MINIREVIEWS

Hepatocellular carcinoma and non-alcoholic steatohepatitis: The state of play

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Abstract

Hepatocellular carcinoma (HCC) is now the fifth cancer of greatest frequency and the second leading cause of cancer related deaths worldwide. Chief amongst the risks of HCC are hepatitis B and C infection, aflatoxin B1 ingestion, alcoholism and obesity. The latter can promote non-alcoholic fatty liver disease (NAFLD), that can lead to the inflammatory form non-alcoholic steatohepatitis (NASH), and can in turn promote HCC. The mechanisms by which NASH promotes HCC are only beginning to be characterized. Here in this review, we give a summary of the recent findings that describe and associate NAFLD and NASH with the subsequent HCC progression. We will focus our discussion on clinical and genomic associations that describe new risks for NAFLD and NASH promoted HCC. In addition, we will consider novel murine models that clarify some of the mechanisms that drive NASH HCC formation.

Key words: Non-alcoholic steatohepatitis; Hepatocellular carcinoma; Non-alcoholic fatty liver disease; Models; Mice

Core tip: Non-alcoholic steatohepatitis (NASH) is a metabolic inflammatory disease that can advance to liver cancer. Clinical studies have suggested links between non-alcoholic fatty liver disease, NASH and progression to hepatocellular carcinoma (HCC). Herein, we discuss genomic screens that have illustrated new candidate genes as markers for increased HCC risk. In addition, we present the latest murine models concerning cellular stress and inflammation, which have now been shown to have a role in promoting liver tumor growth.

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INTRODUCTION

Liver cancer is now the fifth cancer of greatest incidence and second leading cause of cancer related deaths worldwide[1]. The major risk factors are hepatitis B (HBV) and hepatitis C (HCV) that promote fibrosis and cirrhosis and ultimately hepatocellular carcinoma (HCC). However, in the last 20 years the rising rates of obesity have led to the development of the metabolic syndrome and in turn non-alcoholic fatty liver disease (NAFLD) and the progressed and inflammatory form non-alcoholic steatohepatitis (NASH). Experimental and population studies have shown links between NAFLD/NASH and HCC formation. However the mechanisms by which NASH transitions to HCC are only beginning to be elucidated. NASH has histological features similar to alcoholic hepatitis, with prominent fatty deposition and fat storage in the liver parenchymal cells, that can promote inflammation and necrosis[2,3]. The initial theory for the pathogenesis of NASH was known as the "two-hit hypothesis"[4]. Here it was suggested that hepatic triglyceride accumulation or steatosis, increased the susceptibility of the liver to the "second injury hit" of changes in inflammatory cytokines and/or adipokines, mitochrondrial dysfunction and elevated oxidative stress, that together promote steatohepatitis and fibrosis. Although still relevant it is now clear that additional factors including genetic disposition, endoplasmic stress, and altered inflammation can contribute to the generation of NASH and HCC. Thus, within this review we give an update of the relevant concepts that have been published in the last 12 mo.

NASH AND ITS RELATIONSHIP TO HUMAN HCC RISK

The rates of obesity and the metabolic syndrome are increasing worldwide, therefore clinical studies have been undertaken to examine for links with NASH and HCC. Indeed, in two separate studies completed by Wong *et al*[5,6], have shown that NASH is the most rapid growing risk for liver transplantation in patients with HCC. In the first study they considered 61868 patients over the period 2002-2012 and found that the proportion of HCV-related HCC increases from 43.4% to 49.9%, whereas NASH-related HCC increased from 8.3% to 13.5%, an increase of near 63%. Furthermore, they found that the number of NASH-HCC patients undergoing liver transplantation increased nearly 4-fold during this period. In their second and following study, they analysed the number of adults awaiting liver transplantation and found that new registrants with NASH increased by 270%, compared to alcoholic liver disease (ALD) 45% and HCV 14%. Importantly, NASH-HCC patients are less likely to undergo liver transplantation and to survive for 90 d on the waitlist than patients with HCV, ALD, or HCV and ALD.

Reports concerning US Veterans revealed differing trends. In Beste *et al*[7], they studied the burden of cirrhosis and HCC from US Veterans for the period 2001-2013. They found during this period the incidence of HCC increased from 17 per 100000 in 2002 to 45 per 100000 in 2012, a rise of 265%, and within this group the increases were largely driven by HCV-related[8].

GENETIC MARKERS OF NASH HCC

As illustrated above HCC is detected later in NASH-HCC patients and treated less effectively. Thus, there is an emphasis within the field to find new predictors of HCC and HCC risk in individuals with NASH. Evidence supports the concept that genetic factors are involved in the predisposition to NAFLD and NASH, and given that up to 25% of NASH can progress to HCC, the identification of these factors could lead to better patient outcomes[9].

Genome wide association studies (GWAS) and candidate gene studies have imparted greatly on our knowledge of the role of genetics in NAFLD/NASH pathogenesis. One of the most studied candidates to date is a single-nucleotide polymorphism (SNP) variant of the gene encoding the enzyme patatin-like phospholipase domain containing protein 3 rs738409 (*PNPLA3*; missense mutation resulting in an isoleucine to methionine substitution at residue 148 [I148M]) that has been shown to be associated with NAFLD pathogenesis across different patient cohorts[10,11]. Critically, in mechanistic studies the overexpression of *PNPLA3*-I148M in cells and mice promoted triglyceride accumulation through the disruption of hydrolysis[12]. In agreement, the ablation of the *PNPLA3* gene in mice resulted in reduced hepatic steatosis and triglyceride content[13]. Separately as well, this SNP has been strongly associated with clinically important factors, which include the strength of liver fibrosis and cirrhosis as well as the risk of developing NAFLD/NASH-related HCC[14-17]. Of special interest is the work of Liu *et al*[16], who showed that the carriage of each copy of the rs738409 (G) allele promoted an additive risk of HCC, where GG homozygouts have a 5-fold greater risk than CC. The link with HCC has been further enforced by a recent Japanese study where the authors showed that SNP rs738409 located in *PNPLA3* was the highest risk factor in their patient cohort. Further stratification of their group showed that the *PNPLA3* G allele was significantly higher amongst HCC patients with type 2 diabetes mellitus. From this group they found a significant association between the *PNPLA3* G allele and the gene for the Juxtaposed with another Zinc Finger Protein 1 (*JAZF1*) rs864745 G allele. JAZF1

functions as a transcriptional repressor and has been associated with the increased risk of prostate cancer and diabetes[18]. Importantly, a recent study has shown that JAZF1 overexpression in mice suppressed lipid accumulation and decreased droplet size, suggesting that JAZF1 plays a critical role in the regulation of lipid homeostasis and possibly in the prevention of NAFLD and NASH, and thus progression to HCC[19]. Taken together these data suggest strong links between SNP rs738409 and increased HCC risk, but the mechanism through which this SNP can promote HCC risk remains open and will require the development of genetic murine models.

In 2014 two separate studies identified a new rs58542926 C>T genetic variant of the transmembrane 6 superfamily member 2 gene (*TM6SF2*), which encoded a loss-function substitution of lysine (E) to glutamic acid (K) at position 167 (E167K)[20,21]. These studies showed that the *TM6SF2* variant E167K was associated with greater serum levels of alanine transaminase, liver injury, and lower levels of low-density lipoprotein-cholesterol (LDL-C), triglycerides and alkaline phosphatase in patients with NAFLD/NASH. Additionally, both papers illustrated mechanistically that *Tm6sf2* regulates lipogenesis, while the treatment of mice with adeno-associated virus-mediated short hairpin mRNA reduced plasma cholesterol and triglyceride levels (VLDL). Also, it was alternatively shown that the overexpression of *TM6SF2* in mice increased fasting cholesterol. In subsequent independent studies it was illustrated that the inhibition of *TM6SF2* in cellular models was associated with reduced secretion of triglyceride rich lipoproteins and increased triglyceride cellular concentration. Conversely *TM6SF2* overexpression decreased cellular triglyceride concentration and decreased the number and size of intracellular lipid droplets[22].

A recent independent study found that *TM6SF2* rs5842926 could be associated with the severity of NAFLD related fibrosis, and this was independent of the potentially cofounding factors that include gender, age, body mass index (BMI), type 2 diabetes and the *PNPLA3* rs738409 genotype[23]. Given that NASH and fibrosis are risk factors for HCC the authors then extended their analyses to determine the contribution of *TM6SF2* rs5842926 to HCC risk. Here they found in univariate analyses that *TM6SF2* was associated with increased risk of progression to NASH-HCC. However, this link was not maintained when the cofounding factors age, type 2 diabetes and the presence of underlying cirrhosis was incorporated into their model.

Despite these interesting findings, separate studies have suggested that *TM6SF2* rs5842926 may in fact represent a special type of NAFLD. Zhou *et al*[24] examined how *TM6SF2* rs5842926 influenced the circulating triacylglycerol lipid signatures and hepatic or adipose insulin sensitivity in patients. They compared the liver fat and circulating fat levels between *TM6SF2* rs5842926 carriers and non-carriers,

and found that the liver fat content was 34% higher in the carriers than the non-carrier group. However, in further investigations the increases in hepatic fat content did not coincide with a decrease in whole body or hepatic or adipose insulin sensitivity. It was in fact associated with reductions in the circulating total levels of triacylglycerol lipids when compared to non-carriers. This led the authors to suggest that the *TM6SF2* variant represents a metabolically silent variant of NAFLD, as the allele is clearly not associated with hypertriglyceridemia that is typically associated with NAFLD patients[25]. Interestingly, these observations also suggest that as *TM6SF2* rs5842926 carriers have reduced levels of circulating lipids, they could also have a reduced risk of cardiovascular disease. In this light, the publication of Dongiovanni *et al*[26], showed that *TM6SF2* rs5842926 carriers had lower serum lipid levels, more-severe steatosis, necroinflammation, ballooning and fibrosis, but were in fact protected from cardiovascular disease. Taken together these data suggest that carriers have greater prevalence for NAFLD related liver disease. Whether these patients go on and develop a higher risk of NASH HCC will need to be determined from larger patients data sets. In comparison, those that have the normal allele of *TM6SF2* have a reduced risk of NAFLD and increased risk of cardiovascular disease. Despite these indifferent data, studies now need to be performed to evaluate whether *TM6SF2* rs5842926 carriers have associations with other SNPs that can then potentiate the characteristic metabolic phenotype observed in NAFLD and NASH patients. The combination of these additional factors may be a harbinger for increased HCC risk.

BIOMARKERS OF NASH HCC

Given the interest in disregulated lipogenesis in predicting NASH, research has turned to analyzing the proteomic and lipid signature of lipid metabolism in NASH-HCC animal models and patients. An interesting study considered the change of these factors in the hepatocyte-specific PTEN (phosphate and tensin homolog deleted from chromosome 10)-deficient mouse model, which generates spontaneous NASH and HCC[27] and the relationship to human NASH-HCC. They identified a HCC signature where there was upregulated specific lipids within the liver and plasma (see within[27] for more detail), and a NASH signature of upregulated hepatic C18 fatty acid producing elongase (*ELOVL6;* is responsible for the elongation of C16 fatty acids) that was associated with elevated oleic, adrenic, and osbond acids, and reduced cervonic acid in the liver and plasma and with tumor burden. However in response, Kessler *et al*[28] found reduced *ELOVL6* gene expression in a majority of human liver tumours in a separate human cohort when compared to non-tumour tissue. They also saw reduced expression of *ELOVL6* gene expression in the

diethylnitrosamine (DEN) HCC model. Nonetheless, the same group then went and extended their observations to the methionine and choline deficient diet model of NASH, and an elevated C18/C16 fatty acid ratio was found. They further focused their experiments to human NASH and NASH-HCC patients and there they observed that the gene expression of ELOVL6 was significantly elevated[29]. Taken together these data suggest that lipidomic analyses may be a useful way to identify NASH patients who may have a higher HCC risk.

Other markers have also been considered for NAFLD/NASH. A Chinese group found that the serum levels of cytokeratin-18 M30 fragment (Ck-18-M30; a marker of hepatocyte apoptosis) fibroblast-21 (FGF-21; related to decreasing blood glucose, lipid and insulin levels, reversing hepatic steatosis and bettering insulin sensitivity), interleukin 1 receptor antagonist (Il-1Ra; reduces liver inflammation) and pigment epithelium-derived factor (PEDF; an independent risk factor of fatty liver disease) increased in NAFLD patients, while the serum levels of osteoprotegerin [a decoy receptor for the receptor activator of nuclear factor kappa B ligand (RANKL)] decreased[30].

MOLECULAR MOUSE MODELS

Given these findings and the numerous mechanisms that have been proposed to promote NASH-HCC a number of interesting and seminal studies have been published in the last 12 mo outlining new models and explanations of NASH HCC biology.

New models

One of the major hypotheses of the field is that HCC develops from NASH through obesity, insulin resistance, the increased release of inflammatory factors and autophagy[31]. However, there is no standard mouse model that addresses some of these changes. Recently, De Minicis *et al*[32], published a new murine model of NASH HCC development that was associated with peripheral insulin resistance. Here they treated mice with combinations of a choline-deficient L-amino-acid-defined-diet (CDAA) and a low dose of carbon tetrachloride (CCl4) and observed that after one month the mice developed peripheral insulin resistance and at 3 mo extensive steatosis in CDAA and CDAA + CCl4 groups. At 6 mo both groups had increased and equal levels of fibrosis and steatosis and HCC. However, by 9 mo all CDAA+CCl4 treated mice had HCC, whereas only 40% of the CDAA group had HCC. Significantly, cirrhosis was absent and there was decreased gene expression of PTEN and positive histology for AKT, c-Myc and glypican-3, similar to that seen in human NASH HCC, suggesting that this model may be valuable for examining the role of potential gene candidates in HCC development.

Another model that has been recently published removed the gene *Trim24* (tripartite motif-containing 24) from all tissues or only from the liver of mice[33]. *Trim24* gene mediates transcriptional control by interacting with the activation function 2 (AF2) region of varied nuclear receptors, such as the estrogen, retinoic acid, and vitamin D3 receptors and is thought to associate with chromatin and heterochromatin-associated factors. Here the authors found that the global loss of Trim24 protein decreased the expression of genes involved in oxidation and reduction, steroid, fatty acid and lipid metabolism and increased the gene expression of genes regulating the unfolded protein response, endoplasmic reticulum stress and cell cycle pathways. Both the total knock-out and liver specific knock-out mice spontaneously developed liver lipid filled lesions, steatosis, injury, fibrosis and HCC. In contrast in humans elevated histopathological expression of Trim24 protein has been associated with multiple cancers, including HCC[34]. Nevertheless, follow-up studies considering the relative gene expression of *Trim24* in human HCC and NASH-HCC have not yet been published. Thus *Trim24*'s association with human HCC remains open.

New mechanisms

Continuing with same theme of gene regulation, new evidence has suggested that over-nutrition and metabolic pathways can promote the dysregulation of chromatin modifiers and histone post-translational modifications to generate abnormal transcriptional activity. Through quantitative expression profiling of 115 chromatin regulators from dietary and genetic obesity-promoted HCC, the histone deacetylase 8 (HDAC8) was found to be significantly upregulated[35]. Promoter analyses revealed the *HDAC8* promoter to have binding sites for the sterol regulatory element-binding protein-1 (SREBP-1), a master regulator of lipogenesis, and both *HDAC8* and *SREBP-1* were upregulated in the livers of mice fed a high fructose high carbohydrate diet. Further work revealed that HDAC8 promoted insulin resistance and tumour growth *in vivo*. Mechanistically, it was found that HDAC8 promoted tumour growth through the activation of β-catenin signaling and the repression of Wnt antagonists, and was associated with primary human NAFLD-associated HCC. Taken together these data suggest that targeting cancer-specific critical chromatin regulators with small molecular inhibitors may aid in treating obesity related HCC.

Fructose

Fructose has been suggested to be a promoter of liver cancer. Earlier studies have suggested links (see our recent review[36]) but a recent report added weight to this concept, showing in mice using the DEN model that fructose can promote HCC. Here the authors injected DEN and subsequently fed the mice with various diets from 6 wk of age until completion at 32

wk. It was found that high fat diets containing lard or coconut oil increased tumor burden by 1.5- and 2.2-fold, respectively. Mice fed a high fructose diet had approximately 2-fold more tumor burden than control mice. In all diet groups, AMPK and mTORC1 activity was detected, but no diet specific changes were noted for sirtuin 1 (SIRT1) and p53, suggesting that other mechanisms are responsible for the enhanced tumor growth[37].

Endoplasmic stress

The DEN model of HCC has been well utilized and has been shown in conjunction with a high fat diet (HFD) to promote low-grade liver inflammation in association with tumor necrosis factor (TNF) and interleukin-6 (IL-6) expression. The absence of these factors in mice further enhances HCC growth, however in wild-type mice the progression to NASH does not occur[38]. In an effort to identify factors that promote the progression to NASH, Nakagawa and colleagues[39] studied the role of endoplasmic stress (ER) in HCC progression. To do this they utilized the major urinary protein-urokinase plasminogen activator (*MUP-uPA*) transgenic mouse model. This mouse overexpresses the uPA protein that then accumulates in the hepatocyte endoplasmic reticulum, to promote ER stress and hepatic lesions. Compared to wild-type mice the MUP-uPA mice when fed a HFD developed NASH-like disease that spontaneously progressed to HCC. They observed the upregulation of several stress ER markers in the hepatocytes of *MUP-uPA* mice. These included C/EBP homologous protein (CHOP), glucose-regulated protein 78 (GRP78), spliced x-box binding protein (sXBP1), phosphorylated Eukaryotic Initiation Factor 2 (p-eIF2), phosphorylated inositol-requiring enzyme 1α (p-IRE1α), and phosphorylated janus kinase (p-JNK). ER stress also promoted SREBP1 expression, to enhance lipogenesis and ultimately reactive oxygen species production that in turn affects genomic instability. The authors also identified that inflammation played a role as TNF production was increased in HFD-fed *MUP-uPA* livers and was localized to macrophages. Subsequent breeding of the *MUP-uPA* mice with TNF receptor (TNFR1)-deficient mice to generate *MUP-uPA* TNFR1^{-/-} mice, and subsequent HFD feeding reduced HCC development as compared to *MUP-uPA* mice. Finally, the treatment of *MUP-uPA* mice with the TNF antagonist Etanercept (commercial name: Enbrel) reduced tumour growth by 2-fold, suggesting that the application of such a therapy may be useful for NASH and NASH-HCC patients.

In a separate report the role of Gp78, an E3 Ubiquitin Ligase has been examined in NASH and liver cancer[40]. Gp78 regulates endoplasmic reticulum-associated degradation by ubiquitinating misfolded ER proteins. Normally, misfolded proteins are exported from the ER to the cytosol where they are degraded by the ubiquitin-proteasome machinery, of which Gp78 is a part. If the unfolded protein response is delayed or insufficient there may be pathological outcomes such as that seen in the CHOP knock-out mouse model where HCC is potentiated after treatment with DEN[41]. Here the authors found that the ablation of Gp78 induced obesity, hepatic steatosis and inflammation in aged mice. Furthermore they observed that the loss of Gp78 induced steatosis through SREBP-1 activation. Finally, they found that 20% of the mice developed liver tumours, suggesting that Gp78 could be considered as a tumour suppressor. Given these data they decided to explore whether Gp78 could be used as a progression marker and from a tissue array of human HCC, they found that Gp78 expression was significantly lower in the tumour than in normal liver tissues. In sum these data suggest a role for Gp78 in NASH HCC, and further studies are required to readily associate it with human NASH HCC pathology.

Inflammation

A major feature in the transition from NAFLD to NASH is the appearance of hepatic inflammation. A factor that is known to exacerbate NAFLD and NASH is choline deficiency. In humans, low levels of choline intake are associated with reduced hepatic lipoprotein secretion, mitochondrial dysfunction and thus oxidative damage, and ER stress. Given that the injection of mice with DEN combined with HFD promoted more HCC and increased hepatic inflammation, a study was instigated where mice were given a choline deficient diet in combination with a HFD diet (CD-HFD)[42]. It was found that these mice attained features of the metabolic syndrome, hepatic damage and NASH, and the hepatic infiltration and activation of immune cells. Most notably there were increased numbers of CD8^{+} T cells and natural killer T cells (NKT), similar to that seen in NASH patients. Through a series of elegant mouse experiments the authors showed that NKT cells mediate hepatic lipid uptake through the secretion of LIGHT (lymphotoxin-like, exhibits inducible expression, and competes with HSC *g*lycoprotein D 9(gD) for HVEM, a receptor expressed by T lymphocytes) and subsequent activation of the lymphotoxin-β receptor on hepatocytes. Importantly, they showed that the CD8^{+} T cells and NKT cells regulate the formation of NASH and NASH induced HCC in mice, and that the elevated expression of LIGHT, CD8^{+} T cells and NKT was associated with steatosis related human liver diseases. In this light, the data suggest that a therapy targeting the reduction of CD8^{+} T cells and/or NKT in NASH patients has the potential to reduce disease progression, minimize hepatic damage and ultimately NASH HCC.

New concepts

Normally, hepatocytes have the ability to increase the number of chromosome sets, a process called polyploidization, to result in an increase in the number

of nuclei per cell, being diploid (binuclear), tetratploid (4 nuclei) and octoploid (8 nuclei). This phenomenon is not completely understood, but has also been shown to be associated with genomic stress and pathologies like cancer[43,44]. Apart from promoting ER stress, NAFLD and NASH can promote oxidative stress and this is believed to be in part through cytochrome P450 2E1 (CYP2E1) as it metabolises C_{10}-C_{20} fatty acids, that in turn generates ROS[45]. In a recent report it was shown that oxidative stress can promote pathologic polyploidization in murine models of NASH and patients samples[46], and was associated with the activation of a G_2/M DNA damage checkpoint. Interestingly, they treated leptin deficient mice with an antioxidant *in vivo* and the extent of oxidative stress and polyploidy was greatly reduced. Taken together these data suggest that polyploidy may be one of the early events of NAFLD and could contribute to the progression to NASH.

Potential therapeutics

Currently only one pharmacological agent the multi-kinase inhibitor Sorafenib, prolongs the survival of patients with HCC. Nonetheless, median survival is only improved by a paltry 12 wk, emphasizing an urgent need to develop new and improved therapies[47,48]. A factor that has been shown to contribute to NASH pathogenesis is excess free cholesterol. Given that statins are readily prescribed to reduce cholesterol synthesis and thus serum levels, a recent study has collated statin use and NASH risk[49]. Here the authors examined the relationship between statin use, genetic risk factors and hepatic damage of 1201 European patients. Statin use was noted in 107 patients, and it was found that these patients were protected from steatosis, NASH and fibrosis stage F2-F4. Significantly, in statin treated patients, steatosis was reduced by 48%-91% and NASH decreased by 36%-76% and significant fibrosis was nearly halved. Nevertheless, with regards to genetic predisposition, the presence of the I148M *PNPLA3* allele blunted the effect of statins. Taken together, the use of statins is beneficial in NASH patients without the I148M *PNPLA3* allele and will thus likely reduce NASH HCC risk.

The mammalian target of rapamycin (mTOR) has been identified to promote growth in a majority of liver cancers[50]. mTOR consists of two distinct complexes, mTORC1 and mTORC2, and mTORC1 is sensitive to rapamycin and activates down-stream targets to regulate cellular growth and metabolism that is activated by nutrients and growth factors[51]. Moreover, studies have shown that chronic mTORC1 activation is associated with obesity, hepatosteatosis and insulin resistance[52]. Likewise mTOR has been shown to play a key role in liver cancer growth, and agents such as rapamycin and its analogs Everolimus and Temsirolimus, which suppress mTORC1 have been trialed to treat HCC[53]. However, the results to date using rapalogs as a single-agent cancer agent in human HCC have not been encouraging. By example, a phase 3 study showed that Everolimus increased the incidence of hepatic injury and did not extend survival in patients with advanced HCC[54-57]. To examine for reasons why mTORC1 inhibition is not suitable in patients, Umemura *et al*[58] treated mice with rapamycin and generated mice with the hepatocyte specific deletion of the mTORC1 subunit raptor. They found that rapamycin treatment increased hepatocyte cell death, IL-6 expression and signal transducer and activator of transcription 3 (STAT3) activation, and reduced SREBP1 and IL-10 expression, and in sum increased hepatic inflammation. Similarly, the loss of Raptor in the hepatocytes promoted cell hepatocyte cell death and inflammation, and significantly after the injection of DEN, enhanced obesity-promoted hepatocarcinogenesis. Further, they found in these mice, that Raptor deficiency promotes liver fibrosis and does not improve metabolic parameters in obese mice. Given these data and the clinical trial data, single mTOR inhibition approaches are not useful for treating HCC and NASH HCC. However, it is likely that due to feedback mechanisms that AKT activation caused by mTOR inhibition can be a culprit of these phenotypes. Moreover, it remains to be determined whether a more targeted or a combinatorial approach could be useful to treat NASH HCC.

Finally, given that NASH HCC is related to over-nutrition an obvious therapeutic approach would be to increase energy utilisation. Here hepatocyte specific PTEN-deficient mice that spontaneously develop steatohepatitis and HCC were divided into sedentary or exercise groups[59]. After 32 wk of regular exercise, 71% of the exercised groups developed tumor nodules greater than 15 mm^3, whereas 100% of the sedentary group developed tumors of this size. Significantly, the number of tumours and tumour volume was reduced in the exercise group. There was no change in hepatic steatosis in the exercise group, tumor cell proliferation decreased and the phosphorylation of AMPK and raptor increased. Taken together, these data suggest that exercise can reduce HCC development and load in a mouse model, and suggest that patients who have a higher risk for NASH HCC, could increase their physical activity to prevent HCC development.

CONCLUSION

Taken together here we have reflected on the strength of clinical data and genetic mutations found in patients that illustrate the connection between NAFLD and NASH, and increased liver cancer risk. Significantly, in the last 12 mo studies have come together to suggest that *TM6SF2* rs5842926 carriers have a silent phenotype. It will be interesting to see if other mutations are associated with these carriers to promote progression towards HCC. Significantly,

at the molecular level, cellular and murine models have shown the importance of gene modifications, cellular stress and inflammation in driving the HCC progression. It remains open whether therapeutics can be developed with limited off target effects to limit HCC growth. In this light, for many years it was assumed that limiting the activity of the mTOR pathway would be a strategy to treat liver cancer. Clinical trials targeting mTOR in HCC have been disappointing. Moreover, studies presented here now show that targeting mTOR can promote inflammation and in the instance of genetic ablation of Raptor, increase HCC load after DEN injection. Nevertheless, these studies have not addressed the issues of feedback through the mTOR pathway and thus it remains open, whether mTOR targeted therapy in conjunction with other therapeutics or simply a better delivery system could be used to treat NASH HCC patients. In sum, much is needed to be done to further our understanding of the mechanisms in play that promote NASH HCC and to develop new therapeutics to treat this devastating disease.

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