Metformin and Hepatocellular Carcinoma

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Metformin and hepatocellular carcinoma

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Abstract

There is ample evidence that type 2 diabetes is an independent risk factor for the development of various cancers including hepatocellular carcinoma (HCC). Metformin, a widely used antidiabetic agent, has been shown to have chemopreventive properties that may reduce the risk of cancer in diabetes. Here we summarize clinical and experimental data concerning the role of metformin in diabetes-associated hepatocarcinogenesis and review the key molecular mechanisms implicated in this action. By modulating mitochondrial function, metformin activates adenosine monophosphate–activated protein kinase (AMPK), a master regulator of energy metabolism that corrects most diabetes-associated derangements and mitigates the impact of insulin resistance on tumor growth. Moreover, metformin acts through additional AMPK targets in various pathways of oncogenesis and tumor suppression. Finally, metformin may also hinder hepatocarcinogenesis through AMPK-independent mechanisms. Controversies about the use of metformin as a chemopreventive agent include an efficacy bias related to duration and severity of diabetes, a lack of convincing impact on nonalcoholic fatty liver disease (NAFLD) representing the liver manifestation of diabetes, and a disputed role in lactic acidosis as a major adverse event. Future research may help develop randomized clinical trials on the impact of HCC by metformin, explore its utility in the non-diabetic and non-cirrhotic population, elucidate its precise mechanisms of action to identify new molecular targets, and evaluate safer and more efficient derivatives of this intriguing compound.

Keywords: diabetes; nonalcoholic fatty liver disease; metformin; hepatocellular carcinoma; adenosine monophosphate–activated protein kinase; insulin resistance.

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Introduction

The anti-diabetic agent metformin was approved 20 years ago for use in the treatment of type 2 diabetes. Metformin is a biguanide compound derived from the French lilac (Galega officinalis), a forage plant commonly grown in Europe and Asia, known for centuries to relieve the symptoms of diabetes mellitus [1, 2]. Although G. officinalis has been used as a galactagogue to boost milk production in small domestic animals (hence it has also been named ‘goat’s rue’), metformin does not exert its beneficial effect as a secretagogue for insulin [3]. Rather, the primary action of metformin lies in promoting sensitivity to insulin in peripheral tissues such as the liver and skeletal muscle. The mechanisms by which metformin exerts its beneficial metabolic effects in diabetes are subject to intense research but remain incompletely understood.
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Observations that metformin may prevent the development of cancer make this agent all the more interesting. There is substantial evidence that diabetes is an independent risk factor for the development of many different cancers and metformin has been associated with chemopreventive effects in this clinical setting [2, 4, 5]. Accordingly, hepatocellular carcinoma (HCC), the most common form of primary liver cancer that may complicate viral hepatitis and other chronic liver disease with high global prevalence [6], has been linked to diabetes and related metabolic conditions, and epidemiologic, clinical, and experimental data indicate that metformin may reduce the risk of HCC among patients with diabetes. Here we summarize current evidence for the prevention of hepatocarcinogenesis by metformin and review the molecular mechanisms implicated in this action.

Diabetes-associated Liver Disease and Development of HCC

Type 2 diabetes, a common metabolic disorder strongly linked to obesity, is characterized by hyperglycemia in the context of insulin resistance and hyperinsulinemia, while it also represents a complex disorder of lipid metabolism [7]. Adipose tissue expansion and remodeling, deposition of surplus fatty acids in the liver, enhanced de novo lipogenesis boosted by insulin, and harmful effects of ectopically accumulated lipids (lipotoxicity) are key elements of the pathogenesis leading to nonalcoholic fatty liver disease (NAFLD), defined as the hepatic manifestation of diabetes and related metabolic disorders [8–10]. Several studies indicate that some degree of NAFLD is present in most patients with diabetes [11, 12]. In addition, the majority of obese patients who exhibit insulin resistance but may not have developed manifest diabetes are also characterized by the presence of NAFLD [13–15]. Accordingly, NAFLD has become the most common liver condition in developed societies [16, 17] with similar trends in most geographical areas [18, 19]. In most instances, NAFLD is simply characterized by increased hepatic fat content (steatosis), but 20% of all cases feature the more complex disorder of nonalcoholic steatohepatitis (NASH) that involves hepatocellular injury, inflammation, and fibrosis [10]. While all forms of NAFLD have been associated with an adverse cardiovascular risk profile [20, 21], patients with NASH also carry a substantial risk of progression into advanced liver disease including cirrhosis and the development of HCC [10]. In the past 30 years, the incidence of HCC has tripled in the United States [22] and the contribution of NAFLD to this trend is now increasingly recognized [23, 24].

Diabetes has been associated with the promotion of several cancers and there is now ample evidence that diabetes is an independent risk factor for the development of HCC [4]. In the US, several large population-based studies and subsequent meta-analyses estimated that there is a 2–3 fold increased risk for HCC among patients with diabetes [25, 26]. Evidence for a higher risk of HCC in diabetes has also been obtained from other parts of the world [27–32]. A causal association is suggested given that diabetes most often precedes the development of liver disease, along with a significant duration-response relationship [26, 33]. Moreover, diabetes often coexists with chronic liver injury of viral or toxic etiology and may accelerate hepatocarcinogenesis even in the absence of established cirrhosis due to interactions between various oncogenic pathways. For instance, a retrospective study conducted in Japan on a cohort of non-cirrhotic, interferon-treated patients with chronic hepatitis C found that cumulative rates of HCC in diabetic patients were 2–4 fold higher than in non-diabetic patients [34]. Interestingly, increased risk of HCC by diabetes was only observed among patients with non-sustained virologic response, suggesting the importance of synergistic effects in this setting [34]. Several other groups described similar findings regarding the contribution of diabetes to increased risk of HCC in chronic hepatitis C [27, 35].

Prevention of HCC by Metformin: Clinical Observations

Pharmacological correction of the derangements in carbohydrate and lipid metabolism may be essential for preventing the development of HCC associated with diabetes. As reviewed below, there is good evidence that the risk of HCC in NAFLD is significantly reduced by the use of insulin-sensitizing agents such as metformin and thiazolidinediones, while an opposing trend with increased risk of HCC has been observed among patients with diabetes treated with insulin and the insu-
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lin secretagogue sulfonylureas. A retrospective case control study from Italy analyzed 610 cases of HCC and found that the odds ratio for developing HCC in metformin-treated diabetic patients with cirrhosis was 0.16 when compared to those treated with sulphonylureas or insulin [33]. In another study, the development of HCC was prospectively monitored in 100 consecutive French patients with diabetes and chronic hepatitis C [35]. With a median follow-up of 5.7 years, the authors reported that the 5-year incidence of HCC was significantly lower in patients who received metformin compared to those who did not (9.5% vs. 31.2%, P<0.001). In the same study, multivariate analysis indicated that metformin therapy was independently associated with both diminished HCC occurrence (hazard ratio [HR], 0.19; 95% confidence interval [CI], 0.04–0.79) and liver-related death or transplantation (HR, 0.22; 95% CI, 0.05–0.99) [35].

Further confirmation for the favorable effect of metformin in lowering the risk of HCC comes from a case-control study conducted in a large US cancer center [36]. In this study, 420 patients diagnosed with HCC and 1,104 matched controls were enrolled to determine the impact of diabetes treatment in the risk of HCC. The odds ratio of having diabetes was 4.2 in the HCC group and diabetes was present prior to the diagnosis of HCC in 87% of cases. The adjusted odds ratio for HCC was 0.3 among diabetics taking metformin of thiazolidinediones, while it was over 7.0 for those taking insulin secretagogues or relying on dietary control only [36].

A nationwide case-control study from Taiwan used a health insurance database to identify close to 100,000 patients with HCC and found that each incremental year increase in metformin use was associated with 7% decrease of HCC risk in diabetic patients [37]. Finally, a recent meta-analysis evaluated the impact of metformin on HCC development among patients with diabetes. Although the 5 studies encompassing a total of 105,495 patients included in this review showed considerable methodological variations, statistical analysis indicated that the risk of primary liver cancer was reduced by 62% in those who were regularly taking metformin [38].

Intriguingly, while most cases of diabetes-associated HCC develop in the setting of advanced NAFLD, several studies reported that metformin appears to have no impact on the progression of NAFLD. Anti-cancer effects of metformin are therefore not necessarily linked to the prevention of fatty liver disease. While metformin prevented and reversed steatosis and inflammation in a non-diabetic mouse model of NASH, as well as improved liver histology and alanine aminotransferase levels in patients with NASH [39, 40], other studies reported conflicting outcomes. In a small, randomized controlled trial of 36 patients with biopsy-proven NASH, the metformin-treated group showed no change in necroinflammatory activity or fibrosis in comparison to a group treated with diet alone [41]. Thus, 48 patients with biopsy-proven NAFLD were randomized in a clinical trial to treatment with metformin or placebo for 6 months, and no significant differences between the treatment arms were observed for changes in liver transaminases, steatosis, and markers of insulin resistance or inflammation, though body weight and serum parameters of lipid and glucose homeostasis were affected beneficially by metformin [42].

There is also some controversy with regard to the effect of metformin on preventing diabetes-associated cancer in general, and HCC in particular. In a retrospective study based on the UK Clinical Practice Research Datalink, 95,820 participants with diabetes initiated on oral antidiabetic agents within 12 months of their diagnosis were looked up for first-incident cancer with no regard to any subsequent changes in their treatment. Interestingly, cancer risk over a maximum follow-up of 24 years was similar among participants who first started using metformin or a sulfonylurea during the initial 12-month pharmacotherapy [43]. These data suggest that the effect of metformin on overall cancer risk might reflect the increased risk of cancer in patients with long-term diabetes who are ultimately in need of insulin. Similarly, no significant difference in cancer risk of participants taking metformin vs. sulfonylureas was reported in the ADOPT (‘A diabetes outcome progression trial’) and the RECORD (‘Rosiglitazone evaluated for cardiovascular outcomes and regulation of glycemia in diabetes’) trials [44, 45]. Simply put, hyperinsulinemia in advanced diabetes may be more of a risk factor for HCC than metformin could protect against, underscoring the difficulties in reaching solid conclusions on cancer prevention by metformin in the context of diabetes.
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Figure 1. Molecular mechanisms of cancer prevention by metformin. Simplified diagram of key molecular pathways and mediators affected by metformin and implicated in preventing the development and progression of hepatocellular carcinoma (HCC). Mitochondrial complex I is a direct target of metformin, resulting in increased AMP/ATP ratio and activation of adenosine monophosphate–activated protein kinase (AMPK), a master regulator that is able to correct most diabetes-associated derangements of energy metabolism, while it also has specific molecular targets to promote tumor suppression. Thus, metformin may provide an alternative mechanism of AMPK activation when the adiponectin/AMPK axis fails in obesity. In addition, there is evidence for AMPK-independent effects of metformin on hepatocarcinogenesis, which may be conveyed by mild uncoupling properties that protect from oxidative stress due to less reactive oxygen species (ROS) production in the mitochondria. Consequently, metformin seems to have the ability to simultaneously mitigate the impact of insulin resistance on tumor growth and exert independent effects on various oncogenic pathways.

Prevention of HCC by Metformin: Animal Studies

Animal models have greatly contributed to our understanding of the role of metformin in hepatocarcinogenesis. Simultaneous administration of metformin with high-fat diet for 60 weeks in C57BL6 mice decreased hepatic inflammation and fibrosis scores in parallel with a significantly lower proportion of animals developing dysplastic liver nodules [46]. In the short term, metformin also diminished lipid accumulation in the liver and suppressed inflammation in the adipose tissue. No such effects could be observed, however, when metformin therapy was initiated 30 weeks after the beginning of high-fat diet, by which time the animals already developed NAFLD, suggesting that metformin may be useful as an early intervention to protect against adipose tissue remodeling, lipotoxicity, and liver tumor formation [46]. This suggests that metformin therapy and subsequent correction of the metabolic derangements may not inhibit hepatocarcinogenesis if NAFLD is already present, suggesting that the timing of medication initiation is highly relevant [47].

In other experiments, C57BL6 mice were injected with the chemical carcinogen diethylnitrosamine (DEN) before weaning and then were given chow that contained metformin for up to 36 weeks of age. Metformin-treated mice developed 57% less liver tumors than the control group and this effect was associated with significant reduction in the expression of genes that determine the rate of hepatic de novo lipogenesis [48]. Although metformin doses in these experiments were much higher than those applied in the clinical setting, it is important to note that there is significant difference in sensitivity to metformin between rodents and humans [49, 50].
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In a mouse xenograft model, tumor-bearing nude mice inoculated with HepG3 or Hep3B human hepatoma cells were given doxorubicin or metformin alone or in combination to reduce tumor growth [37]. Interestingly, addition of metformin to doxorubicin greatly accelerated hepatoma regression, in line with in vitro evidence for the chemopreventive effect of metformin demonstrated by causing G0/G1 cell cycle arrest in hepatoma cells and enhanced the effect of doxorubicin on tumor cell apoptosis rates [37]. An alternative xenograft transplantation model using non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice indicated that treatment with metformin or the tyrosine kinase inhibitor sorafenib similarly suppressed the growth of subcutaneous tumors derived from transplanted Huh1 and Huh7 human HCC cell lines, while co-treatment appeared to be even more effective [51]. Additional analysis indicated that the action of metformin involves the reduction of cells positive for the tumor-initiating epithelial cell adhesion molecule (EpCAM) [51], a marker recently associated with HCC progenitor cells [52].

Prevention of HCC by Metformin:
Molecular Mechanisms

A number of molecular mechanisms have been proposed to account for the activity of metformin as a cancer preventive agent. In general, these anti-proliferative effects are the result of at least two different mechanisms: they either stem from improved glucose and lipid homeostasis leading to the correction of insulin resistance and hyperinsulinemia, or from direct interference with specific oncogenic pathways [53]. This is an important distinction to make since metformin could have a broader impact as a chemopreventive drug if we had evidence for its anti-cancer activity in the absence of manifest diabetes or insulin resistance (Figure 1). In fact, recent research indicates that metformin is a beneficial addition to neo-adjuvant therapy in breast cancer of non-diabetic patients [54, 55] and other clinical trials to evaluate the anti-cancer effect of metformin in colorectal and breast cancer are underway [54, 56]. The impact of metformin on the development of HCC in non-diabetics, however, remains to be seen.

A key molecular target underlying the beneficial metabolic effects of metformin is the adenosine mono-phosphate-activated protein kinase (AMPK), known as a master regulator of the cell’s bioenergetic homeostasis [57]. While a number of mechanisms are known to stimulate AMPK besides metformin and some effects of metformin have been shown to occur without the activation of AMPK, the metformin-AMPK link provides a major platform for drug development in diabetes [58]. The precise mechanism by which metformin activates AMPK is not fully elucidated, but relates to the modulation of mitochondrial ATP synthesis as AMPK responds to an increased AMP/ATP ratio [59]. In response, AMPK modulates the activity of several enzymes to enhance catabolic pathways that generate ATP and limit ATP-consuming mechanisms in order to restore intracellular energy balance [59]. Accordingly, AMPK activation results in marked metabolic changes such as inhibition of glucose output from the liver, promotion of glucose uptake in the skeletal muscle, decreased lipolysis from peripheral fat cells, and enhanced fatty acid oxidation in liver mitochondria [1, 59]. These changes not only correct diabetes-associated derangements of glucose and lipid metabolism, but also mitigate the oncogenic impact of prolonged hyperinsulinemia. Elevated insulin levels boost the production of insulin-like growth factor (IGF)-binding protein and lead to increased bioavailability of IGF1 and IGF2 [60], which are implicated in the activation of various oncogenic pathways that involve phosphatidylinositol-3-kinase (PI3K)/Akt, mitogen-activated protein kinase (MAPK), and vascular endothelial growth factor (VEGF) [61, 62]. Reversing insulin resistance and hyperinsulinemia via AMPK activation may therefore greatly contribute to the anti-cancer effects of metformin.

AMPK is also under the regulation of adiponectin, a major adipokine with potent anti-inflammatory, antiangiogenic and tumor growth-limiting properties, which has diminished bioavailability in obesity-associated conditions [63]. Adiponectin primarily acts in the liver through the adiponectin receptor 2 (AdipoR2), which has been recently associated with the activation of LKB1, a serine/threonine kinase involved in multiple pathways with a major role in tumor suppression [57, 64]. Accordingly, AMPK is not only a key regulator of energy metabolism, but this role extends to the regulation of cell growth and proliferation. Importantly, if the adiponectin-AdipoR2-LKB1-AMPK axis fails in diabe-
The cancer preventing properties of metformin have also been demonstrated by its impact on the cell cycle. Administration of the AMPK-activating agents AICAR and metformin inhibited the proliferation of PLC/PRF/5 and HepG2 human hepatoma cell lines and induced cell cycle arrest at the G1/S checkpoint [80]. Analysis of HCC tissue samples revealed that the amount of phosphorylated AMPK was significantly decreased in comparison with peritumoral liver tissues [80]. In addition, metformin was shown to inhibit cell growth and promote cell senescence by inhibiting cyclin D1 expression and pRb phosphorylation [81]. Induction of cell cycle arrest in HepG2 and Hep3B by metformin occurs through inhibition of cyclin D1 and upregulation of p21/Cip1 and p27/Kip1 in an LKB1/AMPK-dependent manner [37]. However, the anti-proliferative effects of metformin are not necessarily tied to the activation of AMPK. As recently shown, treatment of the human
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HCC cell lines Huh1 and Huh7 with metformin markedly enhanced the rate of apoptosis and reduced the number of cells positive for CD133 and EpCAM, while these effects did not correlate with changes in the activity of AMPK/mTOR pathway, suggesting the role of additional molecular mechanisms [51].

Current Concerns and Future Directions

There are several clinical and experimental observations to indicate that it is not yet prime time for metformin as a generally recommended chemopreventive agent. As discussed above, some findings could not confirm that reduced risk of HCC in diabetes is due to metformin therapy rather than to a disease with less severity or of shorter duration [43–45]. Furthermore, even when convincing data implicate metformin in the reduced risk of HCC, this effect may not be linked necessarily to the prevention or delay in NAFLD progression, providing therefore little clue for the mechanisms of anti-cancer action [41, 42]. Importantly, more randomized controlled trials are needed to provide robust investigational evidence in support of the observational studies on cancer prevention by metformin.

Recent research indicates that the redox shuttle enzyme mitochondrial glycerophosphate dehydrogenase (mGPD) is another mitochondrial target of metformin, allowing the modulation of hepatocellular redox state and limiting hepatic glucose production [82]. While the role of mGPD as a molecular target of metformin in devising novel anti-cancer strategies remains to be seen, non-competitive inhibition of mGPD by metformin was shown to promote the development of lactic acidosis, which is a rare but significant adverse event associated with the use of biguanides explained by the blockade of mitochondrial complex I [83]. Lactic acidosis may develop as a combined result of diminished gluconeogenesis and higher rates of glycolysis in the liver, while glucose utilization is increased in peripheral tissues [72]. Historically, high incidence of lactic acidosis was seen with phenformin therapy (roughly 64 cases per 100,000 patient-years), which led to withdrawal of the drug in 1976 [84]. Phenformin was an earlier guanidine derivative with higher affinity to mitochondrial membranes and a more potent complex I inhibition [85]. There is often clinician concern about inducing lactic acidosis in diabetic patients with chronic liver disease, although a large Cochrane review in 2010 found that the incidence of lactic acidosis in patients taking metformin was 4.3 cases per 100,000 patient-years compared to 5.4 cases in the non-metformin group based on pooled data from 347 comparative trials and cohort studies [86]. In concurrence, lactic acidosis was never observed in the course of a recent multicenter study analyzing the clinical outcomes in a cohort of 172 diabetic patients who continued metformin therapy after being diagnosed with cirrhosis for up to 13 years [87], indicating that the use of metformin is safe even in this advanced condition.

A recent meta-analysis revealed that the chemopreventive effect of metformin in diabetics was strongest in Western as opposed to Asian cohorts [88]. Notably, the primary etiologies of HCC differ according to geographical areas. HCC associated with alcoholic liver disease and NAFLD predominates in the West, whereas chronic hepatitis C is by far the most common etiology of HCC in Japan, and hepatitis B with or without aflatoxin accounts for many cases of HCC in Asia and sub-Saharan Africa. These differences may reflect on the variable contribution of diabetes-associated liver disease, and hence of metformin therapy, to the regional prevalence of HCC.

Recent data on the potential role of statin therapy in the reduction of HCC risk [89] indicate that, given the large number of individuals afflicted by both hyperlipidemia and diabetes, clinical studies on the concurrent use of statins and insulin-sensitizing agents is warranted to explore the benefits of a combination therapy. Finally, given that a non-trivial proportion of NAFLD-associated HCC can develop in non-cirrhotic livers [24, 90], it will be important to establish HCC surveillance guidelines for non-cirrhotic liver disease of patients with long-standing diabetes. Well-designed randomized controlled trials and longitudinal studies will be necessary to provide convincing evidence related to the efficacy and safety of metformin as a chemopreventive agent in various clinical settings.

Conclusions

Current estimates indicate that 29.1 million Americans, or 9.3% of the population, were afflicted by diabetes in 2012 [91]. This number has been increasing at a considerable rate in recent years. Besides the crippling ef-
effects of macro- and microvascular complications in diabetes, there is overwhelming evidence for higher cancer risk associated with this condition. One of the prominent diabetes-associated cancers is HCC, which is the most common form of primary liver cancer, with a high global prevalence and an age-adjusted incidence in the US that has tripled over the past 3 decades [22]. The recent revolution in the treatment of chronic hepatitis C with the use of highly efficient direct acting antivirals carries the promise of eradicating the hepatitis C virus, thus eliminating a key etiology of HCC. These long-awaited advances will increasingly shift medical attention to NAFLD as an emerging cause of HCC, making it imperative to develop new ways of screening, surveillance, and chemoprevention in diabetes-associated chronic liver disease, which represents an emerging condition with increased risk for hepatocarcinogenesis.

Metformin, an anti-diabetes drug that targets multiple oncogenic pathways, appears to be a promising cancer preventing agent against HCC and several other cancers. However, the role of metformin may be biased due to its different use depending on diabetes duration and severity. In addition, concerns about lactic acidosis, albeit a rare adverse event, has been limiting the pool of patients eligible for metformin and may dampen enthusiasm about its widespread use for preventing the development and progression of HCC. Accordingly, the focus of future research may be on developing randomized clinical trials to evaluate metformin unequivocally in the chemoprevention of HCC, gathering evidence for its use in non-diabetic and non-cirrhotic cohorts, elucidating its precise mechanisms of action to identify new molecular targets, and developing safer and more efficient derivatives of this intriguing compound.

Disclosure
There are no conflicts of interest.

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