC, enabling sustained virological response (SVR) rates of well above 95–98% in the majority of patients. That being said, a high rate of early tumour recurrence was recently observed in patients with HCV-related HCC who had undergone DAA therapy.⁸ While this was a relatively small cohort of patients, it does suggest that there are long-term carcinogenic consequences of HCV infection that are independent of active viral

proteins. Metabolic impairment can persist after SVR, increasing the risk of liver disease progression, cancer development and cardiovascular disease, as seen in patients with non-alcoholic fatty liver disease (NAFLD), a form of fatty liver associated with MetS which many experts propose renaming as “metabolic dysfunction-associated fatty liver disease” (MAFLD).^{9,10} Therefore, whilst eradication of HCV is an important goal, consideration and treatment of the metabolic consequences of HCV are equally important in order to improve patient outcomes. It is therefore crucial to be aware of the high prevalence of metabolic disturbances in patients with HCC and to treat them alongside HCV infection, in order to prevent progression of liver disease, and mortality from HCC or CVD. Herein, we explore how insulin resistance (IR) and MetS worsen fatty liver and promote cancer development in CHC, and we look at the implications for cancer treatment and prevention in patients with CHC.

Viral influences on HCV-mediated steatosis development

Accumulation of lipids in the liver, known as steatosis, results from imbalances in hepatic metabolic fluxes¹¹; steatosis is a prominent histological characteristic of CHC, occurring in 40% to 80% of patients depending on the prevalence of co-factors (such as alcohol consumption, obesity and MetS) and the HCV genotype.⁴ Patients with concurrent CHC and steatosis have an increased risk of

Keywords: Hepatitis C virus; HCV; Hepatocellular carcinoma; HCC; metabolic dysfunction; steatosis; senescence.

Received 31 August 2021; received in revised form 12 January 2022; accepted 31 January 2022; available online 12 February 2022

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<https://doi.org/10.1016/j.jhep.2022.01.029>

Key point

HCV rewires hepatic metabolism, driving liver steatosis and contributing to the development of metabolic dysfunction.

HCC development and mortality compared to those with no steatosis.¹²

An intriguing feature of HCV infection is its tight connection with hepatic and systemic metabolism. Viral HCV proteins, especially in genotype 3, have been proven to hijack the host's glucose and lipid metabolism at multiple levels (Fig. 1) since: i) HCV stimulates *de novo* lipogenesis (DNL) to favour HCV lipovirion assembly via the activation of multiple lipogenic transcription factors^{13,14,17,18}; ii) HCV promotes the synthesis of phospholipids and sphingomyelins (that play important roles in viral replication and HCV assembly) and of lipotoxic ceramides¹⁹; iii) HCV inhibits mitochondrial fatty acid oxidation;^{17,20–26} (and) iv) lipovirion assembly interferes with VLDL assembly and export by hijacking the VLDL secretion pathway.^{20,27–29} Moreover, other possible HCV-mediated effects involve the rewiring of nuclear receptor transcriptional machinery²³ and the upregulation of the pentose phosphate pathway.³⁰ All together these effects have a major impact on hepatic fat content and liver function as described below.

On top of direct viral protein effects on hepatic metabolism, patients with CHC frequently present with IR and T2DM; these metabolic features have been associated with an increased risk of progression toward fibrosis (Fig. 2). Intriguingly IR can develop independently from obesity and correlate with HCV replication rates.^{31,32} Indeed, deep metabolic profiling (including clamps and indirect calorimetry) of lean patients with CHC at different stages of liver disease have suggested that IR primarily affects peripheral organs, while hepatic IR seems to depend on the stage of hepatic damage and/or HCV genotype.^{33–36} Interestingly, especially in lean patients with CHC, HCV-mediated peripheral IR does not seem to be associated with the canonical effects of peripheral insulin on lipid metabolism in adipose tissue (AT; e.g. suppression of AT lipolysis), indicating substantial mechanistic differences from forms of IR that are AT dysfunction-led, and implicating a possible muscle involvement.^{33–38} Since HCV does not infect extrahepatic (metabolically-relevant) tissues, it is conceivable that HCV infection might exert some indirect peripheral effects (e.g. by unbalancing the secretion of hepatokines involved in tuning peripheral insulin sensitivity and/or systemic inflammation) and that part of these effects can be reversed by viral eradication.^{34,36}

However the following issues are still unresolved: i) what is the mechanism of peripheral IR in HCV infection? ii) is peripheral IR a direct consequence of HCV infection *per se*, or is it an outcome of chronic disease progression? 3) is this change in (AT-independent) IR pathophysiologically relevant for the progression of chronic liver disease? Future research should focus on identifying the

mechanism responsible for the liver-to-periphery communication that drives IR in CHC. Moreover, it should be said that the direct association of HCV infection and IR/T2DM is still disputed and conflicting results have been reported even in consecutive studies on the same large cohorts (e.g. US NHANES studies have not continuously reported the association).^{39,40} This raises i) concerns that the specific composition of the study populations might influence the results and interpretation; ii) the question of how best to characterise co-factors that could potentially impact IR (such as age, race, BMI, disease stage, or socio-economic status) in large cohort studies, to allow for a metabolically meaningful interpretation of the data and to definitively address this issue.

In the following paragraphs we will discuss how the combination of HCV infection, obesity and its metabolic complications alter the natural history of CHC and its progression toward HCC, as well as looking at their impact on patient management.

The role of host obesity and adipose tissue dysfunction in HCV-mediated steatosis

It is estimated that up to 78% of patients with CHC are also overweight or obese^{4,41}; obesity is known to potentiate fatty liver development and boost CHC progression in those patients where both co-exist. Obesity is a global epidemic characterised by increased energy intake and/or reduced energy expenditure leading to adipocyte dysfunction and causing a cluster of cardiometabolic risk factors (IR and T2DM, atherogenic dyslipidaemia, hypertension) grouped under the umbrella definition of MetS.¹¹ Obesity and MetS are associated with increased all-cause mortality and enhanced cancer risk.⁴² Obesity is also the main driver of the increasing NAFLD burden and HCC rates in developed countries.⁴³

Metabolic dysfunction develops when AT reaches its expandability limit (e.g. in overweight/obesity or in lipodystrophy): AT expands under nutritional/metabolic pressure as a result of a fine balance of both adipocyte hyperplasia and hypertrophy, but has a finite storage capacity; if adipocyte hyperplasia (increased number of functional and insulin sensitive adipocytes) is “metabolically healthy”, AT hypertrophy (that prevails when adipocyte's hyperplasia is impaired) results in adipocyte dysfunction and IR with subsequent increased AT lipolysis (leading to the spill over of fat to other organs including the liver and muscle).^{11,44} Different clinical studies have confirmed that AT health and function and systemic/AT IR are much better predictors of systemic metabolic disturbances and hepatic steatosis than BMI or waist circumference (WC); moreover AT dysfunction and systemic/AT IR, prominent features of both non-alcoholic steatohepatitis (NASH) and CHC, have been associated with severe steatohepatitis and

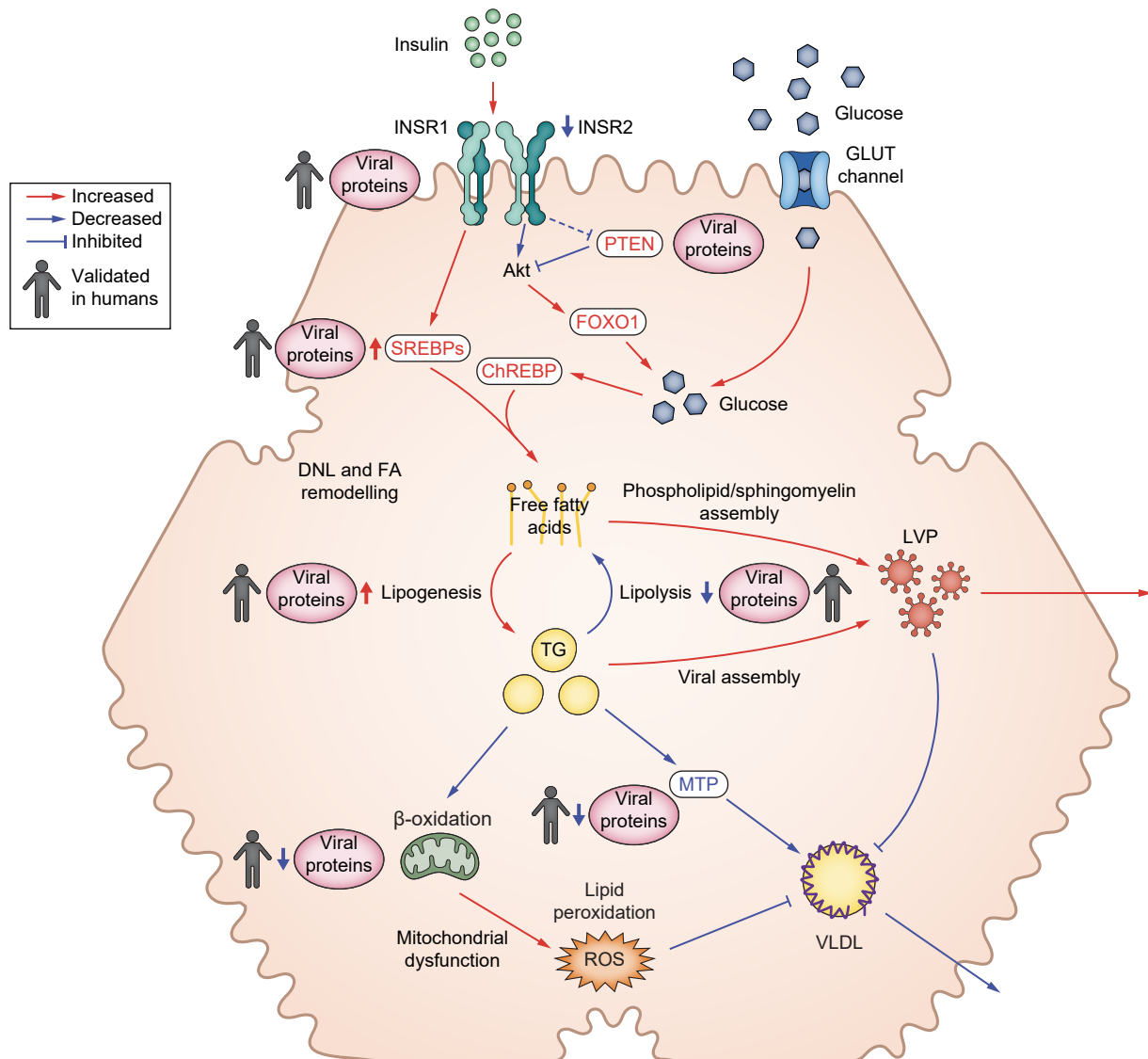


Fig. 1. Mechanisms of HCV-induced steatosis and hepatic insulin resistance. HCV hijacks hepatocyte metabolism at multiple levels either directly, via a direct action exerted by viral proteins on intracellular signalling/metabolic pathways, or indirectly by promoting peripheral insulin resistance, hyperinsulinemia and hyperglycaemia. ChREBP, carbohydrate response element-binding protein; DNL, *de novo* lipogenesis; FA, fatty acid; FOXO1, forkhead box O1; GLUT, glucose transporter; INSR1/2, insulin receptor 1/2; LVP, lipovirion; MTP, microsomal triglyceride transfer protein; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; SREBPs, sterol regulatory element-binding proteins; TG, triglyceride.

fibrosis.^{5,6,45} Additionally, ethnic-specific differences in body composition mandate the redefinition of cut-offs for overweight/obesity.^{11,46,47} Altogether a better understanding of these complex underlying mechanisms necessitate a critical re-evaluation of clinical studies where the BMI (or WC) has solely been used to define the role of metabolic dysfunction as a co-factor in HCV-related liver disease progression. Ideally, a better clinical assessment of AT dysfunction and IR would be preferable to anthropometrics, as it would also explain the apparent “lean fatty liver” paradox whereby individuals within “normal” BMI/WC ranges, who also exhibit IR and MetS, can still develop fatty liver and steatohepatitis.^{46,48,49} This

redefinition would also help to solve the puzzle of apparently conflicting results described in lean patients with CHC as discussed above.

There is also increasing evidence that obesity, IR and fatty liver are associated with AT (especially visceral) inflammation, systemic low grade inflammation and fibrosis, which correlate with the degree of metabolic impairment and complications (e.g. NASH, atherosclerosis). Despite this, it is still debated if the activation of these processes in AT are cause or consequence of AT dysfunction/IR and metabolic disease (the results of clinical trials targeting these pathways have been disappointing from a metabolic perspective). AT (especially visceral) inflammation and systemic low

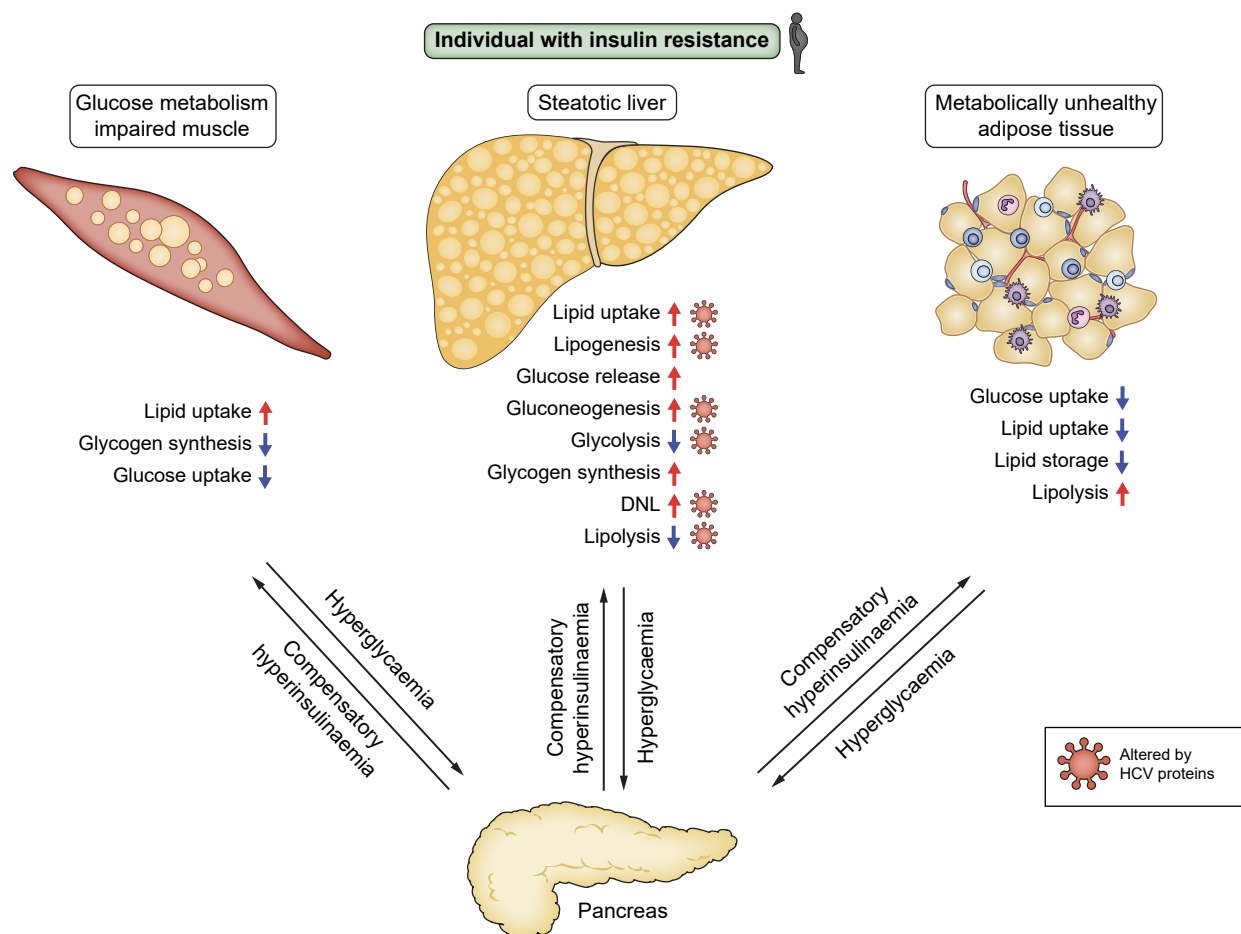


Fig. 2. HCV promotes systemic metabolic dysfunction. Schematic summarising the rewiring of metabolic pathways in metabolically active organs in subjects with insulin resistance (e.g. obesity, metabolic syndrome, and chronic hepatitis C); direct actions exerted by HCV proteins are also highlighted to suggest how HCV rewires systemic metabolism. DNL, *de novo* lipogenesis.

grade inflammation might still be an extremely attractive therapeutic target to improve liver disease outcomes considering their strong association with the progression of steatohepatitis.^{50–56} It is therefore our opinion that AT/systemic inflammation might be an important co-factor for the stratification of patients when assessing disease outcomes in clinical studies.

In the interferon era of HCV management, it was recognised that obesity was associated with decreased rates of SVR⁵⁷ and as such, weight reduction used to play a significant role in HCV management. This is no longer the case in the DAA era where SVR rates do not appear to be affected by obesity.⁵⁸ A recent large study examined the effect of DAA therapy on weight after 2 years of therapy.⁴¹ It is important to note that 78% of the 11,469 patients included were overweight or obese at treatment initiation (defined as a BMI ≥ 25) and that SVR rates were 97% in the cohort. Those who achieved SVR gained 0.56 ± 12.8 lbs vs. a -3.43 ± 14.6 lbs weight loss in those who did not achieve SVR ($p < 0.0001$). Furthermore, 22% of those who had a BMI ≤ 25 at treatment initiation

became overweight during the follow-up period. Weight gain is common with some medications (e.g. anti-psychotics, anti-depressants, glucocorticoids and beta blockers) including antiviral medications (e.g. anti-HIV), but why DAA-treated patients gain weight is not fully understood; it is likely the result of a combination of factors including improved quality of life and increased nutritional intake following SVR.⁵⁹ The worsening of the systemic metabolic profile associated with body weight gain might thus explain, at least in part, the blunted clinical response (with regards to hepatic outcomes) associated with SVR in patients with advanced fibrosis, and/or the lack of protection from HCC in patients who achieved SVR; this is of particular concern in the presence of significant fibrosis and/or other comorbidities associated with liver injury. However, it should be mentioned that the effects of DAAs on systemic IR are debated since some studies have shown an improvement of extrahepatic insulin sensitivity after SVR.^{33,36} Also, it is important to note that alcohol intake should always be considered as a co-factor when assessing post-SVR data: heavy

alcohol consumption promotes steatosis *per se*, and drives liver disease progression toward HCC thus making the picture even more complex.⁶⁰

In summary, these authors wonder if HCV-associated hepatic steatosis is best considered an “overlap syndrome” between HCV infection (and its metabolic/chronic liver disease implications) and NAFLD where, under the metabolic pressure of a positive energy balance and systemic/AT-IR, hepatic fat accumulation is boosted, leading to enhanced lipotoxicity-mediated wound healing with potential for a fast progression toward fibrosis and increased HCC risk. Moreover, it is our opinion that patients should be carefully metabolically re-phenotyped post-SVR: the persistence/worsening of metabolic disturbances and alcohol intake after treatment should be carefully characterised and therapeutically targeted to prevent the progression of liver disease and HCC once HCV has been eradicated.^{61–64}

Host metabolic cross-talk with HCV-mediated steatosis – the role of hyperinsulinemia and hyperglycaemia

As a result of peripheral IR, increased pancreatic insulin secretion, and reduced insulin clearance (due to the progressive development of steatosis), hyperinsulinemia occurs. Muscle-IR also increases glucose levels since it reduces muscle uptake of glucose and conversion to glycogen, therefore favouring hyperglycaemia.¹¹ Elevated circulating levels of glucose (that activate carbohydrate response element-binding protein [ChREBP]) and insulin (that promote sterol regulatory element-binding protein [SREBP]-1c) have profound metabolic effects on lipid uptake, DNL and lipid remodelling in the liver, resulting in increased hepatic fat influx.¹¹ Increased DNL has indeed been described by transcriptomic and/or lipidomic studies in both patients with NAFLD^{65–68} and CHC,^{69–71} and this has been shown to facilitate virus entry into hepatocytes.^{19,21,72} Despite the fact that it has been established that SREBP1/ChREBP1-mediated transcriptional programmes are activated in NAFLD and CHC, it should be noted that it is still debated whether these pathways are maladaptive responses that cause damage, or whether they represent a protective mechanism favouring lipid remodelling (e.g. the formation of monounsaturated fatty acids) and aimed at maintaining hepatic health/homeostasis when the liver is metabolically challenged by an abnormal calorie load.^{68,73–76}

HCV also suppresses insulin receptor substrates (IRS-1 and IRS-2^{37,77,78}), thereby directly causing hepatic IR.^{13,22,79} However, hepatic IR is known to be “selective” (partial), as some pathways still remain insulin sensitive and DNL is not impacted. This phenomenon, yet to be fully understood, occurs because of the complex nature of the insulin

signalling pathway: glucose metabolism is controlled mainly by AKT2-mediated inhibition of forkhead box O1 (FOXO1) function (AKT2 signalling is impaired by IR).^{80,81} On the other hand, SREBP1-mediated lipogenesis remains activated because it is controlled by mTORC1 (which is less responsive to IR⁸²). The IR-mediated overactivation of FOXO1 in fatty liver worsens hyperglycaemia; however, FOXO1 also promotes IRS-2 expression⁸³ further favouring a permissive mechanism that keeps DNL active in the face of ongoing steatosis.^{84,85} Intriguingly, insulin also governs a positive feedback loop mediated by phosphatase and tensin homolog (PTEN); PTEN is a negative regulator of the PI3K/AKT pathway that is suppressed by insulin signalling itself⁸⁶ and DNL products (e.g. mono-unsaturated fatty acids that promote miR-21)^{87,88} to partially preserve insulin intracellular signalling in hepatocytes at the expense of worsening the hepatic lipid content and steatohepatitis.⁸⁷ The HCV core protein (genotype 3a) also suppresses PTEN via miR-21, therefore leading to the formation of large lipid droplets and enhancing virus secretion.^{89–91} PTEN is also a well-described tumour suppressor⁹²; PTEN inhibition in CHC and NAFLD might thus have a strong impact on both hepatocellular carcinogenesis^{93,94} and HCC clinical outcomes.⁹⁵

In conclusion, IR/T2DM and steatosis are independently associated with fibrosis and liver disease progression in patients with CHC in cross-sectional and longitudinal studies^{4,96}; the co-occurrence of CHC and systemic metabolic disturbances might increase the risk of CHC progression and HCC.⁹⁷ Unlike genotype 3 (where steatosis is mainly driven by direct virus-mediated effects), the association of obesity with fibrogenesis is more evident in patients carrying HCV genotype 1 where steatosis is more linearly influenced by systemic metabolic unbalances.^{96,98}

The precancerous niche in CHC

The majority of HCC arises on the background of progressive fibrosis and/or cirrhosis which are known drivers of hepatocarcinogenesis. However, HCC can also develop (albeit at a much lower rate) in transgenic HCV-HCC murine models⁹⁹ and in patients with CHC in the absence of significant chronic inflammation and/or cirrhosis.¹⁰⁰ In the next section we will discuss how HCV infection, CHC progression and metabolic influences interact to favour liver disease progression.

Viral influences

HCV is an RNA virus that is predominantly restricted to the cytoplasm and subsequently is unable to integrate into the host genome and exert direct mutagenic effects.⁴ However, HCV proteins have been implicated in promoting cell proliferation and survival, epithelial-mesenchymal

Key point

HCV infection and obesity promote lipotoxicity, senescence, chronic inflammation and fibrosis which drive malignant hepatocyte transformation and HCC development.

transition, and angiogenesis, through modulation of various signalling cascades including: Raf1/MAPK,¹⁰¹ Wnt/ β -catenin pathway,¹⁰² transforming growth factor- β (TGF- β) signaling,¹⁰³ PI3K/PTEN,¹⁰⁴ and insulin signalling/AKT,¹⁰⁵ thus contributing directly to HCC development. Furthermore, CHC leads to genome-wide epigenetic changes that persist in patients even after SVR,^{106,107} sustaining metabolic dysregulation and inflammation following viral eradication.

Lipotoxicity

Dysregulation of hepatic metabolism leads to the build-up of toxic lipids and intermediates in hepatocytes ("lipotoxicity") resulting in cell dysfunction and injury. CHC is associated with up to a 5-fold increase in liver reactive oxygen species (ROS) levels.^{108,109} HCV core proteins can directly increase ROS levels through increased Nox1 and Nox4 production of superoxide,^{110,111} and also indirectly by inducing mitochondrial dysfunction,^{110,112–114} endoplasmic reticulum stress^{115–117} as well as impairing antioxidant mechanisms (glutathione and small metabolite antioxidants).^{118,120} ROS overproduction also occurs when mitochondrial fatty acid oxidation supplies excessive respiratory substrates causing stalling of the respiratory fluxes: saturation of the oxidative system in steatosis leads to mitochondrial dysfunction and promotion of extra-mitochondrial (i.e. microsomal and peroxisomal) oxidation.^{121,122} Also, fatty acid ω -oxidation, a minor pathway of fatty acid oxidation usually inhibited by insulin in the healthy liver, is upregulated by IR and leads to long-chain dicarboxylic acid production, further worsening mitochondrial dysfunction; long-chain dicarboxylic acids (and very-long-chain fatty acids) can then be partially metabolised by peroxisomal β -oxidation, further inducing ROS production.^{121,122} Multiple reports suggest that HCV worsens these events as it drives mitochondrial dysfunction.^{13,22,79,123,124} Lipotoxicity drives cell death and promotes the chronic activation of wound healing responses, thereby promoting CHC progression and a pre-cancerous niche in CHC (Fig. 3).

Inflammation and immunosurveillance

Oxidative stress, metabolic dysfunction and lipotoxicity can have a profound effect on the immune system in response to both viral infection and also cancerous cells. Effective clearance of HCV infection typically requires the coordinated function of multiple arms of the immune system, including the innate immune system (interferons and natural killer [NK] and NKT cells), as well as the adaptive immune response specific to a given pathogen (CD4+ and CD8+ T cells).¹²⁵ For the majority of patients, these mechanism are unable to eradicate the virus, leading to chronic infection and CHC. The

immunological mechanisms (immunosurveillance) that identify and clear HCV-infected cells are similar to those that detect and destroy malignant cells.¹²⁶ Immunosurveillance can be impaired by a number of HCV-dependent mechanisms that do not completely resolve following DAA treatment¹²⁷ as well as by systemic metabolic influences that confer increased risk of HCC.^{128,129}

Cytotoxic CD8+ T cells are the predominant cell type responsible for clearing HCV-infected hepatocytes. During HCV infection, effector CD8+ T cells become progressively exhausted due to persistent antigen exposure and as a result exhibit reduced antiviral activity.^{130,131} This T-cell exhaustion does not resolve following DAA treatment.¹³² The reduced activity of T cells in CHC may predispose to HCC, as T cells are thought to orchestrate the clearance of senescent pre-malignant hepatocytes.¹³³ T-cell exhaustion is also a feature of HCC, with T cells exhibiting impaired cytotoxic and anti-tumoural activity.¹³⁴ Furthermore, the anti-tumoural activity of CD8+ T cells is impaired as a result of the systemic effects of obesity¹³⁵ which may in part explain why patients with NASH may be less responsive to immunotherapy, although this is a recent observation that requires further validation.¹²⁸

CD4+ T cells are required to maintain antiviral CD8+ T-cell responses and are lost in CHC.¹³⁶ HCV causes CD4+ T-cell dysfunction, reducing the cytokine production by T helper 1 cells that is required for antiviral immunity.¹³⁷ These effects are not limited to HCV-reactive T cells¹³⁷ suggesting that HCV infection may limit the effectiveness of other CD4+ T-cell roles including supporting CD8 T cell-mediated clearance of pre-malignant hepatocytes. Metabolic dysfunction can compound this with hepatic lipotoxicity reducing antigen-specific CD4+ T cell-dependent senescence surveillance of pre-malignant hepatocytes.¹³⁸ Furthermore, in CHC, CD4+ regulatory T cells, which suppress the immunosurveillance activities of CD8+ T cells¹³⁹ and NK cells,¹⁴⁰ expand and remain elevated even after DAA-induced SVR.¹³⁹ Regulatory T cells are also increased in patients with NASH and are reported to be critical for NASH progression and HCC initiation in mice.^{141,142}

NK cells have also been implicated in the clearance of HCV-infected hepatocytes.¹⁴³ In CHC, decreased NK cell levels, altered NK cell subset distribution, activation marker expression and function are observed and not completely resolved following DAA treatment.^{143,144} To date, the presence, regulation and function of NK cells during NAFLD remains controversial, with reports of both increased and decreased numbers as well as activation in patients who are obese or have NAFLD.¹⁴⁵ NK cells are able to kill malignant cells directly through cytotoxic perforin-mediated lysis or indirectly through modulation of other immune cells.

Key point

DAA treatment can result in weight gain in a proportion of patients with CHC and also causes metabolic changes associated with HCC development.

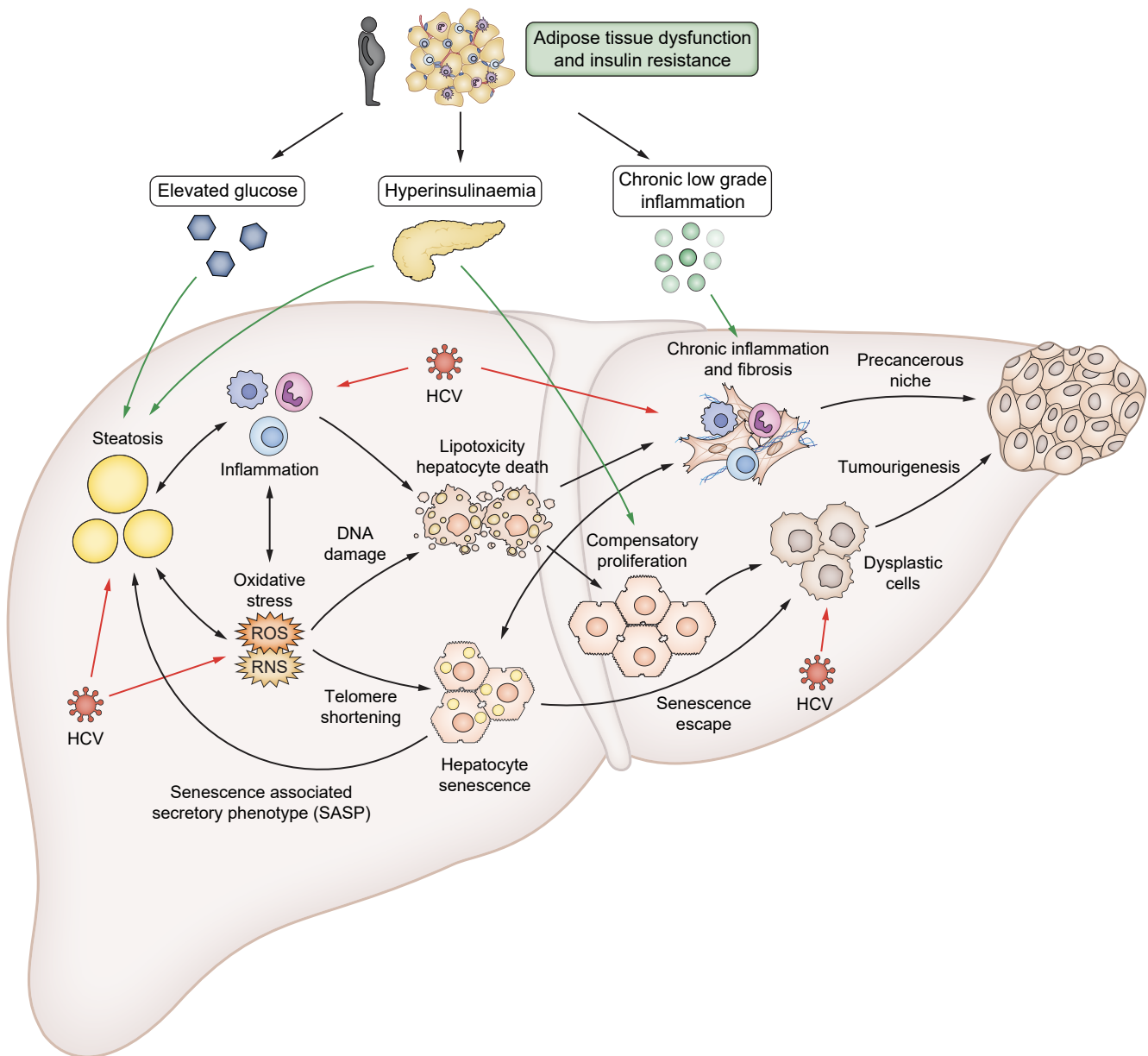


Fig. 3. Potential mechanisms of liver tumorigenesis in HCV-infected patients. In patients with chronic hepatitis C, hepatocellular carcinogenesis is promoted by a tight interplay between systemic metabolic/pro-inflammatory effects and direct/indirect actions of HCV in the liver where it i) boosts lipotoxicity, oxidative stress, DNA damage, cell death and senescence; ii) promotes wound healing; therefore promoting tumorigenesis and a precancerous microenvironment favouring hepatocellular carcinoma development. RNS, reactive nitrogen species; ROS, reactive oxygen species.

Both of these functions may be impacted by HCV infection and metabolic dysfunction.

Therefore, HCV infection and steatosis not only increase the likelihood of developing pre-malignant hepatocytes, but can also impair the host's long-term ability to clear these potentially malignant cells effectively.

Senescence

Oxidative stress in patients with CHC drives chronic injury in the liver, leading to increased compensatory proliferation of hepatocytes which,

over multiple rounds of cell division, will risk telomere shortening and senescence (Fig. 3).^{146,147} Hepatic ROS levels,¹⁴⁸ HCV progression^{149–151} and NASH^{152–154} are associated with telomere shortening/mutations (that are also common in HCC¹⁵⁵ and, more broadly, in cancer¹⁵⁴).

Induction of senescence due to telomere shortening is tumour suppressive if cleared by immunosurveillance mechanisms.¹³³ However, chronic liver disease can result in an enormous senescence burden, with up to 80% of hepatocytes being senescent, which correlates not only with hepatic

Key point

After successful DAA treatment in advanced CHC, hepatic manifestations and established comorbidities continue to injure the liver and HCC risk remains elevated.

inflammation but also with the stage of fibrosis.¹⁵⁶ Failure to clear these senescent cells can result in significant genomic instability, ultimately leading to the formation of cancer clones.¹⁵⁷ Furthermore, senescence can promote a precancerous niche through the production of senescence-associated secretory phenotype (SASP) which can promote adjacent cells to become senescent.^{158,159} The SASP is also highly proinflammatory,¹⁶⁰ recruiting immune cells that can cause further hepatocyte telomere damage and shortening,¹⁶¹ and establishing a vicious cycle of senescence and inflammation (Fig. 4). The effect of HCV infection on the induction or components of SASP is unclear, however, current evidence suggests that HCV infection promotes senescence indirectly through ROS-driven hepatocyte injury.¹⁴⁶

Senescence also has a profound effect on systemic¹⁶² and hepatic metabolism resulting in a worsening of steatosis.¹⁶³ Fat accumulation in senescent hepatocytes was associated with mitochondrial dysfunction and impaired lipid metabolism.¹⁶³ As a result of enhanced lipotoxicity, further liver injury, compensatory proliferation and ultimately replicative senescence establish a vicious cycle that leads to a worsening of steatosis (Fig. 4). Telomeric dysfunction may also play a wider role in the development of metabolic disease: mice with hyper-long telomeres display less metabolic aging, including lower levels of DNA damage, lower body weight, lower cholesterol and LDL, and also improved glucose and insulin tolerance compared to their wild-type counterparts.¹⁶⁴ Short-term cellular senescence might be an attempt to limit cancer development by engaging

immunosurveillance mechanisms, however, the long-term metabolic consequence of this may ultimately lead to an increased risk of HCC. In the future, treatment with senolytics might suppress these vicious cycles of senescence, inflammation and metabolic dysfunction to reduce steatosis and progression to fibrosis and HCC after SVR.

Fibrogenesis

Liver fibrosis stage is the strongest predictive factor for liver-related (and all-cause) mortality, independent of any other histological features¹⁶⁵ and the presence of fibrosis promotes a precancerous liver microenvironment.^{166,167} Fibrogenesis is mediated by activation of hepatic stellate cells that differentiate into collagen-producing myofibroblasts in response to liver injury. Accumulating evidence suggests that fibrosis and activated myofibroblasts contribute to the development of HCC through multiple pathways (reviewed extensively elsewhere^{166,167}). Briefly, fibrosis leads to the stiffening of the liver microenvironment which can promote malignant transformation of hepatocytes as well as tumour cell proliferation and invasion.^{166,167} Activated myofibroblasts release a plethora of growth factors and cytokines, such as TGF- β , platelet-derived growth factor and hepatocyte growth factor, that suppress immune surveillance and promote tumour angiogenesis, proliferation and invasion.^{166,167}

HCV infection primarily promotes fibrogenesis indirectly through chronic liver injury. However, there is some evidence that HCV-infected hepatocytes upregulate the key fibrogenic factor TGF- β through the generation of ROS as well as activation of p38 MAPK, JNK, ERK1/2 and NF- κ B-dependent pathways.¹⁴ Furthermore, TGF- β is also upregulated *in vitro* by HCV in an endoplasmic reticulum stress-dependant manner involving ROS, JNK and IRE1 signalling pathways¹⁶⁸; however, whether this mechanism occurs in patients is yet to be confirmed. TGF- β signalling plays a critical role in the progression of fibrosis in NASH,¹⁶⁹ as well as promoting epithelial-mesenchymal transition which enables tumour cells to invade and metastasise.^{103,170}

Systemic metabolic influences can promote fibrosis and myofibroblast activation. To enable activation, hepatic myofibroblasts undergo significant metabolic reprogramming, including upregulation of DNL, glycolysis and oxidative phosphorylation.^{171–173} Myofibroblast activation can be further altered by systemic factors upregulated in metabolic disease: i) myofibroblasts express the insulin receptor and, in response to both insulin and IGF1 (insulin-like growth factor 1), upregulate TGF- β and CTGF (connective tissue growth factor) release, exacerbating fibrogenesis in the context of hyperinsulinemia and NASH^{174–176}; ii) myofibroblasts also express functional leptin receptors: increased circulating levels of leptin have been described in

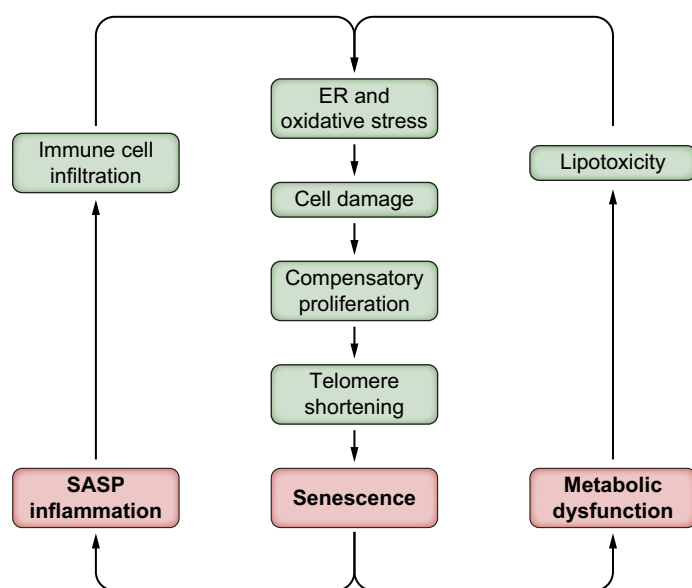


Fig. 4. The vicious cycles of senescence in chronic liver disease. Herein, we propose a model to explain how senescence might contribute to metabolic dysfunction and hepatic inflammation, and thereby a vicious cycle favouring chronic hepatitis C progression and hepatocellular carcinoma. ER, endoplasmic reticulum; SASP, senescence-associated secretory phenotype.

both obesity¹⁷⁷ and CHC¹⁷⁸ and can promote myofibroblast proliferation, collagen secretion and suppression of apoptosis,¹⁷⁹ thereby potentially worsening CHC progression. In a chronic pro-fibrotic niche led by HCV, steatosis, lipotoxicity and adipokines can worsen the chronic activation of wound healing responses and potentiate the mechanisms that lead to HCC development and progression.

HCC in CHC: Systemic metabolic changes and steatosis fuel tumour development and progression

The aforementioned metabolic impairment associated with CHC might also directly contribute to cancer.¹⁸⁰ Obesity, hyperinsulinemia and diabetes are associated with a greater risk of developing a number of cancers, including colorectal, breast, endometrial, pancreatic, ovarian, liver and gastric cancer.^{181–184}

Indeed hyperinsulinemia has a direct effect on cell proliferation and HCC behaviour. First, in the context of fatty liver and hyperinsulinemia, transformed hepatocytes that escape apoptosis/necrosis are exposed (like the surrounding liver parenchyma) to intense metabolic pressure (imposed by hyperglycaemia, hyperinsulinemia, lipotoxicity, and oxidative stress) that will preserve partial intracellular insulin signalling (providing selective advantages to pre-malignant hepatocytes).^{37,77,78,185} In other words, transformed cells find avenues to improve insulin signalling and glucose uptake to fulfil their metabolic needs.¹⁸⁶ Indeed, HCC features the activation of insulin signalling^{187–189} and the main downstream effectors of the insulin receptor (the PI3K-AKT-mTOR and the RAS-MAPK pathways) are also known drivers of cell proliferation^{37,77,78,185} and survival^{187,190,191} in hepatocytes and HCC cells, as well as being promoters of angiogenesis.¹⁹²

Second, both proliferating hepatocytes and HCC cells similarly rewire cell metabolism, reprogramming (glucose/lipid/cholesterol) biosynthetic pathways to generate specific lipid species (e.g. phosphatidylcholine enriched in monounsaturated fatty acid) to fuel cell division and create new membranes, or to limit oxidative stress, thereby ensuring proliferation and survival.^{188,193,194} The upregulation of DNL and the formation of complex lipids, potentiated by obesity, are therefore adaptive metabolic responses that may fuel HCC cells, as shown by multiple lines of evidence: i) the upregulation of DNL and fatty acid desaturation has been widely described in HCC (including HCV-associated HCC) and is considered a prognostic factor for patient survival^{19,70,195–197}; ii) HCC growth/malignancy can be stratified on the basis of acetate metabolism and acetyl-CoA synthase 1 expression¹⁹⁸; iii) the ablation/inhibition of key DNL enzymes (e.g. fatty acid synthase, or acetyl-

coenzyme A carboxylase [ACC]) protects mice from HCC.^{193,199} Moreover, the fact that several genes encoding metabolic enzymes involved in fatty acid uptake (e.g. fatty acid binding protein 4/5) strongly correlate with cancer survival confirms how crucial lipid supply is for HCC biology.²⁰⁰ As such, lifestyle interventions (e.g. low carbohydrate diets) or therapies improving systemic IR and/or directly targeting DNL (such as metformin or ACC inhibitors), are attractive and show efficacy in rodent models of HCC^{193,201} and, more generally, in cancer.¹⁹⁷ However, robust clinical evidence of the efficacy of such agents in the prevention of *de novo* carcinogenesis or of HCC progression is lacking.

All the aforementioned phenomena are intrinsically linked and, through the process of amplifying each other, might provide (part of) the basis for the high prevalence of HCC in HCV. In patients with HCV genotype 3 these processes will be mainly driven by the virus and should be partially reversed by antiviral treatment; in patients with HCV genotypes 1/2, the hepatic/systemic metabolic disturbances will persist, ultimately conferring an increased risk of cancer (unless pre-existing obesity, IR and diabetes are appropriately managed).

Last, it should be kept in mind that diabetes is a major driver of hepatocellular carcinogenesis with a 2–3-fold increase in the risk of HCC independent of the presence of other major HCC risk factors including HCV infection¹⁸¹; this suggests that systemic metabolic disturbances might play a greater role than HCV *per se* in the development of HCC in patients with CHC.

Strategies aimed at reversing metabolic dysfunction and cancer prevention

As already described, a subset of patients with CHC remain at risk of liver-related morbidity despite SVR. In particular, metabolic risk factors appear to play a dominant role in this persisting risk. Lifestyle interventions such as exercise and diet could therefore be effective in modifying the risk of progression of chronic liver disease, preventing fibrotic progression and the development of HCC.^{202–204} Several large cohort studies (although not specific to HCV) have demonstrated an inverse association between physical activity rates and risk of HCC.^{202,205,206} Mechanistic studies have revealed that exercise modulates key signalling and metabolic pathways involved in proliferation and angiogenesis.^{207–210} A case control study reported that the Mediterranean diet appears to be protective against HCC, especially in CHC.²⁰³ The mechanistic link between diet and HCC risk is likely multifactorial; the effect of limiting consumption of foods with a high (refined) carbohydrate content is known to be beneficial in i) reducing the steatotic burden and the progression of liver disease²¹¹; ii) decreasing the metabolic pressure on cell

proliferation induced by insulin signalling, and iii) preventing the intracellular metabolic changes observed in proliferating/HCC cells. Moreover, as has also been proposed in NASH, reports suggest that dietary cholesterol intake is associated with an increased risk of disease progression in patients with CHC and advanced fibrosis or cirrhosis.^{204,212,213}

A systematic evaluation of the benefit of pharmacological interventions (aimed at treating specific features of MetS) in reducing the risk of fibrotic progression and HCC is in its infancy, especially in CHC; however, there is a growing body of evidence to suggest lipophilic statins, metformin and aspirin may be beneficial.^{214–217,219} Lipophilic statins such as simvastatin and atorvastatin modulate cholesterol synthesis and have been shown to reduce the risk of CHC-HCC in a large prospective propensity score matched cohort study,²¹⁷ as well as reducing the risk of fibrotic progression in a prospective study.²¹⁹ Metformin has been shown to be well tolerated and safe in patients with CHC and T2DM, and it may also reduce the risk of liver-related mortality and HCC development beyond its effect on diabetic control;^{214,215,220,221} however, its use in non-diabetic patients warrants further investigation. Low dose aspirin may also protect against HCC development and liver-related mortality in CHC,²¹⁶ with cyclooxygenase 2 inhibition leading to reduced systemic inflammation and modulation of immune cell recruitment into the liver.^{222,223} An additional benefit of all of these medications is that they modify cardiovascular risk, which is important considering that both MetS and CHC are risk factors for cardiovascular disease.^{224,225} However, their use primarily for liver protection and HCC prevention requires further investigation in large prospective studies designed to evaluate benefits and risks.

In many ways, patients with CHC and features of MetS who continue to progress following SVR might be classified as having “NAFLD”. Therefore drugs currently under development for the treatment of NASH may well be useful in preventing disease progression in patients with CHC. Glucagon-like peptide 1 (GLP1) agonists are a commonly used anti-glycaemic class for the treatment of T2DM and have shown quite promising signs of clinical efficacy in NASH,^{226–228} with pre-clinical data demonstrating potential protection from HCC development.^{229,230} Further advances in the field will likely be provided by novel GLP1/glucagon receptor dual agonists currently being tested.²³¹ Another class of medications showing benefit in NASH are the farnesoid X receptor (FXR) agonists, currently in phase II/III NASH clinical trials. FXR improves hepatic function and promotes the expression of small heterodimer protein 1, thereby indirectly inhibiting SREBP, DNL and hepatic fat accumulation. The activation of the FXR-

FGF19 gut-liver axis exerts pleiotropic effects on hepatic/systemic metabolism: reducing the burden of cholesterol and bile acid accumulation, improving hepatic glucose metabolism, and reducing hepatic inflammation.^{233–235} Obeticholic acid is the most advanced FXR agonist under development (currently in phase III trials) and has been shown to prevent fibrotic progression in NASH in a phase III trial.²³⁶ Interestingly, the intestinal/systemic activation of FXR also prevents HCC development in murine models of cholestasis^{234,237,238} and NASH,²³⁹ and might interfere with HCC progression²⁴⁰ but clinical effectiveness of obeticholic acid on HCC prevention/treatment is yet to be clearly established.

As discussed, drugs that inhibit DNL are being investigated for the treatment of NASH and may also be beneficial in patients with CHC who have been cured. ACC inhibitors that halt DNL have shown potential in improving steatosis and possibly reducing fibrotic progression in NASH.^{241–243} ACC inhibition has also been shown to improve survival and response to sorafenib therapy in a pre-clinical model of HCC,¹⁹³ but has yet to be tested in clinical trials of either HCV-associated or non-HCV-associated HCC. Given the overlap in the metabolic dysfunction between CHC and NASH, they warrant further investigation.

In summary, multiple lines of evidence suggest that targeting metabolic pathways for the treatment of NASH and CHC might be a useful strategy in the future to prevent chronic liver disease progression, and prevent/treat HCC.

HCC surveillance following SVR

International guidelines currently recommend continued HCC surveillance in patients with cirrhosis following SVR.^{244–246} In addition European guidelines recommend continued surveillance in those with advanced fibrosis (Metavir F3).^{244,245} There is much debate over the cost effectiveness of this approach, especially in those with advanced fibrosis. Indeed, better ways of stratifying HCC risk following SVR are much needed.²⁴⁷ A number of studies have proposed various risk scoring systems based on markers of fibrosis and/or portal hypertension, liver function, alpha-fetoprotein and high-risk demographics, however, these have not yet been established in routine clinic practice.²⁴⁸ Obesity, steatosis and T2DM have all been shown to increase the risk of liver-related events including the development of HCC post SVR.^{249–253} Research looking at incorporating these metabolic comorbidities into risk stratification algorithms is certainly warranted.

Therapeutic considerations for systemic HCC treatment

HCC remains the most common cancer in patients with CHC and, as discussed, there is considerable

Key point

Targeting metabolic comorbidities may be a useful strategy to prevent CHC-initiated liver disease progression, and to prevent/treat HCC.

overlap with NASH-associated HCC.²⁵⁴ The landmark IMBrave 150 study, which showed superiority of bevacizumab/atezolizumab (a combination treatment which targets angiogenesis and the immune checkpoint mediated by programmed cell death 1 [PD-1] and programmed cell death ligand 1 [PDL-1]) over sorafenib for the treatment of advanced HCC, demonstrated that patients with a viral aetiology had improved post-treatment survival compared to patients with a non-viral aetiology, most of whom had NAFLD.²⁵⁵ This suggests that the metabolic dysfunction associated with NAFLD-HCC may be a driver of poor response to immunotherapy in HCC. This has recently been further explored in a study showing that aberrant T-cell activation in the context of NAFLD resulted in impaired immune surveillance and poor response to immune checkpoint blockade.¹²⁸ Therefore, although CHC-HCC may be a predictor of good response to immunotherapy in HCC, those CHC-HCC patients who develop HCC after SVR due to metabolic dysfunction may behave more like NAFLD-HCC in terms of treatment response to immunotherapy. Further research is warranted to explore this, especially as greater numbers of patients who achieve SVR are followed up. In addition to NAFLD, obesity also appears to influence treatment response to systemic immunotherapy,²⁵⁶ either improving^{257–259} or worsening²⁶⁰ therapeutic responses depending on cancer type. Obesity is thought to upregulate T-cell PD-1 expression through leptin-induced STAT3 signalling,²⁵⁷ with the effect on immunotherapy response being determined by the frequency of tumour PD-1+ T cells as well as changes in other immune populations such as myeloid-derived suppressor cells.^{260,261} Therefore, HCV-associated obesity and metabolic alterations could either improve or worsen response to systemic immunotherapies depending on cancer type and may become an important consideration when making treatment decisions. Whether modulation of metabolic disturbances, using some of the agents currently under development for the treatment of NAFLD, can improve immunotherapy response rates in patients with HCV-HCC warrants further investigation.

Conclusion

In the DAA era, high SVR rates mean that the direct consequences of HCV intracellular functions are not a primary concern. However, alcohol intake, obesity and the lasting metabolic complications of CHC need to be considered as they will impact on the clinical management of patients following a SVR and probably play the primary role in chronic liver disease progression toward HCC (even when HCV has been eradicated). It is now vital that we understand the mechanisms which underpin this metabolic dysfunction, how this favours cancer progression, and how they influence therapeutic

responses both during the course of CHC and after SVR. Ultimately, the systemic/hepatic metabolic dysfunction (initiated by the virus and/or associated with obesity), worsens HCV-mediated steatosis, driving chronic liver injury and predisposing to HCC development. Underpinning this vicious cycle of senescence, SASP inflammation and metabolic dysfunction that will worsen with age but that might be targetable therapeutically is therefore crucial. The effects of an integrated management of the surrounding MetS in CHC should therefore be considered and systematically investigated in patients with CHC, whether they achieve SVR or not.

Abbreviations

ACC, acetyl-coenzyme A carboxylase; AT, adipose tissue; CHC, chronic hepatitis C; ChREBP, carbohydrate response element-binding protein; DAA, direct-acting antiviral; DNL, *de novo* lipogenesis; FOXO1, forkhead box O1; FXR, farnesoid X receptor; GLP1, glucagon-like peptide 1; HCC, hepatocellular carcinoma; IR, insulin resistance; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NK, natural killer; PD-1, programmed cell death 1; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; SREBP, sterol regulatory element-binding protein; SVR, sustained virological response; T2DM, type 2 diabetes mellitus; TGF- β , transforming growth factor; WC, waist circumference.

Financial support

JL, DG and DAM are supported by UK Medical Research Council program grants MR/K0019494/1, MR/R023026/1 and CRUK program grants (C9380/A26813) and (C18342/A23390). DG is funded by the Newcastle CRUK Clinical Academic Training Programme.

Conflicts of interest

D.A.M. is a director of Fibrofind limited. J.L. and D.A.M. are shareholders in Fibrofind limited. The other authors report no conflicts of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

JL, DG and MV drafted the manuscript. AME and DAM reviewed, provided criticism and edited the manuscript. All the authors reviewed and approved the final manuscript.

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2022.01.029>.

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