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**Diabetes mellitus and metformin in hepatocellular carcinoma**

Fujita K *et al.* Metformin in HCC

Koji Fujita, Hisakazu Iwama, Hisaaki Miyoshi, Joji Tani, Kyoko Oura, Tomoko Tadokoro, Teppei Sakamoto, Takako Nomura, Morishita Asahiro, Hirohito Yoneyama, Tsutomu Masaki

**Koji Fujita, Hisaaki Miyoshi, Joji Tani, Kyoko Oura, Tomoko Tadokoro, Teppei Sakamoto, Takako Nomura, Morishita Asahiro, Hirohito Yoneyama, Tsutomu Masaki,** Department of Gastroenterology and Neurology, Faculty of Medicine, Kagawa University, Kagawa 760-8521, Japan

**Hisakazu Iwama,** Life Science Research Center, Kagawa University, Kagawa 760-8521, Japan

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**Correspondence to: Tsutomu Masaki, Professor,** Department of Gastroenterology and Neurology, Faculty of Medicine, Kagawa University, Ikenobe 1750-1, Miki, Kita, Kagawa 760-8521, Japan. tmasaki@med.kagawa-u.ac.jp

**Telephone:** +81-87-891-2156

**Fax:** +81-87-891-2158

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**Abstract**

Hepatocellular carcinoma (HCC) is the leading cause of cancer-related death worldwide. Diabetes mellitus, a risk factor for cancer, is also globally endemic. The clinical link between these two diseases has been the subject of investigation for a century, and diabetes mellitus has been established as a risk factor for HCC. Accordingly, metformin, a first-line oral anti-diabetic, was first proposed as a candidate anti-cancer agent in 2005 in a cohort study in Scotland. Several subsequent large cohort studies and randomized controlled trials have not demonstrated significant efficacy for metformin in suppressing HCC incidence and mortality in diabetic patients; however, two recent randomized controlled trials have reported positive data for the tumor-preventive potential of metformin in non-diabetic subjects. The search for biological links between cancer and diabetes has revealed intracellular pathways that are shared by cancer and diabetes. The signal transduction mechanisms by which metformin suppresses carcinogenesis in cell lines or xenograft tissues and improves chemoresistance in cancer stem cells have also been elucidated. This review addresses the clinical and biological links between HCC and diabetes mellitus and the anti-cancer activity of metformin in clinical studies and basic experiments.

**Key words:** Hepatocellular carcinoma; Diabetes mellitus; Metformin; Risk

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**Core tip:** Diabetes mellitus, an increasing risk factor for hepatocellular carcinoma (HCC), shares pathological mechanisms with HCC. Thus, the first-line anti-diabetic metformin was anticipated to reduce cancer risk. Though basic research has provided evidence of its anti-cancer effect, clinical studies of diabetic patients have not provided conclusive data that metformin reduces HCC risk. Clinical studies have suggested that metformin may suppress cancer in non-diabetic subjects. Basic research on cancer stem cell-targeting therapies has also examined the potential of metformin.

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**INTRODUCTION**

Hepatocellular carcinoma (HCC) is the sixth most frequent cancer worldwide and the third most common cause of cancer death[1,2]. This primary liver cancer originating from hepatocytes is characterized by poor prognosis, and patients with HCC incident risks must be monitored closely for HCC occurrence[3]. Patients in the early stage of malignancy onset can be cured by surgery[4-6] or radiofrequency ablation therapy[7,8] However, HCC recurs in 60%–80% of patients within 5 years of curative treatments[9-12] due to intrahepatic metastasis or multicentric occurrence facilitated by the long-term exposure of the liver to chronic viral hepatitis, fibrotic changes and hyperinsulinemia. Patients who do not meet the criteria for surgical resection or radiofrequency ablation therapy can be treated with either transcatheter arterial chemoembolization therapy[13,14], an anti-cancer drug and lipiodol emulsion[15], or drug-eluting beads[16] with the intention of downstaging the tumor to enable hepatic resection or await liver transplantation. Liver transplantation, a radical curative surgery with a 3-year overall survival rate of approximately 40%, has been established as a standard treatment for HCC using the Milan criteria[17]. However, patients who require liver transplantation must confront a shortage of donors and progression of their cancer stage while awaiting a donor, particularly in Asia[18]. Patients who fail or are excluded from any of the above treatments might receive sorafenib, a multi-kinase inhibitor that has exhibited effectiveness for improving HCC patient prognosis in randomized control trials[19,20] but improves survival by only 3 mo.

Therefore, the prevention of HCC and the intense screening of high-risk individuals are of great importance. The risk factors for HCC are now well-established, and great efforts have been made to decrease HCC prevalence, recurrence and death. The medical histories of HCC patients range from liver-specific viral infections, such as HBV and HCV, and alcohol consumption to metabolic disorders including diabetes mellitus and obesity[21]. Effective anti-viral agents for HBV and HCV significantly suppressed HCC prevalence in clinical trials[22-25]. However, type 2 diabetes mellitus, characterized by hyperinsulinemia in its early stage and usually linked to obesity, is increasing worldwide.

Metformin, an oral anti-diabetic drug that is less expensive than any other anti-cancer agent in use, first attracted attention in 2005 for its potential to suppress not only serum glucose levels but also the incidence of various cancers in an observational study[26]. Metformin has subsequently been investigated as an anti-cancer medicine for malignancies including HCC in diabetic and non-diabetic subjects. This review describes the links between HCC and type 2 diabetes mellitus in terms of their epidemiology and pathology and addresses the benefits and limitations of metformin in the prevention and treatment of HCC.

**DIABETES MELLITUS IN HCC EPIDEMIOLOGICAL TRENDS**

Some trends of HCC epidemiology have recently attracted attention. First, the increase in HCC incidence rates has ceased in two nations, Japan and the United States[27-29], though the prevalence of HCC and HCC-related deaths will continue to increase in the future[2,30]. The restriction of these phenomena to these two developed countries reflects the diversity of HCC incidence with geography, socioeconomic condition, race, generation and gender in these two countries[31]. In Japan, liver cancer incidence rates rapidly increased between the mid-1970s and 1990s but then leveled off and began to decrease by 2003[27,32]. This trend in a Japanese city has been attributed to a decline in HCV infection, previously the major cause of HCC in Japan, partly by avoiding HCV-contaminated blood transfusion and suppression and by restraining injections of drugs of abuse after the Second World War. In the US, the absence of a significant increase in HCC incidence rates is due in part to the decreased prevalence of HCC among the largest group with an HCC risk caused by HBV, Asians/Pacific islanders, particularly men aged 35-49 years[28].

Second, a frequent risk factor for HCC, the hepatitis C virus, can now be more easily and thoroughly eradicated by direct anti-viral agents[24,25], which are newly developed oral medications that might affect HCC prevalence more strongly than interferon[33,34]. HBV is another hepatocarcinogenic virus that infects hundreds of millions of people globally. The replication and inflammatory activity of HBV in a HBV-infected liver can be inhibited by the newly developed nucleotide analogues entecavir[22,23] and tenofovir[35]. These two retrograde transcriptional viral inhibitors have largely overcome the disadvantages of the previous nucleotide analogues, lamivudine and adefovir, which usually caused viral mutations and drug resistance in 70% of patients who took lamivudine for 4 years[36] and in 20% of patients who were prescribed adefovir for 5 years[37,38].

Third, the prevalence of another HCC risk factor, type 2 diabetes mellitus, continues to increase[39,40] with the increase in the incidence of obesity[41]. The number of diabetic patients is estimated to increase to 300–400 million worldwide by 2030[39,40,42]. Such predictions are based on increases in populations living in urban areas in developing countries, in senior diabetic patients and in obesity.

**DIABETES MELLITUS AS A RISK FACTOR FOR HCC**

Diabetes mellitus was first investigated as a risk factor for cancer death at the beginning of the 20th century, when the etiologies of these two major deadly diseases were unknown. An observational study addressing cancer deaths in U.S. cities in 1910 concluded that the correlation between cancer and diabetes mellitus was not fortuitous nor due merely to errors of observation[43], although cancer prevalence at that time was biased by the availability of medical schools at which post-mortem examinations and cancer diagnoses could be performed, as discussed by Greenwood[44]. Greenwood himself analyzed the correlation between death rates due to diabetes and cancer. He concluded that cancer mortality was significantly associated with diabetes in the US and not in Europe, but he did not assess the correlation between organ-specific cancer deaths and diabetes[44].

Among various organ-specific cancers, pancreatic cancer was initially determined to have coincidence with diabetic conditions in an observation of 10000 diabetic patients in 1934, although that conclusion may have been due to reverse causation[45]. In 1970, Kessler reported an association between pancreatic cancer mortality and diabetes mellitus prevalence in human males, although no excess deaths *via* any other type of cancer were observed among diabetic patients; this phenomenon occurred in part because diabetic patients died from diabetes itself or from cardiovascular diseases before cancers other than those of pancreatic origin became fatal[46].

The strength of the association between cancers and diabetes depends on the cancer species; however, a hospital-based case control study in 1986 demonstrated that more than 4 times as many HCC patients suffered from diabetes mellitus than colorectal tumor patients and femoral bone fracture patients[47]. A large population-based cohort study in Uppsala, Sweden, confirmed the significantly increased risk of HCC as well as pancreatic cancer in diabetic patients with a relative ratio of approximately 1.5, which was higher in males than in females[48]. The link between diabetes mellitus and the larger HCC population is supported by two major prospective cohort studies, one in Sweden[49] and the other in Denmark[50], followed by another case-control study in Italy[51]. Diabetes mellitus has subsequently been investigated as a risk factor for the prevalence[52,53], recurrence[54-57] and mortality[58,59] of HCC.

Diabetes mellitus is now considered an independent risk factor for HCC[60,61] and has been proven to increase the risk of HCC even in those not infected with HBV or HCV[57,62,63]. Increased incidence and mortality of several malignancies other than HCC, including pancreatic cancer, endometrial cancer and colon cancer, have been observed among diabetic patients in a series of studies[48,58,64], partly based on obesity, which has also been identified as a risk factor for cancers including HCC[65,66]. An association between post-load plasma glucose in a non-diabetic individual who has the potential to develop diabetes mellitus and cancer mortality due to HCC has also been suggested[67,68].

These observational studies assessing the potential role of diabetes mellitus as a risk factor for cancers were not free from detection bias and reverse causation; the cancer risk was highest immediately after the diabetes cases were registered or diagnosed in each study and then decreased gradually with time[69]. However, the risks of HCC, pancreatic cancer and endometrial cancer remained significant after adjusting for detection bias and reverse causation[166]. Major clinical studies of the relationships between diabetes mellitus and HCC and other cancers are illustrated in Table 1.

**A BIOLOGICAL LINKAGE BETWEEN DIABETES MELLITUS AND CANCER**

In 1910, Maynard hypothesized that cancer occurrence might be due to meteorological conditions, such as hours of sunshine, mean temperature, rainfall and other indicators, but observed no significant correlations[43]. He subsequently focused on diabetes mellitus as a possible cause of cancer because the two diseases occurred at similar ages, were increasing in prevalence and had no known etiologies at that time, as discussed by Greenwood[44]. The subsequent body of research has since established that the etiologies of diabetes mellitus and cancer share a number of biological pathways[61], some of which are based on central obesity and insulin resistance, common risk factors for both diseases[70].

***The insulin/IGF-1 axis involves over-activation of mTOR***

The classical pathways shared by diabetes mellitus and cancer are the Insulin/IGF- axis, including over-activation of mTOR. Type 2 diabetes mellitus is characterized by hyperinsulinemia. Insulin exerts its proliferative effects directly through the insulin receptor (IR) and indirectly by increasing circulating levels of IGF-1[71]. Insulin increases circulating IGF-1 by decreasing hepatic production of IGF-binding protein 1, a ligand of IGF-1, thus increasing levels of free IGF-1[72,73]. In hyperinsulinemia, the activity of insulin becomes less metabolic and more mitogenic. Insulin decreases its metabolic activity by over-activation of mTOR, which phosphorylates IR-substrate-1 and attenuates metabolic pathways downstream of insulin signals. Simultaneously, insulin up-regulates IR-substrate-2 and induces the mitogen-activated kinase pathway, thereby enhancing cell survival[74].

Hyperinsulinemia is regarded as an independent risk factor for HCC, and major dysregulations of insulin-dependent pathways have been reported in HCC[75]. The effect of HCC on development also depends on excess signals from IGF-I but more strongly on signals from IGF-II[76]. Aberrant mTOR signaling in HCC has been confirmed in human tumor samples[77].

***Chronic inflammation caused by adipokines***

Central obesity, which usually accompanies type 2 diabetes mellitus, may be a trigger for carcinogenesis *via* pro-inflammatory cytokines secreted from visceral adipose tissues. Adipokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are produced in the excess visceral fatty compartment and perpetuate chronic low-grade inflammation in peripheral tissues, which provides microenvironments suitable for tumorigenesis[78]. Another adipose tissue-derived hormone, leptin, promotes or suppresses cell proliferation[79]. Adiponectin, which is produced most highly by adipokines, presents both anti-inflammatory and anti-tumor activities[80].

However, in the case of HCC, the carcinogenic or anti-tumor effects of the two hormones, leptin and adiponectin, have been the subject of contradictory reports, and more conclusive data are needed[81].

***Hyperglycemia***

Hyperglycemia is a feature of diabetes mellitus, and Warburg first hypothesized that hyperglycemia itself might have carcinogenic potential[82]. In general, cancer cells produce ATP by anaerophilic glycolysis. Under aerophilic conditions, cytoplasmic ATP production *via* glycolysis is less efficient than synthesis *via* oxidative phosphorylation in mitochondria. Cancer cells are consequently assumed to require more glucose than normal cells, and several types of cancers have been detected by positron emission tomography (PET) based on this theory[83].

Direct carcinogenic effects of hyperglycemia combined with the Wnt signaling pathway were recently proposed to promote carcinogenesis, resulting in nuclear beta-catenin accumulation[84] *via* aberrant acetylation of beta-catenins. A national cohort study in Taiwan revealed a linear relationship between HCC occurrence and HbA1c in hyperglycemia[85]. In addition, a case-control study in Japan demonstrated that post-challenge hyperglycemia was an independent risk factor for HCC[86].

However, hyperglycemia has been considered subordinate to hyperinsulinemia as a carcinogen, and a meta-analysis of large randomized controlled trials did not indicate definitive cancer risk reduction by intensive glycemic controls in patients with type 2 diabetes mellitus[87,88].

***Estrogen***

Estrogen is produced primarily in the body fat of postmenopausal women and obesity, a background metabolic disorder of diabetes mellitus, is linked to elevated serum estrogen levels. Therefore, estrogen is recognized as a carcinogenic risk factor for breast, endometrial and ovarian cancers in post-menopausal women[89].

In HCC, however, primary liver malignancies occur predominantly in males, and male HCC patients usually present with a poorer prognosis than female HCC patients[90]. Among women, post-menopausal women suffer from an elevated incidence of HCC, which is epidemiologically suppressed by estrogen therapy[91]. The relatively lower estrogen levels in males compared to females and in post-menopausal women compared to estrogen-supplemented women suggests that low estrogen might contribute to the more frequent cancer prevalence in men, particularly for HCC, because estrogen appears to suppress HCC development by inactivating chronic low-grade inflammation in the liver[92].

**METFORMIN, A DRUG TO POTENTIALLY PREVENT HCC OCCURRENCE, RECURRENCE AND DEATH**

Metformin, a first-line oral anti-diabetic[93], was associated with reduced prevalence of cancers in type 2 diabetic patients by Evans in 2005[26]. This pilot case-control study, which did not include site-specific cancer data, provided the foundation for epidemiological studies on the anti-tumor effects of metformin. An herb called Galega Officinalis, which contains large amounts of guanidine, the original molecule of metformin, was prescribed as long ago as the 17th century to relieve diabetic symptoms[94].

A population-based cohort study by Bowker *et al*[95] in 2006 demonstrated that cancer mortality in the type 2 diabetic group decreased when metformin was prescribed compared to insulin injection. A Scottish cohort study also demonstrated a protective effect of metformin against total cancers[96].

In the case of HCC, a case-control study suggested that HCC risk was reduced in male type 2 diabetic patients prescribed metformin[97] and a subsequent cohort study including male and female subjects[98]. A hospital-based case-control study in the US also indicated that metformin reduces the incidence of HCC in type 2 diabetic patients[99]. A prospective cohort study in Taiwan performed by Lee *et al*[53] identified benefits of metformin for HCC prevention compared to other anti-diabetics, with a reduced risk of other tumors, pancreatic and colorectal cancer as well. Another cohort study in Taiwan also demonstrated that the development of HCC was suppressed by metformin administration[100].

However, observational studies of cancer incidence and mortality are subject to analyses of time-related biases, which have led to debate and controversy[101,102]. Two retrospective cohort studies observed no influence of metformin on cancer risk[103,104]. A meta-analysis excluding studies with time-related biases stated that metformin did not significantly reduce the risk of HCC. However, colon cancer was the only type of cancer that remained significant in a site-specific cancer risk analysis, and a 10% risk reduction for total cancers remained[105]. A randomized controlled trial comparing metformin to rosiglitazone failed to support a significant difference in cancer occurrence between the two oral anti-diabetics[106]. A meta-analysis of randomized controlled trials concluded that metformin provided patients with little benefit with respect to overall mortality compared to other anti-diabetics or insulin therapy and a 10% reduction in mortality compared to placebo or usual care that did not reach statistical significance[107].

 In summary, the limited epidemiological research on the anti-cancer activity of metformin in diabetic patients indicates that this drug definitely exhibited an association with deceased cancer prevalence in case-control studies and cohort studies but has failed all randomized controlled trials in diabetic subjects[108,109]. No conclusive data from clinical trials regarding the prevention of cancers, including HCC in diabetic subjects, by metformin are available.

Clinical studies of metformin as an adjuvant to conventional chemotherapy and radiotherapy have reported promising data in case-control studies intended for patients with pancreatic cancers[110], breast cancers[111], lung cancers[112] and colorectal cancers[113]. However, a randomized controlled trial investigating the adjuvant use of metformin with conventional chemotherapy intended for pancreatic cancer failed to demonstrate a significant improvement of overall survival[114]. As an adjuvant to an estrogen-synthesis inhibitor, metformin is being prescribed to estrogen receptor-positive post-menopausal breast cancer patients without diabetes mellitus in a phase II randomized controlled trial[115]. No randomized controlled trials are available to demonstrate the feasibility of metformin as an adjuvant to non-surgical therapy.

For HCC, a combination of metformin and radiation therapy yielded prolonged overall survival compared to controls[116], although adjuvant use of sorafenib resulted in shorter progression-free survival and poorer overall survival[117]. As for primary cancer prevention, results on the adjuvant use of metformin in clinical trials are not conclusive, and further investigations are needed.

 The application of metformin as an agent against premalignant tumor activity is being explored in non-diabetic cases to prevent tumor incidence. Metformin suppressed the incidence of colorectal aberrant crypt foci, surrogate markers of colon cancer, in non-diabetic subjects in a small randomized controlled trial[118]. A double-blind randomized controlled trial in Japan demonstrated a significant reduction in colorectal polyp formation in non-diabetic patients after 1 year of administration of low-dose metformin[119]. Similar trials on HCC have not been performed, and the anti-tumor activity of metformin in non-diabetic cases requires further elucidation. The major clinical studies of the anti-cancer effect of metformin against HCC and other cancer types are illustrated in Table 2.

**ANTI-CANCER MECHANISM OF METFORMIN**

Studies of the anti-cancer mechanism of metformin have followed basic research on the intracellular signaling downstream of metformin to improve hyperglycemia, hyperlipidemia and hyperinsulinemia. An anti-cancer role of metformin had not been proposed when AMPK was identified as a major target molecule of metformin[120]. The identification of LKB1, a tumor suppressor gene, upstream of AMPK highlighted the biguanides as candidate anti-cancer drugs[121,122]. Downstream of AMPK, mTOR, an energy sensor and a gene that plays multiple roles in cell proliferation, was identified[123,124]. An *in vivo* study employing LKB1 knockout mice clarified that metformin signals in the liver *via* the LKB1/AMPK axis in the context of glucose homeostasis[125]. A role of the LKB1/AMPK/mTOR axis in carcinogenesis and mediating the anti-cancer signaling of metformin was subsequently identified. Another study in LKB1-AMPK double knockout mice identified an AMPK-independent pathway that improves the diabetic state[126]. AMPK-independent anti-carcinogenic pathways of metformin have also been investigated.

***LKB1/AMPK/mTOR axis***

Metformin halts the respiratory chain in mitochondria and increases cell energy stress, which activates LKB1 and AMPK. AMPK activation inhibits mTOR and suppresses cell proliferation[123,124]. Furthermore, LKB1/AMPK disturbs insulin signals by degrading IR-substrate-1, resulting in the suppression of insulin/IGF-1 signaling[127], a pathway shared by diabetes and cancer. In lipid metabolism, which is indispensable for tumor growth[128], metformin directly inhibits fatty acid synthase[129]. Metformin arrests the cell cycle in malignant cells *via* activated AMPK, which is correlated with the downregulation of cyclin D1 and the upregulation of p21CIP and p27KIP[130,131].

***AMPK-independent pathways***

AMPK-independent pathways downstream of metformin vary. Metformin is thought to protect against DNA damage from reactive oxygen species (ROS) by inhibiting ROS production when metformin inhibits the mitochondrial respiratory chain[132]. Metformin can also bypass AMPK and inhibit mTOR signaling[133] and induce cell cycle arrest by down-regulating cyclin D1 *via* p53[134,135]. An AMPK knockdown study demonstrated that metformin up-regulates apoptosis and autophagy *via* a Stat3/Bcl2 pathway[136]. Metformin decreases glucose uptake into cancer cell decreases *via* a direct allosteric effect on hexokinase II[137].

***MicroRNAs as mediators of the anti-cancer activity of metformin***

Metformin exerts its anti-carcinogenic activity by regulating microRNA (miRNA) expression to down-regulate target messenger RNAs. miRNAs are small non-coding RNAs with a length of 20–25 nucleotides. miRNAs can bind to messenger RNAs at their 3’-UTR and inhibit their translation, that is, they regulate gene expression at the post-transcriptional level and modulate biological processes, such as intracellular metabolism, cell proliferation, differentiation, apoptosis and angiogenesis[138].

 Metformin inhibits the cell cycle of various gastrointestinal tumors, including HCC, by up-regulating the let-7 family *in vitro* and *in vivo*[139-144]. For HCC in particular, metformin’s anti-cancer activities are mediated through let-7c, which targets RAS[145]; miR-140-5p, which targets TGFR1, FGF9[146] and DNMT1[147]; and miR-222, which targets PTEN and p57[148]. In pancreatic cancer cell lines, metformin suppresses HMGA1, a pseudogene gene highly expressed in cancer cells, by up-regulating miR-26a, which binds to and degrades the HMGA1 messenger RNA[149]. MiRNAs and their target messenger RNAs in cancers originating from other organs have been well summarized by Pulito[150].

**CANCER STEM CELLS AS A TARGET OF METFORMIN IN ADJUVANT THERAPY**

Cancer stem cells (CSCs) or tumor-initiating stem cells are a minor subset of the cancer cell population and have been hypothesized to exist among cancer cells. These cells should self-renew indefinitely to generate cancer clones hierarchically and resist chemotherapy and radiotherapy more strongly than any other cancerous daughter cells[151,152]. CSCs do not express definitive cell surface markers and have not been well defined due to extensive heterogeneity[153]. However, using surface markers and the enhanced ALDH1 activity of normal stem cells[154,155], research on CSCs has developed, and a small subset of cells that might include CSCs has been isolated and subjected to further analysis. In the case of HCC, several cell surface markers, such as CD 133, CD90, CD44, EpCAM, OV6 and SP, have been employed to focus on specific cells, including hepatic CSCs[156,157].

Tumors have the potential to be resistant to chemotherapy and radiotherapy, and this potential has been attributed to CSCs[158]. Targeting CSCs in cancer that is refractory to non-surgical treatments may provide a cure. Metformin as an adjuvant to conventional chemotherapy was determined to be effective against CSCs *in vitro* and *in vivo*[158]. For hepatic CSCs, metformin administration reduced EpCAM-positive cells, partly depending on the AMPK/mTOR pathway in cell lines and xenograft tumors[159].

**CONCLUSION**

Diabetes mellitus is globally endemic and has been established as a risk factor for HCC incidence in a large number of observational studies in which researchers critically analyzed study data and adjusted for as many biases as possible. Thus, future increases in diabetes mellitus will likely result in increases in the incidence of HCC. Metformin, a first-line oral anti-diabetic, has been shown to prevent cancer and reduce cancer mortality among diabetic patients in observational studies. Further investigations, particularly randomized controlled trials involving diabetic and non-diabetic subjects, remain necessary. *In vitro* and *in vivo* experiments have already provided evidence of the anti-tumor activity of metformin. Newly developed topics that are being investigated further include the AMPK-independent pathway represented by the LKB1/AMPK/mTOR axis; miRNAs downstream of this biguanide and their messenger RNAs that are pivotal to cell survival and proliferation; and cancer stem cells in HCC that are nearly completely identified using cell surface markers.

In daily clinical practice, the administration of metformin to cancer patients, including those with HCC, is associated with few complications. The biguanides exhibit good tolerance in diabetic patients, even those suffering from cirrhosis[160]. Lactic acidosis is not significantly associated with metformin[161]. Although a recent study proposed that metformin might impair cognitive function[162], causality between metformin prescription and cognitive impairment, including Alzheimer’s dementia, has not been confirmed. In summary, metformin is a safe drug for cancer patients as well as diabetic patients. Further clinical evidence of the anti-cancer activity of metformin would have implications for many patients suffering from cancer with or without diabetes mellitus.

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**Table 1 The influence of diabetes mellitus on the incidence, recurrence, and mortality of hepatocellular carcinoma**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Author** | **Year** | **Study design** | **Type of diabetes** | **Results** |
| Maynard[43] | 1910 | Case-control | Not differentiated | Cancer mortality increased. |
| Greenwood and Wood[44] | 1914 | Case-control | Not differentiated | Cancer mortality increased in American cities; no significant correlation was observed in European cities. |
| Marble[45] | 1934 | Case-control | Not differentiated | Pancreatic cancer incidence increased. |
| Kessler　II[46] | 1970 | Case-control | Not differentiated | Pancreatic cancer deaths increased. |
| Lawson *et al*[47] | 1986 | Case-control | Not differentiated | HCC incidence increased (HR = 3.9). |
| Levine *et al*[67] | 1990 | Cohort | IGT | HCC deaths increased in men; post-load plasma glucose increased. |
| Adami *et al*[48] | 1991 | Cohort | Not differentiated | Incidences of primary liver (RR = 1.5), pancreatic (RR = 1.4) and endometrial (RR = 1.5) cancers increased. |
| Smith *et al*[163] | 1992 | Cohort | IGT | Pancreatic cancer increased (RR = 2.25); post-load plasma glucose increased in IGT men. HCC was not analyzed in organ-specific statistics. |
| La Vecchia *et al*[51] | 1994 | Case-control | Not differentiated | Liver cancer incidence remained elevated 10 years after the diagnosis of diabetes (RR = 2.6). |
| Adami *et al*[49] | 1996 | Cohort | Not differentiated | Primary liver cancer incidence increased (SIR = 4.7 in men and 3.4 in women). |
| Wideroff *et al*[50] | 1997 | Cohort | Not differentiated | Primary liver cancer incidence increased (SIR = 4.0 in men and 2.1 in women). |
| La Vecchia *et al*[164] | 1997 | Case-control | Not differentiated | Liver cancer incidence increased (OR = 2.2) for at least 10 years after the diagnosis of diabetes. |
| Ikeda *et al*[54] | 1998 | Cohort | Not differentiated | Recurrence-free survival after hepatic resection decreased in diabetic cases. |
| Balkau *et al*[52] | 2001 | Cohort | Not differentiated | HCC incidence increased with fasting hyperinsulinemia (HR = 2.72) and 2-hour hyperinsulinemia (HR = 3.41). |
| Huo *et al*[55] | 2003 | Cohort | Not differentiated | HCC recurrence increased in HBV-seropositive cases. |
| Coughlin *et al*[58] | 2004 | Cohort | Not differentiated | Liver cancer mortality increased in men (RR = 2.19). |
| Batty *et al*[68] | 2004 | Cohort | IGT | HCC (HR = 2.47) and pancreatic cancer (HR = 1.35) increased; post-load plasma glucose increased in IGT men. |
| El-Serag *et al*[60] | 2006 | Meta-analysis | Not differentiated | HCC incidence increased in 9 case-control studies (OR = 2.5) and 7 cohort studies (OR = 2.5). |
| Inoue *et al*[64] | 2006 | Cohort | Not differentiated | HCC incidence increased (HR = 2.24 in men and 1.94 in women). |
| Komura *et al*[56] | 2007 | Cohort | Not differentiated | Postoperative recurrence-free survival decreased in diabetic cases. |
| Kawamura *et al*[57] | 2008 | Cohort | Not differentiated | HCC recurrence increased (HR = 4.61). |
| Landman *et al*[59] | 2010 | Cohort | Type 2 | HCC death increased (SMR = 1.47). |
| Lee *et al*[53] | 2011 | Cohort | Type 2 | Incidences of total cancer, HCC and pancreatic cancer increased. |
| Hense *et al*[165] | 2011 | Cohort | Type 2 | HCC incidence increased (SIR = 1.94). |
| Johnson *et al*[166] | 2011 | Cohort | Type 2 | After detection biases were excluded, incidences of HCC (HR = 2.53), pancreatic (HR = 1.65) and endometrial (HR = 1.58) cancers increased. |
| P. Wang *et al*[167] | 2012 | Meta-analysis | Type 1 and type 2 | HCC incidence (RR = 2.23) and mortality (RR = 2.43) increased in cohort studies. |
| C. Wang *et al*[168] | 2012 | Meta-analysis | Not differentiated | HCC incidence (RR = 2.01) and mortality (RR = 1.56) increased. |
| Lai *et al*[100] | 2012 | Cohort | Not differentiated | HCC incidence increased (HR = 1.73). |
| Schlesinger *et al*[62] | 2013 | Cohort | Not differentiated | HCC incidence increased (RR = 2.17) in HBV/HCV-negative individuals. |
| Koh *et al*[63] | 2013 | Cohort | Not differentiated | HCC incidence increased (HR = 2.14), particularly in non-viral cases (HR = 5.15). |
| Y-G Wang *et al*[169] | 2014 | Meta-analysis | Not differentiated | HCC in diabetic cases was related to overall survival (RR = 1.46) and disease-free survival (RR = 1.57). |
| Harding *et al*[69] | 2015 | Case-control | Type 1 and type 2 | Incidences of total, liver, pancreatic and endometrial cancer increased in cases involving type 2 diabetes mellitus. |

RR: Relative risk; OR: OR; HR: Hazard ratio; SIR: Standardized incidence ratio; SMR: Standardized mortality ratio; IGT: Impaired glucose tolerance.

**Table 2 The efficacy of metformin on the incidence, recurrence and mortality of hepatocellular carcinoma and other tumors**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Author** | **Year** | **Study design** | **Type of diabetes** | **Results** |
| Evans *et al*[26] | 2005 | Case-control | Type 2 | HCC incidence decreased (OR = 0.79). |
| Bowker *et al*[95] | 2006 | Cohort | Type 2 | Mortality was lower among metformin users than among insulin or sulfonylurea users (HR = 0.77). |
| Libby *et al*[96] | 2009 | Cohort | Type 2 | Total cancer incidence decreased (HR = 0.63). |
| Donadon *et al*[97] | 2009 | Case-control | Type 2 | HCC incidence was lower among metformin users (OR = 0.33) than among insulin users (OR = 2.99). |
| Donadon *et al*[98] | 2010 | Cohort | Type 2 | HCC incidence was lower among metformin users (OR = 0.15) than among insulin or sulfonylurea users. |
| Hassan *et al*[99] | 2010 | Case-control | Not differentiated | HCC incidence decreased (OR = 0.30). |
| Home *et al*[106] | 2010 | Randomized controlled trial | Type 2 | Total cancer incidence did not decrease compared with rosiglitazone users. |
| Landman *et al*[59] | 2010 | Cohort | Type 2 | HCC deaths decreased (HR = 0.43). |
| Hosono *et al*[118] | 2010 | Randomized controlled trial | Non-diabetic | A surrogate marker of colorectal cancer incidence decreased. |
| Ferrara *et al*[103] | 2011 | Cohort | Not differentiated | No decreases in the incidence of any cancer; no data on HCC were available. |
| Lee *et al*[53] | 2011 | Cohort | Type 2 | Incidences of total cancer (HR = 0.12), HCC (HR = 0.06) and colorectal cancer (HR = 0.36) decreased. |
| Hense *et al*[165] | 2011 | Cohort | Type 2 | HCC incidence did not decrease. |
| Lai *et al*[100] | 2012 | Cohort | Not differentiated | HCC incidence was decreased by metformin (HR = 0.49) and thiazolidinedione (HR = 0.56). |
| Ruiter *et al*[170] | 2012 | Cohort | Not differentiated | Incidences of total cancer (HR = 0.90) and HCC (HR-0.67) were lower among metformin users than among sulfonylurea users. |
| Stevens *et al*[107] | 2012 | Meta-analysis | Type 2 and at-risk for diabetes | The summary RR for cancer outcomes was 1.02 across all trials. |
| Thakkar *et al*[108] | 2013 | Meta-analysis | Type 2 | Total cancer incidence decreased in case-control studies (RR = 0.90) and cohort studies (RR = 0.70) but did not significantly decrease in randomized controlled trials. |
| Yin *et al*[109] | 2013 | Meta-analysis | Type 2 | Overall survival (HR = 0.65) and cancer-specific survival (HR = 0.62) for total cancers were better for metformin than for other glucose-lowering medications. |
| Tsilidis *et al*[104] | 2014 | Cohort | Type 2 | Incidences of total cancer and HCC were not significantly lower among metformin users than among sulfonylurea users |
| Gandini *et al*[105] | 2014 | Meta-analysis | Not differentiated | After adjusting for time-related biases, total cancer incidence decreased (RR = 0.90), but this decrease became insignificant after adjusting for BMI in addition to time-related biases. Total cancer mortality and HCC incidence did not decrease after adjusting for time-related biases. |
| Higurashi *et al*[119] | 2016 | Randomized controlled trial | Non-diabetic | Incidences of metachronous colorectal adenomas (HR = 0.60) and total polyps (HR = 0.67) decreased. |

RR: Relative risk; OR: Odds ratio; HR: Hazard ratio.