Metformin and cancer: An existing drug for cancer prevention and therapy (Review)

FUMING ZI¹, HUAPU ZI², YI LI³, JINGSONG HE³, QINGZHI SHI¹ and ZHEN CAI³

¹Department of Hematology, The Second Affiliated Hospital of Nanchang University, Nanchang, Jiangxi 330008; ²Department of Oncology, Rizhao Traditional Chinese Medicine Hospital of Shandong Traditional Chinese Medicine University, Rizhao, Shandong 276800; ³Bone Marrow Transplantation Center, The First Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang 310003, P.R. China

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Abstract. Metformin is a standard clinical drug used to treat type 2 diabetes mellitus (T2DM) and polycystic ovary syndrome. Recently, epidemiological studies and meta-analyses have revealed that patients with T2DM have a lower incidence of tumor development than healthy controls and that patients diagnosed with cancer have a lower risk of mortality when treated with metformin, demonstrating an association between metformin and tumorigenesis. In vivo and in vitro studies have revealed that metformin has a direct antitumor effect, which may depress tumor proliferation and induce the apoptosis, autophagy and cell cycle arrest of tumor cells. The mechanism underpinning the antitumor effect of metformin has not been well established. Studies have demonstrated that reducing insulin and insulin-like growth factor levels in the peripheral blood circulation may lead to the inhibition of phosphoinositide 3-kinase/Akt/mechanistic target of rapamycin (mTOR) signaling or activation of AMP-activated protein kinase, which inhibits mTOR signaling, a process that may be associated with the antitumor effect of metformin. The present review primarily focuses on the recent progress in understanding the function of metformin in tumor development.

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1. Introduction

Tumorigenesis is a chronic process involving numerous factors, including genetic, environmental, life-style and psychological factors. Prophylaxis and etiological treatments are important methods used to control cancer progression. In the early 1930s, Marble (1) first demonstrated the association between diabetes and cancer. Over the past 15 years, an increasing number of studies have demonstrated that the incidence of tumor development is higher in diabetic patients than in healthy controls (2-4), and that cancer patients with diabetes mellitus (DM) are less sensitive to chemotherapy and exhibit a higher risk of mortality (5-10). Epidemiological studies revealed that the incidence of tumorigenesis and the mortality rate of patients with diabetes were significantly reduced in the metformin-treated group compared with that in patients treated with insulin or sulfonylureas (4,10-12). Further studies have demonstrated that metformin has a direct antitumor effect in vivo and in vitro, which may repress the proliferation of tumor cells, and induce apoptosis, autophagy and cell cycle arrest (13-15). Taken together, these results suggest that metformin may become an alternative adjuvant therapy for the treatment of cancer.

2. Diabetes and cancer: Epidemiological evidence

In 1934, Marble indicated an association between diabetes and cancer (1). Over the past 15 years, this association has been increasingly recognized, and has become an area of interest for endocrinologists and oncologists. In 2003, Meyerhardt et al (5) observed that colon cancer patients with DM have a higher rate of mortality and tumor recurrence. In 2005, Yang et al (16) reported that the risk of colorectal cancer was increased in type 2 DM (T2DM) patients. Studies have revealed that diabetes is recognized as an independent prognostic factor...
for colon, pancreatic, breast, liver and bladder cancer (6). A meta-analysis revealed that cancer patients with pre-existing diabetes have an increased risk of mortality compared with those without diabetes [hazard ratio (HR), 1.4; 95% confidence interval (CI), 1.28-1.55] (7). Subgroup analyses revealed an increased risk for certain types of cancer, including endometrial (HR, 1.76; 95% CI, 1.34-2.31), breast (HR, 1.61; 95% CI, 1.46-1.78) and colorectal (HR, 1.52; 95% CI, 1.42-1.41) cancer, respectively. Lee et al. (17) reported that, following adjustment for sex, age, hypertension, dyslipidemia and gout, a total of 104,343 Taiwanese patients with diabetes, who were followed up between 1998 and 2009, had an increased incidence of liver, colon, lung, breast cancer and prostate cancer. Furthermore, another meta-analysis demonstrated that patients with diabetes exhibited a 23% increased risk of breast cancer and a 26% increased risk of colorectal cancer (18).

Controversy remains regarding the association between diabetes and prostate cancer. A meta-analysis demonstrated that the risk of prostate cancer in patients with diabetes was lower than that in those without diabetes (19). Gong et al. (20) also discovered that the risk of prostate cancer was lower in patients with diabetes, when compared with that in those without diabetes. Subsequently, a prospective study of 328,316 males who were followed up for 5 years observed that a history of diabetes is associated with a lower incidence of prostate cancer (21). The biological mechanisms of this association remain unknown, but may be associated with increased physical activity resulting in lower circulating levels of insulin and testosterone, or with changes in the transcription factor 2 hepatic gene (21-24). A multi-ethnic cohort-based prospective study revealed that patients with diabetes were at a lower risk of developing prostate cancer and that this was not associated with their ethnicity (25). However, whether or not the mortality of men with prostate cancer is associated with diabetes remains unknown. It has previously been demonstrated that pre-existing diabetes affects the mortality rate of patients with prostate cancer (25), however this hypothesis is contested by a different study observing no significant association between these two factors (26).

Epidemiological evidence demonstrates an association between diabetes and hematological malignancies (27,28). Using a random-effects model, a meta-analysis of observational studies revealed that T2DM is associated with an increased risk of developing non-Hodgkin’s lymphoma, leukemia and myeloma (28). Table I summarizes the meta-analyses of the associations between diabetes and different types of cancer over a number of years (29-41).

3. Molecular relationship between diabetes mellitus and tumorigenesis

The mechanisms behind the fact that patients with T2DM are more likely to develop tumors have not yet been fully elucidated. Previous studies have suggested that there are three factors serving important functions in this process.

Insulin resistance. T2DM, which is characterized by insulin resistance and hyperinsulinemia, causes an increased level of insulin and insulin-like growth factor (I/IGF), which could bind to receptors and activate the downstream phosphatidylinositol 3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK) signaling pathways, ultimately leading to the proliferation of cells (11,37,42-45). It has been confirmed that I/IGF and its downstream signaling pathway have an important function in tumor development, and thus, may serve as targets for tumor therapy (46,47). I/IGF signaling pathways are also recognized as playing an important role in the relationship between diabetes and cancer (48).

Diabetes and inflammation. Previously, certain researchers believed diabetes to be an inflammatory disease (49-51). Metabolic disturbances and enhanced oxidative stress in patients with diabetes promote a continuous pro-inflammatory state, resulting in the decreased antioxidant capacity of cells. The insulin resistance that characterizes T2DM may produce large numbers of cytokines, including tumor necrosis factor α (TNF-α), interleukin (IL)-6 and IL-1β (49,52). TNF-α and IL-6 may activate nuclear factor-κB and Janus kinase/signal transducer and activator of transcription 3 pathways, which are important signaling pathways in tumorigenesis (53,54).

Hypoinnunity of diabetic patients. Patients with DM are more likely to have persistent infections, suggesting that these patients may be immunodeficient and therefore more susceptible to opportunistic infections (55).

4. Antitumor effect of metformin: Epidemiological evidence

In 1957, the FDA approved the use of metformin for the treatment of T2DM. Following this, metformin became recognized as the first-line treatment for diabetes due to its excellent hypoglycemic and cardiovascular protective effects. In 2005, Evans et al. (56), in a case-controlled study that included 11,876 T2DM patients, identified for the first time that metformin may reduce the risk of cancer in patients with diabetes (unadjusted odds ratio, 0.79; 95% CI, 0.67-0.93), and that this effect was positively correlated with the dosage of metformin. In 2006, a population-based retrospective cohort study by Bowker et al. (57) revealed that the metformin treatment group had a lower cancer-associated mortality rate compared with that of the sulfonylurea group and the insulin treatment group consisting of other patients with cancer and DM. In 2009, Evans and colleagues re-highlighted the association between metformin treatment and tumorigenesis in patients with T2DM. The tumor incidence in 4,085 patients with diabetes treated with metformin was lower than that in the control group (7.3 vs. 11.6%), and the adjusted odds ratio was 0.63 (95% CI, 0.53-0.75), further demonstrating that metformin may reduce the risk of tumorigenesis (58).

In 2009, a case-controlled study reported by Li et al. (59) from the MD Anderson Cancer Center (Houston, TX, USA) revealed that metformin was associated with a reduced risk of pancreatic cancer in patients with diabetes. Compared with those who were not treated with metformin, diabetic patients treated with metformin exhibited an ~62% reduced risk of developing pancreatic cancer. A retrospective cohort study of 62,809 diabetic patients undertaken by Currie et al. (60) demonstrated that monotherapy with metformin was associated with the lowest risk of solid tumor genesis compared
with insulin or sulfonylurea treatment. However, combined treatment with metformin and either insulin or sulfonylurea may reduce the insulin- or sulfonylurea-induced tumor risk. In the aforementioned study, it was observed that, compared with metformin treatment, insulin treatment increased the risk of colorectal and pancreatic cancer, and the HR was 1.69 (95% CI, 1.23-2.33) and 4.63 (95% CI, 2.64-8.10), respectively. However, metformin therapy did not reduce the risk of breast or prostate cancer (60).

Another case-controlled study undertaken by Donadon et al (61) demonstrated that treatment with metformin significantly reduced the risk of developing hepatocellular carcinoma (HCC) by >80% compared with sulphonylurea and insulin therapy. Additionally, another study revealed that metformin may reduce the risk of HCC in diabetic patients with chronic liver disease (62).

In 2010, a prospectively-followed cohort study assessed the association between the use of metformin and cancer mortality in 1,353 patients with T2DM (63). Metformin-treated patients were revealed to exhibit a reduced cancer mortality time compared with that of the controls with a median of 9.6 years and an adjusted HR of 0.43 (95% CI, 0.23-0.80) (63). Diabetic patients taking metformin had a 31% reduced tumor risk compared with those taking any other antidiabetic drug, particularly for pancreatic cancer and HCC, but not for colon, breast or prostate cancer (64). Jiralerspong et al (65) observed that diabetic patients with breast cancer treated with metformin and neoadjuvant chemotherapy acquired a higher pathological complete response rate than those not being treated with metformin. A nested case-controlled analysis including 22,621 female patients with T2DM demonstrated that patients with diabetes who used metformin for ≥5 years had a decreased risk of developing breast cancer (adjusted odds ratio, 0.44; 95% CI, 0.24-0.82) (66).

Certain studies have also revealed that diabetic patients with thyroid cancer treated with metformin exhibit a higher rate of remission. Tumor size in the metformin-treated group is significantly smaller than that in the control groups. An in vitro study demonstrated that metformin may activate AMP- inducible protein kinase (AMPK) and downregulate p70S6K/pS6 protein to inhibit the growth of tumor cells (67). Kumar et al (12) revealed that metformin treatment was associated with an improved survival time in patients with ovarian cancer. The progression-free survival time of patients with ovarian cancer and T2DM was longer in the metformin group (68).

Another prospective study (21) and a meta-analysis (19) suggested that pre-existing diabetes may reduce the risk of prostate cancer. Prostate cancer patients with diabetes exhibited a higher rate of mortality compared with those without diabetes (26). Furthermore, Patel et al (69) revealed that prostate cancer patients with diabetes had a higher rate of relapse following prostatectomy, and the use of metformin did not prove to be of any benefit. Additionally, Azoulay et al (70) discovered that the use of metformin did not decrease the risk of prostate cancer in patients with diabetes. These results conflicted with those of Wright and Stanford (71), which observed that metformin may reduce the risk of prostate cancer in patients with diabetes, while He et al (72) suggested that the use of metformin may improve the overall survival of prostate cancer patients with diabetes.

In 2012, Sadeghi et al (73) demonstrated that metformin was associated with an increased survival time in pancreatic cancer patients with diabetes. Furthermore, Noto et al (74) revealed that the use of metformin in patients with diabetes may reduce the risk of cancer incidence and mortality, and indicated that analysis based upon observational studies and long-term randomized controlled trials should be performed to confirm this result. With regards to patients without diabetes, one study discovered that treatment with metformin was associated with a lower risk of colorectal carcinogenesis (75).

5. Mechanism through which metformin exerts its antitumor effect

A large volume of epidemiological data has suggested that metformin may benefit cancer patients. In vitro and in vivo experiments have confirmed that metformin may inhibit the proliferation of a variety of tumor cells, but the mechanism underpinning this has not yet been fully elucidated. At present, two major pathways are recognized as the main ways in which metformin exerts its antitumor effect. The first pathway, the I/IGF pathway, may reduce the level of I/IGF-1 in the blood circulation, thereby inactivating its downstream PI3K/Akt/mTOR signaling pathways to inhibit tumor cell proliferation. The second pathway, the AMPK signaling pathway, may facilitate metformin to directly act on tumor cells, upregulate AMPK and inhibit downstream mTOR (42).

I/IGF and its downstream signaling pathway. I/IGF promote cell mitosis, stimulate cell growth and inhibit cell apoptosis, all of which serve important functions in tumor genesis and
which inhibits phosphorylation of mTOR and its downstream binding partner raptor on 722 and 792 serine residues, which inactivates raptor and mTOR (98). Goodwin et al (78) demonstrated that, in breast cancer patients without overt DM, the use of metformin may significantly decrease insulin levels and improve insulin resistance. Memmott et al (79) observed that when mice were exposed to the tobacco carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane, the use of metformin reduced lung tumor burden by up to 53%. This mechanistic study revealed that metformin may directly inhibit mTOR by activating AMPK in liver tissue and may indirectly inhibit mTOR by decreasing activation of insulin receptor/IGF-1 receptor and Akt in lung tissue (79). Algire et al (80) observed that, when colon carcinoma MC38 cells or Lewis lung carcinoma 1 (LLC1) cells were transfected with short hairpin RNA (shRNA) against liver kinase B1 (LKB1), the growth of MC38 and LLC1 cells was significantly inhibited and the phosphorylation of AMPK was markedly increased by metformin in vitro. Additionally, under low-glucose conditions in vitro, MC38 and LLC1 cells transfected with shRNA against LKB1 were more sensitive to metformin. LKB1+ and LKB1− MC38 cells were subcutaneously transplanted in the high-fat diet and normal diet mice respectively. The results showed that regardless of the expression of LKB, mice with a high-fat diet were more likely to have tumors, and metformin was able to significantly inhibit insulin levels in high-fat diet mice (80). Pollak (81) demonstrated that, while metformin reduced the insulin level, no effect on the IGF-I/IGF-II level was observed (47,77,81). Karnevi et al (82) demonstrated that metformin may inhibit IGF-1R and activate AMPK in pancreatic cancer cells.

**AMPK signaling pathway.** AMPK, which is a cellular energy sensor, may be activated by an increased AMP/ATP ratio. Metformin may inhibit the effect of respiratory complex I, leading to reduced oxidative phosphorylation and reduced ATP production, resulting in a reduction in cellular ATP and activation of AMPK (47). In 2006, Zakikhani et al (83) demonstrated that metformin inhibited the proliferation of breast cancer cells through activation of AMPK, leading to the inhibition of mTOR. This growth inhibition was AMPK-dependent and was blocked by small interfering RNA against AMPK (83). There are two pathways known to inhibit mTOR following activation of AMPK. Firstly, AMPK may directly phosphorylate tuberous sclerosis complex 2 (TSC2) on T1227 and S1345, and activate the TSC1/TSC2 compounds, which inhibit the activity of Ras homolog enriched in brain and mTOR (84). Secondly, AMPK directly phosphorylates the mTOR binding partner raptor on 722 and 792 serine residues, which inactivates raptor and mTOR (85,86). Dowling et al (87) revealed that metformin may activate the expression of AMPK, which inhibits phosphorylation of mTOR and its downstream ribosome S6 protein kinase (p70S6K) and eIF4E-binding proteins (4E-BP1). Similarly, other studies observed that the proliferation of cells in leukemia, lymphoma, and prostate, ovarian, colon, endometrial and liver cancer was inhibited by metformin through the AMPK/mTOR pathway (88-93).

Metformin also exerts its antitumor effect in an AMPK-independent manner. Ben Sahra et al (94) reported that metformin may inhibit the cell proliferation and induce the cell-cycle arrest of prostate cancer cell lines by increasing regulated in development and DNA damage response 1 (REDD1) expression in a p53-dependent manner in the absence of AMPK. Inhibition of REDD1 reverses metformin-induced cell-cycle arrest (94). Kalender et al (95) demonstrated that metformin may inhibit mTORC1 in a rag GTPase-dependent manner in the absence of AMPK and TSC1/2. Certain studies have also revealed that metformin may induce the apoptosis and cell-cycle arrest of melanoma cells (96) and epithelial ovarian cancer cell lines (OVCA-3 and OVCAR-4) (97) in an AMPK-independent manner. Zi et al (98) revealed that metformin may inhibit melanoma cell proliferation through the P13K/Akt/mTOR signaling pathway, but not through the AMPK/mTOR pathway (98). Taken together, these results demonstrate that metformin exerts an anti-proliferation function through a range of mechanisms.

Whether or not AMPK is an oncogene or a tumor suppressor gene remains to be fully elucidated (99,100). Studies have revealed that AMPK is overactivated in multiple myeloma cells and prostate cancer cells, which may result in cell apoptosis (101,102). At present, metformin is considered to be an AMPK activator by the majority of researchers. However, it is difficult to confirm whether or not metformin is more effective in the treatment of tumors without the functional LKB1-AMPK pathway (80,103). Liu et al (104) observed that, compared with that in normal tissue, AMPK is constitutively activated in human and mouse gliomas (104). However, using an AMPK direct activator, A769662, did not induce glioma cell apoptosis, suggesting that metformin may not exert its antitumor effect through the AMPK pathway (104). Metformin may increase glucose consumption and inhibit the production of ATP in cells. As an ‘energy sensor’, the activation of AMPK may be a result of cells adapting under survival pressure (80). The authors of the present review hypothesize that when the time or concentration of metformin exposure are increased, cells may have been unable to adapt to compensate for energy deprivation, AMPK phosphorylation may have been suppressed and tumor cell apoptosis may eventually have been induced. Furthermore, it was observed that when AMPK was knocked down, the tolerance of myeloma cells for nutritional deficiency was decreased when compared with that of the control group. Therefore, the effects of metformin on AMPK require further investigation. Fig. 1 summarizes the mechanisms through which metformin exerts its antitumor effect.

### 6. Combination of metformin and other drugs

Combined treatment with metformin may enhance the curative effect of and reduce the adverse reactions associated with chemotherapy. Therefore, combining metformin with traditional chemotherapy drugs has become a novel aspect of cancer therapy. Jiralerspong et al (65) observed that breast cancer patients with DM treated with metformin and neoadjuvant chemotherapy had a higher complete pathological response.
rate when compared with the control group (65). Another study reported that this enhanced antitumor effect of chemotherapy may have been due to the inactivation of vitamin B12 induced by metformin through the N,O pathway (105). Ben Sahra et al (106) discovered that metformin combined with 2-deoxyglucose (2-DG) may inhibit mitochondrial respiration and glycolysis through tumor protein p53 (p53)-dependent apoptosis via the AMPK pathway. Furthermore, the re-expression of a functional p53 in p53-deficient prostate cancer cells may restore caspase-3 activity (106). A later study observed that combined therapy with metformin and 2-DG may inhibit autophagy and induce AMPK-dependent apoptosis in prostate cancer cells (107). Colquhoun et al (108) revealed that bicalutamide combined with metformin may significantly reduce prostate cancer cell growth compared with single-agent monotherapy. In androgen receptor (AR)-positive cells, this effect appeared to be mediated by enhanced antiproliferation, while the same effect appeared to be mediated by enhanced apoptosis in AR-negative cells (108).

Rocha et al (109) revealed that treating MCF-7 and A549 cells with metformin and paclitaxel markedly inhibited cell viability and increased the number of cells arrested in the G2-M phase of the cell cycle, compared with individual drug treatments, and that the AMPK signaling pathway was involved in this process (109). In an in vitro study, Rattan et al (110) discovered that metformin may inhibit the proliferation, metastasis and angiogenesis of ovarian tumors in xenograft models of A2780 ovarian cancer cells and that it may potentiate cisplatin-induced cytotoxicity by inhibiting tumor growth. Another study observed that, in mouse xenograft models of breast, lung and prostate cancer cell lines, metformin had a synergistic effect when combined with paclitaxel, carboplatin or doxorubicin (111). Metformin also potentiates the effects of paclitaxel by inhibiting the cell proliferation of endometrial cancer cell lines via the mTOR pathway (112). Our previous study also demonstrated that metformin displays anti-myeloma activity and a synergistic effect when combined with dexamethasone in in vitro and in vivo xenograft models (98).

Meanwhile, in an in vitro study, Janjetovic et al (113) demonstrated that metformin reduced the anticancer activity of cisplatin in U251 human glioma, C6 rat glioma, SHSY5Y human neuroblastoma, L929 mouse fibrosarcoma and HL-60 human leukemia cell lines through AMPK-independent upregulation of the Akt pathway.

7. Influence of metformin on cancer stem cells (CSCs)

CSCs are considered as one of the causes of tumor resistance and relapse (114-116). Hirsch et al (117) revealed that low doses of metformin may selectively kill breast CSCs (cluster of differentiation (CD)44high /CD24low ) in four genetically different types of breast cancer (MCF-7, SKBR3, MDA-MB-468 and MCF-10A). Additionally, metformin combined with doxorubicin may kill both CSCs and non-stem cancer cells in vitro, and the remission time was longer when compared with single-agent therapy in a xenograft mouse model (117). A further two studies also demonstrated that metformin may kill breast CSCs (118,119). Shank et al (120) discovered that metformin may restrict the growth and proliferation of ovarian CSCs in vitro and in vivo. Additionally, by re-expressing the miRNAs (let-7a, let-7b, miR-26a, miR-101, miR-200b and miR-200c) that are typically lost in pancreatic cancer and decreasing the expression of CSC-specific genes (including CD44, epithelial cell adhesion molecule, enhancer of zeste homolog 2, Notch-1, Nanog and octamer-binding transcription factor 4), metformin may attenuate the function of CSCs and may be useful for overcoming therapeutic resistance in pancreatic cancer cells (121). Recently, certain studies revealed that metformin may inhibit the growth of prostate and gastrointestinal CSCs (122,123). Florio (124) discovered that metformin selectively inhibits the proliferation of human glioblastoma CSCs through the direct inhibition of chloride intracellular
channel-I. Therefore, metformin has become recognized as a novel therapeutic option for targeting CSCs (125,126).

8. Conclusion

Metformin is the first-line drug for the treatment of T2DM. Epidemiological and basic studies have demonstrated that it may also inhibit the growth of a variety of tumor cells, and an increasing number of ongoing clinical trials on the antitumor effects of metformin are being processed for the treatment of cancer. Metformin has been proven to be safe as a treatment drug for T2DM and has subsequently been used clinically for a number of years. If large-scale clinical trials are able to attest to the antitumor effects of metformin, this drug may become an alternative cancer adjuvant therapy, providing a novel approach for cancer prevention and treatment.

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