

Metformin: a review of its potential indications

Yi-Wei Wang^{1,*}

Si-Jia He^{1,*}

Xiao Feng¹

Jin Cheng¹

Yun-Tao Luo¹

Ling Tian²

Qian Huang¹

¹The Comprehensive Cancer Center and Shanghai Key Laboratory for Pancreatic Diseases, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China; ²Institute of Translational Medicine, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China

*These authors contributed equally to this work

Abstract: Metformin is the most commonly prescribed drug for type 2 diabetes mellitus. In recent years, in addition to glucose lowering, several studies have presented evidence suggesting some potential role for metformin, such as antitumor effect, antiaging effect, cardiovascular protective effect, neuroprotective effect or an optional treatment for polycystic ovary syndrome. This paper will critically review the role of metformin to provide reference for doctors and researchers.

Keywords: metformin, antitumor effect, antiaging effect, cardiovascular protective effect, neuroprotective effect, PCOS

Introduction

Metformin has become one of the most widely used drugs in the treatment of type 2 diabetes mellitus (T2DM) since its approval in the United Kingdom in 1958 and in the United States in 1995, with doses ranging from 500 to 2,500 mg/day.¹ It is the first-line therapy for patients with T2DM according to the American Diabetes Association/European Association for Study of Diabetes guidelines.² Metformin works by decreasing intestinal glucose absorption, improving peripheral glucose uptake, lowering fasting plasma insulin levels and increasing insulin sensitivity, which result in a reduction of blood glucose concentrations without causing overt hypoglycemia.³ Additionally, metformin can inhibit gluconeogenesis with the activation of AMP-activated protein kinase (AMPK).⁴ AMPK is an important player in the regulation of energy metabolism, which plays a key role in diabetes and related metabolic diseases. It is demonstrated that AMPK is required for maintaining glucose homeostasis.⁵ Metformin has few adverse side effects, the most common adverse side effects being gastrointestinal symptoms (incidence rate 20%–30%), including nausea and vomiting,⁶ and the most serious adverse effects being lactic acidosis (incidence rate 1/30,000), mainly in diabetic patients with liver and kidney dysfunction.⁷

Since metformin's worldwide spread for over 50 years, numerous studies concerning other potential indications have emerged, which showed that metformin can also be used as an anticancer agent,⁸ an antiaging agent,⁹ a cardiovascular protective agent,¹⁰ a neuroprotective agent¹¹ or an optional drug for polycystic ovary syndrome (PCOS).¹² In this review, we summarized the currently potential indications and possible mechanism of metformin, and expect many more studies for further verification.

Antitumor effect of metformin

Metformin was first discovered as an antitumor agent on hamsters in 2001. In this experiment, there were two groups of high-fat (HF)-fed hamsters. One group received metformin in drinking water for life (HF + Met group), and the other group served as the control group (HF group). All hamsters were treated with N-nitrosobis-(2-oxopropyl)

Correspondence: Qian Huang
The Comprehensive Cancer Center
and Shanghai Key Laboratory for
Pancreatic Diseases, Shanghai General
Hospital, Shanghai Jiao Tong University
School of Medicine, No 650, Xin Song
Jiang Road, Shanghai 201620, People's
Republic of China
Tel +86 21 3779 8906
Email qhuang@sjtu.edu.cn

amine, a pancreatic carcinogen, and after 42 weeks, 50% of the hamsters in the high-fat group developed malignant lesions; however, none was found in the HF + Met group ($P < 0.05$).¹³ A large case-control study in Scotland first showed that metformin reduced the risk of cancer in patients with T2DM (odds ratio [OR] 0.77, 95% CI 0.64–0.92 for any metformin exposure versus no metformin exposure).¹⁴ A representative population prospective cohort study of 800,000 individuals showed that metformin reduced the incidences of several gastroenterological cancers in treated diabetes (total 0.12 (0.08–0.19), colorectal 0.36 (0.13–0.98), liver 0.06 (0.02–0.16), pancreas 0.15 (0.03–0.79)), the dose of metformin is shown in Table 1.¹⁵ In addition to the reduction of cancer incidence,^{16,17} metformin intake was also associated with a decrease of cancer mortality. Landman et al showed that metformin was associated with lower cancer mortality (hazard ratio [HR] 0.43 [0.23–0.80]) and that the effect was dose dependent (Table 1).¹⁸ A recent meta-analysis concluded that metformin reduced cancer incidence and mortality in patients with diabetes, with overall cancer incidence reduced by 31% and cancer mortality reduced by 34%.⁸ Furthermore, a meta-analysis¹⁹ suggested that metformin had the greatest benefits as an adjuvant agent in colorectal and prostate cancer treatment, particularly in those receiving radiotherapy. However, the dose of metformin needs to be further explored. So far, several epidemiologic studies have reported the antitumor effect of metformin in different tumors, such as ovarian,^{20,21} breast,^{22,23} prostate²⁴ and colorectal.²⁵

However, some studies also considered that there was no significant effect of metformin on cancer risk, survival

time and mortality risk of cancer in T2DM patients, such as lung cancer,²⁶ breast cancer²⁷ and prostate cancer.²⁸ A recent study also reported that metformin has no protective association with survival in colorectal cancer patients with T2DM (HR 1.06, 95% CI 0.80–1.40).²⁹

Therefore, whether metformin has antitumor effect or not has attracted much attention. Accumulating evidence showed metformin's role in attenuating tumorigenesis. First, Wu et al³⁰ uncovered that metformin's antitumor properties rely on two elements of a single genetic pathway – the nuclear pore complex (NPC), which allows the passage of molecules into and out of the nucleus, and an enzyme called acyl-CoA dehydrogenase family member-10 (ACAD10) (Figure 1). Basically, metformin's suppression of mitochondrial respiratory capacity reduces cellular energy, restricting transit of the RagA-RagCGTPase heterodimer through the NPC. This shuts off an important cellular growth molecule called mTORC1, and the inactivation of mTORC1 subsequently inhibits growth and extends the lifespan of *Caenorhabditis elegans* through transcriptional induction of ACAD10. Moreover, in human melanoma and pancreatic cancer cells, the investigators confirmed that application of biguanides restricted nuclear pore transit and induced ACAD10 expression. After all, the experiments showed that metformin can no longer block the growth of cancer cells, if we force the nuclear pore to remain open, or if we permanently close the ACAD10. This pathway provides a unified mechanism by which metformin can kill cancer cells and extend lifespan, and in specific environments, the nuclear pore and ACAD10 may be manipulated for the prevention or even treatment of certain cancers. Second, metformin can significantly reduce the risk factors of tumor in patients with T2DM, including glucose, insulin and insulin-like growth factor 1 (IGF-1). In order to create a fuel-rich environment for cancer progression, cancer cells usually uptake high levels of glucose.³¹ Metformin, as a glucose-lowering agent, can cut off supplies for cancer cells and inhibit tumor growth.³² Insulin and IGF-1 may act as potential growth factors capable of stimulating cell survival and mitogenesis, protecting cells from apoptosis to promote cancer development and progression.^{31,33,34} This effect is mediated by the insulin receptor and the insulin-like growth factor 1 receptor (IGF-1R), which are expressed on many cancer cells,³⁵ through Ras/Raf/MEK/ERK signaling and PI3K/Akt/mTORC1 signaling.^{36–38} Moreover, hyperinsulinemia has been shown to increase free or bioactive IGF-1 levels by the downregulation of insulin-like growth factor binding protein, resulting in the activation of IGF-1R.³⁹ Metformin treatment can reduce the levels of insulin and IGF-1, thereby

Table 1 Summary of effective dose of metformin in studies

Antitumor effect of metformin	500 mg/day ¹⁵ The greater the metformin exposure, the stronger the risk reduction ¹⁸
Antianging effect of metformin	0.1% metformin in diet ⁵⁴
Cardiovascular protective effects of metformin	Starting with one 850 mg tablet per day, then 850 mg twice a day, and then 1,700 mg in the morning and 850 mg with the evening meal (maximum dose = 2,550 mg) ⁵⁶ 1.4±0.2 g ¹⁰
Neuroprotective effect of metformin	Starting with 0.5 g tablet, two tablets a day with meals. If the blood glucose level is not controlled for 7 days after taking metformin, it should be increased to 1.5 g/day over 2 weeks (maximum dose < 2.0 g/day) ⁸⁵ 1,000 mg twice a day ⁸⁶
Polycystic ovary syndrome	Range from 850 to 1,700 mg

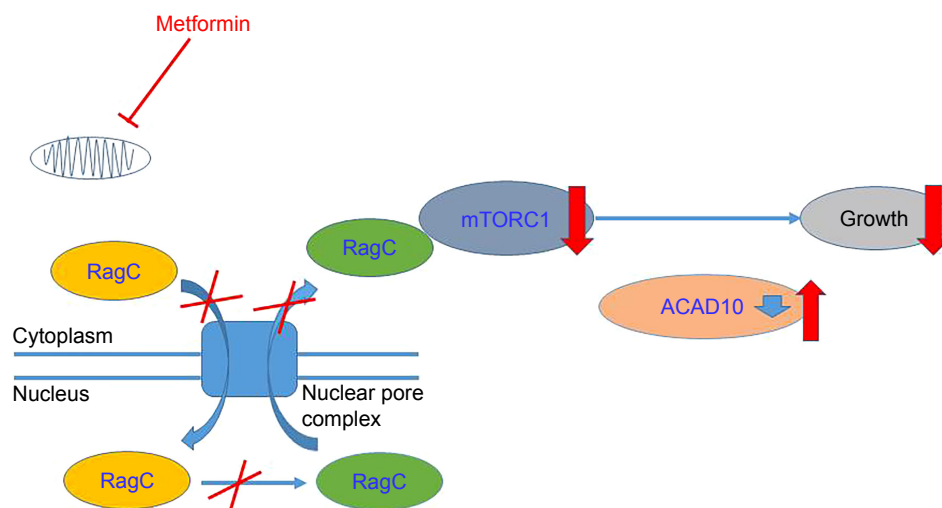


Figure 1 Model of the ancient pathway by which metformin extends lifespan and inhibits growth.

Notes: The figure shows that metformin's suppression of mitochondrial respiratory capacity reduces cellular energy, restricting transit of the RagA-RagCGTPase heterodimer through the NPC. Nuclear exclusion renders RagC incapable of gaining the guanosine diphosphate-bound state necessary to activate mTORC1, subsequently inhibiting growth through transcriptional induction of ACAD10.

Abbreviations: NPC, nuclear pore complex; ACAD10, acyl-CoA dehydrogenase family member-10.

reducing the growth of cell.⁴⁰ Third, activating the AMPK signaling pathway is also an important anticancer mechanism of metformin. The activated AMPK leads to energy preservation processes for cell survival at the expense of growth and proliferation. It can phosphorylate tuberous sclerosis complex 1 and 2, leading to the suppression of mTORC1 activation by inhibiting Ras homolog enrichment in brain (an mTORC1 activator).^{41–43} This inhibition of mTORC1 ultimately decreases protein synthesis and cell growth.^{36,44} Metformin can also inhibit mTORC1 by suppressing Rag GTPases⁴⁵ and upregulating the expression of REDD1 in a p53-dependent manner.⁴⁶ Meanwhile, metformin has other AMPK-mediated actions that may be implicated in cancer, such as reduced lipogenesis,^{47,48} decreased angiogenesis,⁴⁹ inhibition of the synthesis of proinflammatory cytokines⁵⁰ and increase in the number of CD8(+) tumor-infiltrating lymphocytes.⁵¹ Metformin's multiple tumor-relevant actions are depicted in Figures 1 and 2. However, most of the evidence for the treatment of metformin has been derived from retrospective cohort studies and case–control studies, instead of rigorous prospective or randomized controlled trials. There is still a lack of clinical evidence for metformin's antitumor activity in non-diabetic patients. Therefore, more clinical trials are needed to evaluate the role of metformin in different tumors.

Antiaging effect of metformin

Aging is a complex process, which is associated with accumulation of damage, loss of function and increased vulnerability

to disease, ultimately leading to death. Human aging and age-related diseases are becoming one of the greatest challenges and financial burdens faced by developed and developing countries.⁵² A growing body of evidence showed that metformin could delay aging and increase lifespan in vivo, specifically in nematodes and mice. Cabreiro et al found that metformin at 25, 50 and 100 mM concentration increased mean lifespan by 18%, 36% and 3%, respectively, in *C. elegans*.⁵³ Martin-Montalvo et al showed that metformin increased lifespan by 4%–6% in different mouse breeds, and the long-term treatment with metformin (0.1% metformin in diet) extended lifespan, while a higher dose (1% metformin) was toxic (Table 1).⁵⁴ A recent study determined that mean lifespan would be increased by 14% and maximum lifespan by 1 month if treatment with metformin is started early in life, but at older age, this effect would be declined.⁵⁵ Additionally, there are also many studies that focus on whether antiaging effects of metformin can be demonstrated in patients with T2DM. In the United Kingdom Prospective Diabetes Study (UKPDS), the use of metformin decreased the risk of cardiovascular disease,⁵⁶ cancer incidence and overall mortality,⁵⁷ compared with other antidiabetic drugs. Furthermore, a large retrospective observational study including over 180,000 subjects showed that patients with T2DM initiated with metformin monotherapy had longer survival than did matched, non-diabetic controls; however, in this study they did not investigate for a dose-response association.⁹ On the contrary, Slack et al⁵⁸ found that metformin can activate AMPK and reduce lipid stores, but cannot extend lifespan

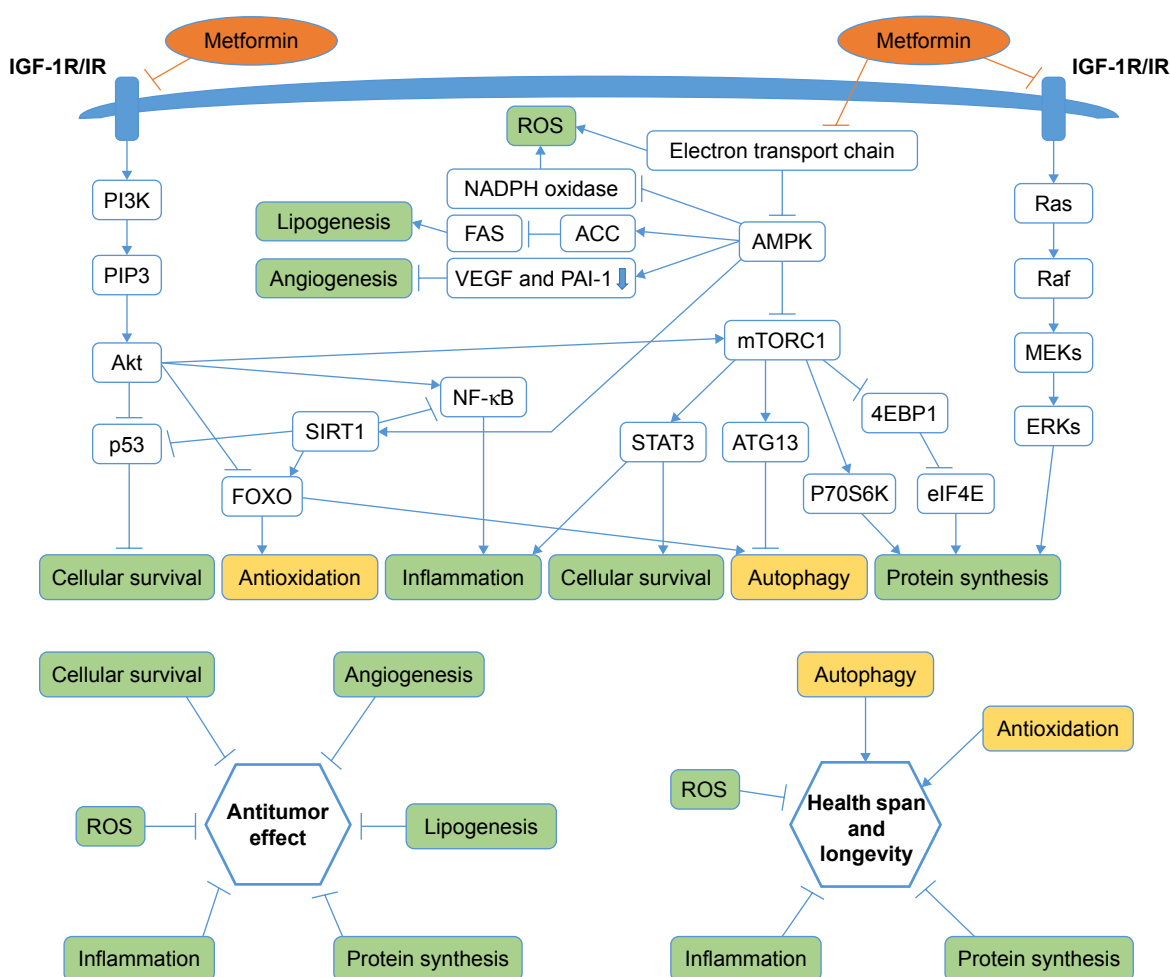


Figure 2 Metformin targets multiple pathways of oncogenesis and aging.

Notes: The figure schematically depicts the pathways that are important to target oncogenesis and aging, and indicates at which points metformin has been shown to have effects. These processes jointly affect cellular survival, antioxidation, inflammation, autophagy and protein synthesis, which are major biological outcomes associated with oncogenesis and aging/longevity. The green background means that metformin has a negative effect on the step. The yellow background means that metformin has an active effect on the step.

Abbreviations: IGF-1R, insulin-like growth factor 1 receptor; IR, insulin receptor; ROS, reactive oxygen species; AMPK, AMP-activated protein kinase.

in *Drosophila* (the final concentrations are 1, 2.5, 5, 10, 25, 50 and 100 nM). One possible reason is that the dose of metformin in the study is toxic. Similarly, a study confirmed that diet supplementation with 0.1% metformin led to a 5.83% extension of mean lifespan of C57BL/6 mice, while a higher concentration of metformin (1%) was toxic and significantly shortened mean lifespan by 14.4%.⁵⁴

Interventions that target aging-related pathways are capable of extending lifespan dramatically, especially health span, a period of life during which an individual is fully functional and free of chronic illness.⁵⁹ These include intermittent or prolonged fasting, mild caloric restriction combined with a low glycemic index diet and protein restriction, inhibition of the GH/IGF-I axis, inhibition of TOR-S6K signaling, activation of sirtuins or AMPK and chronic metformin use. Metformin's multiple aging-relevant actions are also depicted

in Figures 1 and 2. On one hand, as previously described, NPC and ACAD10 mediate biguanide-induced growth inhibition and lifespan extension.³⁰ On the other hand, specifically for aging, metformin can not only decrease insulin and IGF-1 levels,⁶⁰ reduce the endogenous production of reactive oxygen species (ROS)^{61,62} and active AMPK,⁶²⁻⁶⁵ and inhibit mTOR,^{66,67} but also influence metabolic and cellular processes such as inflammation⁶⁸ and autophagy.⁶⁹ The United States intends to carry out a big clinical trial about the antiaging effect of metformin enrolling 3,000 non-diabetics aged 70-80 years at roughly 15 centers. The follow-up will last for 5-7 years, and the situation of disease suffering and death after metformin treatment will be emphatically studied. The result of the clinical trial aimed to prove that metformin has a positive impact on human lifespan in non-diabetics and healthy people.

Cardiovascular protective effects of metformin

Diabetic patients mainly die of cardiovascular complications,⁷⁰ including macrovascular complications (such as stroke, coronary artery disease [CAD] and myocardial infarction) and microvascular complications (such as kidney disease, retinal injury and peripheral nerve disease), of which approximately 70% of all diabetic patients die of heart and brain macrovascular diseases. A number of clinical studies have shown that metformin has cardiovascular protective effects and reduces the incidence and mortality of cardiovascular events. In 1998, UKPDS, a randomized, prospective, multicenter trial, was the first trial to determine that metformin could significantly reduce the risk of all-cause mortality and acute myocardial infarction in overweight patients with T2DM; the dose of metformin is shown in Table 1.⁵⁶ In addition, a 10-year post-interventional follow-up of the UKPDS survivor cohort further examined that metformin treatment had a long-term benefit on cardiovascular risk in overweight patients. Compared with sulfonylurea and insulin treatment, metformin treatment can effectively reduce the risk of myocardial infarction and death.⁷¹ Similarly, Roumie et al also showed that compared with sulfonylurea therapy, metformin treatment was associated with a decreased hazard of cardiovascular disease events or death in T2DM.⁷² Moreover, data from the Reduction of Atherothrombosis for Continued Health Registry indicated that the use of metformin as a means of secondary prevention was associated with a 24% reduction in all-cause mortality after 2-year follow-up among patients with atherothrombosis. Thus, metformin has cardiovascular protective effects independent of glucose-lowering effects.⁷³ Furthermore, in a multicenter, randomized, double-blind, placebo-controlled clinical trial, Hong et al found that among type 2 diabetic patients with CAD, compared with glipizide, metformin treatment for 3 years (mean daily dose was 1.4±0.2 g; Table 1) substantially reduced major cardiovascular events in a median follow-up of 5 years, which indicated a potential benefit of metformin treatment on cardiovascular outcomes in high-risk patients.¹⁰ After all, metformin is the only antidiabetic drug to be recommended by the 2013 AACE guidelines for cardiovascular benefit.

Metformin may exert beneficial effects to prevent cardiovascular disease. The risk factors of cardiovascular disease include dyslipidemia, obesity, hypertension, insulin resistance and so on. First, metformin may improve lipometabolism and reduce the level of LDL cholesterol by activation of AMPK.⁷⁴ Second, metformin was associated with weight loss or less weight gain,^{75–77} the mechanism of which

is thought to be the decreased perceived hunger resulting in diminished food intake.⁷⁸ Third, a recent meta-analysis suggested that metformin could effectively lower systolic blood pressure in non-diabetic patients;⁷⁹ possible mechanisms of blood pressure lowering by metformin include reduction of insulin resistance and plasma insulin, adrenergic receptor deactivation, reduction of intracytoplasmic calcium, inhibition of sympathetic drive especially in conditions of high dietary salt intake and increase of glomerular filtration rate and sodium excretion.⁸⁰ In addition, metformin can alleviate oxidative stress and inflammatory response as well as improve endothelial cell function.^{81,82}

The neuroprotective effect of metformin

Clinical studies concerning whether metformin could improve cognitive function and reduce the incidence of dementia in patients with T2DM are inconsistent. A Singapore Longitudinal Aging Study by Ng et al found that long-term treatment (>6 years) with metformin among T2DM patients was significantly associated with lowest risk of cognitive impairment in both cross-sectional analysis (OR 0.30, 95% CI 0.11–0.80) and in longitudinal analysis (OR 0.27, 95% CI 0.12–0.60).⁸³ Herath et al also showed that metformin has better protective effect on domain of verbal learning, working memory and executive function, compared to other diabetic treatments.⁸⁴ A small clinical trial by Guo et al found that the treatment with metformin for 24 weeks significantly improved cognitive performance and reduced depressive symptoms in T2DM patients with depression; the dose of metformin is shown in Table 1.⁸⁵ Similarly, significant improvements can be found in subjects without treated diabetes with mild cognitive impairment after 12 months of metformin treatment (1,000 mg twice a day, Table 1).⁸⁶ In addition, Cheng et al reported that T2DM patients with metformin have lower risk of dementia than those with other diabetes medications.¹¹ Furthermore, it is reported that compared with no metformin use, 1 year, 2 years, 2–4 years, and >4 years of metformin exposure among elderly veterans with diabetes increased 7% ($P=0.61$) and decreased 29% ($P=0.08$), 41% ($P=0.0026$) and 84% ($P<0.0001$) risk of neurodegenerative diseases (ND), including Alzheimer, Huntington, Parkinson and dementia among elder adults, which concluded that the long-term metformin treatment has protective effect on the incidence of ND (American Diabetes Association, 2016).¹¹⁴ However, some studies have different results. A case-control study from the United Kingdom found that long-term use of metformin was associated with a slightly higher risk of AD

(OR 1.71, 95% CI 1.12–2.60).⁸⁷ A recent Australian study suggested that T2DM patients treated with metformin had increased risk for impaired cognitive performance (OR 2.23, 95% CI 1.05–4.75), but metformin users who were taking vitamin B12 and calcium may have alleviated metformin-induced vitamin B12 deficiency and improved cognitive outcomes (OR 0.41, 95% CI 0.91–0.92).⁸⁸ Therefore, a larger trial seems warranted to evaluate the efficacy of metformin in neuroprotective effect.

Studies about the effect of metformin mostly focus on the A β production and tau level. Metformin may decrease tau phosphorylation and total tau level,^{89,90} but its effect on A β production is still inconsistent.^{91,92} Besides, it is reported that AMPK play an important role in various ND;⁹³ the activation of AMPK via an AMPK activator (metformin) may be neuroprotective, via the enhancement of angiogenesis, neurogenesis and induction of autophagy.^{93–96} Metformin can also prevent brain mitochondrial dysfunction, decrease oxidative stress, increase brain-derived neurotrophic factor levels, ameliorate cognitive impairment and improve neurological deficits.^{97–100}

About PCOS

PCOS is an endocrine and metabolic disorder found among women of reproductive age, which is characterized by hyperandrogenism, ovulatory dysfunction, altered LH/FSH ratio (>2/3:1), oligomenorrhea/amenorrhea and polycystic ovaries.^{101,102} Approximately 50%–70% of PCOS patients

suffer from insulin resistance and resulting hyperinsulinemia.^{103–105} Patients with PCOS are predisposed to many complications such as cardiovascular and cerebrovascular diseases, hypertension, metabolic syndrome and T2DM.^{106–109} Metformin has been used for PCOS treatment since 1994,¹¹⁰ by which most of the metabolic abnormalities of PCOS can be reversed.¹¹¹ Metformin dose ranged from 850 to 1,700 mg in different studies (Table 1). The mechanism is thought to be mediated through increased insulin sensitivity, increased ovarian secretion of estrogen, decreased ovarian production of androgen and augmentation of the production of sex hormone binding globulin.^{111,112} A recent meta-analysis by Tang et al demonstrated that metformin can reduce testosterone and insulin in PCOS women.¹¹³

Conclusion

Metformin is the most commonly prescribed therapy for patients with T2DM. It has a good safety profile and is associated with low cost. With further exploration of the clinical effect and possible mechanism of metformin, its indications have been extended to antitumor effect, antiaging effect, cardiovascular protective effects, neuroprotective effects and an optional treatment for PCOS; the linkage of these effects is shown in Figure 3. Furthermore, many questions such as whether these potential indications of metformin can be observed in non-diabetics and whether genetic factors have an influence on the effect of metformin need to be clarified by substantial basic experiments and clinical trials.

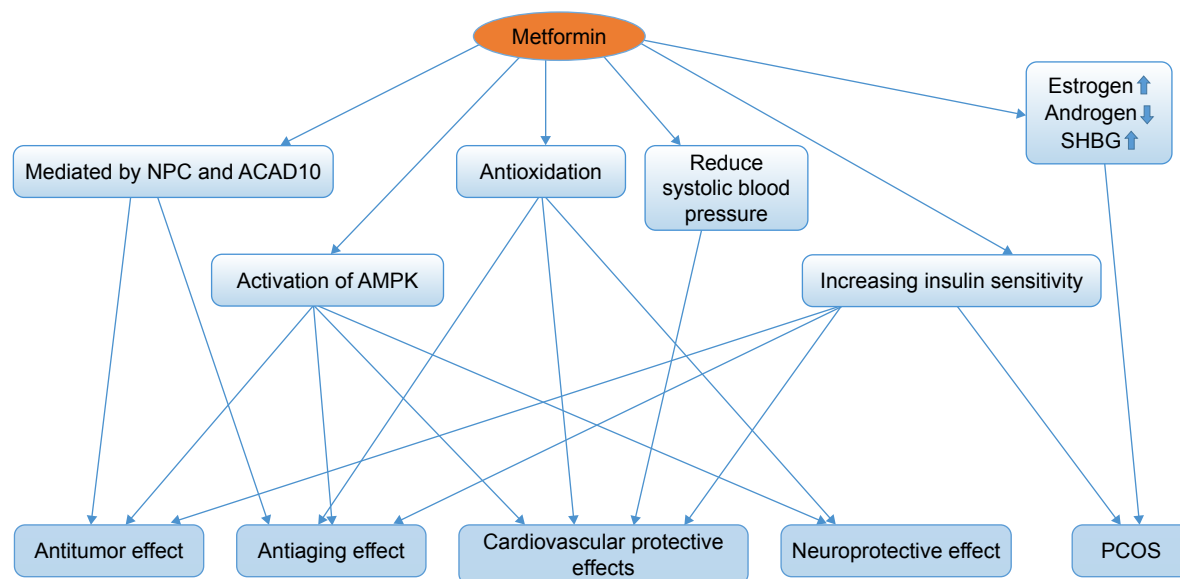


Figure 3 The linkage of the potential indications of metformin.

Abbreviations: NPC, nuclear pore complex; ACAD10, acyl-CoA dehydrogenase family member-10; AMPK, AMP-activated protein kinase; SHBG, sex hormone binding globulin; PCOS, polycystic ovary syndrome.

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Disclosure

The authors report no conflicts of interest in this work.

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