

Type 2 diabetes mellitus and risk of hepatocellular carcinoma: spotlight on nonalcoholic fatty liver disease

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Abstract: The incidence of both type 2 diabetes mellitus (T2DM) and multiple cancer types are rapidly increasing worldwide. Several studies documented that T2DM is closely associated with an increased incidence of cancer. However, while some methodological considerations preclude a definitive association between T2DM and the risk of certain cancers, the relationship between T2DM and increased risk of incident hepatocellular carcinoma (HCC) remains significant even after adjustment for detection bias and reverse causation, indicating that such association is clinically reliable and robust. In addition, a number of observational studies also showed that T2DM is associated with higher mortality among persons with HCC. Some recent meta-analyses suggested that treatment with metformin may be associated with a lower risk of HCC, and may also beneficially influence HCC prognosis, whereas treatment with sulphonylureas or insulin seems to be related to a higher HCC risk. The underlying biological mechanisms linking T2DM and HCC are complex and difficult to elucidate, but the existence of close inter-connections among T2DM, obesity and nonalcoholic fatty liver disease (NAFLD) induces hepatic/systemic insulin resistance and causes the release of multiple pro-inflammatory cytokines, vasoactive factors and pro-oxidant molecules, which are all potentially implicated in the development and progression of HCC. In this clinical review, we discuss the epidemiological evidence linking T2DM to the risk of HCC. Moreover, we also briefly discuss the putative underlying mechanisms linking T2DM, NAFLD and HCC, and the potential effect of certain hypoglycemic agents on the risk of developing HCC.

Keywords: Diabetes; cancer; hepatocellular carcinoma (HCC); nonalcoholic fatty liver disease (NAFLD)

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Introduction

The French surgeon Theodore Tuffier was probably the first one to hypothesize the existence of a relationship of type 2 diabetes mellitus (T2DM) with cancer risk in the second half of nineteenth century (1,2). He observed that patients with established T2DM exhibited a greater risk of certain cancers than those without diabetes (1,2). Consequently, he formulated the following key questions:

(I) could diabetes affect the incidence of cancer? (II) could diabetes influence the natural history of cancer? and (III) could cancer affect the natural history of diabetes? (1,2).

To date, seeing that the incidence of both T2DM and cancer are rapidly increasing worldwide, the above-mentioned questions remain of considerable clinical importance for investigating the epidemiological and pathophysiological associations between T2DM and cancer risk (3-6). These questions are particularly important for the

relationship between T2DM and the risk of hepatocellular carcinoma (HCC), because the incidence of this cancer has rapidly increased over the last decade and it represents now the third cause of cancer-related mortality worldwide (7,8). Moreover, there is now clear evidence that T2DM and HCC are closely linked, owing to their association with obesity, impaired insulin sensitivity and nonalcoholic fatty liver disease (NAFLD) (9-14). In particular, the increasing incidence of HCC is, in large part, attributable to the rising prevalence and incidence of NAFLD worldwide. Indeed, the prognostic impact of NAFLD *per se* on HCC risk is currently taking increasingly greater clinical importance. NAFLD encompasses a spectrum of progressive hepatic disease (ranging from pure steatosis to steatohepatitis [NASH] and cirrhosis) that is often observed in patients with T2DM (occurring in up to 70% of these patients) (15-19). Over the last decade, strong evidence demonstrated that patients with T2DM are more likely to develop the more severe histologic forms of NAFLD, including cirrhosis and HCC (15-19).

Given the sharp rise in the global incidence of T2DM, NAFLD and HCC, it is therefore unsurprising that in clinical practice, diabetologists often have to manage diabetes in patients who are being treated for HCC and oncologists/gastroenterologists are increasingly required to plan cancer treatment for patients with pre-existing diabetes. It is hence very important that diabetologists, oncologists, gastroenterologists but also general practitioners are aware of the strong relationship between T2DM and the risk of incident HCC.

In this clinical review, we will examine the evidence for a link between T2DM, NAFLD and HCC from an epidemiological and a pathophysiological perspective. Moreover, we will also briefly discuss the potential effect of certain hypoglycemic agents on HCC risk.

Epidemiological studies linking T2DM to cancer risk

Currently, the estimated global prevalence of T2DM is approximately 9% worldwide, with a worrying tendency to increase sharply in the next years (3,4,20,21). In parallel, the total number of deaths attributed to cancer is estimated to rise over time, mainly in developing countries (5). For instance, it was calculated that there were approximately 8 million cancer-related deaths worldwide in 2012 (5). There is also strong evidence indicating that cancer risk and mortality are progressively increasing with the rise of

T2DM incidence (22-24). For example, on the basis of a pooled analysis including 97 prospective cohort studies with a total of nearly 800,000 individuals, the Emerging Risk Factors Collaboration group investigators showed that individuals with established diabetes showed a remarkably greater risk of mortality from cancer than those without diabetes (25). Interestingly, the observed relationships between the presence of diabetes and the risk of site-specific cancer deaths were strongest for the risk of liver and pancreatic cancers, intermediate for ovarian and colorectal cancers, and lowest for lung and breast cancers (25). Death from site-specific cancers was also examined in a cohort of nearly 7,200 T2DM patients from Verona (Italy) over a 10-year follow-up by reviewing death certificates (26). The authors found that mortality rates from site-specific cancers were significantly higher in T2DM patients than in the general population (26). In particular, increased risk of death from liver cancer [standard mortality ratio (SMR) =1.86, 95% confidence interval (CI): 1.44–2.38] was observed in both sexes. In addition, women with diabetes also showed higher risk of death from both pancreatic (SMR =1.78, 95% CI: 1.13–2.67) and breast cancers (SMR =1.40, 95% CI: 1.06–1.81). Other investigators subsequently reported similar findings in other countries (27-30). On the basis of all these observations, a 2010 consensus statement of experts assembled jointly by the American Diabetes Association (ADA) and the American Cancer Society (ACS) concluded that the incidence rates of liver, pancreatic, colorectal, breast, endometrial and bladder cancers were increased among individuals with T2DM (31). However, it is important to note that while some methodological considerations preclude a definitive association between T2DM and risk of some cancers, the increased HCC risk observed in patients with T2DM remains statistically significant even after adjustment for detection bias and reverse causation, so indicating that such association is clinically reliable and robust (1,26,27,32,33).

Epidemiological studies linking T2DM to HCC risk

HCC represents the commonest form of primary liver cancer (5,6). The worldwide number of incident cases of HCC has been estimated to be approximately 600,000 in 2002, 80% of which were recorded in developing countries. *Table S1* summarizes the principal observational studies and meta-analyses examining the relationship between pre-existing T2DM and the risk of HCC. An early description of the existence of an association between T2DM and HCC

has been reported approximately 30 years ago (34). In 1986, in a case-control study involving 105 HCC patients and 105 persons with either colorectal cancer or femoral bone fractures who were matched by age and sex, Lawson *et al.* firstly documented that there was an approximately four-fold risk of prevalent T2DM among patients with HCC compared with those with colorectal cancer or femoral bone fractures, irrespective of chronic viral hepatitis, alcoholic cirrhosis and hemochromatosis (34). Subsequently, in a systematic analysis based on 242 cases of primary liver cancers and 1,169 controls recorded between 1984 and 1989, La Vecchia *et al.* found that patients with T2DM had an approximately 2.5-fold increased risk of developing liver cancers (mainly owing to HCC), independent of metabolic factors and other potential confounding variables (35). Notably, such increased risk of HCC was also observed in patients with a diagnosis of T2DM that occurred 5 or more years before the diagnosis of HCC (35). After these pioneering reports, several other case-control and prospective studies as well as some meta-analyses have been conducted, all confirming the existence of a strong relationship between T2DM and HCC risk, irrespective of several potential confounders, including overweight/obesity, excessive alcohol consumption and chronic viral hepatitis (36-39). For example, in a large meta-analysis involving approximately 3 million people, El-Serag *et al.* showed that among 13 case-control studies, pre-existing diabetes was associated with risk of prevalent HCC in 9 studies (odds ratio 2.50; 95% CI: 1.8-3.5). Among 13 cohort studies, diabetes was associated with risk of incident HCC in 7 studies (hazard risk 2.50; 95% CI: 1.9-3.2). The findings were consistent in different populations, different geographic locations, and a variety of control groups. The relationship between diabetes and HCC risk remained statistically significant after adjusting for alcohol use or chronic viral hepatitis in the 10 studies that examined these risk factors (40). In another meta-analysis published 6 years later, involving 49 observational studies (32 cohort studies and 17 case-control studies), Wang *et al.* confirmed that pre-existing T2DM was strongly associated with increased HCC prevalence and mortality, independent of several confounding factors and metabolic variables (41). In 2013, in a large cohort study of 363,426 non-diabetic individuals and 8,588 T2DM patients without any cancer or metastasis at baseline, Schlesinger *et al.* reported that T2DM was associated with an increased incidence of both HCC and bile tract cancer over a follow-up of 8 years, independent of age, sex, body mass index (BMI), waist-to-

height ratio, center, education level, smoking and alcohol consumption (42). In a community-based cohort study of 63,257 Chinese individuals followed-up for 10 years, Koh *et al.* showed that T2DM was associated with increased incidence of non-viral HCC, irrespective of age, sex, BMI, recruitment year, dialect group, education level, smoking and consumption of alcohol, coffee or tea (43). In 2015, in an Italian hospital-based case-control study of 224 HCC patients and 389 control subjects, Miele *et al.* found that the risk of HCC was significantly increased among patients with T2DM, especially among those with a longer duration of disease (44). Recently, in a meta-analysis of 9 studies (7 cohort studies and 2 case-control studies), Dyal *et al.* examined the inter-relationships of T2DM, obesity and hepatic steatosis with incidence rates of HCC in patients with chronic hepatitis C virus (HCV) (45). These authors documented that T2DM was closely associated with increased risk of HCC in this patient population, independent of age, sex, obesity, hypertension, smoking, alcohol intake, serum liver enzymes, albumin, lipids, platelet count, and presence of cirrhosis and hepatic steatosis (45). Notably, the presence of obesity and hepatic steatosis (along with T2DM) were also found to be independent predictors of incident HCC (45). These findings are clinically relevant, because they would suggest a potential synergistic, additive interaction between T2DM and other major metabolic risk factor for HCC.

Epidemiological studies linking NAFLD to HCC risk

Over the last years, it has become increasingly clear that NAFLD is strongly associated with increased risk of incident HCC. For instance, in a US population-based cohort study of 4,406 HCC cases followed-up for approximately 6 years, Sanyal *et al.* reported that the most common risk factor for HCC was NAFLD, followed by T2DM and HCV chronic infection (46). Almost identical findings were found in a smaller study showing that NAFLD was the most common etiology for HCC, thus outstripping chronic viral hepatitis and alcoholic liver disease (47).

Although there is now epidemiologic evidence to indicate a close relationship between NAFLD or NASH and the HCC risk, this risk appears to be particularly elevated in patients with cirrhosis, which is a well-established risk factor for HCC (11,12,48). In a national cohort of 1,500 patients who developed HCC from 2005 through 2010 from Veterans Administration hospitals, Mittal *et al.* showed that NAFLD was the third most common risk factor

for HCC, and that more than half of these patients had cirrhosis (49). In a large meta-analysis of 17 cohort studies, 18 case-control or cross-sectional studies and 26 case-series, White *et al.* reported that the cohorts of NAFLD or NASH patients with few or no cases of cirrhosis showed a minimal risk of HCC (HCC mortality of 0–3% for study follow-ups up to 20 years). The cohorts of patients with NASH and cirrhosis had a substantially greater risk (HCC incidence ranging from 2.4% over 7 years to 12.8% over 3 years). However, the HCC risk was lower in the cohorts of patients with NASH-cirrhosis than for cohorts with HCV-related cirrhosis (50). Similarly, in a prospective study enrolling 315 patients with HCV-cirrhosis and 195 patients with NASH-cirrhosis who were followed-up for approximately 4 years, Ascha *et al.* found that the yearly cumulative HCC incidence among patients with NASH-cirrhosis was 2.6% compared to 4.0% among those with HCV-cirrhosis (51). It is important to remark that in all the above-mentioned studies other major risk factors for development of HCC were older age, excessive alcohol consumption, pre-existing T2DM and presence of hepatic iron accumulation. Notably, some studies also suggested that the overall survival in cirrhotic NAFLD patients with HCC was significantly shorter compared with that observed in patients with HCC secondary to HCV cirrhosis (48,52,53). A plausible explanation for this intriguing finding is that cirrhotic NAFLD patients with HCC were older, and more likely to have larger tumor diameters and less surveillance compared to patients with HCC secondary to HCV cirrhosis (48). Conversely, the overall survival of patients with NAFLD-related HCC and neoplastic lesions eligible for curative treatment appeared to be superimposable to that observed in patients with HCC secondary to HCV cirrhosis (48).

Interestingly, however, in the last years the concept that HCC develops only in patients with cirrhotic NAFLD has been challenged, as HCC has been increasingly recognized in non-cirrhotic patients with NASH (11,48,54–57). For example, in a small cross-sectional study of 54 patients with HCC resulting by NAFLD, Leung *et al.* reported that approximately 15% of them were not cirrhotic, and that non-cirrhotic NAFLD patients had a significantly larger mean tumor diameter at diagnosis compared to those with cirrhosis (58). In another small study of 36 patients without cirrhosis and 47 NAFLD-HCC patients with cirrhosis, Mohamad *et al.* reported that patients with NAFLD-HCC in the absence of cirrhosis had larger tumor diameters at diagnosis, higher tumor recurrence rates and worse survival outcomes compared to NAFLD-

HCC patients with cirrhosis (59). To further increase the complexity of this problem, some cases of HCC in patients with imaging-diagnosed NAFLD (hepatic steatosis) have been also recently described. In a Japanese study involving 6,508 individuals with ultrasound-diagnosed NAFLD and followed for a median of nearly 6 years, Kawamura *et al.* found that the cumulative rates of HCC were 0.02% after 4 years, 0.20% after 8 years, and 0.51% after 12 years, respectively (60). These authors also observed that patients with advanced NAFLD fibrosis, as detected by aspartate aminotransferase (AST) to platelet ratio index (i.e., a non-invasive clinical score for advanced fibrosis), had an approximately 25-fold increased incidence of HCC compared with those without advanced NAFLD fibrosis (60).

Given that NAFLD is considered to be the “hepatic manifestation” of metabolic syndrome, it is unsurprising that several epidemiological studies also reported a strong association between other metabolic syndrome features, such as overweight or obesity, and the risk of developing HCC. Over the last decade, some large meta-analyses documented that individuals with overweight or obesity had a 50% to 85% increased risk of incident HCC compared with non-obese individuals (61–63). Similarly, in a large follow-up study, including nearly 360,000 individuals from the Prospective Investigation into Cancer and Nutrition Study, Schlesinger *et al.* reported a strong and positive association between abdominal obesity and HCC incidence over a mean follow-up period of 8.6 years (64).

Putative biological mechanisms linking T2DM and NAFLD with HCC risk

The exact pathophysiological mechanisms linking T2DM, NAFLD and HCC are not completely understood. However, although the pathophysiology underpinning these associations are not entirely clear, understanding of HCC pathophysiology in this context has improved in recent years.

Figure 1 schematically summarizes the putative pathophysiological mechanisms that might link T2DM, NAFLD and HCC.

It is well known that T2DM and NAFLD are strongly associated with increased hepatic/peripheral insulin resistance, lipotoxicity, increased oxidative stress and chronic low-grade inflammatory state. For instance, when insulin resistance and lipotoxicity develop, there is an increased release of multiple pro-inflammatory cytokines

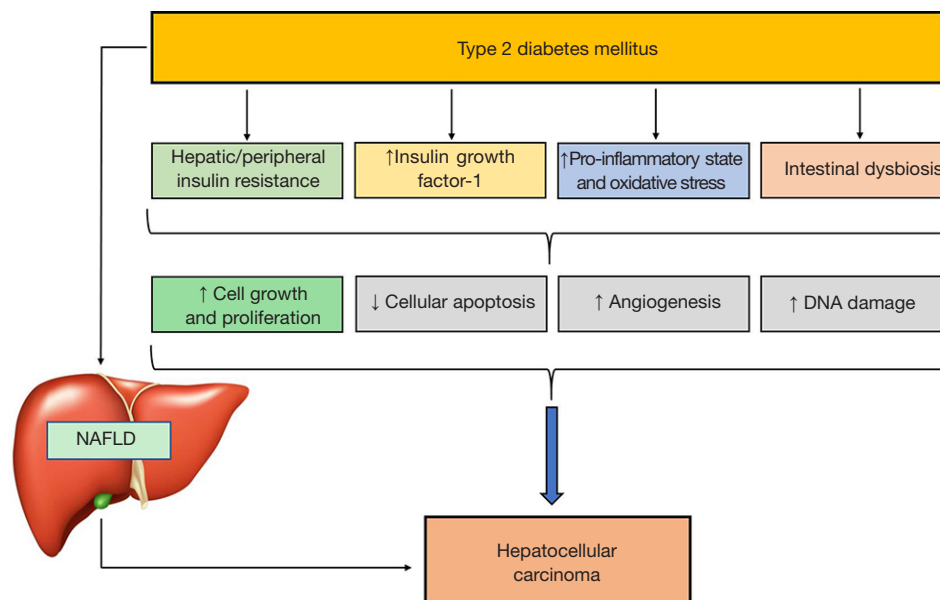


Figure 1 Putative biological mechanisms linking type 2 diabetes mellitus and nonalcoholic fatty liver disease (NAFLD) to the development and progression of hepatocellular carcinoma. The biological mechanisms underlying the association between type 2 diabetes, NAFLD and hepatocellular carcinoma are complex and not entirely understood. Both type 2 diabetes and NAFLD are strongly associated with abdominal obesity, hepatic/peripheral insulin resistance, low-grade chronic inflammation and increased oxidative stress that may contribute to the development and progression of hepatocellular carcinoma, promoting increased cellular growth and proliferation, inhibition of cellular apoptosis, increased angiogenesis and DNA damage. Insulin resistance and coexistent chronic hyperinsulinemia lead to increased production of insulin-like growth factor 1 (IGF-1), which further promotes hepatic cellular growth/proliferation and inhibits cellular apoptosis within the liver. In addition, chronic hyperinsulinemia also activates insulin receptor substrate-1 (IRS-1), which plays a key role in the regulation of multiple cytokine pathways potentially implicated in the pathophysiology of hepatocellular carcinoma. Evidence also suggests that insulin resistance alters gut microbiota (dysbiosis) and increases circulating levels of free fatty acids, which promote hepatic steatosis (NAFLD); both of these pathologic conditions play a part in the development and progression of hepatocellular carcinoma. In particular, clear evidence indicates that NAFLD, especially in its necro-inflammatory form, exacerbates hepatic/peripheral insulin resistance and causes the intrahepatic release of multiple hepatokines and mediators that increase the risk of hepatic carcinogenesis.

(e.g., C-reactive protein, interleukin-1, interleukin-6, tumor necrosis factor-alpha, tumor growth factor-beta), vasoactive factors and pro-oxidant molecules into bloodstream (13,14,19,65). Several studies suggest that all these factors may contribute to the development of HCC by promoting hepatic cellular growth/proliferation and by inhibiting cellular apoptosis (65-71). In addition, in the presence of insulin resistance, insulin concentrations rise in blood, so resulting in increased insulin-like growth factor-1 (IGF-1) production, i.e., a well-known hormone capable to stimulate hepatic cellular growth/proliferation and inhibit cellular apoptosis within the liver. Hyperinsulinemia also stimulates insulin receptor substrate-1 (IRS-1), which plays a key role in the activation of some intracellular cytokine signalling pathways implicated in hepatic carcinogenesis (65,72).

Both T2DM and NAFLD are associated with increased oxidative stress and release of reactive oxygen species (ROS) (64,70,72). Experimental studies reported that when hepatocytes are steatotic, they are able to produce ROS (65,73-76). It is known that increased ROS and oxidative stress may promote the development of many types of cancer, including HCC (65,71,73-76). Indeed, ROS can lead to cytotoxicity, DNA damage as well as activation and suppression of multiple genes that are potentially implicated in the cellular proliferation and growth (e.g., c-Jun amino terminal kinase 1 [c-JNK], p53, D-cycline, c-Fos, c-RAS, and c-Myc), thus further promoting hepatic carcinogenesis (65,71,73-76). In addition, increased production of ROS can be due to mitochondrial dysfunction (i.e., structural mitochondrial lesions, decreased activity of the respiratory

chain enzymes and abnormal mitochondrial beta-oxidation) (65,71,73-76). Interestingly, many experimental studies reported that T2DM and NAFLD are associated with the presence of mitochondrial dysfunction, thus resulting in increased ROS production (65,71,73-76). For an updated review on the molecular mechanisms of NAFLD-induced hepatocarcinogenesis the interested readers are referred to Zoller *et al.* (14).

Recently, accumulating evidence also suggests that gut microbiota alterations might play a part in the pathogenesis of T2DM, obesity and NAFLD (77-80). To note, there is now some evidence suggesting that altered gut microbiota may be also implicated in hepatic carcinogenesis (77-80). The putative pathophysiological processes that link altered gut microbiota and HCC development are complex, but they might include abnormalities in Toll Like Receptors (TLRs), increased levels of gut bacterial metabolites, increased levels of secondary bile acids with subsequent development of intestinal dysbiosis (77-80). With regard to intestinal dysbiosis, for example, in a recent case-control study including 150 HCC patients (105 with early HCC and 45 with advanced HCC) and 131 control individuals, Ren *et al.* reported that altered gut microbiota (characterized by decreases in *Bacteroidetes* and increases in *Proteobacteria* and *Fusobacteria*) was associated with more advanced HCC (81). It is plausible to assume that alterations in gut microbiota may promote the increased production of multiple fibrotic, inflammatory and cancer growth mediators by the hepatic stellate cells (77-80).

To date, the advent of next-generation sequencing and other “omics” technologies strongly implemented our knowledge(s) about the pathophysiology of HCC (65,71,82-89). For example, several studies reported recurrent mutations in the cell cycle regulator TP53 and CDKN2A, in the gene encoding for albumin, and also in the genes of the β -catenin/WNT signaling pathway (i.e., CTNNB1 and AXIN1) (65,71,82-89). Using the technique of comparative genomic hybridization, other experimental reports documented that patients with NASH have a greater level of genomic instability compared to those without NASH (65,71,82-89). Interestingly, it has been recently reported that some epigenetic alterations might be also important for HCC development (65,71,82-89). For example, the hypermethylation of the E-cadherin-1 (*CDH-1*) gene has been related to increased incidence of NAFLD-related HCC (65,71,82-89). Accumulating evidence also suggests the importance of genetic variation in the patatin like phospholipase domain-containing protein-3

(*PNPLA3*) gene (65,71,82-89). This gene encodes for a protein (named adiponutrin) located in intra-hepatic lipid droplets, which is capable to promote hepatic lipogenesis and lipolysis (65,71,82-89). The genetic variations of *PNPLA3* gene have been found to be closely associated with increased risk of NAFLD progression, especially with risk of hepatic fibrosis (65,71,82-89). It has subsequently been shown that carriers of *PNPLA3* genetic polymorphism encoding for the I148M variant allele are also at higher risk of HCC (65,71,82-89). Therefore, genotyping of the *PNPLA3* I148M polymorphism will reveal mechanisms implicated in hepatic fibrogenesis and carcinogenesis and will possibly inform clinical practice in the future (i.e., contributing to identify those to whom HCC surveillance may be targeted).

Epidemiological studies linking the use of certain glucose-lowering medications to HCC risk

Currently, there is increasing evidence to suggest that certain glucose-lowering medications may have a modifying effect on incidence rates of HCC. In 2005, Evans *et al.* conducted a pilot study suggesting that metformin use may be associated with a lower risk of cancer among individuals with known T2DM (90). After this pioneering study, other larger studies have been performed. For example, in a population-based cohort of 10,309 T2DM patients followed-up for nearly 5 years, Bowker *et al.* reported that cancer mortality significantly differed among the various treatments for diabetes at baseline: 3.5% for metformin users, 4.9% for sulphonylurea users and 5.8% for insulin users, respectively (91). In a longitudinal study of more than 8,000 patients with T2DM followed for a median of 3 years, Libby *et al.* showed that those treated with metformin had a lower incidence of total cancer than those treated with sulphonylureas or insulin, independent of age, sex, BMI, hemoglobin A1c, smoking and use of other medications (92). In a study including 610 HCC patients, 618 cirrhotic patients and 1,696 controls, Donadon *et al.* showed that metformin use was associated with a significant HCC risk reduction compared with the use of sulphonylureas or insulin, in diabetic HCC patients *vs.* control subjects and *vs.* cirrhotic patients (93). In a hospital-based study including 420 HCC patients and 1,104 controls, Hassan *et al.* reported that use of sulphonylureas or insulin conferred the highest HCC risk, whereas use of metformin or glitazones was associated with a 70% risk reduction in HCC in patients with T2DM (94). Similarly, in a large

Taiwanese population-based study enrolling 19,349 patients with newly diagnosed T2DM and 77,396 non-diabetic controls, Lai *et al.* showed that patients with T2DM had a two-fold higher incidence of HCC than those without T2DM, and that this incidence was significantly lower in patients treated with either metformin or glitazones than in those treated with other hypoglycemic agents (95). Some recent meta-analyses confirmed these findings. In a meta-analysis of 5 case-control studies, three cohort studies and two randomized controlled trials with a total of 22,650 HCC cases in 334,307 patients with T2DM, Singh *et al.* documented that patients treated with metformin had a nearly 50% risk reduction in HCC incidence compared with those treated with sulphonylureas, glitazones or insulin (96). More recently, in a network meta-analysis of 13 randomized controlled trials, cohort studies or case-control studies enrolling approximately 480,000 T2DM patients with more than 240,000 HCC cases, Zhou *et al.* showed that metformin use was associated with decreased HCC risk, whereas insulin use was associated with a higher risk of this cancer (97).

That said, it is also important to underline that other observational studies produced conflicting results. In a retrospective cohort study of nearly 96,000 individuals with T2DM who began to take metformin or other oral glucose-lowering agents within 12 months of the diagnosis, Tsilidis *et al.* showed that the users of sulphonylureas or metformin had similar incidence rates of total cancer over 5 years of follow-up (98). Similar results were also found when the analysis was restricted to incidence rates of different types of cancer, such as HCC, colorectal, prostate, lung and breast cancers (98). Interestingly, in a post-hoc analysis of the ADOPT (A Diabetes Outcome Progression Trial) and the RECORD (Rosiglitazone Evaluated for Cardiovascular Outcomes and Regulation of Glycaemia in Diabetes) trials, Home *et al.* showed that the cancer incidence rates did not allow to support the evidence that use of metformin conferred a particular protection against cancer risk compared to rosiglitazone (99). In addition, other studies did not support the conclusion that metformin may reduce the rates of cancer incidence among patients with established T2DM (100-102).

Collectively, there is still uncertainty about whether hypoglycemic drugs used for diabetes treatment may either increase or decrease the risk of incident HCC, or even influence cancer prognosis. Most observational studies suggest that metformin use might have any chemo-

preventive effects against HCC, and a biologically plausible mechanism also exists (this drug activates AMP-activated protein kinase (AMPK) and inhibits the PI3K/AKT/mTOR signaling pathway that is important in regulating the cell cycle) (1,14,96). In contrast, it is uncertain if the observed insulin-related increase in HCC risk is related to toxicity associated with the medication, or if it is simply reflective of increased HCC risk in patients with more severe diabetes. Further larger randomized clinical trials are required in order to confirm a possible chemo-protective effect of metformin on HCC risk, and to find novel metabolic approaches to HCC prevention and treatment in individuals with T2DM.

Conclusions

HCC incidence is rapidly increasing worldwide, and this is likely linked to the increasing incidence of T2DM, NAFLD and metabolic syndrome. There is now robust evidence of an association between T2DM and HCC development. The pathophysiology underlying development of HCC in this context is complex and is likely to involve NAFLD (especially NASH with varying amounts of hepatic fibrosis), increased hepatic/peripheral insulin resistance hyperinsulinemia, increased pro-inflammatory mediators, oxidative stress, JNK-1 activation, increased IGF-1 activity, altered gut microbiota and immunomodulation. However, a greater understanding of the underlying pathophysiology might help in the future development of rational, targeted treatments for patients with both HCC and T2DM.

In clinical practice, clinicians are increasingly required to manage and treat patients with both T2DM and HCC. Although there are still important gaps in our knowledge(s), the use of metformin may be associated with a lower incidence of HCC. To date, studies reporting on the effect of glucose-lowering medications other than metformin on HCC prognosis are both scant and difficult to interpret, owing to the complexity of pharmacotherapy for T2DM, and the many sources of bias that this complexity may generate. Further research is required to clarify the variables that contribute to the complexity of the associations between T2DM, hyperglycemia, diabetes treatment and HCC risk.

Given that clinicians increasingly encounter patients with coexistent T2DM and HCC, it is desirable that the next randomized clinical trials of new drug candidates in oncology do not exclude people with T2DM in order to

better understand the pathophysiological mechanisms linking T2DM and HCC, and to provide new drugs for prevention and treatment of HCC in these patients.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Supplementary

Table S1 Principal observational studies and meta-analyses that have explored the association between type 2 diabetes and risk of hepatocellular carcinoma (ordered by publication year)

Study	Study characteristics	Diabetes diagnosis	Covariate adjustment considered	Main findings
Adami HO <i>et al.</i> <i>Cancer Causes Control</i> 1991;2:307-14	Hospital-based cohort: 51,008 patients with DM. Mean follow-up: 9 years	Hospital discharge diagnosis	None	DM was associated with increased risk of incident HCC
Adami HO <i>et al.</i> <i>J Natl Cancer Inst</i> 1996;88:1472-7	Hospital-based cohort: 153,852 patients with DM. Follow-up: from 1 to 24 years	Hospital discharge diagnosis	None	DM was associated with increased risk of incident HCC
La Vecchia <i>et al.</i> <i>Int J Cancer</i> 1997;73:204-7	Case-control study: 428 HCC cases, 59 with gallbladder and bile duct cancers, and 1,502 control subjects from hospital	Self-reported	Age, sex, area of residence, education level, alcohol intake, BMI, smoking, history of chronic hepatitis and cirrhosis, family history of liver cancer	DM was associated with increased risk of incident HCC
Huo TI <i>et al.</i> <i>Eur J Gastroenterol Hepatol</i> 2003;15:1203-8	Prospective study: 239 HCC patients (16.3% of whom had DM). Mean follow-up: 2.6 years	Fasting glucose ≥ 126 mg/dL or 2-hour post-load glucose ≥ 200 mg/dL, or past history	Age, sex, tumor size, anti-HCV-Ab positivity, HBeAg-positivity, cirrhosis, alcohol intake, alpha-fetoprotein, albumin, bilirubin	DM did not affect long-term survival in HCV-related HCC, but was a recurrence-independent prognostic factor for HBV-related HCC
Coughlin SS <i>et al.</i> <i>Am J Epidemiol</i> 2004;159:1160-7	Population cohort study: 467,922 men and 588,321 women without history of cancer at baseline. Mean follow-up: 16 years	Self-reported	BMI	DM was associated with increased risk of incident HCC only in men
El-Serag HB <i>et al.</i> <i>Gastroenterology</i> 2004;126:460-8	Prospective study: 73,643 patients with DM and 650,620 patients without DM. Mean follow-up: 5 years	Self-reported	Alcoholic liver disease, viral chronic hepatitis, demographic variables	DM was associated with an increased risk of incident HCC. DM carried the highest risk among patients with a follow-up longer than 10 years
Davilla JA <i>et al.</i> <i>Gut</i> 2005;54:533-9	Population based case-control study: 2,061 HCC patients (of whom 43% with DM) and 6,183 non-cancer controls (of whom 19% with DM)	Electronic register	Age, sex, race, HCV, HBV, alcoholic liver disease, and hemochromatosis	DM was associated with a nearly three-fold increased risk of HCC
Inoue M <i>et al.</i> <i>Arch Intern Med</i> 2006;166:1871-7	Prospective study: 97,771 Japanese adult individuals followed-up for cancer incidence over 5 years. At baseline, 4.7% of them had DM	Self-reported	Age, study area, BMI, prior cardiovascular disease, smoking, alcohol intake, leisure-time physical activity, green vegetable intake, coffee intake	DM was associated with increased risk of total cancer and cancer in specific sites, including HCC
El-Serag HB <i>et al.</i> <i>Clin Gastroenterol Hepatol</i> 2006;4:369-80	Meta-analysis: a total of 26 studies (of whom 13 case-control studies and 13 cohort studies), inclusive of approximately 3 million of individuals	Self-reported	Alcohol intake, chronic viral hepatitis, diet, BMI	Among 13 cohort studies, DM was associated with an increased risk of HCC
Kawamura Y <i>et al.</i> <i>J Gastroenterol Hepatol</i> 2008;23:1739-46	Prospective study: 40 consecutive HCC patients (with HCC associated with non-B, non-C hepatitis), and later underwent surgical resection or radiofrequency ablation. Prevalence of DM was 45%. Mean follow-up: 5 years	Fasting glucose ≥ 126 mg/dL or past history	Age, sex, dyslipidemia, smoking, alcohol intake, history of blood transfusion, state of liver disease (chronic hepatitis or cirrhosis), AST, albumin, bilirubin, alpha-fetoprotein, pro-thrombin time, tumor size, multiplicity, hyper-vascularity and portal vein invasion of HCC	DM was a significant predictor of tumor recurrence after potentially curative therapy for HCC
Donadon V <i>et al.</i> <i>World J Gastroenterol</i> 2009;15:2506-11	Case-control study: 465 HCC patients, 618 with cirrhosis and 490 control subjects. The prevalence of DM was 31.2% in HCC, 23.3% in cirrhotic patients and 12.7% in control group	Self-reported	Age, sex, BMI, alcohol abuse, HBV and HCV	DM was an independent risk factor for HCC. Among male patients with DM, there was a positive association of HCC with insulin/sulphonylurea treatment and an inverse association with metformin
Hassan MM <i>et al.</i> <i>Cancer</i> 2010;116:1938-46	Hospital-based case-control study: 420 patients with HCC and 1,104 healthy controls. The prevalence of DM was 33.3% in patients with HCC and 10.4% in controls	Self-reported	Age, sex, race, educational level, smoking, alcohol intake, HCV, HBV, family history of cancer	DM increased the risk of HCC. Treatments with sulfonylureas or insulin were associated with higher HCC risk, whereas treatments with metformin or glitazones were associated with lower HCC risk
Hense HW <i>et al.</i> <i>Diabetol Metab Syndr</i> 2011;3:15	Community-based study: 26,742 DM patients, who were 40 to 79 years old, resided in the Muenster district. Mean follow-up: 3.3 years	Self-reported	Sex, diabetes duration, BMI, insulin treatment	Risk of any incident cancer in DM was increased, in particular for HCC. Insulin therapy was related to higher cancer risk, while metformin was not
Johnson <i>et al.</i> <i>Diabetologia</i> 2011;54:2263-71	Population-based retrospective cohort study: 185,100 individuals with DM and 185,100 without DM, matched by sex and age. Mean follow-up: 10 years	Electronic register	Age, sex, socio-economic status, number of physician visits, year of diagnosis	DM was associated with increased risk of selected cancers, including HCC
Li Q <i>et al.</i> <i>Int J Canc</i> 2012;131:1197-202	Hospital-based case-control study: 1,105 patients with HBV-related HCC and 5,170 patients with chronic HBV. The whole prevalence of DM was 6.7%	Fasting glucose ≥ 126 mg/dL or past history	Age, family history of HCC, city of residence, HBV-Ag and cirrhosis	DM was associated with increased risk of HCC, only in women
Wang C <i>et al.</i> <i>Int J Cancer</i> 2012;130:1639-48	Meta-analysis: a total of 25 cohort studies, enrolling 1,283,112 persons. Mean follow-up: 8.8 years	Self-report, medical records	Geographic location, alcohol intake, history of cirrhosis, or HBV and HCV infections	DM was associated with increased risk of incident HCC and higher HCC mortality. Longer diabetes duration and use of sulphonylureas or insulin were associated with increased risk of HCC. Metformin treatment was protective
Wang P <i>et al.</i> <i>Diabetes Metab Res Rev</i> 2012;28:109-22	Meta-analysis: 17 case-control studies (a total of nearly 6,000 HCC cases and 74,000 controls) and 32 cohort studies (a total of nearly 6,500,000 individuals)	Self-report, medical records	BMI, prior hepatitis, cirrhosis, alcohol intake, smoking, treatment, duration of diabetes	The combined risk estimate of all studies showed a significant increased risk of HCC among DM individuals. In addition, meta-analysis of 7 cohort studies found a significant increased risk of HCC mortality for individuals with DM compared to those without
Lai SW <i>et al.</i> <i>Am J Gastroenterol</i> 2012;107:46-52	Population-based cohort study: 19,349 newly diagnosed DM patients and 77,396 control subjects without DM. Mean follow-up: 5 years	Electronic register	Age, sex, cirrhosis, alcoholic liver damage, viral hepatitis	DM was associated with increased risk of incident HCC. Use of metformin or glitazones was associated with reduced HCC risk
Schlesinger S <i>et al.</i> <i>Ann Oncol</i> 2013;24:2449-55	Community-based cohort study: 363,426 participants, after excluding those with cancer at baseline. Mean follow-up: 8.5 years	Self-reported	Age, sex, center, education level, smoking, alcohol intake, BMI, waist-to-height ratio	DM was independently associated with higher risk of incident HCC and biliary tract cancer. HCC risk was higher in those treated with insulin. Results were similar in HCV/HBV-negative individuals
Zheng Z <i>et al.</i> <i>PLoS One</i> 2013;8:e84776	Hospital-based retrospective case-control study: 1,568 participants of whom 716 patients were diagnosed with benign liver diseases, and 852 patients were diagnosed with HCC. The prevalence of DM was 7.6%	Fasting glucose ≥ 126 mg/dL or 2-hour post-load glucose ≥ 200 mg/dL, HbA1c $\geq 6.5\%$	Age, sex, HBV and HCV infections, cirrhosis, gallstone disease, cholinesterase, alkaline phosphatase	DM was associated with increased risk of HCC. However, there was significant interaction between DM and HBV on HCC occurrence
Koh WP <i>et al.</i> <i>Br J Cancer</i> 2013;108:1182-8	Community-based cohort study: 63,257 middle-aged and older individuals. The prevalence of DM was 8.6%. Mean follow-up: 14 years	Self-reported	Age, sex, BMI, recruitment year, education level, smoking, alcohol intake, consumption of coffee and tea	DM was associated with an increased risk of incident non-viral HCC
Miele L <i>et al.</i> <i>Gastroenterol Res Pract</i> 2015;2015:570356	Hospital-based case-control study: 224 HCC patients and 389 controls. The prevalence of DM was 19.7%	Self-reported	Age, sex, smoking, alcohol intake	DM was associated with increased risk of HCC. Treatment with any glucose-lowering drugs was not associated with increased HCC risk
Dyal HK <i>et al.</i> <i>Dig Dis Sci</i> 2016;61:636-45	Meta-analysis: 9 studies (7 cohorts and 2 case-controls) with a total of nearly 10,000 adults with chronic HCV. The prevalence of DM was 8%	Self-reported	Age, sex, BMI, hypertension, smoking, alcohol intake, liver enzymes, albumin, lipids, platelet count, cirrhosis, HCV treatment, HCV genotype, hepatic steatosis	DM was associated with increased risk of HCC

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DM, diabetes mellitus; GGT, gamma-glutamyltransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma.