World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v22.i27.6100

World J Gastroenterol 2016 July 21; 22(27): 6100-6113
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2016 Hepatocellular Carcinoma: Global view

Diabetes mellitus and metformin in hepatocellular carcinoma

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

Koji Fujita, Hisaaki Miyoshi, Joji Tani, Kyoko Oura, Tomoko Tadokoro, Teppei Sakamoto, Takako Nomura, Asahiro Morishita, 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

Author contributions: All authors made contributions to this manuscript.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Manuscript source: Invited manuscript

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: +81-87-8912156
Fax: +81-87-8912158

Received: March 28, 2016
Peer-review started: March 29, 2016
First decision: May 12, 2016
Revised: May 25, 2016
Accepted: June 15, 2016
Article in press: June 15, 2016
Published online: July 21, 2016

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

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.

Fujita K, Iwama H, Miyoshi H, Tani J, Oura K, Tadokoro T, Sakamoto T, Nomura T, Morishita A, Yoneyama H, Masaki T. Diabetes mellitus and metformin in hepatocellular carcinoma. *World J Gastroenterol* 2016; 22(27): 6100-6113 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i27/6100.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i27.6100

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 United States, 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 United States 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 United States 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[70]. Major clinical studies of the relationships between diabetes mellitus and HCC and other cancers are illustrated in Table 1.

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[71].

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[72]. Insulin increases circulating IGF-1 by decreasing hepatic production of IGF-binding protein

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

Ref.	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[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*[164]	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 yr 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*[165]	1997	Case-control	Not differentiated	Liver cancer incidence increased (OR = 2.2) for at least 10 yr 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-h 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*[166]	2011	Cohort	Type 2	HCC incidence increased (SIR = 1.94)
Johnson *et al*[70]	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
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
Wang *et al*[168]	2012	Meta-analysis	Not differentiated	HCC incidence (RR = 2.01) and mortality (RR = 1.56) increased
Lai *et al*[101]	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)
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: Odds ratio; HR: Hazard ratio; SIR: Standardized incidence ratio; SMR: Standardized mortality ratio; IGT: Impaired glucose tolerance.

1, a ligand of IGF-1, thus increasing levels of free IGF-1[73,74]. 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[75].

Hyperinsulinemia is regarded as an independent risk factor for HCC, and major dysregulations of insulin-dependent pathways have been reported in HCC[76]. The effect of HCC on development also depends on excess signals from IGF-Ⅰ but more strongly on signals from IGF-Ⅱ[77]. Aberrant mTOR signaling in HCC has been confirmed in human tumor samples[78].

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 and interleukin-6 are produced in the excess visceral fatty compartment and perpetuate chronic low-grade inflammation in peripheral tissues, which provides microenvironments suitable for tumorigenesis[79]. Another adipose tissue-derived hormone, leptin, promotes or suppresses cell proliferation[80]. Adiponectin, which is produced most highly by adipokines, presents both anti-inflammatory and anti-tumor activities[81].

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[82].

Hyperglycemia

Hyperglycemia is a feature of diabetes mellitus, and Warburg first hypothesized that hyperglycemia itself might have carcinogenic potential[83]. 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 based on this theory[84].

Direct carcinogenic effects of hyperglycemia combined with the Wnt signaling pathway were recently proposed to promote carcinogenesis, resulting in nuclear beta-catenin accumulation[85] *via* aberrant acetylation of beta-catenins. A national cohort study in Taiwan revealed a linear relationship between HCC occurrence and HbA1c in hyperglycemia[86]. In addition, a case-control study in Japan demonstrated that post-challenge hyperglycemia was an independent risk factor for HCC[87].

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[88,89].

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[90].

In HCC, however, primary liver malignancies occur predominantly in males, and male HCC patients usually present with a poorer prognosis than female HCC patients[91]. Among women, post-menopausal women suffer from an elevated incidence of HCC, which is epidemiologically suppressed by estrogen therapy[92]. 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[93].

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

Metformin, a first-line oral anti-diabetic[94], 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. As a matter of fact, 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[95].

A population-based cohort study by Bowker *et al*[96] 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[97].

In the case of HCC, a case-control study suggested that HCC risk was reduced in male type 2 diabetic patients prescribed metformin[98] and a subsequent cohort study including male and female subjects[99]. A hospital-based case-control study in the United States also indicated that metformin reduces the incidence of HCC in type 2 diabetic patients[100]. 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[101].

However, observational studies of cancer incidence and mortality are subject to analyses of time-related biases, which have led to debate and controversy[102,103]. Two retrospective cohort studies observed no influence of metformin on cancer risk[104,105]. 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[106]. A randomized controlled trial comparing metformin to rosiglitazone failed to support a significant difference in cancer occurrence between the two oral anti-diabetics[107]. 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[108].

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[109,110]. 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[111], breast cancers[112], lung cancers[113] and colorectal cancers[114]. 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[115]. 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[116]. 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[117], although an adjuvant to sorafenib resulted in shorter progression-free survival and poorer overall survival[118]. 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[119]. 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[120]. 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[121]. The identification of LKB1, a tumor suppressor gene, upstream of AMPK highlighted the biguanides as candidate anti-cancer drugs[122,123]. Downstream of AMPK, mTOR, an energy sensor and a gene that plays multiple roles in cell proliferation, was identified[124,125]. 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[126]. 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[127]. 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[124,125]. Furthermore, LKB1/AMPK disturbs insulin signals by degrading IR-substrate-1, resulting in the suppression of insulin/IGF-1 signaling[128], a pathway shared by diabetes and cancer. In lipid metabolism, which is indispensable for tumor growth[129], metformin directly inhibits fatty acid synthase[130]. Metformin arrests the cell cycle in malignant cells *via* activated AMPK, which is correlated with the downregulation of cyclin D1 and the upregulation of $p21^{CIP}$ and $p27^{KIP}$[131,132].

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[133]. Metformin can also bypass AMPK and inhibit mTOR signaling[134] and induce cell cycle arrest by down-regulating cyclin D1 *via* p53[135,136]. An AMPK knockdown study demonstrated that metformin up-regulates apoptosis and autophagy *via* a Stat3/Bcl2 pathway[137]. Metformin decreases glucose uptake into cancer cell decreases *via* a direct allosteric effect on hexokinase Ⅱ[138].

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

Table 2 Efficacy of metformin on the incidence, recurrence and mortality of hepatocellular carcinoma and other tumors

Ref.	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*[96]	2006	Cohort	Type 2	Mortality was lower among metformin users than among insulin or sulfonylurea users (HR = 0.77)
Libby *et al*[97]	2009	Cohort	Type 2	Total cancer incidence decreased (HR = 0.63)
Donadon *et al*[98]	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*[99]	2010	Cohort	Type 2	HCC incidence was lower among metformin users (OR = 0.15) than among insulin or sulfonylurea users
Hassan *et al*[100]	2010	Case-control	Not differentiated	HCC incidence decreased (OR = 0.30)
Home *et al*[107]	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*[119]	2010	Randomized controlled trial	Non-diabetic	A surrogate marker of colorectal cancer incidence decreased
Ferrara *et al*[104]	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*[166]	2011	Cohort	Type 2	HCC incidence did not decrease
Lai *et al*[101]	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*[108]	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*[109]	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*[110]	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*[105]	2014	Cohort	Type 2	Incidences of total cancer and HCC were not significantly lower among metformin users than among sulfonylurea users
Gandini *et al*[106]	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*[120]	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.

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[139].

Metformin inhibits the cell cycle of various gastrointestinal tumors, including HCC, by up-regulating the let-7 family *in vitro* and *in vivo*[140-145]. For HCC in particular, metformin's anti-cancer activities are mediated through let-7c, which targets RAS[146]; miR-140-5p, which targets TGFR1, FGF9[147] and DNMT1[148]; and miR-222, which targets PTEN and p57[149]. 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[150]. MiRNAs and their target messenger RNAs in cancers originating from other organs have been well summarized by Pulito[151].

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[152,153]. CSCs do not express definitive cell surface markers and have not been well defined due to extensive heterogeneity[154]. However, using surface markers and the enhanced ALDH1 activity of normal stem cells[155,156], 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[157,158].

Tumors have the potential to be resistant to chemotherapy and radiotherapy, and this potential has been attributed to CSCs[159]. 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*[159]. For hepatic CSCs, metformin administration reduced EpCAM-positive cells, partly depending on the AMPK/mTOR pathway in cell lines and xenograft tumors[160].

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[161]. Lactic acidosis is not significantly associated with metformin[162]. Although a recent study proposed that metformin might impair cognitive function[163], 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.

REFERENCES

1 **El-Serag HB**. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273.e1 [PMID: 22537432 DOI: 10.1200/JCO.2008.20.7753]

2 **Altekruse SF**, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol* 2009; **27**: 1485-1491 [PMID: 19224838]

3 **Bruix J**, Sherman M, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

4 **Regimbeau JM**, Kianmanesh R, Farges O, Dondero F, Sauvanet A, Belghiti J. Extent of liver resection influences the outcome in patients with cirrhosis and small hepatocellular carcinoma. *Surgery* 2002; **131**: 311-317 [PMID: 11894036 DOI: 10.1067/msy.2002.121892]

5 **Hasegawa K**, Kokudo N, Imamura H, Matsuyama Y, Aoki T, Minagawa M, Sano K, Sugawara Y, Takayama T, Makuuchi M. Prognostic impact of anatomic resection for hepatocellular carcinoma. *Ann Surg* 2005; **242**: 252-259 [PMID: 16041216 DOI: 10.1097/01.sla.0000171307.37401.db]

6 **Hasegawa K**, Kokudo N, Makuuchi M, Izumi N, Ichida T, Kudo M, Ku Y, Sakamoto M, Nakashima O, Matsui O, Matsuyama Y. Comparison of resection and ablation for hepatocellular carcinoma: a cohort study based on a Japanese nationwide survey. *J Hepatol* 2013; **58**: 724-729 [PMID: 23178708 DOI: 10.1016/j.jhep.2012.11.009]

7 **Shiina S**, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, Ishikawa T, Koike Y, Yoshida H, Kawabe T, Omata M. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. *Gastroenterology* 2005; **129**: 122-130 [PMID: 16012942 DOI: 10.1053/j.gastro.2005.04.009]

8 **Lin SM**, Lin CJ, Lin CC, Hsu CW, Chen YC. Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. *Gut* 2005; **54**: 1151-1156 [PMID: 16009687 DOI: 10.1136/gut.2004.045203]

9 **Poon RT**, Fan ST, Lo CM, Liu CL, Wong J. Long-term survival and pattern of recurrence after resection of small hepatocellular carcinoma in patients with preserved liver function: implications for a strategy of salvage transplantation. *Ann Surg* 2002; **235**: 373-382 [PMID: 11882759 DOI: 10.1097/00000658-200203000-00009]

10 **Zhou L**, Rui JA, Wang SB, Chen SG, Qu Q, Chi TY, Wei X, Han K, Zhang N, Zhao HT. Factors predictive for long-term survival of male patients with hepatocellular carcinoma after curative resection. *J Surg Oncol* 2007; **95**: 298-303 [PMID: 17326130 DOI: 10.1002/jso.20678]

11 **Naito S**, Imamura H, Tukada A, Matsuyama Y, Yoshimoto J, Sugo H, Ishizaki Y, Kawasaki S. Postoperative recurrence pattern and prognosis of patients with hepatocellular carcinoma, with particular reference to the hepatitis viral infection status. *Liver Int* 2014; **34**: 802-813 [PMID: 24350618 DOI: 10.1111/liv.12447]

12 **Guo R**, Feng X, Xiao S, Yan J, Xia F, Ma K, Li X. Short- and long-term outcomes of hepatectomy with or without radiofrequency-assist for the treatment of hepatocellular carcinomas: a retrospective comparative cohort study. *Biosci Trends* 2015; **9**: 65-72 [PMID: 25787911 DOI: 10.5582/bst.2014.01142]

13 **Llovet JM**, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862 DOI: 10.1016/S0140-6736(02)08649-X]

14 **Lo CM**, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, Fan ST, Wong J. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; **35**: 1164-1171 [PMID: 11981766 DOI: 10.1053/jhep.2002.33156]

15 **Matsui O**, Kadoya M, Yoshikawa J, Gabata T, Arai K, Demachi H, Miyayama S, Takashima T, Unoura M, Kogayashi K. Small hepatocellular carcinoma: treatment with subsegmental transcatheter arterial embolization. *Radiology* 1993; **188**: 79-83 [PMID: 8390073 DOI: 10.1148/radiology.188.1.8390073]

16 **Lammer J**, Malagari K, Vogl T, Pilleul F, Denys A, Watkinson A, Pitton M, Sergent G, Pfammatter T, Terraz S, Benhamou Y, Avajon Y, Gruenberger T, Pomoni M, Langenberger H, Schuchmann M, Dumortier J, Mueller C, Chevallier P, Lencioni R. Prospective randomized study of doxorubicin-eluting-bead embolization in the

treatment of hepatocellular carcinoma: results of the PRECISION V study. *Cardiovasc Intervent Radiol* 2010; **33**: 41-52 [PMID: 19908093 DOI: 10.1007/s00270-009-9711-7]

17 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]

18 **de Villa V**, Lo CM. Liver transplantation for hepatocellular carcinoma in Asia. *Oncologist* 2007; **12**: 1321-1331 [PMID: 18055852 DOI: 10.1634/theoncologist.12-11-1321]

19 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

20 **Cheng AL**, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]

21 **de Lope CR**, Tremosini S, Forner A, Reig M, Bruix J. Management of HCC. *J Hepatol* 2012; **56** Suppl 1: S75-S87 [PMID: 22300468 DOI: 10.1016/S0168-8278(12)60009-9]

22 **Hosaka T**, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, Akuta N, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Kumada H. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. *Hepatology* 2013; **58**: 98-107 [PMID: 23213040 DOI: 10.1002/hep.26180]

23 **Wong GL**, Chan HL, Mak CW, Lee SK, Ip ZM, Lam AT, Iu HW, Leung JM, Lai JW, Lo AO, Chan HY, Wong VW. Entecavir treatment reduces hepatic events and deaths in chronic hepatitis B patients with liver cirrhosis. *Hepatology* 2013; **58**: 1537-1547 [PMID: 23389810 DOI: 10.1002/hep.26301]

24 **Lawitz E**, Poordad FF, Pang PS, Hyland RH, Ding X, Mo H, Symonds WT, McHutchison JG, Membreno FE. Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naive and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial. *Lancet* 2014; **383**: 515-523 [PMID: 24209977 DOI: 10.1016/S0140-6736(13)62121-2]

25 **Afdhal N**, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, Nahass R, Ghalib R, Gitlin N, Herring R, Lalezari J, Younes ZH, Pockros PJ, Di Bisceglie AM, Arora S, Subramanian GM, Zhu Y, Dvory-Sobol H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Sulkowski M, Kwo P. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483-1493 [PMID: 24725238 DOI: 10.1056/NEJMoa1316366]

26 **Evans JM**, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. *BMJ* 2005; **330**: 1304-1305 [PMID: 15849206 DOI: 10.1136/bmj.38415.708634.F7]

27 **Tanaka H**, Imai Y, Hiramatsu N, Ito Y, Imanaka K, Oshita M, Hijioka T, Katayama K, Yabuuchi I, Yoshihara H, Inoue A, Kato M, Takehara T, Tamura S, Kasahara A, Hayashi N, Tsukuma H. Declining incidence of hepatocellular carcinoma in Osaka, Japan, from 1990 to 2003. *Ann Intern Med* 2008; **148**: 820-826 [PMID: 18519928 DOI: 10.7326/0003-4819-148-11-200806030-00004]

28 **Altekruse SF**, Henley SJ, Cucinelli JE, McGlynn KA. Changing hepatocellular carcinoma incidence and liver cancer mortality rates in the United States. *Am J Gastroenterol* 2014; **109**: 542-553 [PMID: 24513805 DOI: 10.1038/ajg.2014.11]

29 **Njei B**, Rotman Y, Ditah I, Lim JK. Emerging trends in hepatocellular carcinoma incidence and mortality. *Hepatology* 2015; **61**: 191-199 [PMID: 25142309 DOI: 10.1002/hep.27388]

30 **Venook AP**, Papandreou C, Furuse J, de Guevara LL. The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. *Oncologist* 2010; **15** Suppl 4: 5-13 [PMID: 21115576 DOI: 10.1634/theoncologist.2010-S4-05]

31 **Major JM**, Sargent JD, Graubard BI, Carlos HA, Hollenbeck AR, Altekruse SF, Freedman ND, McGlynn KA. Local geographic variation in chronic liver disease and hepatocellular carcinoma: contributions of socioeconomic deprivation, alcohol retail outlets, and lifestyle. *Ann Epidemiol* 2014; **24**: 104-110 [PMID: 24332863 DOI: 10.1016/j.annepidem.2013.11.006]

32 **Tanaka H**, Uera F, Tsukuma H, Ioka A, Oshima A. Distinctive change in male liver cancer incidence rate between the 1970s and 1990s in Japan: comparison with Japanese-Americans and US whites. *Jpn J Clin Oncol* 2007; **37**: 193-196 [PMID: 17332055 DOI: 10.1093/jjco/hyl148]

33 **Kasahara A**, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, Iijima A, Urushihara A, Kiyosawa K, Okuda M, Hino K, Okita K. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology* 1998; **27**: 1394-1402 [PMID: 9581697 DOI: 10.1002/hep.510270529]

34 **Lok AS**, Everhart JE, Wright EC, Di Bisceglie AM, Kim HY, Sterling RK, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Morgan TR. Maintenance peginterferon therapy and other factors associated with hepatocellular carcinoma in patients with advanced hepatitis C. *Gastroenterology* 2011; **140**: 840-849; quiz e12 [PMID: 21129375 DOI: 10.1053/j.gastro.2010.11.050]

35 **Snow-Lampart A**, Chappell B, Curtis M, Zhu Y, Myrick F, Schawalder J, Kitrinos K, Svarovskaia ES, Miller MD, Sorbel J, Heathcote J, Marcellin P, Borroto-Esoda K. No resistance to tenofovir disoproxil fumarate detected after up to 144 weeks of therapy in patients monoinfected with chronic hepatitis B virus. *Hepatology* 2011; **53**: 763-773 [PMID: 21374657 DOI: 10.1002/hep.24078]

36 **Liaw YF**, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Chien RN, Dent J, Roman L, Edmundson S, Lai CL. Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *Gastroenterology* 2000; **119**: 172-180 [PMID: 10889166 DOI: 10.1053/gast.2000.8559]

37 **Hadziyannis SJ**, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Ma J, Brosgart CL, Borroto-Esoda K, Arterburn S, Chuck SL. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. *Gastroenterology* 2006; **131**: 1743-1751 [PMID: 17087951 DOI: 10.1053/j.gastro.2006.09.020]

38 **Marcellin P**, Chang TT, Lim SG, Sievert W, Tong M, Arterburn S, Borroto-Esoda K, Frederick D, Rousseau F. Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. *Hepatology* 2008; **48**: 750-758 [PMID: 18752330 DOI: 10.1002/hep.22414]

39 **King H**, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* 1998; **21**: 1414-1431 [PMID: 9727886 DOI: 10.2337/diacare.21.9.1414]

40 **Shaw JE**, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010; **87**: 4-14 [PMID: 19896746 DOI: 10.1016/j.diabres.2009.10.007]

41 **Kelly T**, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. *Int J Obes (Lond)* 2008; **32**: 1431-1437 [PMID: 18607383 DOI: 10.1038/ijo.2008.102]

42 **Wild S**, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; **27**: 1047-1053 [PMID: 15111519 DOI: 10.2337/diacare.27.5.1047]

43 **Maynard GD**. A statistical study in cancer death-rates. *Biometrik* 1910; **7**: 276-304 [DOI: 10.1093/biomet/7.3.276]

44 **Greenwood M**, Wood F. The Relation between the Cancer and Diabetes Death-rates. *J Hyg* (Lond) 1914; **14**: 83-118 [PMID: 20474569]

45 **Marble A**. Diabetes and cancer. *N Engl J Med* 1934; **211**: 339-349 [DOI: 10.1056/NEJM193408232110801]

46 **Kessler II**. Cancer mortality among diabetics. *J Natl Cancer Inst* 1970; **44**: 673-686 [PMID: 11515436]

47 **Lawson DH**, Gray JM, McKillop C, Clarke J, Lee FD, Patrick RS. Diabetes mellitus and primary hepatocellular carcinoma. *Q J Med* 1986; **61**: 945-955 [PMID: 2819932]

48 **Adami HO**, McLaughlin J, Ekbom A, Berne C, Silverman D, Hacker D, Persson I. Cancer risk in patients with diabetes mellitus. *Cancer Causes Control* 1991; **2**: 307-314 [PMID: 1932543 DOI: 10.1007/BF00051670]

49 **Adami HO**, Chow WH, Nyrén O, Berne C, Linet MS, Ekbom A, Wolk A, McLaughlin JK, Fraumeni JF. Excess risk of primary liver cancer in patients with diabetes mellitus. *J Natl Cancer Inst* 1996; **88**: 1472-1477 [PMID: 8841022 DOI: 10.1093/jnci/88.20.1472]

50 **Wideroff L**, Gridley G, Mellemkjaer L, Chow WH, Linet M, Keehn S, Borch-Johnsen K, Olsen JH. Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. *J Natl Cancer Inst* 1997; **89**: 1360-1365 [PMID: 9308706 DOI: 10.1093/jnci/89.18.1360]

51 **La Vecchia C**, Negri E, Franceschi S, D'Avanzo B, Boyle P. A case-control study of diabetes mellitus and cancer risk. *Br J Cancer* 1994; **70**: 950-953 [PMID: 7947103 DOI: 10.1038/bjc.1994.427]

52 **Balkau B**, Kahn HS, Courbon D, Eschwège E, Ducimetière P. Hyperinsulinemia predicts fatal liver cancer but is inversely associated with fatal cancer at some other sites: the Paris Prospective Study. *Diabetes Care* 2001; **24**: 843-849 [PMID: 11347741 DOI: 10.2337/diacare.24.5.843]

53 **Lee MS**, Hsu CC, Wahlqvist ML, Tsai HN, Chang YH, Huang YC. Type 2 diabetes increases and metformin reduces total, colorectal, liver and pancreatic cancer incidences in Taiwanese: a representative population prospective cohort study of 800,000 individuals. *BMC Cancer* 2011; **11**: 20 [PMID: 21241523 DOI: 10.1186/1471-2407-11-20]

54 **Ikeda Y**, Shimada M, Hasegawa H, Gion T, Kajiyama K, Shirabe K, Yanaga K, Takenaka K, Sugimachi K. Prognosis of hepatocellular carcinoma with diabetes mellitus after hepatic resection. *Hepatology* 1998; **27**: 1567-1571 [PMID: 9620328 DOI: 10.1002/hep.510270615]

55 **Huo TI**, Wu JC, Lui WY, Lee PC, Huang YH, Chau GY, Tsay SH, Chang FY, Lee SD. Diabetes mellitus is a recurrence-independent risk factor in patients with hepatitis B virus-related hepatocellular carcinoma undergoing resection. *Eur J Gastroenterol Hepatol* 2003; **15**: 1203-1208 [PMID: 14560154 DOI: 10.1097/01.meg.0000085498.01212.6a]

56 **Komura T**, Mizukoshi E, Kita Y, Sakurai M, Takata Y, Arai K, Yamashita T, Ohta T, Shimizu K, Nakamoto Y, Honda M, Takamura T, Kaneko S. Impact of diabetes on recurrence of hepatocellular carcinoma after surgical treatment in patients with viral hepatitis. *Am J Gastroenterol* 2007; **102**: 1939-1946 [PMID: 17573788 DOI: 10.1111/j.1572-0241.2007.01354.x]

57 **Kawamura Y**, Ikeda K, Arase Y, Yatsuji H, Sezaki H, Hosaka T, Akuta N, Kobayashi M, Saitoh S, Suzuki F, Suzuki Y, Kumada H. Diabetes mellitus worsens the recurrence rate after potentially curative therapy in patients with hepatocellular carcinoma associated with nonviral hepatitis. *J Gastroenterol Hepatol* 2008; **23**: 1739-1746 [PMID: 18713301 DOI: 10.1111/j.1440-1746.2008.05436.x]

58 **Coughlin SS**, Calle EE, Teras LR, Petrelli J, Thun MJ. Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults. *Am J Epidemiol* 2004; **159**: 1160-1167 [PMID: 15191933 DOI: 10.1093/aje/kwh161]

59 **Landman GW**, van Hateren KJ, Kleefstra N, Groenier KH, Gans RO, Bilo HJ. The relationship between glycaemic control and mortality in patients with type 2 diabetes in general practice (ZODIAC-11). *Br J Gen Pract* 2010; **60**: 172-175 [PMID: 20202363 DOI: 10.3399/bjgp10X483517]

60 **El-Serag HB**, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clin Gastroenterol Hepatol* 2006; **4**: 369-380 [PMID: 16527702 DOI: 10.1016/j.cgh.2005.12.007]

61 **Giovannucci E**, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M, Regensteiner JG, Yee D. Diabetes and cancer: a consensus report. *CA Cancer J Clin* 2010; **60**: 207-221 [PMID: 20554718 DOI: 10.3322/caac.20078]

62 **Schlesinger S**, Aleksandrova K, Pischon T, Jenab M, Fedirko V, Trepo E, Overvad K, Roswall N, Tjønneland A, Boutron-Ruault MC, Fagherazzi G, Racine A, Kaaks R, Grote VA, Boeing H, Trichopoulou A, Pantzalis M, Kritikou M, Mattiello A, Sieri S, Sacerdote C, Palli D, Tumino R, Peeters PH, Bueno-de-Mesquita HB, Weiderpass E, Quirós JR, Zamora-Ros R, Sánchez MJ, Arriola L, Ardanaz E, Tormo MJ, Nilsson P, Lindkvist B, Sund M, Rolandsson O, Khaw KT, Wareham N, Travis RC, Riboli E, Nöthlings U. Diabetes mellitus, insulin treatment, diabetes duration, and risk of biliary tract cancer and hepatocellular carcinoma in a European cohort. *Ann Oncol* 2013; **24**: 2449-2455 [PMID: 23720454 DOI: 10.1093/annonc/mdt204]

63 **Koh WP**, Wang R, Jin A, Yu MC, Yuan JM. Diabetes mellitus and risk of hepatocellular carcinoma: findings from the Singapore Chinese Health Study. *Br J Cancer* 2013; **108**: 1182-1188 [PMID: 23370206 DOI: 10.1038/bjc.2013.25]

64 **Inoue M**, Iwasaki M, Otani T, Sasazuki S, Noda M, Tsugane S. Diabetes mellitus and the risk of cancer: results from a large-scale population-based cohort study in Japan. *Arch Intern Med* 2006; **166**: 1871-1877 [PMID: 17000944 DOI: 10.1001/archinte.166.17.1871]

65 **Møller H**, Mellemgaard A, Lindvig K, Olsen JH. Obesity and cancer risk: a Danish record-linkage study. *Eur J Cancer* 1994; **30A**: 344-350 [PMID: 8204357]

66 **Wolk A**, Gridley G, Svensson M, Nyrén O, McLaughlin JK, Fraumeni JF, Adam HO. A prospective study of obesity and cancer risk (Sweden). *Cancer Causes Control* 2001; **12**: 13-21 [PMID: 11227921 DOI: 10.1023/A:1008995217664]

67 **Levine W**, Dyer AR, Shekelle RB, Schoenberger JA, Stamler J. Post-load plasma glucose and cancer mortality in middle-aged men and women. 12-year follow-up findings of the Chicago Heart Association Detection Project in Industry. *Am J Epidemiol* 1990; **131**: 254-262 [PMID: 2296978]

68 **Batty GD**, Shipley MJ, Marmot M, Smith GD. Diabetes status and post-load plasma glucose concentration in relation to site-specific cancer mortality: findings from the original Whitehall study. *Cancer Causes Control* 2004; **15**: 873-881 [PMID: 15577289 DOI: 10.1007/s10552-004-1050-z]

69 **Harding JL**, Shaw JE, Peeters A, Cartensen B, Magliano DJ. Cancer risk among people with type 1 and type 2 diabetes: disentangling true associations, detection bias, and reverse causation. *Diabetes Care* 2015; **38**: 264-270 [PMID: 25488912 DOI: 10.2337/dc14-1996]

70 **Johnson JA**, Bowker SL, Richardson K, Marra CA. Time-varying incidence of cancer after the onset of type 2 diabetes: evidence of potential detection bias. *Diabetologia* 2011; **54**: 2263-2271 [PMID: 21748485 DOI: 10.1007/s00125-011-2242-1]

71 **Hjartåker A**, Langseth H, Weiderpass E. Obesity and diabetes epidemics: cancer repercussions. *Adv Exp Med Biol* 2008; **630**: 72-93 [PMID: 18637486]

72 **Pollak M**. Insulin, insulin-like growth factors and neoplasia. *Best Pract Res Clin Endocrinol Metab* 2008; **22**: 625-638 [PMID: 18971123 DOI: 10.1016/j.beem.2008.08.004]

73 **Powell DR**, Suwanichkul A, Cubbage ML, DePaolis LA, Snuggs MB, Lee PD. Insulin inhibits transcription of the human gene for insulin-like growth factor-binding protein-1. *J Biol Chem* 1991; **266**: 18868-18876 [PMID: 1717456]

74 **Ooi GT**, Tseng LY, Tran MQ, Rechler MM. Insulin rapidly decreases insulin-like growth factor-binding protein-1 gene transcription in streptozotocin-diabetic rats. *Mol Endocrinol* 1992; **6**: 2219-2228 [PMID: 1283442 DOI: 10.1210/mend.6.12.1283442]

75 **Laplante M**, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012; **149**: 274-293 [PMID: 22500797 DOI: 10.1016/j.cell.2012.03.017]

76 **Chettouh H**, Lequoy M, Fartoux L, Vigouroux C, Desbois-Mouthon C. Hyperinsulinaemia and insulin signalling in the pathogenesis and the clinical course of hepatocellular carcinoma. *Liver Int* 2015; **35**: 2203-2217 [PMID: 26123841 DOI: 10.1111/liv.12903]

77 **Enguita-Germán M**, Fortes P. Targeting the insulin-like growth factor pathway in hepatocellular carcinoma. *World J Hepatol* 2014; **6**: 716-737 [PMID: 25349643 DOI: 10.4254/wjh.v6.i10.716]

78 **Villanueva A**, Chiang DY, Newell P, Peix J, Thung S, Alsinet C, Tovar V, Roayaie S, Minguez B, Sole M, Battiston C, Van Laarhoven S, Fiel MI, Di Feo A, Hoshida Y, Yea S, Toffanin S, Ramos A, Martignetti JA, Mazzaferro V, Bruix J, Waxman S, Schwartz M, Meyerson M, Friedman SL, Llovet JM. Pivotal role of mTOR signaling in hepatocellular carcinoma. *Gastroenterology* 2008; **135**: 1972-1983, 1983.e1-11 [PMID: 18929564 DOI: 10.1053/j.gastro.2008.08.008]

79 **Garg SK**, Maurer H, Reed K, Selagamsetty R. Diabetes and cancer: two diseases with obesity as a common risk factor. *Diabetes Obes Metab* 2014; **16**: 97-110 [PMID: 23668396 DOI: 10.1111/dom.12124]

80 **Guo S**, Liu M, Wang G, Torroella-Kouri M, Gonzalez-Perez RR. Oncogenic role and therapeutic target of leptin signaling in breast cancer and cancer stem cells. *Biochim Biophys Acta* 2012; **1825**: 207-222 [PMID: 22289780 DOI: 10.1016/j.bbcan.2012.01.002]

81 **Becker S**, Dossus L, Kaaks R. Obesity related hyperinsulinaemia and hyperglycaemia and cancer development. *Arch Physiol Biochem* 2009; **115**: 86-96 [PMID: 19485704 DOI: 10.1080/13813450902878054]

82 **Duan XF**, Tang P, Li Q, Yu ZT. Obesity, adipokines and hepatocellular carcinoma. *Int J Cancer* 2013; **133**: 1776-1783 [PMID: 23404222 DOI: 10.1002/ijc.28105]

83 **Warburg O**. On the origin of cancer cells. *Science* 1956; **123**: 309-314 [PMID: 13298683 DOI: 10.1126/science.123.3191.309]

84 **Miles KA**, Williams RE. Warburg revisited: imaging tumour blood flow and metabolism. *Cancer Imaging* 2008; **8**: 81-86 [PMID: 18390391 DOI: 10.1102/1470-7330.2008.0011]

85 **Chocarro-Calvo A**, García-Martínez JM, Ardila-González S, De la Vieja A, García-Jiménez C. Glucose-induced β-catenin acetylation enhances Wnt signaling in cancer. *Mol Cell* 2013; **49**: 474-486 [PMID: 23273980 DOI: 10.1016/j.molcel.2012.11.022]

86 **Li CI**, Chen HJ, Lai HC, Liu CS, Lin WY, Li TC, Lin CC. Hyperglycemia and chronic liver diseases on risk of hepatocellular carcinoma in Chinese patients with type 2 diabetes--National cohort of Taiwan Diabetes Study. *Int J Cancer* 2015; **136**: 2668-2679 [PMID: 25387451 DOI: 10.1002/ijc.29321]

87 **Takahashi H**, Mizuta T, Eguchi Y, Kawaguchi Y, Kuwashiro T, Oeda S, Isoda H, Oza N, Iwane S, Izumi K, Anzai K, Ozaki I, Fujimoto K. Post-challenge hyperglycemia is a significant risk factor for the development of hepatocellular carcinoma in patients with chronic hepatitis C. *J Gastroenterol* 2011; **46**: 790-798 [PMID: 21331763 DOI: 10.1007/s00535-011-0381-2]

88 **Johnson JA**, Bowker SL. Intensive glycaemic control and cancer risk in type 2 diabetes: a meta-analysis of major trials. *Diabetologia* 2011; **54**: 25-31 [PMID: 20959956 DOI: 10.1007/s00125-010-1933-3]

89 **Yang XL**, Ma RC, Chan JC. Meta-analysis of trial data may support a causal role of hyperglycaemia in cancer. *Diabetologia* 2011; **54**: 709-710; author reply 711-712 [PMID: 21153895 DOI: 10.1007/s00125-010-2017-0]

90 **Brown SB**, Hankinson SE. Endogenous estrogens and the risk of breast, endometrial, and ovarian cancers. *Steroids* 2015; **99**: 8-10 [PMID: 25555473 DOI: 10.1016/j.steroids.2014.12.013]

91 **El-Serag HB**, Mason AC, Key C. Trends in survival of patients with hepatocellular carcinoma between 1977 and 1996 in the United States. *Hepatology* 2001; **33**: 62-65 [PMID: 11124821 DOI: 10.1053/jhep.2001.21041]

92 **Yu MW**, Chang HC, Chang SC, Liaw YF, Lin SM, Liu CJ, Lee SD, Lin CL, Chen PJ, Lin SC, Chen CJ. Role of reproductive factors in hepatocellular carcinoma: Impact on hepatitis B- and C-related risk. *Hepatology* 2003; **38**: 1393-1400 [PMID: 14647050 DOI: 10.1016/j.hep.2003.09.041]

93 **Shi L**, Feng Y, Lin H, Ma R, Cai X. Role of estrogen in hepatocellular carcinoma: is inflammation the key? *J Transl Med* 2014; **12**: 93 [PMID: 24708807 DOI: 10.1186/1479-5876-12-93]

94 **Nathan DM**, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, Zinman B. Medical management of hyperglycaemia in type 2 diabetes mellitus: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetologia* 2009; **52**: 17-30 [PMID: 18941734 DOI: 10.1007/s00125-008-1157-y]

95 **Bailey CJ**, Day C. Traditional plant medicines as treatments for diabetes. *Diabetes Care* 1989; **12**: 553-564 [PMID: 2673695 DOI: 10.2337/diacare.12.8.553]

96 **Bowker SL**, Majumdar SR, Veugelers P, Johnson JA. Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. *Diabetes Care* 2006; **29**: 254-258 [PMID: 16443869 DOI: 10.2337/diacare.29.02.06.dc05-1558]

97 **Libby G**, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. *Diabetes Care* 2009; **32**: 1620-1625 [PMID: 19564453 DOI: 10.2337/dc08-2175]

98 **Donadon V**, Balbi M, Ghersetti M, Grazioli S, Perciaccante A, Della Valentina G, Gardenal R, Dal Mas M, Casarin P, Zanette G, Miranda C. Antidiabetic therapy and increased risk of hepatocellular carcinoma in chronic liver disease. *World J Gastroenterol* 2009; **15**: 2506-2511 [PMID: 19469001 DOI: 10.3748/wjg.15.2506]

99 **Donadon V**, Balbi M, Mas MD, Casarin P, Zanette G. Metformin and reduced risk of hepatocellular carcinoma in diabetic patients with chronic liver disease. *Liver Int* 2010; **30**: 750-758 [PMID: 20331505 DOI: 10.1111/j.1478-3231.2010.02223.x]

100 **Hassan MM**, Curley SA, Li D, Kaseb A, Davila M, Abdalla EK, Javle M, Moghazy DM, Lozano RD, Abbruzzese JL, Vauthey JN. Association of diabetes duration and diabetes treatment with the risk of hepatocellular carcinoma. *Cancer* 2010; **116**: 1938-1946 [PMID: 20166205 DOI: 10.1002/cncr.24982]

101 **Lai SW**, Chen PC, Liao KF, Muo CH, Lin CC, Sung FC. Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. *Am J Gastroenterol* 2012; **107**: 46-52 [PMID: 22085817 DOI: 10.1038/ajg.2011.384]

102 **Suissa S**, Azoulay L. Metformin and the risk of cancer: time-related biases in observational studies. *Diabetes Care* 2012; **35**: 2665-2673 [PMID: 23173135 DOI: 10.2337/dc12-0788]

103 **Johnson JA**, Carstensen B, Witte D, Bowker SL, Lipscombe L, Renehan AG. Diabetes and cancer (1): evaluating the temporal relationship between type 2 diabetes and cancer incidence. *Diabetologia* 2012; **55**: 1607-1618 [PMID: 22476947 DOI: 10.1007/s00125-012-2525-1]

104 **Ferrara A**, Lewis JD, Quesenberry CP, Peng T, Strom BL, Van Den Eeden SK, Ehrlich SF, Habel LA. Cohort study of pioglitazone and cancer incidence in patients with diabetes. *Diabetes Care* 2011; **34**: 923-929 [PMID: 21447664 DOI: 10.2337/dc10-1067]

105 **Tsilidis KK**, Capothanassi D, Allen NE, Rizos EC, Lopez DS, van Veldhoven K, Sacerdote C, Ashby D, Vineis P, Tzoulaki I, Ioannidis JP. Metformin does not affect cancer risk: a cohort study in the U.K. Clinical Practice Research Datalink analyzed like an intention-to-treat trial. *Diabetes Care* 2014; **37**: 2522-2532 [PMID: 24898303 DOI: 10.2337/dc14-0584]

106 **Gandini S**, Puntoni M, Heckman-Stoddard BM, Dunn BK, Ford L, DeCensi A, Szabo E. Metformin and cancer risk and mortality: a systematic review and meta-analysis taking into account biases and confounders. *Cancer Prev Res* (Phila) 2014; **7**: 867-885 [PMID: 24985407 DOI: 10.1158/1940-6207.CAPR-13-0424]

107 **Home PD**, Kahn SE, Jones NP, Noronha D, Beck-Nielsen H, Viberti G. Experience of malignancies with oral glucose-lowering drugs in the randomised controlled ADOPT (A Diabetes Outcome Progression Trial) and RECORD (Rosiglitazone Evaluated for Cardiovascular Outcomes and Regulation of Glycaemia in Diabetes) clinical trials. *Diabetologia* 2010; **53**: 1838-1845 [PMID: 20532476 DOI: 10.1007/s00125-010-1804-y]

108 **Stevens RJ**, Ali R, Bankhead CR, Bethel MA, Cairns BJ, Camisasca RP, Crowe FL, Farmer AJ, Harrison S, Hirst JA, Home P, Kahn SE, McLellan JH, Perera R, Plüddemann A, Ramachandran A, Roberts NW, Rose PW, Schweizer A, Viberti G, Holman RR. Cancer

outcomes and all-cause mortality in adults allocated to metformin: systematic review and collaborative meta-analysis of randomised clinical trials. *Diabetologia* 2012; **55**: 2593-2603 [PMID: 22875195 DOI: 10.1007/s00125-012-2653-7]

109 **Thakkar B**, Aronis KN, Vamvini MT, Shields K, Mantzoros CS. Metformin and sulfonylureas in relation to cancer risk in type II diabetes patients: a meta-analysis using primary data of published studies. *Metabolism* 2013; **62**: 922-934 [PMID: 23419783 DOI: 10.1016/j.metabol.2013.01.014]

110 **Yin M**, Zhou J, Gorak EJ, Quddus F. Metformin is associated with survival benefit in cancer patients with concurrent type 2 diabetes: a systematic review and meta-analysis. *Oncologist* 2013; **18**: 1248-1255 [PMID: 24258613 DOI: 10.1634/theoncologist.2013-0111]

111 **Choi Y**, Kim TY, Oh DY, Lee KH, Han SW, Im SA, Kim TY, Bang YJ. The Impact of Diabetes Mellitus and Metformin Treatment on Survival of Patients with Advanced Pancreatic Cancer Undergoing Chemotherapy. *Cancer Res Treat* 2016; **48**: 171-179 [PMID: 25779362 DOI: 10.4143/crt.2014.292]

112 **Jiralerspong S**, Palla SL, Giordano SH, Meric-Bernstam F, Liedtke C, Barnett CM, Hsu L, Hung MC, Hortobagyi GN, Gonzalez-Angulo AM. Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. *J Clin Oncol* 2009; **27**: 3297-3302 [PMID: 19487376 DOI: 10.1200/JCO.2009.19.6410]

113 **Tan BX**, Yao WX, Ge J, Peng XC, Du XB, Zhang R, Yao B, Xie K, Li LH, Dong H, Gao F, Zhao F, Hou JM, Su JM, Liu JY. Prognostic influence of metformin as first-line chemotherapy for advanced nonsmall cell lung cancer in patients with type 2 diabetes. *Cancer* 2011; **117**: 5103-5111 [PMID: 21523768 DOI: 10.1002/cncr.26151]

114 **Lee JH**, Kim TI, Jeon SM, Hong SP, Cheon JH, Kim WH. The effects of metformin on the survival of colorectal cancer patients with diabetes mellitus. *Int J Cancer* 2012; **131**: 752-759 [PMID: 21913184 DOI: 10.1002/ijc.26421]

115 **Kordes S**, Pollak MN, Zwinderman AH, Mathôt RA, Weterman MJ, Beeker A, Punt CJ, Richel DJ, Wilmink JW. Metformin in patients with advanced pancreatic cancer: a double-blind, randomised, placebo-controlled phase 2 trial. *Lancet Oncol* 2015; **16**: 839-847 [PMID: 26067687 DOI: 10.1016/S1470-2045(15)00027-3]

116 **Kim J**, Lim W, Kim EK, Kim MK, Paik NS, Jeong SS, Yoon JH, Park CH, Ahn SH, Kim LS, Han S, Nam SJ, Kang HS, Kim SI, Yoo YB, Jeong J, Kim TH, Kang T, Kim SW, Jung Y, Lee JE, Kim KS, Yu JH, Chae BJ, Jung SY, Kang E, Choi SY, Moon HG, Noh DY, Han W. Phase II randomized trial of neoadjuvant metformin plus letrozole versus placebo plus letrozole for estrogen receptor positive postmenopausal breast cancer (METEOR). *BMC Cancer* 2014; **14**: 170 [PMID: 24612502 DOI: 10.1186/1471-2407-14-170]

117 **Jang WI**, Kim MS, Lim JS, Yoo HJ, Seo YS, Han CJ, Park SC, Kay CS, Kim M, Jang HS, Lee DS, Chang AR, Park HJ. Survival Advantage Associated with Metformin Usage in Hepatocellular Carcinoma Patients Receiving Radiotherapy: A Propensity Score Matching Analysis. *Anticancer Res* 2015; **35**: 5047-5054 [PMID: 26254406]

118 **Casadei Gardini A**, Marisi G, Scarpi E, Scartozzi M, Faloppi L, Silvestris N, Masi G, Vivaldi C, Brunetti O, Tamberi S, Foschi FG, Tamburini E, Tenti E, Ricca Rosellini S, Ulivi P, Cascinu S, Nanni O, Frassineti GL. Effects of metformin on clinical outcome in diabetic patients with advanced HCC receiving sorafenib. *Expert Opin Pharmacother* 2015; **16**: 2719-2725 [PMID: 26513009 DOI: 10.1517/14656566.2015.1102887]

119 **Hosono K**, Endo H, Takahashi H, Sugiyama M, Sakai E, Uchiyama T, Suzuki K, Iida H, Sakamoto Y, Yoneda K, Koide T, Tokoro C, Abe Y, Inamori M, Nakagama H, Nakajima A. Metformin suppresses colorectal aberrant crypt foci in a short-term clinical trial. *Cancer Prev Res* (Phila) 2010; **3**: 1077-1083 [PMID: 20810669 DOI: 10.1158/1940-6207.CAPR-10-0186]

120 **Higurashi T**, Hosono K, Takahashi H, Komiya Y, Umezawa S, Sakai E, Uchiyama T, Taniguchi L, Hata Y, Uchiyama S, Hattori A, Nagase H, Kessoku T, Arimoto J, Matsuhashi N, Inayama Y, Yamanaka S, Taguri M, Nakajima A. Metformin for chemoprevention of metachronous colorectal adenoma or polyps in post-polypectomy patients without diabetes: a multicentre double-blind, placebo-controlled, randomised phase 3 trial. *Lancet Oncol* 2016; **17**: 475-483 [PMID: 26947328 DOI: 10.1016/S1470-2045(15)00565-3]

121 **Zhou G**, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 2001; **108**: 1167-1174 [PMID: 11602624 DOI: 10.1172/JCI13505]

122 **Hawley SA**, Boudeau J, Reid JL, Mustard KJ, Udd L, Mäkelä TP, Alessi DR, Hardie DG. Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. *J Biol* 2003; **2**: 28 [PMID: 14511394 DOI: 10.1186/1475-4924-2-28]

123 **Lizcano JM**, Göransson O, Toth R, Deak M, Morrice NA, Boudeau J, Hawley SA, Udd L, Mäkelä TP, Hardie DG, Alessi DR. LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *EMBO J* 2004; **23**: 833-843 [PMID: 14976552 DOI: 10.1038/sj.emboj.7600110]

124 **Kimura N**, Tokunaga C, Dalal S, Richardson C, Yoshino K, Hara K, Kemp BE, Witters LA, Mimura O, Yonezawa K. A possible linkage between AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) signalling pathway. *Genes Cells* 2003; **8**: 65-79 [PMID: 12558800 DOI: 10.1046/j.1365-2443.2003.00615.x]

125 **Inoki K**, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 2003; **115**: 577-590 [PMID: 14651849 DOI: 10.1016/S0092-8674(03)00929-2]

126 **Shaw RJ**, Lamia KA, Vasquez D, Koo SH, Bardeesy N, Depinho RA, Montminy M, Cantley LC. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. *Science* 2005; **310**: 1642-1646 [PMID: 16308421 DOI: 10.1126/science.1120781]

127 **Foretz M**, Hébrard S, Leclerc J, Zarrinpashneh E, Soty M, Mithieux G, Sakamoto K, Andreelli F, Viollet B. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *J Clin Invest* 2010; **120**: 2355-2369 [PMID: 20577053 DOI: 10.1172/JCI40671]

128 **Takano A**, Usui I, Haruta T, Kawahara J, Uno T, Iwata M, Kobayashi M. Mammalian target of rapamycin pathway regulates insulin signaling via subcellular redistribution of insulin receptor substrate 1 and integrates nutritional signals and metabolic signals of insulin. *Mol Cell Biol* 2001; **21**: 5050-5062 [PMID: 11438661 DOI: 10.1128/MCB.21.15.5050-5062.2001]

129 **Menendez JA**, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat Rev Cancer* 2007; **7**: 763-777 [PMID: 17882277 DOI: 10.1038/nrc2222]

130 **Cantoria MJ**, Boros LG, Meuillet EJ. Contextual inhibition of fatty acid synthesis by metformin involves glucose-derived acetyl-CoA and cholesterol in pancreatic tumor cells. *Metabolomics* 2014; **10**: 91-104 [PMID: 24482631 DOI: 10.1007/s11306-013-0555-4]

131 **Cai X**, Hu X, Cai B, Wang Q, Li Y, Tan X, Hu H, Chen X, Huang J, Cheng J, Jing X. Metformin suppresses hepatocellular carcinoma cell growth through induction of cell cycle G1/G0 phase arrest and p21CIP and p27KIP expression and downregulation of cyclin D1 in vitro and in vivo. *Oncol Rep* 2013; **30**: 2449-2457 [PMID: 24008375 DOI: 10.3892/or.2013.2718]

132 **Cai X**, Hu X, Tan X, Cheng W, Wang Q, Chen X, Guan Y, Chen C, Jing X. Metformin Induced AMPK Activation, G0/G1 Phase Cell Cycle Arrest and the Inhibition of Growth of Esophageal Squamous Cell Carcinomas In Vitro and In Vivo. *PLoS One* 2015; **10**: e0133349 [PMID: 26196392 DOI: 10.1371/journal.pone.0133349]

133 **Algire C**, Moiseeva O, Deschênes-Simard X, Amrein L, Petruccelli L, Birman E, Viollet B, Ferbeyre G, Pollak MN. Metformin reduces endogenous reactive oxygen species and associated DNA damage. *Cancer Prev Res* (Phila) 2012; **5**: 536-543 [PMID: 22262811 DOI: 10.1158/1940-6207.CAPR-11-0536]

134 **Kalender A**, Selvaraj A, Kim SY, Gulati P, Brûlé S, Viollet B, Kemp BE, Bardeesy N, Dennis P, Schlager JJ, Marette A, Kozma SC, Thomas G. Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. *Cell Metab* 2010; **11**: 390-401 [PMID: 20444419 DOI: 10.1016/j.cmet.2010.03.014]

135 **Ben Sahra I**, Laurent K, Loubat A, Giorgetti-Peraldi S, Colosetti P, Auberger P, Tanti JF, Le Marchand-Brustel Y, Bost F. The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. *Oncogene* 2008; **27**: 3576-3586 [PMID: 18212742 DOI: 10.1038/sj.onc.1211024]

136 **Ben Sahra I**, Regazzetti C, Robert G, Laurent K, Le Marchand-Brustel Y, Auberger P, Tanti JF, Giorgetti-Peraldi S, Bost F. Metformin, independent of AMPK, induces mTOR inhibition and cell-cycle arrest through REDD1. *Cancer Res* 2011; **71**: 4366-4372 [PMID: 21540236 DOI: 10.1158/0008-5472.CAN-10-1769]

137 **Feng Y**, Ke C, Tang Q, Dong H, Zheng X, Lin W, Ke J, Huang J, Yeung SC, Zhang H. Metformin promotes autophagy and apoptosis in esophageal squamous cell carcinoma by downregulating Stat3 signaling. *Cell Death Dis* 2014; **5**: e1088 [PMID: 24577086 DOI: 10.1038/cddis.2014.59]

138 **Salani B**, Marini C, Rio AD, Ravera S, Massollo M, Orengo AM, Amaro A, Passalacqua M, Maffioli S, Pfeffer U, Cordera R, Maggi D, Sambuceti G. Metformin impairs glucose consumption and survival in Calu-1 cells by direct inhibition of hexokinase-II. *Sci Rep* 2013; **3**: 2070 [PMID: 23797762 DOI: 10.1038/srep02070]

139 **Morishita A**, Masaki T. miRNA in hepatocellular carcinoma. *Hepatol Res* 2015; **45**: 128-141 [PMID: 25040738 DOI: 10.1111/hepr.12386]

140 **Kato K**, Gong J, Iwama H, Kitanaka A, Tani J, Miyoshi H, Nomura K, Mimura S, Kobayashi M, Aritomo Y, Kobara H, Mori H, Himoto T, Okano K, Suzuki Y, Murao K, Masaki T. The antidiabetic drug metformin inhibits gastric cancer cell proliferation in vitro and in vivo. *Mol Cancer Ther* 2012; **11**: 549-560 [PMID: 22222629 DOI: 10.1158/1535-7163.MCT-11-0594]

141 **Miyoshi H**, Kato K, Iwama H, Maeda E, Sakamoto T, Fujita K, Toyota Y, Tani J, Nomura T, Mimura S, Kobayashi M, Morishita A, Kobara H, Mori H, Yoneyama H, Deguchi A, Himoto T, Kurokohchi K, Okano K, Suzuki Y, Murao K, Masaki T. Effect of the anti-diabetic drug metformin in hepatocellular carcinoma in vitro and in vivo. *Int J Oncol* 2014; **45**: 322-332 [PMID: 24806290 DOI: 10.3892/ijo.2014.2419]

142 **Kobayashi M**, Kato K, Iwama H, Fujihara S, Nishiyama N, Mimura S, Toyota Y, Nomura T, Nomura K, Tani J, Miyoshi H, Kobara H, Mori H, Murao K, Masaki T. Antitumor effect of metformin in esophageal cancer: in vitro study. *Int J Oncol* 2013; **42**: 517-524 [PMID: 23229592 DOI: 10.3892/ijo.2012.1722]

143 **Fujimori T**, Kato K, Fujihara S, Iwama H, Yamashita T, Kobayashi K, Kamada H, Morishita A, Kobara H, Mori H, Okano K, Suzuki Y, Masaki T. Antitumor effect of metformin on cholangiocarcinoma: In vitro and in vivo studies. *Oncol Rep* 2015; **34**: 2987-2996 [PMID: 26398221 DOI: 10.3892/or.2015.4284]

144 **Fujihara S**, Kato K, Morishita A, Iwama H, Nishioka T, Chiyo T, Nishiyama N, Miyoshi H, Kobayashi M, Kobara H, Mori H, Okano K, Suzuki Y, Masaki T. Antidiabetic drug metformin inhibits esophageal adenocarcinoma cell proliferation in vitro and in vivo. *Int J Oncol* 2015; **46**: 2172-2180 [PMID: 25709052 DOI: 10.3892/ijo.2015.2903]

145 **Kato K**, Iwama H, Yamashita T, Kobayashi K, Fujihara S, Fujimori T, Kamada H, Kobara H, Masaki T. The anti-diabetic drug metformin inhibits pancreatic cancer cell proliferation in vitro and in vivo: Study of the microRNAs associated with the antitumor effect of metformin. *Oncol Rep* 2016; **35**: 1582-1592 [PMID: 26708419 DOI: 10.3892/or.2015.4496]

146 **Johnson SM**, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. RAS is regulated by the let-7 microRNA family. *Cell* 2005; **120**: 635-647 [PMID: 15766527]

147 **Yang H**, Fang F, Chang R, Yang L. MicroRNA-140-5p suppresses tumor growth and metastasis by targeting transforming growth factor β receptor 1 and fibroblast growth factor 9 in hepatocellular carcinoma. *Hepatology* 2013; **58**: 205-217 [PMID: 23401231 DOI: 10.1002/hep.26315]

148 **Takata A**, Otsuka M, Yoshikawa T, Kishikawa T, Hikiba Y, Obi S, Goto T, Kang YJ, Maeda S, Yoshida H, Omata M, Asahara H, Koike K. MicroRNA-140 acts as a liver tumor suppressor by controlling NF-κB activity by directly targeting DNA methyltransferase 1 (Dnmt1) expression. *Hepatology* 2013; **57**: 162-170 [PMID: 22898998 DOI: 10.1002/hep.26011]

149 **Garofalo M**, Di Leva G, Romano G, Nuovo G, Suh SS, Ngankeu A, Taccioli C, Pichiorri F, Alder H, Secchiero P, Gasparini P, Gonelli A, Costinean S, Acunzo M, Condorelli G, Croce CM. miR-221& amp; 222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. *Cancer Cell* 2009; **16**: 498-509 [PMID: 19962668 DOI: 10.1016/j.ccr.2009.10.014]

150 **Li W**, Yuan Y, Huang L, Qiao M, Zhang Y. Metformin alters the expression profiles of microRNAs in human pancreatic cancer cells. *Diabetes Res Clin Pract* 2012; **96**: 187-195 [PMID: 22245693 DOI: 10.1016/j.diabres.2011.12.028]

151 **Pulito C**, Donzelli S, Muti P, Puzzo L, Strano S, Blandino G. microRNAs and cancer metabolism reprogramming: the paradigm of metformin. *Ann Transl Med* 2014; **2**: 58 [PMID: 25333033 DOI: 10.3978/j.issn.2305-5839.2014.06.03]

152 **Reya T**, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001; **414**: 105-111 [PMID: 11689955 DOI: 10.1038/35102167]

153 **Visvader JE**, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer* 2008; **8**: 755-768 [PMID: 18784658 DOI: 10.1038/nrc2499]

154 **Sell S**, Leffert HL. Liver cancer stem cells. *J Clin Oncol* 2008; **26**: 2800-2805 [PMID: 18539957 DOI: 10.1200/JCO.2007.15.5945]

155 **Boman BM**, Wicha MS. Cancer stem cells: a step toward the cure. *J Clin Oncol* 2008; **26**: 2795-2799 [PMID: 18539956 DOI: 10.1200/JCO.2008.17.7436]

156 **Ginestier C**, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS, Dontu G. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* 2007; **1**: 555-567 [PMID: 18371393 DOI: 10.1016/j.stem.2007.08.014]

157 **Zhu CP**, Wang AQ, Zhang HH, Wan XS, Yang XB, Chen SG, Zhao HT. Research progress and prospects of markers for liver cancer stem cells. *World J Gastroenterol* 2015; **21**: 12190-12196 [PMID: 26576103 DOI: 10.3748/wjg.v21.i42.12190]

158 **Chiba T**, Iwama A, Yokosuka O. Cancer stem cells in hepatocellular carcinoma: Therapeutic implications based on stem cell biology. *Hepatol Res* 2016; **46**: 50-57 [PMID: 26123821 DOI: 10.1111/hepr.12548]

159 **Hirsch HA**, Iliopoulos D, Tsichlis PN, Struhl K. Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. *Cancer Res* 2009; **69**: 7507-7511 [PMID: 19752085 DOI: 10.1158/0008-5472.CAN-09-2994]

160 **Saito T**, Chiba T, Yuki K, Zen Y, Oshima M, Koide S, Motoyama T, Ogasawara S, Suzuki E, Ooka Y, Tawada A, Tada M, Kanai F, Takiguchi Y, Iwama A, Yokosuka O. Metformin, a diabetes drug, eliminates tumor-initiating hepatocellular carcinoma cells. *PLoS One* 2013; **8**: e70010 [PMID: 23922888 DOI: 10.1371/journal.pone.0070010]

161 **Zhang X**, Harmsen WS, Mettler TA, Kim WR, Roberts RO, Therneau TM, Roberts LR, Chaiteerakij R. Continuation of metformin use after a diagnosis of cirrhosis significantly improves survival of patients with diabetes. *Hepatology* 2014; **60**: 2008-2016 [PMID: 24798175 DOI: 10.1002/hep.27199]

162 **Salpeter SR**, Greyber E, Pasternak GA, Salpeter EE. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2010; **(4)**: CD002967 [PMID: 20393934 DOI: 10.1001/archinte.163.21.2594]

163 **Moore EM**, Mander AG, Ames D, Kotowicz MA, Carne RP, Brodaty H, Woodward M, Boundy K, Ellis KA, Bush AI, Faux NG, Martins R, Szoeke C, Rowe C, Watters DA. Increased risk of cognitive impairment in patients with diabetes is associated with metformin. *Diabetes Care* 2013; **36**: 2981-2987 [PMID: 24009301 DOI: 10.2337/dc13-0229]

164 **Smith GD**, Egger M, Shipley MJ, Marmot MG. Post-challenge glucose concentration, impaired glucose tolerance, diabetes, and

cancer mortality in men. *Am J Epidemiol* 1992; **136**: 1110-1114 [PMID: 1462971]

165 **La Vecchia C**, Negri E, Decarli A, Franceschi S. Diabetes mellitus and the risk of primary liver cancer. *Int J Cancer* 1997; **73**: 204-207 [PMID: 9335443]

166 **Hense HW**, Kajüter H, Wellmann J, Batzler WU. Cancer incidence in type 2 diabetes patients - first results from a feasibility study of the D2C cohort. *Diabetol Metab Syndr* 2011; **3**: 15 [PMID: 21752291 DOI: 10.1186/1758-5996-3-15]

167 **Wang P**, Kang D, Cao W, Wang Y, Liu Z. Diabetes mellitus and risk of hepatocellular carcinoma: a systematic review and meta-analysis. *Diabetes Metab Res Rev* 2012; **28**: 109-122 [PMID: 21898753 DOI: 10.1002/dmrr.1291]

168 **Wang C**, Wang X, Gong G, Ben Q, Qiu W, Chen Y, Li G, Wang L. Increased risk of hepatocellular carcinoma in patients with diabetes mellitus: a systematic review and meta-analysis of cohort studies. *Int J Cancer* 2012; **130**: 1639-1648 [PMID: 21544812 DOI: 10.1002/ijc.26165]

169 **Wang YG**, Wang P, Wang B, Fu ZJ, Zhao WJ, Yan SL. Diabetes mellitus and poorer prognosis in hepatocellular carcinoma: a systematic review and meta-analysis. *PLoS One* 2014; **9**: e95485 [PMID: 24830459 DOI: 10.1371/journal.pone.0095485]

170 **Ruiter R**, Visser LE, van Herk-Sukel MP, Coebergh JW, Haak HR, Geelhoed-Duijvestijn PH, Straus SM, Herings RM, Stricker BH. Lower risk of cancer in patients on metformin in comparison with those on sulfonylurea derivatives: results from a large population-based follow-up study. *Diabetes Care* 2012; **35**: 119-124 [PMID: 22100960 DOI: 10.2337/dc11-0857]

P- Reviewer: Guerrieri F, Li YX **S- Editor**: Yu J **L- Editor**: A
E- Editor: Wang CH

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327