Carcinogenesis on the background of liver fibrosis: Implications for the management of hepatocellular cancer

Joanne Marie O'Rourke, Vandana Mridhu Sagar, Tahir Shah, Shishir Shetty

Joanne Marie O'Rourke, Vandana Mridhu Sagar, Shishir Shetty, Centre for Liver Research, Institute of Biomedical Research, Birmingham B15 2TT, United Kingdom

Joanne Marie O'Rourke, Vandana Mridhu Sagar, Tahir Shah, Shishir Shetty, NIHR Birmingham Biomedical Research Centre, University of Birmingham and University Hospitals Birmingham NHS Foundation Trust, United Kingdom

ORCID number: Joanne Marie O'Rourke (0000-0001-7851 -0703); Vandana Mridhu Sagar (0000-0003-2662-755X); Tahir Shah (0000-0002-0420-0304); Shishir Shetty (0000-0002 -4729-2173).

Abstract

Hepatocellular carcinoma (HCC) is now the second leading cause of cancer-related deaths globally and many patients have incurable disease. HCC predominantly occurs in the setting of liver cirrhosis and is a paradigm for inflammation-induced cancer. The causes of chronic liver disease promote the development of transformed or premalignant hepatocytes and predisposes to the development of HCC. For HCC to grow and progress it is now clear that it requires an immunosuppressive niche within the fibrogenic microenvironment of cirrhosis. The rationale for targeting this immunosuppression is supported by responses seen in recent trials with checkpoint inhibitors. With the impact of immunotherapy, HCC progression may be delayed and long term durable responses may be seen. This makes the management of the underlying liver cirrhosis in HCC even more crucial as studies demonstrate that measures of liver function are a major prognostic factor in HCC. In this review, we discuss the development of cancer in the setting of liver inflammation and fibrosis, reviewing the microenvironment that leads to this tumourigenic climate and the implications this has for patient management.

Key words: Hepatocellular cancer; Carcinogenesis; Inflammation; Fibrosis; Immunotherapy

Core tip: In this review, we discuss the development of hepatocellular carcinoma in the setting of liver inflammation and fibrosis, reviewing the microenviron-

ment that leads to this tumorigenic climate and the implications this has for patient management.

INTRODUCTION

Hepatocellular carcinoma (HCC) is now the fifth most commonly diagnosed cancer in men worldwide, and in women it is ranked ninth. HCC is the second most common cause of cancer related deaths and is reported to have been responsible for nearly 745000 deaths in 2012[1]. Incidence rates are highest in Asia and Africa with Central Europe having intermediate rates[2]. Different risk factors predominate depending on the region of the world. In Africa and Asia infection with hepatitis B virus (HBV) and aflatoxin B1 exposure are the major risk factors. In developed countries the hepatitis C virus (HCV), alcohol and the metabolic syndrome have predominated[3].

Despite increasing knowledge on the aetiologies of cirrhosis and progress in diagnosing and managing risk factors, the incidence rates for HCC are increasing. In England, HCC incidence increased from 0.63 per 100000 in 1990 to 2.48 in 2009[4]. In the United States (US), HCC incidence increased by 4.5% (95%CI: 4.3-4.7) annually between 2000 and 2009 but only 0.7% annually (95%CI: 0.2-1.6) after that. The post 2009 slowing in overall rates, seen in the US, may represent a plateau created from increases in vaccination against HBV and improved chronic HBV antiviral treatment[5].

It is uncommon to see HCC in the absence of liver fibrosis but it does occur. Table 1 lists some of the aetiologies associated with non-cirrhotic HCC[6,7]. Chronic hepatitis B is a major risk factor for the development of HCC in the non-cirrhotic setting[8]. In Europe and the United States the 5-year cumulative incidence of developing HCC was found to be 1% in non-cirrhotic chronic HBV hepatitis. This incidence increased to 10% in HBV with cirrhosis[9]. Other causes of HCC in the non-cirrhotic setting include hereditary conditions for example porphyria and type 1 glycogen storage disease, metabolic syndromes and genotoxin exposure. Genotoxins are agents which damage the genetic information within a cell. For example, the aflatoxin B1, which is produced by Aspergillus flavus, is a pathogenic fungus and can lead to non-cirrhotic HCC induction[10]. The global epidemic of non-alcoholic fatty liver disease (NAFLD) which is characterised by macrovesicular steatosis can lead to cirrhosis. It is however observed that a significant proportion of patients with NAFLD develop HCC in the non-cirrhotic setting[11,12]. However

Table 1 Conditions which have been associated with hepatocellular carcinoma development in the non-cirrhotic liver[6,7]

Viral	HBV
Metabolic	Porphyria
	Type 1 glycogen storage disease
	NAFLD
	A1 antitrypsin
	Haemochromatosis
	Type 1 hypercitrullinemia
Genotoxins	Aflatoxin B1
Congenital	Alagille syndrome
	Congenital hepatic fibrosis
Sex hormones	Anabolic steroids
	Hepatic adenoma transformation
Vascular	Hepatic vascular pathology, *e.g.*, Budd Chiari

HBV: Hepatitis B virus; NAFLD: Non-alcoholic fatty liver disease.

worldwide at present the majority (70%-90%) of HCC cases occur on a background of cirrhosis[13].

When data from the World Health Organization (WHO) mortality database was examined by Ascione *et al*[14], the age-standardised death rate for liver cirrhosis in European countries between 1970 and 2010 showed cause for concern for the United Kingdom (UK), Finland, Ireland and Denmark. Looking at percentage change in mortality, the UK in those four decades showed a high increase (+284.8%), Finland, Ireland and Denmark also saw increases. However these countries were the exceptions and in all other countries in Europe there was a reduction in mortality for liver cirrhosis. The same database provided comparable data, between 1980-2010 with a 85.4% increase in death from HCC over this period[14]. The overall decrease in liver cirrhosis related deaths in Europe and the increasing mortality for HCC is confounding and concerning.

Cirrhosis mortality in the UK has been the subject of extensive discussion and patterns of alcohol consumption may account for the discrepancies between the UK and other parts of Europe. The rise in HCC cases in Europe over the last 30 years seems confounding when it is reported that in many countries mortality from cirrhosis is reducing. However, our knowledge and the management of chronic liver disease has over this timeframe improved, and it is suggested that with increased survival we are seeing increased development of HCC[14]. This would be in keeping with our knowledge that cirrhosis creates a microenvironment for tumour development and is considered a precursor for HCC.

Over 3 decades ago the 5-year survival for HCC was 3%. Despite improvements in earlier detection 5-year survival is less than 20% for this cancer[15].

PATHOGENESIS

Setting of inflammation and fibrosis

Liver fibrosis is a risk factor for the development of HCC with up to 90% of cases occurring on the background

of a cirrhotic liver[16] and is a leading cause of death in this population. The major global causes of liver disease which are associated with HCC include viral hepatitis, alcoholism and non-alcoholic steatohepatitis (NASH). The effects of HBV infection have started to decline due to increased use of antivirals and immunisation programs. It is hoped that in the age of new direct acting antiviral agents with time we will see a reduction in HCV associated cirrhosis. The impact of alcohol and the development of NASH cirrhosis will prove to be more challenging to prevent and cases are predicted to continue to rise.

Fibrosis occurs when the liver is repeatedly and continuously injured. Liver volume is formed from 80% parenchymal and 20% non-parenchymal cells[17]. Hepatocytes are the parenchymal cells and they are the target for hepatotoxic agents. Damage to hepatocytes triggers the release of reactive oxygen species (ROS) and mediators of fibrosis inducing activation of hepatic stellate cells (HSCs). HSCs with phagocytic Kupffer cells (KCs) and liver sinusoidal endothelial cells (LSECs) are central players in fibrosis development[18]. The activation of HSCs, extracellular matrix (ECM) producing myofibroblasts, is said to be the key step in fibrosis development. Paracrine signals from injured hepatocytes and activated KCs play a prominent role in HSC activation. KCs also generate ROS in the liver and this enhances HSC activation and collagen synthesis leading to fibrosis[19,20].

In addition to the multitude of cells involved in the development of cirrhosis there are also several cytokines that have been identified to play significant roles. They include platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β), tumour necrosis factor-α (TNF-α), interferon and interleukins (ILs). A variety of hepatotoxic agents can induce KC to synthesise PDGF[21] which binds to the HSC membrane and activates them. There are different isoforms of PDGF and two of these, PDGF B and D have been shown to have a role in activating HSCs leading to liver fibrosis[22]. TGF-β is the most potent stimulator of fibrogenesis and is produced by a variety of cells in the liver: HSCs, KCs, LSECs, and hepatocytes. The TGF-β family has multiple members and the one that has been implicated as a notable player in hepatic fibrosis is TGF-β1. It is reported to contribute not only to activation of HSCs but also the inhibition of ECM breakdown[23] and the induction of apoptosis of hepatocytes[24]. TNF-α has also been shown to activate HSCs to synthesise ECM[25], however, results from murine studies on TNF-α are complex and it appears to also have antifibrogenic effects in some reports[26]. ILs are expressed by many cells with the majority of ILs being produced by helper CD4 T lymphocytes. In the liver ILs have both pro-fibrogenic and antifibrogenic roles[18]. IL-1 can activate HSCs[27] and IL-17 has a role inducing fibrosis through the activation of HSCs and KCs[28]. ILs with antifibrogenic roles have been identified as IL-6, IL-10 and IL-22[29-31].

Setting for tumour development and progression in fibrosis

The inception and progression of HCC is described as being largely influenced by the microenvironment of the liver. This includes influences from chronic inflammation, liver remodelling, changes in genetics and cellular signalling. These pathways can be affected by chemical toxins, viruses, immune cells, hypoxia, ECM changes, microflora from the gastrointestinal tract and extra cellular microvesicles which carry altering signals, cytokines and oncogenic miRNAs.

Chronic inflammation and fibrosis are seen in the background of many HCCs and the most common aetiologies are viruses and ethanol. The immune mediated cell death seen in viral infections leads to increased production of ROS. This leads to increased hepatocellular oxidative stress which induces DNA mutations contributing to HCC development. Ethanol consumption is associated with increased ROS concentrations in hepatocytes resulting in hepatic DNA damage[32]. Chronic inflammation leads to increased proliferation of hepatocytes, shortening of telomeres and therefore chromosomal instability and a predisposition to malignant transformation[33]. Genomic alterations which have been identified in HCC and are considered to be drivers in progression include mutations affecting telomere maintenance, Wnt pathway activation, inactivation of p53, chromatin remodelling, Ras signalling, mechanistic target of rapamycin (mTOR) signalling and ROS pathway initiation[34].

Chronic inflammation can progress to fibrosis and cirrhosis and this in turn induces several further changes in the microenvironment. Firstly, it creates altered blood flow and hypoxic hepatocytes which produce reactive nitrogen species[35]. Areas of hypoxia in the liver parenchyma lead to changes in molecular signalling and we know that the response is to upregulate angiogenic factors including vascular endothelial growth factor (VEGF)[36]. In a tumour this facilitates angiogenesis and tumour growth. The hepatocytes may provide the genetic mutation but it is the unique surrounding microenvironment that enables the tumour to establish.

We have explained that chronic inflammation has effects on cytokine expression within the liver, ECM production by HSC, TNF-α receptors and also the mitogenic cytokine IL6 is significantly increased in advanced cirrhosis leading to a propensity towards cancer[37]. IL6 regulates immune cells and the growth of tumour cells[38] and this dual role therefore is an example of the association between the tumour and the microenvironment. The effects of IL6 are controlled by nuclear factor-κB signalling. Both pathways are altered in liver inflammation and hepatocarcinogenesis[39]. EGFR overexpression also promotes liver cancer progression when present in macrophages[40]. It has also been shown that hepatic stellate cells can promote the pro-

tumourigenic change in macrophages[41]. The expansion of liver progenitor cells to replace hepatocytes, in the presence of cytokines and increases in oxidative stress promotes the accruing of mutations[42].

Hepatocytes have great potential to regenerate but we know this predisposes cells to malignant transformation[43]. The cell underlying the inception of HCC is also critical to understand. The human liver is not just made up of hepatocytes but also adult stem cells and progenitor cells which maybe potential cells of origin for cancer[44].

The gut microbiota has in recent years received much attention in many disease processes including liver disease. It has been described that the microbes in our bodies encompass 100 trillion cells, with the majority residing in the gut[45]. It is increasingly recognised that the gastrointestinal tract plays a pivotal role in liver diseases, including HCC. As we have previously described HCC usually occurs in inflamed and fibrotic livers and intensive immune cell infiltration is seen. *Via* the portal vein the liver is exposed to gut-derived bacterial products and in advanced liver diseases there is increased intestinal permeability to gut-derived bacterial products including lipopolysaccharides (LPS)[46]. Accumulation of LPS is said to contribute to HCC development by generating inflammatory reactions in the hepatic environment[47], activating KCs and endothelial cells to release pro-inflammatory cytokines which contributes to liver injury. Levels of LPS are increased in animal models of hepatocarcinogenesis and in patients with HCC[47-49]. Dapito *et al*[50] found that toll like receptor 4 activation by LPS contributed to driving inflammation and tumour progression and that gut sterilisation supressed hepatocarcinogenesis. To date studies have been on preclinical animal models but there is potential that manipulating the microbiome may one day be an option in the prevention and perhaps treatment of HCC[51].

Tumour antigen tolerance promotes carcinogenesis
Dysregulation of the immune system has been implicated in the pathogenesis of HCC. Changes in the innate and adaptive immune system makes the immune system tolerant to cancer and facilitates tumour progression. Understanding these processes is therefore essential to tailor therapeutic approaches. Key cells implicated include T lymphocytes, myeloid-derived suppressor cells (MDSCs), dendritic cells and natural killer (NK) cells[52]. The innate immune system key players are dendritic cells, macrophages, MDSCs and NK cells. The adaptive immune system comprises the T lymphocyte subsets. Failure of HCC antigen presentation by dendritic cells is one defect in the immune system seen in HCC. Activated dendritic cells in HCC are not able to infiltrate cancer tissue effectively[53] and tumour associated macrophages express cytokines that favour tumour growth, invasion and suppress the anti-tumour immune response[54]. MDSCs possess strong immunosuppressive activities and expand in cancer and regulate T cell responses,

increased quantities of these cells are seen in the tumour environment of a HCC[55]. NK cells are cytotoxic lymphocytes and they can modulate the activity of other immune cells, including dendritic cells and macrophages, *via* cytokine release. They are critical to the innate immune system and are capable of rapid responses and can destroy tumour cells without prior priming. In HCC a reduction in NK cell subsets has been reported with reduced cytotoxic ability[56].

The adaptive immune system has a significant role in thwarting the development and advancement of cancer. CD8+ cytotoxic T cells play a salient part in anti-tumour mechanisms and CD4+ helper cells have a role in generating CD8+memory T cells[57] which assist in the destruction of tumour cells. In the setting of cirrhosis there is a reduction in CD4+ cells[58]. Tregs expressing CD4+, CD25+ and forkhead box P3 (Foxp3) have an inhibitory role and they can suppress effective anti-tumour responses[59]. There are increased Tregs seen in patients with HCC and depletion can increase anti-tumour responses[60] and lead to a reduction in tumour growth[61]. In advanced HCC there are increased numbers of CD8+FoxP3+ regulatory T cells perhaps helping the tumour evade the immune system[62]. NK T cells accumulate in the tumour environment and they appear to be able to function either as anti-tumour cells or can promote tumour tolerance depending on the subset[63]. We can therefore conclude that a complex, partially understood, dysregulated immune environment has a key role in the development and evolution of liver tumours. An overview of the key responses to hepatic injury leading to fibrosis and HCC development together with therapeutic strategies are summarised in Figure 1.

THE SAME FIBROTIC ENVIRONMENT WHICH PROMOTES THE DEVELOPMENT OF HCC ALSO IMPACTS NEGATIVELY ON TREATMENT OPTIONS

Management of advanced HCC and the impact of cirrhosis
The fact that the majority of HCC arise in the cirrhotic liver, which affects liver function, can severely impact on therapeutics. A detailed review of the management of HCC has been covered elsewhere[64]. There are several algorithms for the management of HCC including TNM stage, the Japanese integrated system, Cancer of the Liver Italian group and the Hong Kong Liver Cancer staging system. The most well recognised being the Barcelona Liver Cancer (BCLC) criteria which is recommended by several international guidelines[65,66]. Considering the underlying liver disease is vital in HCC, the BCLC guidelines include both tumour stage and the severity of underlying liver cirrhosis (Child Pugh score) and helps guide treatment and to predict overall prognosis. The seminal study by Hoshida *et al*[67]
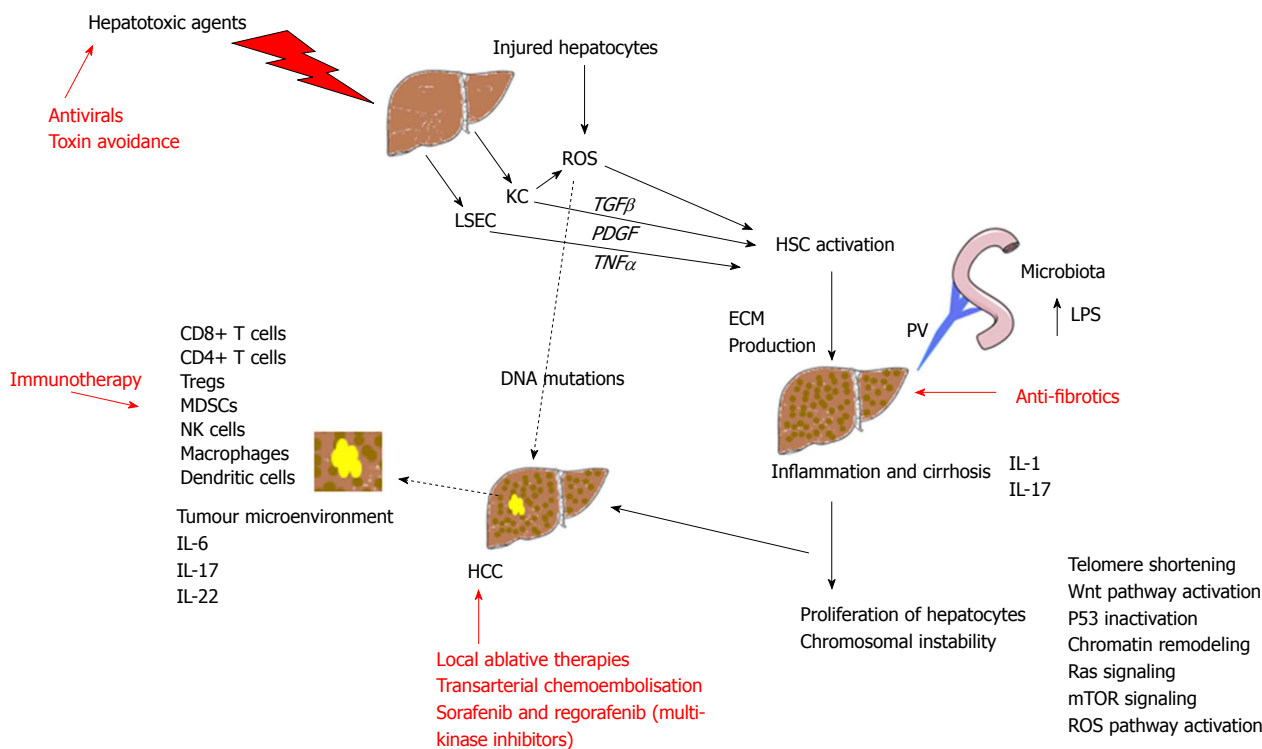
Figure 1 Overview of the key factors associated with fibrosis development and progression to hepatocellular carcinoma. Hepatotoxic agents damage key liver cells triggering reactive oxygen species and cytokine release culminating in hepatic stellate cell activation, the key step in fibrosis development. Chronic inflammation and fibrosis instigates several changes in the microenvironment predisposing to hepatocellular carcinoma (HCC) and creating distinct immune changes which promote HCC progression. Key therapeutic strategies are highlighted in red. LSEC: Liver sinusoidal endothelial cells; KC: Kupffer cells; ROS: Reactive oxygen species; HCC: Hepatocellular carcinoma; TGFβ: Transforming growth factor β; PDGF: Platelet-derived growth factor; HSC: Hepatic stellate cell; ECM: Extracellular matrix; PV: Portal vein; LPS: Lipopolysaccharides; IL: Interleukins; Treg: Regulatory T cell; MDSC: Myeloid-derived suppressor cells; NK: Natural killer cells.

highlighted the importance of underlying liver disease to overall prognosis in HCC. In this study the gene expression of the tumour was not associated with overall survival but the gene expression in adjacent non-tumour liver tissue correlated strongly with survival. More recently the severity of underlying liver disease as a prognostic marker has been highlighted with a study focusing on a scoring system based on bilirubin levels and albumin values. Johnson *et al*[68] developed a model incorporating just bilirubin and albumin levels called the ALBI score (Figure 2) which was an accurate discriminatory method for assessing liver function in HCC. Within the Child-Pugh class A patients, the ALBI score was able to differentiate patient groups with different prognoses. The model across a database of 3887 patients identified a median ten month difference in survival between ALBI grade 1 and ALBI grade 2 within the Child-Pugh class A group for European and US patients[64].

Patients who present with early HCC are amenable to curative treatments including surgery (resection or transplant) and local ablative therapies. Those with more intermediate stage HCC have non curative options such as transarterial chemoembolisaton which has been shown to improve survival in randomised control trials[69]. A significant proportion of patients present with advanced incurable disease; these patients have a poor prognosis and the only licensed medical treatment has been the multi-kinase inhibitor Sorafenib[70]. The SHARP trial demonstrated an improved survival in patients with advanced HCC who took Sorafenib but this was by only a median of three months. Several other agents have been studied in randomised trials but none have successfully demonstrated superiority to Sorafenib but most recently a phase 3 trial demonstrated Lenvatinib, an inhibitor of VEGF receptors 1-3, FGF receptors 1-4, PDGF receptor alpha, RET and KIT, had similar efficacy to Sorafenib[71]. Trials in which Sorafenib has been combined with other treatment such as TACE have not been successful in improving efficacy of these treatments[72]. Until recently no second line agents in randomised trials had demonstrated clinical benefit after Sorafenib therapy, but a recent trial with Regorafinib, another multikinase inhibitor, has finally demonstrated improved survival with a second line agent[73]. This has led to FDA approval for patients who have failed therapy with Sorafenib but overall survival for advanced HCC remains poor. These experiences have provided impetus to explore immunotherapy in HCC. We have already described that progressive HCC is associated with an immunosuppressive microenvironment. Recent early stage trials with checkpoint inhibitors which activate T cells have shown promising results. Checkpoint inhibitors currently include cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) blockers and inhibitors of programmed cell death protein-1 (PD-1)/programmed cell death protein ligand-1 (PDL-1) interaction with

ALBI-score

[log$_{10}$ bilirubin (μmol/L) $\times$ 0.66] + [albumin (g/L) $\times$ -0.085]

ALBI grade is defined by the resulting score:

Grade 1 $\leqslant$ -2.60

Grade 2 > -2.60 to $\leqslant$ -1.39

Grade 3 > -1.39

Figure 2 Formula to calculate the ALBI-score and translate the result to ALBI grade[64].

three studies being reported in the context of HCC. One study with 30 patients involved the administration of Tremelimumab, a CTLA-4 blocker, combined with ablative therapy, showed some patients did demonstrate immune responses and led to the accumulation of CD8+ T cells in tumours. A further study with the same drug was performed with 20 patients and demonstrated both anti-tumour and antiviral activity[74]. Recently, a large phase II trial (CheckMate-040 trial) with the agent Nivolumab (anti-PD-1 drug) has led to significant attention because of strong anti-tumour responses, leading to improved survival and led to accelerated approval by the FDA in 2017 for the treatment of patients after failure with Sorafenib[75].

Novel therapeutics which alter T-cell regulation are now being pursued for HCC. A study by Sia *et al*[76] looked at over 900 HCC samples and identified that around 25% exhibited high expression levels of programmed death ligand-1 (PD-L1) and programmed cell death protein 1 (PD-1) and a subgroup expressed many genes that are regulated by TGF β1, a cytokine which is linked to aggressive cancers and suppresses the immune response. TGF-β is involved in cell proliferation, angiogenesis, migration, immune infiltration, metastases dissemination, and drug resistance[77]. There are ongoing trials in HCC using the TGFβ1 inhibitor Galunisertib. The phase 2 trial using Galunisertib as monotherapy has shown promise with improved overall survival in AFP responders[78]. We are entering an era where we may be able to identify which tumours are most likely to respond to immunotherapy, tailoring treatment to the tumour biology.

With these advances in treatment of advanced HCC, clinicians will need to consider how best to manage the underlying chronic liver disease that is associated with HCC. Attempts to improve liver function will have the aim of (1) increasing the number of patients eligible for these novel therapies; (2) to minimise the potential liver related side effects of these novel agents and (3) to prolong the overall survival in patients whose tumours respond to these agents.

Management approaches of chronic liver disease for patients with HCC

Targeting the initiating factors of chronic liver disease can significantly improve liver function in patients even when they have established cirrhosis. Animal models have demonstrated that severe fibrosis can undergo resolution and healing through cell-mediated mechanisms including reducing the number of activated HSCs and the contribution of macrophages[79]. Patients with identifiable factors such as excess alcohol intake and metabolic syndrome benefit significantly from becoming abstinent and improving their metabolic risk factors respectively. Those with active chronic hepatitis B undergo significant improvement of liver function by suppressing viral replication. The treatment of HCV has seen dramatic improvements with the advent of direct acting antivirals[80]. Significant improvement of fibrosis has also been demonstrated in patients with HBV and HCV antiviral medication[81]. One assumption would be that the clearance of Hepatitis C would be beneficial in patients to improve liver function and reduction of future HCC recurrence. This is countered by recent reports suggesting that the viral clearance of HCV could alter immunological surveillance of tumour cells. Case series have described aggressive HCC in patients with cirrhosis after completing successful treatment of hepatitis C[82]. It is not possible to draw conclusions from these findings currently and further studies are required to clarify the situation.

In addition to treating the cause of chronic liver disease, there may also be benefit in preventing the complications of cirrhosis. Variceal bleeding is a complication of cirrhosis associated with a very high mortality rate (20%)[83]. It is well established that patients with HCC have a higher mortality rate compared to matched cirrhosis groups[84,85]. Ripoll *et al*[86] in their study confirmed this and furthermore demonstrated that less than half of HCC patients who were eligible for primary prophylaxis for bleeding were actually prescribed this medication. They also suggested that secondary prophylaxis improved survival in HCC patients.

Loss of muscle mass and function is provided the term sarcopenia and is frequently seen in advanced liver disease. The prevalence is estimated to be between 40%-70% in patients with cirrhosis alone[87]. The underlying mechanism is complex and not fully understood but includes inadequate intake, malabsorption and abnormal metabolism favouring proteolysis for gluconeogenesis. The addition of anti-cancer therapies can further compound the situation. A study looking at whether sarcopenia predicts the prognosis of patients treated with Sorafenib showed that skeletal muscle depletion is an independent prognostic factor[88]. The same has also been shown to apply to patients who have undergone transarterial chemoembolisation treatment for their HCC[89]. Although there is no evidence that sarcopenia is impacting on HCC progression, there is an impact on outcomes and therefore it should be recognised and addressed as part of the patient management pathway.

Carcinogenesis: Prevention is better than cure

With the aim to reduce the complications of cirrhosis

Table 2 Summary of some of the antifibrotic agents being actively pursued in clinical trials, outlining mechanism and key outcomes to date

Drug	Mechanism	Comment	Ref.
BMS-986263/ND-LO2-s0201	siRNA that inhibits HSP47, reducing type 1 collagen synthesis	A lipid nanoparticle containing siRNA that inhibits HSP47. Vitamin A conjugated to the nanoparticle surface target facilitating targeted delivery to HSC and preclinical studies suggest disruption of collagen synthesis which may reverse fibrosis. A phase 1 study has demonstrated tolerability	[94]
Simtuzumab	Inhibition of Lysyl oxidases (LOX) mediated collagen cross linking reduces the breakdown of collagen by proteases such as MMPs	Simtuzumab is a humanized monoclonal antibody. It binds to LOXL2 and acts as an immunomodulator. However in a large phase 2 clinical trial in patients with NASH fibrosis the results were disappointing and focus has been diverted to LOXL1 inhibition where expression appears constant in carbon tetrachloride induced fibrosis in mouse models	[95,96]
Selonsertib	Inhibits apoptosis signal-regulating kinase 1 which in the setting of oxidative stress activates pathways which lead to fibrosis	In patients with NASH has shown promise that it may lead to a reduction in fibrosis in a phase 2 trial where it was given with and without Simtuzumab and compared to Simtuzumab alone	[97,98]
Cenicriviroc	Dual antagonist of C-C motif chemokine receptor (CCR) types 2 and 5	Demonstrated anti-fibrotic activity in animal models of liver fibrosis. In a phase 2 study improvements were seen in noninvasive markers of hepatic fibrosis. Antagonism of CCR2 reduces pro-inflammatory monocytes and macrophages. CCR5 antagonism impairs the activation of HSCs	[99-101]
Emricasan	Inhibitor of apoptotic and inflammatory caspases	Emricasan in the murine NASH model attenuated HSC activation. In phase 2 clinical trials for the regression of hepatic fibrosis caused by HCV infection after liver transplantation, the study didn't reach its primary endpoint but the results from a phase 2 trial in NASH are awaited	[102]
GR-MD-02	Targets galectin-3	Phase 3 studies are already planned for GR-MD-02. Phase 1 and 2 have been completed in the NASH cohort. Preclinical data showed some reversal of fibrosis and a reduction in portal pressures in cirrhosis	[103]
Erlotinib	Epidermal growth factor (EGF) receptor inhibitor	In preclinical models the FDA approved inhibitor regressed fibrosis in some animals and blocked the development of HCC. A pilot phase1/2 trial is underway	[104]

including decompensation there has been progress in recent years to try to prevent or reverse liver fibrosis and some evidence that this reduces HCC risk[90]. The WHO recognise that the viral hepatitis pandemic contributes to an estimated 1.4 million deaths per year including HCC and cirrhosis[91]. The published strategy outlines aims of reducing transmission of HCV and creating global access to treatment by 2030.

Identification and addressing of specific aetiologies, such as viral hepatitis and addressing metabolic risk factors in those with NAFLD is clearly essential but there is also a drive to develop specific anti-fibrotic agents. Whether we can reduce carcinogenesis by interrupting or reversing fibrogenic process with specific anti-fibrotic agents remains to be seen but certainly improvement in liver function is desirable to facilitate management and improve outcomes when cancer occurs.

With a better understanding of the processes that lead to fibrogenesis and fibrolysis it is now possible to target the relevant cytokines and effector cells including HSCs, myofibroblasts, profibrogenic immune cells and other ECM targets including collagens and matrix metalloproteinases (MMP) inhibitors like tissue metallopeptidase inhibitor 1. We know that there are collagen types which are increased in liver fibrosis and are potentially targetable by small interfering RNA (siRNA) or antisense oligonucleotides. The therapeutic advantage of these nucleic acid based therapies is that they can now be delivered in vehicles which are preferentially taken up by the cell being targeted achieving knockdown effects only at the site of interest[92,93]

The identification of novel targets in the fibrotic environment has led to the development of therapeutic agents with efficacy in preclinical models and we have seen the first clinical trials take place. We summarise in Table 2[94-104] some of the therapeutic targets being actively pursued for their anti-fibrotic effects. Unfortunately trial design for direct anti-fibrotic agents are not at present designed to assess if they have an effect on HCC prevention.

Pre-clinical experiments have demonstrated inhibiting the pathways for pro-fibrogenic cytokines including

TGF-β and PDGF are also potential anti-fibrotic strategies to be explored further[105]. Integrins are transmembrane receptors that promote ECM adhesion. Some integrins can facilitate MMP activation and activate fibrogenic mediators such as TGF-β1. The identification of integrins which are important in fibrosis has led to mouse studies involving their direct and indirect inhibition[106]. Other preclinical studies are looking at targeting transmembrane collagen receptors, proteins expressed on myofibroblasts and manipulating the inflammatory environment to deactivate matrix producing myofibroblasts and hepatic stellate cells.

CONCLUSION

Hepatocellular cancer is a deadly complication of cirrhosis and remains difficult to diagnose at an early curative stage. The prognosis in advanced cases remains extremely poor despite significant changes in epidemiology and causes of chronic liver disease, with the rising epidemic of obesity. Nevertheless, new studies with novel agents are demonstrating increasing rates of tumour response and stability and there are exciting developments with immunotherapies. The challenge in patients who do respond to anti-cancer therapies is to maintain their underlying liver function in order that intolerable liver related side effects are minimised and patients have the best chance for a prolonged overall survival. Furthermore, standard of care for cirrhosis may not be met in patients with hepatocellular cancer because of assumptions of lack of benefit, but with new treatments leading to improved survival this will need to be reconsidered. There have been significant improvements in elucidating the underlying cellular mechanisms which drive fibrosis and cancer within the liver. Hopefully, new therapies will take advantage of these findings leading to personalised therapeutic combinations for these patients which have the dual effect of promoting fibrosis regression and anti-cancer effects.

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