# Liver Cancer: Connections with Obesity, Fatty Liver, and Cirrhosis

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## Keywords

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## Abstract

The burden of hepatocellular carcinoma (HCC), the most common form of liver cancer, is steadily growing because obesity, type 2 diabetes, and nonalcoholic fatty liver disease (NAFLD) are replacing viral- and alcohol-related liver disease as major pathogenic promoters. The most worrisome aspects of these new risk factors are their large spread in the general population and their link with HCC arising in noncirrhotic livers. HCC may be the presenting feature of an asymptomatic nonalcoholic steatohepatitis (NASH), the progressive form of NAFLD. The HCC risk connected to metabolic factors has been underestimated so far, and a poorer surveillance has prevented an adequate treatment. Systemic and hepatic molecular mechanisms involved in obesity- and NAFLD-induced hepatocarcinogenesis as well as potential early markers of HCC are being extensively investigated. This review summarizes current evidence linking obesity, NAFLD and liver cancer, discusses its clinical impact and describes the main mechanisms underlying this complex relationship.

## INTRODUCTION

**HCC:** hepatocellular carcinoma

**HBV:** hepatitis B virus

**HCV:** hepatitis C virus

**NAFLD:** nonalcoholic fatty liver disease

**NASH:** nonalcoholic steatohepatitis

**MetS:** metabolic syndrome

**LT:** liver transplantation

**BMI:** body mass index

**T2DM:** type 2 diabetes mellitus

The prevalence of obesity has reached epidemic proportions, with a steep increase in recent decades. Besides eliciting metabolic and cardiovascular disorders, obesity is also an established risk factor for several types of cancer, predominantly in the gastrointestinal tract, where the strongest associations are with pancreatic and liver cancer (1). Primary liver cancer is the fifth most common malignancy worldwide, and it has a yearly fatality ratio of approximately 1, indicating that most cases do not survive a year. Hepatocellular carcinoma (HCC) accounts for 70–85% of the total liver cancer burden and usually develops within a background of advanced chronic liver disease, mainly related to hepatitis B virus (HBV), hepatitis C virus (HCV), and alcohol abuse. HCC is increasing in the United States and in other countries, with an age-adjusted incidence rising from 1.5 to 4.9 per 100,000 individuals in the past 30 years (2). Although half of this increase is attributed to long-term HCV exposure between 1960 and 1970, approximately 15–50% of HCC cases remain idiopathic; however, recent studies identified nonalcoholic fatty liver disease (NAFLD) as the underlying cause in 13–38.2% of patients presenting with HCC unrelated to virus and alcohol (3).

The term NAFLD was coined by gastroenterologists almost 20 years ago to define a spectrum of progressive liver disease that encompasses simple steatosis, nonalcoholic steatohepatitis (NASH—histologically characterized by steatosis, necroinflammation, and/or fibrosis) and, ultimately, cirrhosis. NAFLD is tightly associated with the metabolic syndrome (MetS) and affects up to one-third of the general population, whereas NASH is diagnosed in 5–7% of individuals (4). These percentages considerably increase in the high-risk groups of obese and diabetics. Although estimates vary, one large European study found NAFLD in 94% of obese patients [body mass index (BMI) >30 kg/m$^2$], including 25% with NASH, and an overall prevalence of NAFLD in patients with type 2 diabetes mellitus (T2DM) ranging from 40% to 70% (5). NAFLD currently represents the most common liver disease in Western countries, with a burden of NAFLD-related cirrhosis about twice as high as that caused by chronic hepatitis C, and is projected to be the principal reason for liver transplantation (LT) within the decade (6). Although cirrhosis is a major risk factor for liver cancer in humans, a growing burden of evidence demonstrates that HCC can develop in individuals with NAFLD who do not have cirrhosis (7). The aim of this review is to detangle the complicated relationship between liver cancer, obesity, and NAFLD and to describe its clinical impact.

## HEPATOCELLULAR CARCINOMA AND OBESITY

Obesity makes a large contribution to overall HCC burden, either alone or as cofactor, as highlighted by several large-scale epidemiological studies (1, 8–12) (**Table 1**).

In a cohort of 900,000 American adults, the risk of dying from liver cancer was 4.5 times higher in men with a BMI of 35 kg/m$^2$ or above compared to the group with a normal BMI (18.5–25 kg/m$^2$) (1). In a longitudinal cohort study including 578,700 subjects, obesity and MetS predicted the development of HCC over the 12-year observation period, with a relative risk (RR) of 1.39 and 1.35, respectively (13). Besides BMI itself, weight gain in adulthood increases the risk for HCC by up to 2.5-fold (14). A recent meta-analysis including more than seven million participants concluded that the summary RR of liver cancer was 17% for overweight subjects and 89% for the obese, with an average 24% increase in risk for each 5 kg/m$^2$ increase in BMI (15). In this setting, the importance of the pattern of fat accumulation has been addressed by only a few studies. In the EPIC (European Prospective Investigation into Cancer) cohort, the waist-to-hip ratio (WHR), a rough estimate of abdominal fat, predicted better than BMI the incidence of HCC over an 8.6-year follow-up and conferred a threefold HCC risk to subjects in the upper tertile of

**Table 1 Principal studies of the association between hepatocellular carcinoma, obesity, and type 2 diabetes mellitus**

| Reference | Study subjects | Main findings |
|---|---|---|
| ***HCC and obesity*** | | |
| 8 | Population-based cohort of 28,129 obese hospital patients | SIR 2.4 (95% CI 1.6–3.4) in obese patients |
| 9 | 19,271 patients with cirrhosis. HCC incidence was 3.4% (n = 659) | Higher risk of HCC in obese patients with alcoholic or cryptogenic cirrhosis. RR 1.65 (95% CI 1.22–2.22) |
| 1 | Cohort of 900,000 American adults | Risk of dying from HCC was 4.5 times higher in men with BMI >35 kg/m$^2$ compared to the group with normal BMI. RR 2.41 in men (95% CI 1.92–3.01) and 1.47 in women (95% CI 1.08–2) |
| 10 | Prospective cohort studies of London-based government employees (n = 18,403) | RR in obese subjects 3.76 (95% CI 1.36–10.4) |
| 11 | Cohort of 781,283 Korean men with 10 years of follow-up | RR in obese patients 1.56 (95% CI 1.15–2.12) |
| 12 | 362,552 Swedish men followed from 1971 to 1999 | RR in obese patients 3.62 (95% CI 2.62–5) |
| 15 | Meta-analysis of cohort studies of excess body weight and risk of HCC from 1966 to 2007 | Summary RR of HCC was 1.17 (95% CI 1.02–1.34) for overweight subjects and 1.89 (95% CI 1.51–2.36) for the obese |
| 16 | 23,820 Taiwanese subjects followed up for 14 years | Obesity was associated with a RR of HCC of 4.13 in anti-HCV subjects (95% CI 1.38–12.4) and of 2.36 (95% CI 0.91–6.17) in patients without viral infections. The presence of both obesity and diabetes increased more than 100-fold the risk of HCC in HBV or HCV carriers |
| 20 | 185 HCC cases and 404 controls enrolled from 1999 to 2003 | OR in obese patients 1.9 (95% CI 0.9–3.9) |
| 13 | Cohort study with 578,700 subjects; 266 subjects developed HCC during the 12-year observation period | A high BMI and MetS (RR 1.39 and 1.35, respectively; CI 95% 1.24–1.58 and 1.12–1.61) were confirmed as risk factors for HCC |
| 14 | European multicenter trial with 359,525 subjects | Weight increase in adulthood was found to be a risk factor for HCC (RR 2.48, 95% CI 1.49–4.13) |
| ***HCC and type 2 diabetes mellitus*** | | |
| 21 | Danish population-based study including discharge records of 109,581 individuals hospitalized with a diagnosis of diabetes | Increased risk for HCC among patients who had diabetes alone (SIR 4 in males, CI 95% 3.5–4.6; 2.1 in females, 95% CI 1.6–2.7) |
| 19 | 173,643 patients with diabetes and 650,620 patients without diabetes included between 1985 and 1990. Follow-up through 2000 for the occurrence of chronic NAFLD and HCC | The incidence of HCC increased among diabetics (2.39 versus 0.87 per 10.000 person-years, respectively). Diabetes increased the risk for HCC (HR 2.16, 95% CI 1.86–2.52) |
| 18 | 2,061 HCC patients and 6,183 noncancer controls from the Surveillance Epidemiology and End-Results Program-Medicare linked database. Population-based case-control study | The proportion of HCC patients who had diabetes (43%) was significantly greater than controls (19%). Diabetes increased the risk for HCC (adjusted OR 2.87, 95% CI 2.49–3.30) |
| 17 | Questionnaire to 97,771 Japanese subjects | Diabetes led to a higher risk of HCC in males and females (HR 2.24, CI 95%, 1.64–3.04 for males; HR 1.94, CI 95%, 1.00–3.73 for females) and was more strongly associated with development of HCC among malignant tumors |

(*Continued*)

**Table 1** (*Continued*)

| Reference | Study subjects | Main findings |
|---|---|---|
| 20 | 185 HCC cases and 404 controls enrolled from 1999 to 2003 | OR in diabetic patients 3.7 (95% CI 1.7–8.4) |
| 22 | Japanese meta-analysis | Diabetes increased the risk of HCC (OR 3.64, 95% CI 2.61–5.07) |

Abbreviations: BMI, body mass index; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; RR, relative risk; SIR, standardized incidence ratio.

WHR (14). Given this intriguing preliminary evidence, the role of visceral rather than generalized adiposity in HCC risk certainly deserves ad hoc studies.

Overweight and obesity are also well-known cofactors for HCC development in patients with viral or alcohol-related chronic liver disease, although their importance is often underestimated. In a Taiwanese population study, obesity was associated with a twofold risk of HCC in patients without viral infections but with a fourfold risk in anti-HCV-positive subjects. A synergistic effect has been observed: when both obesity and diabetes are present, the risk of HCC in HBV or HCV carriers can increase more than 100-fold, indicating that screening for metabolic conditions in subjects with chronic viral liver disease is not trivial (16).

The contribution of T2DM to the overall risk of HCC seems to be independent of obesity (**Table 1**). In a large cohort study, the risk of malignancy was 20% higher in patients with T2DM than in those without and was mostly due to the development of HCC (17), with a hazard ratio (HR) of 2.24 in males and 1.94 in females. Similar conclusions were drawn in a case-control series (18), where T2DM was twice as prevalent in HCC patients as in controls (43% versus 19%) and increased threefold the risk for HCC. In a longitudinal study (19) on a large cohort of patients with or without T2DM (173,643 and 650,620 subjects respectively), after 10–15 years the incidence of HCC was twofold higher in diabetics (2.39 versus 0.87 per 10,000 person-years, respectively), and was proportional to follow-up duration (RR was 1.55 for patients who had <5 years of follow-up, 1.50 for those with 5–10 years, and 2.03 for those with >10 years). Although further evaluation is required to draw definite conclusions, it is estimated that T2DM can increase the risk of HCC by roughly two- to threefold, and the development of HCC is the most worrisome liver-related complication in diabetic patients.

## HEPATOCELLULAR CARCINOMA AND NONALCOHOLIC FATTY LIVER DISEASE

Both obesity and T2DM per se exert a carcinogenic potential, but the underlying presence of NAFLD and NASH is usually underestimated, and HCC may be the presenting feature of a clinically insidious and asymptomatic liver disease. The evidence that the development of HCC is part of the natural history of NAFLD derives from heterogeneous studies performed in subjects with a clinical diagnosis of NAFLD, with biopsy-proven evidence of NASH, or with NASH-related cirrhosis or cryptogenic cirrhosis (**Table 2**).

### HCC in NAFLD-Related Cirrhosis

The first report on HCC complicating NAFLD with cirrhosis was published in the 1990s. Further studies indicated that although steatosis has an almost negligible effect on liver-related mortality, NASH is a risk factor for the development of cirrhosis and HCC (23).

**Table 2 Principal studies of the association between NAFLD/NASH and hepatocellular carcinoma**

| Reference | Study subjects | Main findings | Comments |
|---|---|---|---|
| ***HCC in NAFLD/NASH-related cirrhosis*** | | | |
| 32 | 44 CCs among 641 cases of cirrhosis-associated HCC compared with viral- and alcohol-associated HCC | The group with CC-related HCC had a higher prevalence of T2DM and obesity. High TG, T2DM, and normal liver enzymes were independent risk factors for CC-associated HCC | NAFLD-related features are more frequent in HCC arising in CC than in HCC of viral or alcoholic origin |
| 3 | 105 HCC patients. HCV (51%) and CC (29%) were the most common etiologies. 50% of CC-related HCC resembled NAFLD | Patients with CC-related HCC were less likely to have undergone HCC surveillance and had larger tumors at diagnosis. NAFLD accounted for at least 13% of the cases | HCV and CC account for the majority of HCC cases. Surveillance is mandatory to detect HCC amenable to surgery but is rarely done in CC-related cases |
| 31 | 210 patients with chronic liver disease who underwent resection for HCC | 18 (8.6%) had CC. The prevalence of obesity, T2DM, AST/ALT ratio <1, and steatosis >20% was higher in CC-related HCC than other etiologies | Obesity and T2DM may be important risk factors for HCC, via NAFLD and CC |
| 36 | Adult patients with cirrhosis secondary to chronic HCV (n = 315) or NASH (n = 195) were evaluated | 25/195 (12.8%) of NASH-cirrhotic and 64/315 (20.3%) of HCV cirrhotic patients developed HCC (yearly cumulative incidence, 2.6% versus 4.0%) | Patients with NASH cirrhosis have an increased risk of HCC |
| 35 | Multicenter Japanese study in 87 HCC cases on histologically proven NASH | Obesity, T2DM, dyslipidemia, and hypertension were present in 62%, 59%, 28%, and 55% of patients, respectively. In nontumor liver tissues, the degree of fibrosis was stage 3–4 in 72% of cases. The prevalence of cirrhosis was lower in male patients (39% versus 70% in women) | Most patients with NASH who develop HCC are men, with features of MetS, who develop HCC at a less advanced stage of liver fibrosis than do women |
| 37 | Retrospective cohort study in a public Japanese hospital (6,508 patients with NAFLD diagnosed by ultrasound) | 16 (0.25%) new cases with HCC were diagnosed. The annual rate of HCC was 0.043%. The independent risk factors for HCC were raised AST level, platelet count $<150 \times 10^3$/ml, age ≥60 years, and T2DM | Low annual incidence of HCC among Japanese with NAFLD. Elderly NAFLD patients with T2DM, high AST, and low platelets are at higher risk |
| 64 | 1,500 Veterans Administration hospital patients who developed HCC from 2005 through 2010 | NAFLD accounted for 8% of total cases. Cirrhosis was less common in NAFLD-related cases than alcoholic or HCV-related HCC. A higher percentage of NAFLD-related HCC patients were not diagnosed during surveillance programs, and a lower proportion received HCC-specific treatments, but 1-year survival was not different | NAFLD is the third most common risk factor for HCC |
| 70 | A retrospective analysis of 10,061 adult LT recipients in the United States (2002–2012) for HCC | NAFLD is the second leading indication for HCC-related LT. From 2002 to 2012, the number of patients undergoing LT for NASH-related HCC increased fourfold, much more than for any other HCC etiology | NAFLD is the most rapidly growing indication for LT in HCC cases in the United States |

(*Continued*)

**Table 2** (*Continued*)

| Reference | Study subjects | Main findings | Comments |
|---|---|---|---|
| 38 | 33,782 cases with HCC in 53 tertiary care centers in Japan from 1991 to 2010 (5,326 non–virus related; 596 NAFLD related) | The proportion of non-virus-related HCC cases increased from 1991 to 2010 (10.0% to 24.1%). The proportion of cirrhosis was lower in NAFLD-related cases | Most cases of nonviral HCC are related to lifestyle factors, including obesity and T2DM |
| ***HCC in noncirrhotic liver with NAFLD/NASH*** | | | |
| 33 | Cohort of 31 HCC patients with MetS as the only risk factor for liver disease | Mild or no fibrosis in most cases compared to those harboring HCC associated with an overt cause of liver disease (65% versus 26%, $p < 0.0001$). HCCs arising in the context of MetS were more often well differentiated (28% versus 64.5%) and, in one-third of cases, developed in a preexisting liver cell adenoma | NAFLD contributes to noncirrhotic HCC |
| 35 | Multicenter Japanese study in 87 HCC cases on histologically proven NASH | No established cirrhosis in 43 cases | Most patients with NASH-related HCC are at a less advanced stage of liver fibrosis |
| 30 | 623 English patients with HCC analyzed between 2000 and 2010 | HCC cases in the absence of cirrhosis most commonly occurred in NAFLD. Patients without cirrhosis were not in surveillance programs, and consequently the majority (62.3%) presented symptomatically, with larger tumors. Their median survival was only 7.2 months | Increased prevalence of HCC in noncirrhotic NAFLD patients |
| 39 | Retrospective study analyzed 54 patients with NAFLD-associated HCC between 2000 and 2012 | 8/54 (15%) were not cirrhotics, and noncirrhotics had a significantly larger mean tumor diameter at diagnosis ($p = 0.041$) | HCC can develop in NAFLD without cirrhosis. At diagnosis such tumors are larger than those in cirrhotics, conferring a poorer prognosis |

Abbreviations: CC, cryptogenic cirrhosis; HCC, hepatocellular carcinoma; BMI, body mass index; NAFLD, nonalcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NASH, nonalcoholic steatohepatitis; LT, liver transplantation; HCV, hepatitis C virus.

The exact burden of HCC related to NAFLD remains uncertain, but NAFLD was the most common underlying etiologic risk factor for HCC (59%) in a US population–based study, with a cumulative incidence of 0.3% over a 6-year follow-up (24). The mortality rates for HCC ranged from 0.25% to 2.3% over 8.3 and 13.7 years of follow-up in two further studies (25, 26) and were up to 3–6% over 21 years in a third one. As expected, the risk of HCC was more elevated in NAFLD patients with advanced liver disease. In the largest prospective community-based study performed so far (27), after a mean follow-up of 7.6 years, only 0.5% of patients developed HCC but the rate among cirrhotics was 10%. Two longitudinal studies on the natural history of NASH-related cirrhosis in the United States (28) and Japan (27) confirmed that HCC was the cause of 47% of deaths in NASH patients, representing an independent risk factor for liver-related mortality (HR 7.96).

Overall, the relative HCC risk and mortality rate in NASH-related cirrhosis seem to be lower than in viral or alcohol-related cirrhosis. In a large cohort study, HCC was significantly more common in HCV than in NAFLD (6.8% versus 2.4%, respectively) (29), and the HCV cohort had

an approximate 0.15% risk per year of HCC development versus 0.05% in NAFLD. However, the common perception that HCC is a rare and late complication of NAFLD has been challenged by recent reports that definitely unveiled what we are going to face in the next decades. In northeastern England, the overall incidence of HCC rose 1.8-fold from 2000 to 2010, but most shocking was a more-than-tenfold increase in HCC associated with NAFLD, accounting for 34.8% of all the cases in 2010 and making it the single most common underlying etiology (30). Not surprisingly, this rising incidence of HCC was associated with an increasing prevalence of overweight and obesity (61% in 2000 and 65.5% in 2010).

**IR:** insulin resistance

**IGF-1:** insulin-like growth factor 1

It is necessary to recall that NAFLD may remain unrecognized in cases of HCC arising in cryptogenic cirrhosis, a condition for which no underlying etiology has been clinically identified. It is estimated that 20–40% of all HCC cases in industrialized countries occur in patients with cryptogenic cirrhosis. Most of the cases have been identified as "burnout NASH," bearing historical or metabolic vestiges of MetS but no longer having classic biopsy features (31, 32), which often disappear in cirrhosis.

In order to foresee the future trends of HCC in the general population, we need to understand that the lower rates of incidence of HCC arising in NAFLD cirrhosis compared with other causes of chronic liver disease are definitely outweighed by the much larger spread of NAFLD in the general population and by the onset of HCC in noncirrhotic livers.

## HCC in Noncirrhotic Liver with NAFLD

The most worrisome issue highlighted by a rapidly growing literature is the onset of HCC in NASH patients who do not have cirrhosis yet. Since 2004, at least 116 such cases have been reported. A French study analyzed a cohort of 31 HCC patients with MetS as the only risk factor for liver disease and found mild or no fibrosis in most cases, compared to those harboring HCC associated with an overt cause of liver disease (65% versus 26%, $p < 0.0001$) (33). HCC cases arising in the context of MetS were more likely to be well differentiated (28% versus 64.5%), and one-third of these cases developed in a preexisting liver cell adenoma (33). These data concur well with larger Japanese studies, where cirrhosis was absent in 38% of 292 (34) and 49% of 87 patients (35) with NAFLD-related HCC, and with an English epidemiological study, where the same observation was made in one-third of the NAFLD cases (30). As these patients were not in surveillance programs, the majority (62.3%) presented symptomatically, with larger tumors, and their median survival was just 7.2 months. Hence, the number of individuals with NAFLD potentially at risk for developing HCC may be much larger than previously thought. This reveals an urgent need to better understand the risk factors linked to the development of HCC in noncirrhotic livers and to update screening programs.

## MOLECULAR MECHANISMS OF HEPATOCARCINOGENESIS

Mechanisms of HCC arising in long-standing cirrhosis have been extensively described (40) and are generally characterized by recurrent cycles of hepatocellular death and compensatory regeneration, accompanied by constant cell growth and proliferation that favor tumor development. However, the mechanisms relating obesity, NAFLD, and HCC, particularly in the absence of cirrhosis, are probably related to the pathogenesis of the underlying disease rather than to fibrosis alone and are still unclear (40). The common soil of insulin resistance (IR) and hepatic steatosis favors liver carcinogenesis by promoting adipose tissue–derived inflammation (41), hormonal changes (42), oxidative stress and lipotoxicity (43–45), and stimulation of the IGF-1 axis by

hyperinsulinemia (46). Others mechanisms involving diet (47, 48), gut microbiome (49, 50) and genetic factors are increasingly important and clinically relevant (51) (**Figure 1**).

**IKK:** inhibitor of nuclear factor kappa-B kinase subunit beta

**JNK:** Jun N-terminal kinase

**NF-κB:** the nuclear factor kappa-B kinase beta subunit

**STAT3:** the signal transducer and activator of transcription 3

**JAK:** Janus kinase

**PI-3K:** phosphoinositide 3-kinase

**Akt:** protein kinase B

**mTOR:** mammalian target of rapamycin

**PTEN:** the phosphatase and tensin homologue

**SOCS3:** the suppressor of cytokine signaling 3

**ChREBP:** carbohydrate response element binding protein

**SREBP-1c:** sterol regulatory element binding protein 1c

**ATP:** adenosine triphosphate

**SASP:** the senescence-associated secretory phenotype

## Dysfunctional Adipose Tissue and Proinflammatory Cytokine Pathways

The low-grade chronic inflammation characteristic of IR, particularly in the setting of obesity, favors macrophage recruitment and massive release of several proinflammatory cytokines (52). Among these, tumor necrosis factor alpha (TNFα) and interleukin (IL)-6 have been more clearly linked to the progression from NASH to HCC (41), mainly through their action on the IKK and JNK signaling pathways, which are the pivotal mediators of obesity-induced inflammation and are also involved in liver tumorigenesis. TNFα can induce activation of JNK, leading to impairment of the normal insulin receptor signaling (53), and interacts with NF-κB to promote the transcription of genes involved in apoptosis, inflammation, proliferation, and angiogenesis. IL-6 is able to activate STAT3, which promotes cell growth and differentiation (54). The higher IL-6 levels in obese subjects and the involvement of STAT3 in different cancers indirectly suggest a role of this pathway in HCC development.

High leptin and low adiponectin levels are two hallmarks of obesity and are both involved in NAFLD progression and carcinogenesis. Leptin contributes to the development of IR, hepatic steatosis, and fibrosis, and plays an important role in the regulation of immune response, glucose homeostasis, and angiogenesis (55). Leptin binding to the Ob receptor leads to the activation of different molecular pathways, such as JAK 2/STAT3 and PI-3K/Akt. One of the most important downstream effectors of Akt is mTOR, a serine-threonine protein kinase implicated in cell growth, proliferation, and survival (56). The activation of the Akt/mTOR pathway has been reported in about 30–40% of HCC patients and can be negatively regulated by PTEN (56). Liver PTEN-deficient mice develop steatosis, inflammation, fibrosis, and HCC similar to human NASH.

Conversely, adiponectin is able to reduce STAT3 and Akt phosphorylation in vitro by upregulating the expression of SOCS3 (57), but in the setting of IR and obesity, low levels of adiponectin hamper its anti-inflammatory activity and its antagonizing effect on leptin-induced hepatocellular carcinogenesis.

## Diet and Gut Microbiome

The composition of diet can significantly influence the above-described pathways. IL-6 and TNFα expression in the liver is strongly induced in response to a high-fat diet, which can also increase NF-κB activation (40). A high carbohydrate intake can activate the transcription factor ChREBP and acts in concert with chronic hyperinsulinemia, through SREBP-1c, to induce hepatic lipogenic gene expression. De novo lipogenesis is upregulated by threefold in obese, diabetic, and NAFLD subjects and produces exclusively saturated fatty acids, which amplify lipotoxic injury (58). Fructose, in particular, seems to favor HCC initiation through a cascade of different events: It increases lipoperoxidation by stimulating de novo lipogenesis and blocking the conversion of free fatty acids (FFAs) into triglycerides for hepatic ATP depletion (59); it downregulates the expression of sirtuin-1, which is involved in the regulation of cellular survival; and finally, it alters the intestinal microbiome composition (60). Changes in composition of the gut microbiota are tightly associated with obesity and contribute to hepatic inflammation through increased intestinal permeability, translocation of bacterial components such as lipopolysaccharides, and activation of the toll-like receptors. Notably, deoxycholic acid, a secondary bile acid produced mainly by the gram-positive bacteria *Clostridium*, can produce reactive oxygen species leading to DNA damage and release of SASP from hepatic stellate cells (HSCs) (51). This HSC molecular signature has

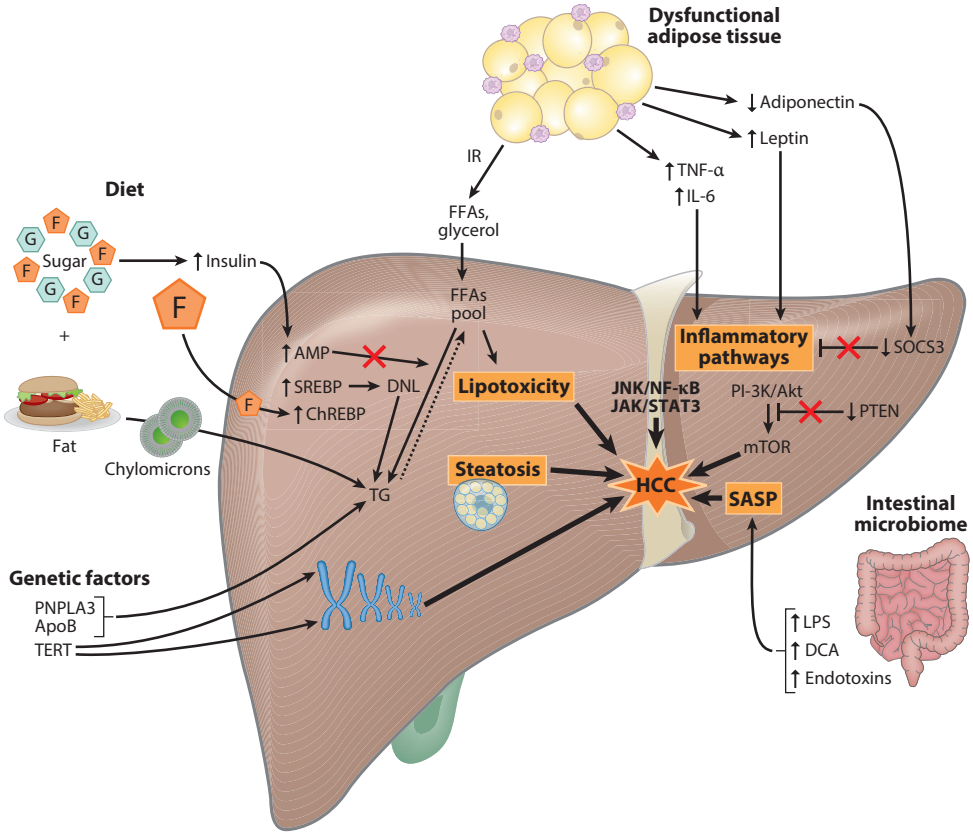


**Figure 1**

In the setting of NAFLD, low-grade chronic inflammation and IR create a microenvironment suitable for HCC growth. TNFα and IL-6 enhance JNK/NF-κB and JAK/STAT3 pathways while leptin activates PI-3K/Akt signaling, leading to the expression of genes involved in cell proliferation, migration, and survival. Other factors such as SOCS3 and PTEN are downregulated and fail to block molecular HCC-related signaling. High-fat diets and high carbohydrate intake (mainly fructose) can worsen the cytokine pattern and increase hepatic DNL, in turn promoting lipoperoxidation. The intestinal dysbiosis associated with obesity alters the gut microbiome and promotes the release of endotoxins, LPS, and DCA, leading to the activation of HSCs and expression of SASP in the liver. Genetic inheritance contributes to increase the risk of HCC, mainly through the *PNPLA3* rs738409 variant. See text for further details. Abbreviations: Akt, protein kinase B; AMP, adenosine monophosphate; ApoB, apolipoprotein B gene; ChREBP, carbohydrate response element binding protein; DCA, deoxycholic acid; DNL, de novo lipogenesis; F, fructose; FFAs, free fatty acids; G, glucose; HCC, hepatocellular carcinoma; IL-6, interleukin-6; HSC, hepatic stellate cells; IR, insulin resistance; JAK, Janus kinase; JNK, c-Jun N-terminal kinases; LPS, lipopolysaccharides; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa-B kinase beta subunit; NAFLD, nonalcoholic fatty liver disease; *PNPLA3*, patatin-like phospholipase domain-containing 3 gene; PTEN, phosphatase and tensin homolog; SASP, senescence-associated secretory phenotype; SOCS3, suppressor of cytokine signaling 3; SREBP, sterol-regulatory element binding protein; STAT3, signal transducer and activator of transcription 3; TERT, telomerase reverse transcriptase gene; TG, triglycerides; TNFα, tumor necrosis factor alpha.

been described also in the absence of severe fibrosis, thus helping to unveil the obscure mechanisms of HCC arising in the absence of cirrhosis.

**ICAM-1:** proinflammatory mediator intercellular adhesion molecule 1

## Genetic Factors

In the past eight years, genome-wide association studies (GWAS) led to the identification of several genes related to NAFLD, NASH, and its complications including HCC. In 2008, Romeo et al. (61) described a single-nucleotide polymorphism (SNP) in the patatin-like phospholipase domain-containing 3 (*PNPLA3*) gene, which encodes the triglyceride lipase adiponutrin and strongly affects fat accumulation in the liver in the absence of IR. The *PNPLA3* rs738409 [G] risk allele, found in ~40% of the European population, can also increase threefold the risk of progression to NASH and, most importantly, twelve-fold that of developing HCC (62). Among HCC patients, GG homozygosity is also associated with younger age, shorter history of cirrhosis or less advanced liver disease, and more diffuse HCC at diagnosis, hence reduced survival. A meta-analysis including 2,503 European cirrhotic patients found that the rs738409[G] allele was strongly associated with overall HCC independent of obesity, particularly in alcohol-related cirrhosis (63). The relationship between this mutation and liver damage or HCC onset remains to be established, but rs738409 has been linked to increasing circulating levels of ICAM-1 and decreased levels of adiponectin. Finally, uncommon genetic variants seem to influence HCC development in a fatty liver. The most important are the nonsense mutation c.6718A>T in the apolipoprotein B (*ApoB*) gene, identified through whole-exome sequencing in a family with hypobetalipoproteinemia, and the human telomerase reverse transcriptase (*TERT*) gene, which is upregulated in human cancer and is a hallmark of HCC in patients carrying loss-of-function *TERT* mutations (51). Although only a few SNPs have been associated with the development of HCC, they are clinically relevant for potential use as markers of HCC in NAFLD.

## HEPATOCELLULAR CARCINOMA SURVEILLANCE AND PREVENTION

Poor surveillance is a constant problem for NAFLD patients. Their risk of HCC is often underestimated, and the disease is often asymptomatic for decades and associated with several comorbidities. In one of the most recent studies, 46.2% of patients with HCV-related HCC, 32.0% of patients with alcohol-related HCC, and just 22.8% of patients with NAFLD had their HCC detected by surveillance (64). The onset of HCC in noncirrhotic NASH has resulted in further uncertainty about the optimal screening strategy for the early detection of HCC, essential for curative therapies. Generalized HCC screening programs adopted in cirrhotic patients (abdominal ultrasound every six months) (68) would not be cost effective because of the high prevalence of NAFLD in the general population (26). Three out of five guidelines on the management of NAFLD support the practice of oncologic follow-up on an individual basis, particularly for patients with obesity and T2DM; the guidelines of the Asia-Pacific region suggest the extension of screening to those "cancers whose incidence is increased by MetS," but specific recommendations in noncirrhotic patients are currently lacking. It is conceivable that the development of polymorphism-based risk scoring algorithms will enable the identification of high-risk groups for HCC.

The use of metformin has been recently linked to antineoplastic effects through both insulin-dependent and -independent mechanisms (65). A meta-analysis including 22,650 cases of HCC in 334,307 patients with T2DM concluded that incidence of HCC was reduced by 50% with metformin use, increased with sulfonylurea or insulin and unchanged with glitazones (66).

Statins may also decrease the risk of cancers through antiproliferative, proapoptotic, antiangiogenic, and immunomodulatory effects. A systematic meta-analysis of 26 randomized controlled

trials, including almost 1.5 million patients and 4,298 cases of HCC, showed that the use of statins was associated with a 37% reduction in HCC incidence after adjusting for potential confounders (67). Although no definite recommendations can be given, diabetic patients at high risk for development of HCC should preferentially be treated with metformin if clinically appropriate, as opposed to insulin or insulin secretagogues. The use of statins should be favored in subjects with NAFLD, not only to decrease HCC risk but also in consideration of their increased risk of cardiovascular events.

## TREATMENT OF HEPATOCELLULAR CARCINOMA

The therapeutic options for NAFLD-related HCC are the same as those for any patient with liver disease (LT, resection, radiofrequency ablation, chemoembolization, sorafenib) (68), but late diagnosis, older age, and concurrent metabolic or vascular disease restrict the options for potentially curative treatments. In the study by Dyson et al. (30), the majority of 136 NAFLD-related HCC patients received only supportive care or transarterial chemoembolization (TACE). Because of the lower prevalence of cirrhosis, resection was more frequent, although LT was performed less often (30). In another study from Germany (69) comparing 45 NASH-related HCC and 1,074 non-NASH-related HCC cases, resection or LT was performed in only 17.8% and 4.4% of patients with NASH-related HCC, despite preserved liver function. In both studies, a larger tumor size and a higher rate of multifocality at the time of diagnosis were the reasons for the exclusion of LT in NASH-related HCC patients; sorafenib was more frequently administered as primary treatment.

Nevertheless, NASH is currently the most rapidly growing indication for LT in patients with HCC. A recent study analyzed the burden of NASH among 10,061 patients who underwent LT for primary HCC (70). The proportion of HCV-related HCC increased steadily from 2002 to 2012, and HCV remained the leading etiology of HCC (43.4% in 2002 and 49.9% in 2012). However, NASH-related HCC rose from 8.3% in 2002 to 13.5% in 2012, thus becoming the second leading etiology of HCC treated by LT. Importantly, the number of patients undergoing LT for NASH-related HCC increased by nearly fourfold, compared to a twofold increase in LT for those with HCV-related HCC. The higher rate of comorbidities in NASH patients may explain the higher mortality among patients on the waiting list for LT, particularly for those with BMI $>40$ kg/m$^2$. In patients with very high BMI, especially morbid obesity, LT might be contraindicated, and in some centers physical exercise and treatment of obesity are recommended as preparation for LT. Bariatric surgery might be difficult or impossible in patients with end-stage liver disease, but preliminary results suggest that combined LT along with sleeve gastrectomy might be considered in appropriate patients with persistent obesity and MetS prior to LT. A recent meta-analysis concluded that five-year survival in NAFLD does not differ from non-NAFLD because the greater risk of death from cardiovascular complications and sepsis is counterbalanced by a lower risk of graft failure (71).

## CONCLUSIONS

In consideration of the spread of obesity and NAFLD in the general population, the growing incidence of HCC linked to these novel risk factors can become a serious challenge for public health, with high costs for surveillance and treatment, including LT. Importantly, a considerable number of NAFLD-associated HCC cases develop in noncirrhotic livers, particularly in patients with multiple metabolic risk factors. Delay in diagnosis and the presence of relevant comorbidities often limit the possibility of therapeutic intervention. Although weight loss can

generally ameliorate obesity-induced complications, the capability to prevent the development of HCC or halt its progression is unknown. Many other questions remain to be answered, including carcinogenesis in noncirrhotic livers and the best strategy for targeting high-risk subjects in the general population. A better understanding of the molecular events leading from obesity to NASH and HCC will allow the discovery of new targets for therapeutic and preventive intervention. In the meanwhile, the best and probably sole effective intervention to address this growing problem is to hinder the spread of obesity and NAFLD through public awareness and education programs.

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