Front. Med. 2023, 17(3): 388−431
https://doi.org/10.1007/s11684-023-0998-6

REVIEW

The development and benefits of metformin in various diseases

Ying Dong[1,*], Yingbei Qi[1,5,*], Haowen Jiang[1,*], Tian Mi[1,*], Yunkai Zhang[1,2], Chang Peng[1,2], Wanchen Li[1,2], Yongmei Zhang[1,5], Yubo Zhou (✉)[1,7], Yi Zang (✉)[1,3,5], Jia Li (✉)[1,2,4,5,6]

[1]State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; [2]University of Chinese Academy of Sciences, Beijing 100049, China; [3]Lingang Laboratory, Shanghai 201203, China; [4]Open Studio for Druggability Research of Marine Natural Products, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao 266237, China; [5]School of Pharmaceutical Science and Technology, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, Hangzhou 310024, China; [6]Shandong Laboratory of Yantai Drug Discovery, Bohai Rim Advanced Research Institute for Drug Discovery, Yantai 264117, China; [7]Zhongshan Institute for Drug Discovery, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Zhongshan 528400, China

Abstract **Metformin has been used for the treatment of type II diabetes mellitus for decades due to its safety, low cost, and outstanding hypoglycemic effect clinically. The mechanisms underlying these benefits are complex and still not fully understood. Inhibition of mitochondrial respiratory-chain complex I is the most described downstream mechanism of metformin, leading to reduced ATP production and activation of AMP-activated protein kinase (AMPK). Meanwhile, many novel targets of metformin have been gradually discovered. In recent years, multiple pre-clinical and clinical studies are committed to extend the indications of metformin in addition to diabetes. Herein, we summarized the benefits of metformin in four types of diseases, including metabolic associated diseases, cancer, aging and age-related diseases, neurological disorders. We comprehensively discussed the pharmacokinetic properties and the mechanisms of action, treatment strategies, the clinical application, the potential risk of metformin in various diseases. This review provides a brief summary of the benefits and concerns of metformin, aiming to interest scientists to consider and explore the common and specific mechanisms and guiding for the further research. Although there have been countless studies of metformin, longitudinal research in each field is still much warranted.**

Keywords metformin; metabolism; cancer; aging; neurological disorder

Introduction

Metformin, a first line oral therapy, is the most widely prescribed clinical drug for the treatment of type 2 diabetes (T2D) [1]. Metformin inhibits glucose production in liver by increasing insulin sensitivity in the peripheral tissues, resulting in elevated glucose uptake and consumption by skeletal muscle and adipose tissues [2]. It is accepted that the central anti-diabetes mechanism of metformin is to inhibit the mitochondrial respiratory-chain complex I, resulting in the decrease of ATP and the increase of AMP. The transient decrease in cellular energy promotes the activation of AMPK, a cellular energy sensor and the target of metformin, to further impair the overall synthesis of glucose, lipid, and protein, but increases fatty acids usage [3,4].

Metformin originates from *Galega officinalis*, which has been served as a traditional medicine for treatment of worms, epilepsy, fever and pestilence in medieval Europe, and the first-known literature was published in *Culpeper's Complete Herbal* in the 18th century, around that time *G. officinalis* was described for reducing thirst and frequent urination [5–7]. In the 20th century, isolation and chemical analysis of the plant identified guanidine and related compounds, and guanidine was later reported to reduce blood glucose in rabbits and dogs, but initial optimism was overshadowed by disappointment because of toxicity profile in clinical use [5,8–11]. In the early 20th century, diguanide (such as synthalin A and synthalin B) and biguanides (such as phenformin, buformin and metformin) with less toxicity, lower blood glucose and more effective were synthesized for diabetes

Received December 23, 2022; accepted April 1, 2023

Correspondence: Yubo Zhou, ybzhou@simm.ac.cn;
Yi Zang, yzang@lglab.ac.cn;
Jia Li, jli@simm.ac.cn

*These authors contributed equally.

treatment [12–16]. The use of diguanides declined in the 1930s and had been forgotten for decades with their toxicity and the availability of insulin [17–21]. Despite that, there is always a way out, and renewed attention was paid to metformin for diabetes treatment because of accidental reports to lower blood glucose in malaria and influenza treatment. In the 1940s, a guanidine derivative, proguanil, was first introduced for malaria treatment, as well metformin was reported to be helpful for influenza outbreak in Philippines, thus interest was sparked for metformin to reduce blood glucose in some of the influenza patients [22,23]. Breakthrough for metformin occurred in the 1950s that Jean Sterne, a French physician-scientist, conducted the first human experiments of metformin and coined the compound "glucophage" (meaning glucose eater), igniting the further research and promoting the clinical translation of metformin. Sterne published his finding that is now widely known as a landmark paper of metformin for diabetes treatment [24,25]. Biguanides became the most prescribed glucose-lowering drugs worldwide. Although approved in the UK in 1958 and in Canada in 1972, metformin was initially of little clinical interest and subjected to doubts because phenformin and buformin, more effective biguanides in clinical use, were rediscovered, published and approved around the same time, but withdrawn from market in 1978 with inducing fatal lactic acidosis [26–29]. To prove the hypoglycemic effects and low toxicity of metformin, the famous UKPDS study (UK Diabetes Prospective Study), a prospective randomized trial of 5100 T2D patients, was conducted to note the long-term benefits of glucose-lowering and cardiovascular protection of metformin. The study was an epoch-making milestone in the history of diabetes treatment, started in 1977 and ended in 1997; median follow-up was 10 years, which brought metformin to the first line for type 2 diabetes treatment [30–33]. Thus, metformin was approved by FDA in 1994 and introduced in 1995 in the USA. In 2013, metformin was accepted by WHO Model List of Essential Medicines (Table 1). Besides hypoglycemic effects, metformin also has several other non-FDA-approved indications, including gestational diabetes, antipsychotic-induced weight gain, polycystic ovary syndrome (PCOS), Alzheimer's disease, and various types of cancer [34–39].

In this review, we present the pharmacokinetics and pharmacology of metformin, discuss the relation between metformin with systemic metabolism, cancer and the combination strategy, aging, and neurological disorders, to direct the potential clinical programs of metformin in the future.

Pharmacokinetics of metformin

Since its publication in 1957, metformin has become one of the most widely used oral drugs for treatment T2D in the world, and increasing attempts to use metformin for treatment of various diseases other than diabetes have been mentioned. It is necessary and meaningful to clarify the pharmacokinetics and pharmacology of metformin in humans and provide insights and ideas for further understanding of metformin. It is well known that metformin is transported across cellular membranes by numerous transporters because of its hydrophilic base chemically. Transporters play an important role in the absorption, distribution, and elimination of metformin, and serve as determinants for its bioavailability, clearance, and pharmacological effects. Here, we summarize the pharmacokinetics of metformin including its absorption, distribution, metabolism, and elimination.

Absorption of metformin

Metformin is slowly and incompletely absorbed. After an

Table 1 The history of metformin

Year	Comment	References
1772	*Galega officinalis* was used to reduce thirst and frequent urination, symptoms of diabetes	[7,40]
1844–1879	Identification of guanidine, synthesis of guanidine and biguanidie	[19,41,42]
1918	Guanidine reduced blood glucose in rabbits and dogs	[8]
1922	Synthesis of diguanide and biguanides	[16]
1926–1929	Diguanide and biguanides reduced blood glucose in animal	[12–14]
1957	Jean Sterne published on the use of metformin to treat diabetes	[24,29]
1957–1959	Phenformin and buformin were reported to treat diabetes	[18,20,26]
1958	Metformin was approved and introduced for treatment diabetes in UK	[25,26]
1994–1995	Metformin was approved and introduced for treatment diabetes in USA	[43,44]
1998	UKPDS reported the long-term benefits of glucose-lowering and cardiovascular protection of metformin	[31]
2002	Metformin was reported to reduce incidence of diabetes	[45]
2013	Metformin was included in the WHO Model List of Essential Medicines	[46]

oral administration of 500 mg or 850 mg metformin hydrochloride tablets (immediate-release formulation), the absolute oral bioavailability (F) is approximately 50% to 60% under fasting conditions while rapidly and largely absorbed from the small intestine [47,48]. Following a single oral administration of immediate-release formulation, peak plasma concentrations (C_{max}) occur approximately at 3 h, and C_{max} ranges from 1.0 to 1.6 mg/L after a 500 mg dose. After a single oral administration of metformin hydrochloride extended-release tablet (extended-release formulation), C_{max} is reached at 7 h, and the bioavailability is very similar to that of immediate-release formulation [47,49,50]. The C_{max} is about 30% higher for taking extended-release formulation 2000 mg once daily than for taking immediate-release formulation 1000 mg twice daily, while the C_{max} of extended-release formulation is lower than the same dose of immediate-release formulation, but the extent of absorption (as measured by plasma concentration-time curve, AUC) is similar for both formulations [51,52]. Under the fed condition, although the extent of absorption is slightly reduced and delayed, and the absorption is increased by about 50% compared to the fasting condition, there is no effect on C_{max} and T_{max} of metformin [49,53].

For the absorption of metformin, the plasma membrane monoamine transporter (PMAT, *SLC29A4*) expressed in the apical membranes of polarized enterocyte has an apparent affinity K_m for metformin of 1.32 mmol/L and acts as a major role in the intestinal absorption [54]. The other important transporters, organic cation transporter 1 (OCT1, *SLC22A1*) and organic cation transporter 3 (OCT3, *SLC22A3*) in the *SLC22* family, have apparent affinity K_m for metformin of 1.47 mmol/L and 1.10 mmol/L, respectively. OCT1 is expressed in the basolateral membrane of the enterocyte and hepatocytes and is assumed to play a major role in metformin transportation into the hepatocytes or the portal vein [55,56]. But the steady-state pharmacokinetics of metformin is independent of the OCT1 genotype in healthy volunteers [57]. OCT3 is expressed in the brush border membrane of enterocytes and is responsible for metformin absorption *in vivo* [58–61]. Carnitine/organic cation transporter (OCTN1, *SLC22A4*) localized to the apical membrane of enterocytes have also been identified to participate in the intestinal absorption of metformin [62]. Organic cation transporter 2 (OCT2, *SLC22A2*) with a K_m of 0.99 mmol/L for metformin is predominantly expressed in the basolateral membrane of renal tubules and facilitates to absorb metformin from circulation into renal epithelial cells [58,63]. The pharmacokinetics of metformin is significantly affected by genetic variants of OCT2-808 G>T and OCTN1-917C>T polymorphisms, and this may influence the clinical response to metformin therapy [64]. Other transporters such as serotonin reuptake transporter (SERT, *SLC6A4*), thiamine transporter 2 (THTR2, *SLC19A3*) may also contribute to the intestinal absorption of metformin [65,66]. In addition to active transport, passive transport has been recently reported to be responsible for metformin distribution dynamics between plasma and red blood cells in humans [67].

Distribution of metformin

Metformin is widely distributed into body tissues, including the intestine, liver and kidney, with negligible binding to plasma proteins [47,48,68]. The volume of distribution (V_d) after intravenous administration has been reported to range from 63 to 276 L [68,69], and the apparent volume of distribution (V_d/F) after oral administration of immediate-release formulation of 850 mg metformin averages at 654 ± 358 L, and the actual V_d after multiple doses of metformin is about 300 L [47]. Following a single oral administration, concentrations in the kidneys, adrenal glands, pancreas and liver are up to seven times higher than in blood, and concentrations in the lung, muscle and spleen are lower. In addition, the concentration of metformin in the liver is higher than that in the portal vein, as well as in general circulation and other organs [3,47]. The higher concentration in the kidney may be due to the higher concentration of metformin in the urinary tract rather than absorption in renal tissue [47]. Since the glucose-lowering effect of metformin is mainly to inhibit hepatic glucose output and the concentration of metformin in the liver is much higher than that in other organs, it is considered that the liver is the primary site of metformin function [70]. Steady-state plasma concentrations (C_{ss}) of metformin are usually reached within 1 to 2 days and are generally less than 1 μg/mL [47,52].

For the distribution of metformin, OCT3 is an important transporter expressed in various tissues including lung, prostate, skeletal muscle, and adipose tissue. Furthermore, OCT3 has also been detected in the blood-brain barrier, placenta, and salivary glands, and studies have shown that OCT3 is responsible for the accumulation and secretion of metformin [58,71]. In addition, multidrug and toxin extrusion transporter 1 (MATE1, *SLC47A1*), which is mainly expressed in the kidney and liver, may contribute to the transport and excretion of metformin [72].

Elimination or excretion of metformin

Metformin undergoes renal clearance rather than goes through hepatic metabolism, biliary excretion or gastrointestinal excretion in humans, with a half-life of 4.0 to 8.7 h [48,69]. Since renal clearance is approximately 4–5 times higher than glomerular filtration rate (GFR), tubular secretion is responsible for the

elimination of metformin [73,74]. Following oral administration, about 90% of the absorbed drug is excreted in the kidney within the first 24 h, while a population mean of renal clearance (CL_r) is 510 ± 130 mL/min and apparent total clearance (CL/F) is 1140 ± 330 mL/min [47,68,75]. Furthermore, metformin is little or undetectable in feces after intravenous administration, and 20%–30% of metformin may recover from feces after oral administration [76]. So, it is clinically recommended to take it with food to minimize gastrointestinal side effects of metformin, such as bloating, flatulence, and diarrhea. The rapid elimination of metformin via kidney was reported to be associated with a lower number of episodes of lactic acidosis than phenformin [77]. While another study also showed that most of the absorbed dose of metformin is excreted very quickly, a small fraction is removed much more slowly. Thus in patients with moderate renal impairment, creatinine clearance is not suggested as a reliable indicator of potential metformin accumulation [78].

For the elimination of metformin, transporters in the kidney, such as MATE1 (*SLC47A1*) and MATE2 (*SLC47A2*), facilitate the clearance of metformin. MATE1 with K_m of 0.23 mmol/L is localized in the bile canaliculus or kidney and is a carrier of metformin, thus possibly transporting metformin into bile or transporting metformin from renal tubule into urine, whereas MATE2 with K_m of 1.05 mmol/L is expressed in the apical membrane of the renal proximal tubule cells and plays a key role in renal excretion of metformin [47,75,79,80].

Conclusions

Metformin is eliminated intactly by renal excretion with high clearance rates. The maximum approved total daily dose of metformin for diabetes treatment is 2.5 g (35 mg/kg body weight). After treatment, the portal vein plasma concentration of animals was between 40 and 70 μmol/L, and the concentration would reduce to 10–40 μmol/L after hepatic uptake [80]. However, very high concentration of metformin *in vitro* (5 mmol/L) has been applied in multiple studies, which may lead to a non-specific effect of the drug or toxic effect. Therefore, given its pharmacokinetic characteristics, the concentration of metformin should be carefully used.

Metformin and systemic metabolism

Metformin has been the first-line drug treatment for type 2 diabetes for several decades. Besides its glucose-lowering effect, metformin also has the potential relevance on systemic metabolism, including regulating gut homeostasis, thermogenesis, appetite, and so on. However, the underlying mechanism of its action remains elusive. Some convincing data have documented that metformin-induced activation of the energy-sensor AMPK is its core mechanism [81,82], but may not account for all effects. Here, we summarize current knowledge for the action of metformin and its potential mechanisms (Fig.1).

Metformin and adipose tissue

White adipose tissue (WAT) and brown adipose tissue (BAT) compose the adipose organ that constitutes almost a quarter of body weight even in a lean state, playing pivot roles in energy homeostasis in mammals through multiple mechanisms [83,84]. Compared with other

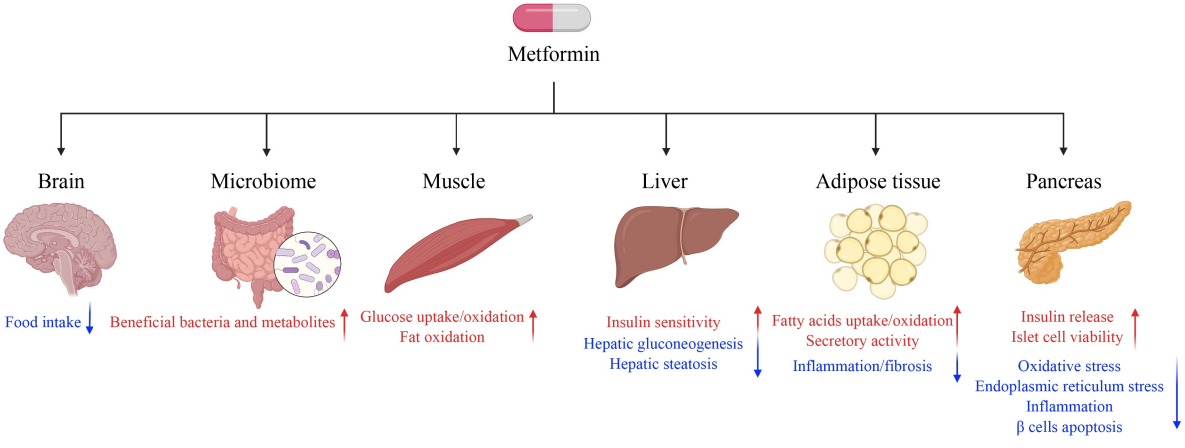

Fig. 1 The effects of metformin on metabolic regulation. Metformin exerts various regulatory effects in systemic metabolism beyond the hypoglycemic effect. Metformin improves the metabolism in the key metabolic organs including the liver, muscle, and adipose tissue. In the pancreas, metformin reduces adverse factors such as oxidative and endoplasmic stress, inflammation, and β cell apoptosis to increase the release of insulin to lower blood glucose. Moreover, even not the direct metabolic organ, the anorexia effect of brain and the changed constitution of the microbe, and the number of beneficial metabolites in the intestine also coordinate body metabolism.

metabolic organs such as the liver, kidney, and intestine, it is impossible that the relatively lower concentration of metformin in adipose tissue can exert the effect of reduction in body fat mass only through the glucose-lowering effect [85]. Metformin has a positive regulation of the lipid metabolism of adipose tissue. It was found that metformin increases the uptake and utilization of fatty acids in adipose tissue, which is related to the decrease of VLDL-TG and adipose tissue mass in HFD-feeding mice and patients [86,87]. Moreover, metformin can reduce visceral fat mass through increased oxidation of fatty acids by adaptive thermogenesis. After the treatment of metformin, the baseline respiratory quotient decreased and the postprandial respiratory quotient increased significantly in healthy subjects and patients with T2D [88], and metformin treatment reduces the content of visceral fat mass accompanied by the upregulation of UCP-1 in the brown adipose tissue [89]. Metformin can also prevent the loss of BAT caused by olanzapine treatment and gene expression analysis found a great change in the expression of multiple key genes controlling energy expenditure, which confirms that the regulation of energy expenditure is a primary effect of metformin [90]. Except for the regulation of the metabolism of adipose tissue, metformin also influences the balance of remodeling of adipose tissue. Metformin can induce macrophages into an anti-inflammatory phenotype and the reduction of inflammation in adipose tissue caused by metformin has been found to improve obesity-related metabolic disorders [91,92]. Adipose tissue fibrosis is another target of metformin. Extracellular matrix (ECM) remodeling, especially adipose tissue fibrosis induced by obesity, can lead to a dysfunctional process of adipose tissue homeostasis [93]. Metformin treatment can activate AMPK and suppress transforming growth factor-β1 (TGF-β1)/Smad3 signaling, therefore decreasing collagen deposition and the expression of fibrotic genes in adipose tissue to inhibit ECM deposition in obese mice, leading to improved systemic insulin sensitivity [94]. Except as the classical metabolic organ to maintain the balance of lipid dynamics, adipose tissue also can act as an endocrine organ to orchestrate metabolic homeostasis. Up to now, more than 100 adipokines are involved in the regulation of local or systematic tissue homeostasis [95]. Two kinds of miRNA secreted by adipose tissue may be the target of metformin. First, metformin can increase the insulin sensitivity of adipose tissue in patients with diabetes by reducing the expression of miR-223 and activating the IRS/Akt/GLUT4 signaling pathways [96]. Moreover, exposure to metformin can decrease the accumulation of intracellular lipid of adipose-derived stem cells that have the potency to differentiate to mature adipocytes by regulating the expression of miR-145 [97]. Collectively, as an active organ with complex properties, adipose tissue is an important target of metformin.

Metformin and microbiome

Although metformin is generally thought to mediate its antihyperglycemic effects by inhibiting hepatic glucose output by activating AMPK-dependent [98–100] and AMPK-independent pathways in the liver, there is increasing evidence that it may also act through the intestinal pathway. Recent evidence suggests that the gut microbiome is a site of action of metformin. Transfer of fecal samples from metformin-treated donors into germ-free mice showed improved glucose tolerance in mice receiving metformin-treated altered microbiota. When administered intravenously, metformin does not lower blood glucose [101]. In addition, a delayed-release formulation of metformin, which delivers the drug to the lower plasma-exposed intestinal segments, exerts the same glucose-lowering effect comparing to the standard immediate release formulation, providing further evidence for a gut-mediated mechanism of metformin [102]. Recent studies in rodents [103–105] and humans [106,107] have suggested that changes in the gut microbiome may contribute to the antidiabetic effects of metformin. In recent years, studies have reported that the clinical benefits of metformin are related to the composition and function of intestinal microbes [108]. The gut-mediated health effects of metformin may originate specifically from alterations in the gut microbiota. A seminal study by Cabreiro *et al*. [109] showed that metformin prolongs the lifespan of *Caenorhabditis elegans* (*C. elegans*) by altering the production of folate and methionine by *Escherichia coli*, providing evidence that metformin affects the composition of the gut microbiota. In a randomized trial, overweight/obese adults had significantly altered microbiota composition after metformin treatment, with the relative abundance of *E. coli* increasing in the metformin group, while that of *Intestinibacter bartlettii* decreased. Circulating SCFAs were altered by metformin. Metformin increases butyrate, acetate, and valerate, and the increase in acetate is associated with a decrease in fasting insulin. Whole-genome metagenomic sequencing revealed that metformin altered 62 metagenomic functional pathways, including one acetate production pathway and three glucose metabolism pathways. Bauer *et al*. identified glucose-sensing pathways in the upper small intestine of rodents that reduce glucose production. The high-fat diet altered the microbiota in the upper small intestine and impaired glucose sensing, whereas metformin treatment in the upper small intestine counteracted the microbiota changes and restored glucose sensing. The high-fat diet reduced glucose-SGLT1 sensing and metformin restored glucose-SGLT1 sensing, whereas increased metformin-treated microbial

transplantation restored glucose-SGLT1 sensing [110]. Metformin treatment in the upper small intestine restored SGLT1 expression and glucose sensing while partially altering the upper small intestinal microbiota by increasing *Lactobacillus* abundance. Transplantation of the upper small intestine microbiota from metformin-treated HFD rats into the upper small intestine of untreated HFD rats also increased *Lactobacillus* abundance and glucose sensing in the upper small intestine by upregulating SGLT1 expression. Thus, we demonstrate that metformin alters the upper small intestine microbiota and affects the glucose-SGLT1-sensing glucose regulation pathway. Some studies have identified a bacterial signaling pathway that integrates metformin and nutrition to alter the microbial metabolites, and the changes produced by the metabolites in turn affect the host fatty acid metabolism. How do gut microbes interact with metformin to affect the host? It has been reported that microbes integrate cues from metformin and diet to alter microbial metabolites through the transcriptional regulator CRP-mediated phosphot-ransferase signaling pathway. The microbiota of metformin-treated patients increased the capacity of agmatine production. Agmatine is a regulator of host lipid metabolism and lifespan, which in turn affects host fatty acid metabolism [111].

Metformin and pancreas

Though there is extensive research focusing on the pivotal roles of metformin in regulating insulin sensitivity and glucose homeostasis in insulin-targeted organs, the exact function of metformin in pancreatic β cells is still controversial. In 2005, a study showed that metformin can increase insulin secretion slightly, in addition to the known function of improvement of insulin sensitivity [112]. In brief, metformin exerts a beneficial influence on β cell function directly in many aspects such as insulin release, transcriptional regulation, and islet cell viability, and these effects are dependent on the presence of glucose [113]. After chronic exposure to free fatty acids or high glucose, pancreatic β cell show decreased insulin secretion which can be restored by metformin treatment. However, the roles of metformin are not observed in normal conditions. Furthermore, this phenomenon can be observed in both human islets and cell lines accompanied with reduced oxidative stress [114,115]. It is known that glucotoxicity-induced oxidative and endoplasmic reticulum (ER) stress are essential in the development of β cell dysfunction. Human islets cultured with high glucose revealed reduced glucose-stimulated insulin secretion. However, these effects can be reversed with metformin treatment via inhibiting the activity of mitochondrial complex I [114]. Research shows that hyperglycemia induces oxidative stress and inflammation which eventually lead to impaired insulin secretion and increased apoptosis in β cells, which can be improved by the treatment of metformin. Human islet cells in culture with palmitate for seven days show reduced expression of p-AMPK and a significantly increased expression of phosphorylated eukaryotic initiation factor-2 (p-EIF2α), C/EBP homologous protein, and cleaved caspase 3, but their expressions were back to a normal state in the presence of metformin at a dose of 25 μmol/L [116]. This suggests that metformin can promote pancreatic β cell function following chronic fatty acid exposure. Also, metformin is able to protect against glucotoxicity-induced reactive oxygen species production and inhibits the cluster determinant 36-mediated free fatty acid influx in pancreatic β cells [117]. In the aspect of inflammation, researchers observed that the pancreatic mRNA expressions of inflammation factors including TLR4, NF-κB, JNK, IL-6, TNF-α along with p-NF-κB p65 protein levels in diabetic KKAy mice were downregulated by metformin, while expressions of genes participating in the insulin secretion including SERCA2 and Kir6.2 are upregulated. These observations indicate that metformin alleviates pancreatic inflammation, and elevates β cell function in diabetic KKAy mice [118]. On the other hand, metformin was also reported to play roles in pancreatic β cell survival under different conditions. Under normal growth condition, metformin suppresses MIN6 β cell proliferation and promotes apoptosis via an AMPK-dependent and autophagy-mediated mechanism. While metformin protects MIN6 and INS-1 cells against palmitic acid (PA)-induced apoptosis, suggesting a dual role of metformin in regulating MIN6 pancreatic β cell survival [119]. Under glucolipotoxicity conditions, low dose metformin was reported to prevent Ca^{2+}-induced PTP opening in permeabilized and intact INS-1 cells to preserve β cell viability [120]. And metformin can inhibit the ER stress-induced apoptosis in NIT-1 cells or pancreatic islets, via the regulation of AMPK-PI3 kinase-JNK pathway in lipotoxicity [121]. In conclusion, metformin exerts beneficial effects on β cell functions such as increased insulin secretion and β cell viability depending on the presence of glucose. Although accumulated evidence shed light on metformin activities, the precise mechanisms of metformin in pancreatic β cells remain unclear, needing further investigations in the future.

Metformin and anorexia effect

The contribution of metformin administration on weight loss is attributed to food intake rather than energy consumption, according to the number of results both in human and experimental animals [122–124]. Although recent studies attempted to assess the role of metformin in weight loss induced by food intake reduction, the precise

mechanism of its anorexigenic effect has not been elucidated. There are proven studies that metformin acts directly on the central nervous system and thus leads to the suppression of appetite. The increasing neuropeptide Y (NPY, a known orexigenic neuropeptide) gene expression resulted from the phosphorylation of AMPK was detected in low-glucose medium cultured primary rat hypothalamic neurons, which was blocked by the addition of metformin [125]. It is implied that metformin inhibits AMPK activity and then regulates the expression of the orexigenic peptide NPY. Other research also demonstrates that metformin inhibits the increased food intake caused by acute ghrelin application [126]. Also, metformin administration restored the impaired response to leptin in HFD-induced obese mice, which suggests that metformin could improve leptin sensitivity [127]. Moreover, in an acute intracerebroventricular leptin injection study, metformin has been identified to enhance the anorexigenic effect mediated by leptin, accompanied by increased phosphorylation of stat3 [128]. In addition to direct action on the neural center, metformin may orchestrate the secretion of some endocrine factor communicating with the central nervous system indirectly. Growth differentiation factor 15 (GDF15), a member of the TGF-β superfamily, has been classified as responsible for the weight loss of metformin. GDF15 binds to the GDNF family receptor α-like (GFRAL) in the hindbrain, thus inhibiting food intake [129–131]. Coll *et al*. present that the circulating GDF15 level significantly increases upon metformin administration in a short-term human study and a series of animal experiments, and the lowering of weight is dependent on the GDF15-GFRAL pathway which has no impact on the hypoglycemic and insulin-sensitizing effects [132]. In addition, they found that GDF15 induced by metformin was predominantly expressed in the small intestine and colon. Hence, its further mechanism remains to be determined. Unexpectedly, Klein *et al*. definitely verify the causal correlation between circulating GDF15 and metformin, while there was no difference in the weight loss and food intake between GDF15 or GFRAL-knockout mice and their littermates, suggesting GDF15 dispensable for the effects of metformin [133]. In fact, it is still controversial whether the weight loss induced by metformin depends on the GDF15-GFRAL pathway. Therefore, whether GDF15 contributes to the anorexigenic effect of metformin requires additional studies. Moreover, Day *et al*. conduct experiments in primary mouse hepatocytes and indicate that the expression of activating transcription factor 4 (ATF4) and C/EBP homologous protein (CHOP; also known as DDIT3) might play an important role in the secretion of GDF15 caused by metformin [134]. Taken together, the molecular mechanism underlying metformin-induced weight loss has remained unclear, making it a big hurdle for the clinical application of metformin. It is essential to illustrate the identified targets or metabolic pathways, which perhaps provide promising strategies for obesity.

Metformin and muscle

Metformin is recommended as the most prescribed antidiabetic in the world taking its safety and efficacy into account, which has been considered the first-line drug of type 2 diabetes mellitus [135]. As a metabolically highly active tissue, skeletal muscle whose metabolic disturbances are associated with metabolism syndrome plays an important role in blood glucose regulation, which uses both circulating plasma glucose as well as stored glycogen as fuel sources, and insulin action, dysregulation of lipid metabolism, reduced mitochondrial oxidation. Metformin is suggested to have an effect on skeletal muscle energy metabolism and is beneficial to glucose homeostasis. Studies have shown that metformin can stimulate skeletal muscle glucose uptake and oxidation [136], and can lower intramuscular triglyceride content and bioactive acyl-chain bioactive lipids by partially increasing fat oxidation [137,138]. On the one hand, metformin can induce the transport of glucose transporter 4 to the cell plasma membrane, thereby increasing glucose uptake and improving insulin resistance [139]. Moreover, skeletal muscle is responsible for 70%–80% of whole-body insulin-stimulated glucose uptake and plays an important role in lipid metabolism, where insulin resistance results from bioactive lipid accumulation such as intramuscular long-chain acyl-CoA (LCACoA), diacylglycerols (DAG), and ceramide (Cer) [138]. And AMPK also can be activated by metformin in skeletal muscle, which can promote increased fatty acid oxidation because of the activation of acetyl-CoA carboxylase 2 and the reduction of malonyl-CoA [140]. Additionally, metformin also can enhance mitochondrial biogenesis and mitochondrial oxidase activity in skeletal muscle by activating PGC-1α through AMPK phosphorylation. In summary, these results illustrate that metformin is of importance in skeletal muscle energy metabolism which will improve glucose homeostasis, lipid metabolism, and insulin resistance.

Metformin and liver

Metformin exerts its glucose-lowering effect primarily by decreasing hepatic glucose production through suppression of gluconeogenesis and enhancing insulin suppression of endogenous glucose production [141]. The role of AMPK in mediating the action of metformin in primary hepatocytes was initially supported by Zhou and colleagues [142]. It was reported that ablation of liver kinase B1 (LKB1) in the liver prevented the antihyperglycemic effects of metformin in high-fat-fed

mice [99]. AMPK activation by metformin has also been reported to be involved in the transcriptional regulation of hepatic gluconeogenic enzyme genes by different mechanisms: (1) dissociation of the CREB-CBP (CREB binding protein)-TORC2 transcription complex, through the phosphorylation of the transcriptional coactivator CBP via atypical protein kinase C i/l [143], (2) increased expression of the orphan nuclear receptor small heterodimer partner [144], and (3) induction of SIRT1-mediated CRTC2 deacetylation [145]. In addition, it was reported that the reduction in hepatic gluconeogenesis by metformin might result from direct inhibition of the mitochondrial glycerophosphate dehydrogenase (mGPD), identifying another putative mitochondrial target of the drug [146]. Another effect of metformin is to improve lipid metabolism by reducing hepatic steatosis as demonstrated in rodent liver [147,148]. Fullerton and colleagues recently showed that metformin-induced improvements in insulin action operate through alterations in hepatic lipid homeostasis via the inhibitory phosphorylation of acetyl CoA carboxylase (ACC) by AMPK, the role of AMPK in the mechanisms of metformin action on lipid metabolism was provided in knock-in mouse models in which ACC1 and ACC2 were rendered insensitive to AMPK phosphorylation [100]. ACC is a rate-determining enzyme for the synthesis of malonyl-CoA, both a critical substrate for fatty acid biosynthesis and a potent inhibitor of fatty acid oxidation. It is generally accepted that the actions of metformin on mitochondria underlie most of the pleiotropic effects of the drug. Two seminal papers published in 2000 reported that metformin decreases cellular respiration by a mild and specific inhibition of the respiratory-chain complex I (NADH: ubiquinone oxidoreductase) [149,150]. The specific inhibition of the mitochondrial respiratory-chain complex I by metformin was confirmed in many cellular models, including rat, mouse, and human primary hepatocytes [149–151]. It was shown that the inhibitory effect of metformin on complex I was not prevented by nitric oxide (NO) synthase inhibitors or reactive oxygen species (ROS) scavengers [149] and was independent of AMPK, at least in primary mouse hepatocytes [151]. To investigate the mechanisms by which metformin affects the cellular energy state, together with the putative involvement of AMPK in this process, they used hepatocytes from wild-type and liver-specific AMPKα1/2$^{-/-}$ mice for permeabilisation of the plasma membrane by digitonin, allowing the mitochondrial OXPHOS pathway to be investigated *in situ* [151]. It is also worth mentioning that the inhibition of complex I activity by metformin is rather mild when compared to the reference inhibitor rotenone [149]. Furthermore, metformin was shown to significantly reduce mitochondrial ROS production by selective inhibition of the reverse electron flow through the respiratory-chain complex I, whereas rotenone triggers ROS production by increasing forward electron flow [152]. However, it is still unclear concerning the mechanism by which metformin inhibits complex I despite its use for 60 years. Some researchers proposed a direct effect of metformin on complex I involving an accumulation of metformin inside the mitochondria while others proposed an indirect effect (the drug no longer having to diffuse into the mitochondria), which is in urgent clarification [153].

Summary and future perspectives

In recent decades, the lack of effective treatment contradicts the rapidly increasing incident rate of metabolic diseases for the reason of the complexity of pathogenesis. The heterogeneity of different metabolic organs makes it a great challenge to find a key common target to improve metabolism-related disorders. However, metformin brings the hope that various metabolic diseases may be improved only by one compound. It is a miracle that metformin exerts its beneficial effects in almost all the metabolic organs including direct metabolic organs, the liver, adipose tissue, and muscle, and indirect regulatory organs, the pancreas, and the brain. This extensive therapeutic effect of metformin may attribute to its simple carbon structure, which makes metformin bind with various proteins to involve in the regulation of key signal transduction or functions. Up to now, metformin is expected to become the panacea in improving metabolic diseases, even though its potential target is still under exploration.

Metformin and cancer

Metformin is a well-tolerated antidiabetic drug, with potential as an anticancer agent [154,155]. It has been reported to reduce the risk of cancer in diabetic patients [156,157]. In 2005, Evans *et al*. suggested that taking metformin may be associated with reduced risk of cancer in patients with type 2 diabetes applied in a cohort study and the potential link with the tumor suppressor role of LKB1, which enhanced great interest in metformin as an anti-cancer drug since then [156]. Metformin possesses beneficial clinical efficacy, including synergistically inhibiting cancer cell or cancer stem cell growth, reducing recurrence, cardiotoxicity, and mortality, as well as increasing the efficacy of other agents in various types of cancer models [156,158,159]. It is used in monotherapy or in combination with other various chemotherapeutic agents [160–162].

The anti-tumor effect of metformin probably attributes to a combination of indirect and direct mechanisms (Fig. 2). Metformin may indirectly contribute to anti-tumor effects by lowering the systemic glucose and

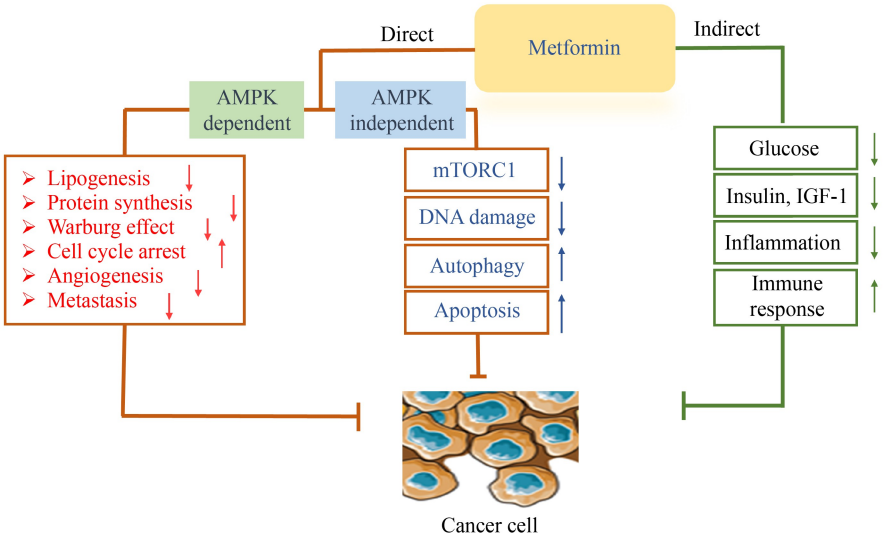

Fig. 2 Potential molecular mechanisms of metformin in cancer. The anti-tumor effect of metformin is probably a combination of indirect and direct effects. In indirect action, metformin lowers systemic glucose and insulin levels, and may reduce cancer risk through anti-inflammatory effects and promoting immune response to tumor cells. In the other direct context, AMPK-dependent and AMPK-independent mechanisms have been described, which are likely to coexist and interact together to suppress tumor development.

insulin levels, and reducing cancer risk [80,163,164]. In the other direct context, AMPK-dependent and AMPK-independent mechanisms have been described previously, which are likely to coexist and interact together to suppress tumor development [154]. LKB1, a tumor suppressor protein, serves as an activator of AMPK in response to cellular stresses and previous reports have demonstrated that LKB1 is essential for the inhibition of cancer cell proliferation [165]. Activation of AMPK can affect multiple signaling in cells, such as mTOR, p53 and NF-κB pathway, to regulate cell proliferation and survival. mTOR, the mammalian target of rapamycin, plays a vital role in tumor proliferation and inhibition of cell death [166,167]. Metformin activates AMPK via activation of LKB1, subsequently inhibits mTOR activity resulting in inhibition of protein synthesis, induction of cell cycle arrest, apoptosis, or autophagy [168–172]. p53, a critical tumor suppressor, regulates cell growth, survival, and development. AMPK activated by metformin induces p53 activity leading to cell cycle arrest and autophagy [173,174]. NF-κB, the nuclear factor kappa B, is a protein complex and is involved in cell migration and proliferation. Metformin inhibits NF-κB pathway dependently on AMPK, reforming chemotherapy resistance and inducing cancer cell pyroptosis, metastasis, and invasion [175–177]. Our team recently found that metformin activates AMPK-PHF2 axis to downregulate H3K9me2 and inhibits lung cancer metastasis [178]. Furthermore, studies also reported that metformin inhibits activation of NF-κB via AMPK activation to inhibit release of cytokines and exert anti-inflammatory effects [179–181]. Among these, LKB1-AMPK-mTOR signaling pathway may be one of the main mechanisms of metformin to exert anticancer effects [169,182]. In addition, metformin also inhibits the occurrence and development of tumor cells through AMPK mediated Warburg effect, fatty acid synthesis, cell cycle regulation, and its regulatory role in tumor metastasis and angiogenesis [183–185]. In AMPK independent manner, metformin, acts as an insulin-sensitizing agent, inhibits insulin and insulin-like growth factor 1 (IGF-1), key regulators of metabolism and growth, to control tumor growth [186–189]. Decreased insulin after administration of metformin also inhibits phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT) pathway, leading to inhibit mTOR [190–192]. And, metformin inhibits mTOR independently on AMPK via activation of REDD1 to exert anticancer effects [193,194]. Besides, mTORC1 activated in the amino acid metabolic signaling pathway could also be directly inhibited by metformin, thereby controlling the energy metabolism of tumor cells [195]. And, metformin directly interferes NF-κB signaling pathway, inducing cancer cell pyroptosis, metastasis, and invasion [196]. In addition, metformin inhibits mitochondrial complex I and reduce the ATP/AMP ratio leading to activation of AMPK and reduce tumorigenesis [197]. Therefore, based on the complex regulatory network of metformin, it is important to further study the anticancer mechanism of metformin.

Though metformin with the favorable safety profile exerts the anti-tumor effects, the drug administrated alone may not achieve the desired results in certain scenes; meanwhile, pleiotropic effects of metformin on cancers also make it a unique adjuvant to combination with other effective anti-cancer drugs [159,161,198–203]. Instead, most of the preclinical and clinical data are exploring and

supporting its role as an adjuvant drug in the treatment of cancer (Tables 2–4), although the action mechanisms are not fully elucidated. Herein, we focus on the combination strategy of metformin with chemotherapy, targeted molecular drugs, and immunotherapy to promote clinical application of metformin in cancer therapy.

Table 2 Clinical trials on metformin in combination with chemotherapeutic agents in cancer (data from ClinicalTrails.gov)

Title (NCT No.)	Phases	Tumor type	Drug	Status
The Effect of Metformin on Breast Cancer Patients (NCT04559308)	Phase 2	Breast cancer	Metformin, doxorubicin, cyclophosphamide, paclitaxel	Unknown
Evaluation of the Effect of Metformin on Metastatic Breast Cancer as Adjuvant Treatment (NCT04143282)	Phase 2	Breast cancer	Metformin, chemotherapy	Completed
Advanced Lung Cancer Treatment With Metformin and Chemo-Radiotherapy (NCT02115464)	Phase 2	Lung cancer	Metformin, cisplatin	Terminated
Role of Adding Metformin to Neoadjuvant Chemotherapy in Patients With Breast Cancer (NCT04170465)	Phase 2	Breast cancer	Metformin, doxorubicin, cyclophosphamide, paclitaxel	Unknown
Metformin-Docetaxel Association in Metastatic Hormone-refractory Prostate Cancer (NCT01796028)	Phase 2	Prostate cancer	Metformin, docetaxel	Completed
Study of Metformin With Carboplatin/Paclitaxel Chemotherapy in Patients With Advanced Ovarian Cancer (NCT02312661)	Phase 1	Epithelial ovarian cancer	Metformin, carboplatin, paclitaxel	Completed
Metformin Combined With Chemotherapy for Pancreatic Cancer (NCT01210911)	Phase 2	Pancreatic cancer	Metformin, gemcitabine, erlotinib	Completed
Safety and Efficacy of Metronomic Cyclophosphamide, Metformin and Olaparib in Endometrial Cancer Patients (NCT02755844)	Phases 1 and 2	Endometrial cancer	Metformin, olaparib, cyclophosphamide	Completed
Combination Chemotherapy With or Without Metformin Hydrochloride in Treating Patients With Metastatic Pancreatic Cancer (NCT01167738)	Phase 2	Pancreatic cancer	Metformin, capecitabine, csplatin, epirubicin, gemcitabine	Terminated
Neoadjuvant FDC With Melatonin or Metformin for Locally Advanced Breast Cancer (NCT02506777)	Phase 2	Breast cancer	Metformin, fluoruracil, doxorubicin, cyclophosphamide, melatonin	Unknown
Metformin Plus Irinotecan for Refractory Colorectal Cancer (NCT01930864)	Phase 2	Colorectal cancer	Metformin, irinotecan	Unknown
Metformin in Children With Relapsed or Refractory Solid Tumors (NCT01528046)	Phase 1	Solid tumors	Metformin, vincristine, irinotecan, temozolomide	Active, not recruiting
Metformin Plus Paclitaxel for Metastatic or Recurrent Head and Neck Cancer (NCT01333852)	Phase 2	Head and neck neoplasms	Metformin, paclitaxel	Terminated
Metformin Plus Modified FOLFOX 6 in Metastatic Pancreatic Cancer (NCT01666730)	Phase 2	Metastatic pancreatic cancer	Metformin, oxaliplatin, leucovorin, fuorouracil	Completed
Metformin Combined With Gemcitabine as Adjuvant Therapy for Pancreatic Cancer After Curative Resection (NCT02005419)	Phase 2	Pancreatic cancer	Metformin, gemcitabine	Completed
Paclitaxel and Carboplatin With or Without Metformin Hydrochloride in Treating Patients With Stage III, IV, or Recurrent Endometrial Cancer (NCT02065687)	Phases 2 and 3	Endometrial cancer	Metformin, carboplatin, paclitaxel	Active, not recruiting
Myocet + Cyclophosphamide + Metformin Vs Myocet + Cyclophosphamide in 1st Line Treatment of HER2 Neg. Metastatic Breast Cancer Patients (NCT01885013)	Phase 2	Breast cancer	Metformin, myocet, cyclophosphamide	Completed
Dose-finding Study of Metformin With Chemoradiation in Locally Advanced Head and Neck Squamous Cell Carcinoma (NCT02325401)	Phase 1	Head and neck squamous cell carcinoma	Metformin, cisplatin	Completed
NeoMET Study in Neoadjuvant Treatment of Breast Cancer (NCT01929811)	Phase 2	Breast cancer	Metformin, docetaxel, epirubicin, cyclophosphomide	Terminated
Chemotherapy and Radiation Therapy With or Without Metformin Hydrochloride in Treating Patients With Stage III Non-small Cell Lung Cancer (NCT02186847)	Phase 2	Non small cell lung cancer	Metformin, carboplatin, paclitaxel	Active, not recruiting
Treatment of Patients With Advanced Pancreatic Cancer After Gemcitabine Failure (NCT01971034)	Phase 2	Pancreatic cancer	Metformin, paclitaxel	Completed
Study of Paclitaxel, Carboplatin and Oral Metformin in the Treatment of Advanced Stage Ovarian Carcinoma (NCT02437812)	Phase 2	Ovarian carcinoma	Metformin, paclitaxel, carboplatin	Unknown
Oxidative Phosphorylation Targeting In Malignant Glioma Using Metformin Plus Radiotherapy Temozolomide (NCT04945148)	Phase 2	Glioblastoma	Metformin, temozolomide	Not yet recruiting
Metformin in Combined With Cisplatin Plus Paclitaxel With Advanced Esophageal Squamous Cell Carcinoma (NCT03833466)	Phase 2	Esophageal squamous cell carcinoma	Metformin, paclitaxel, cisplatin	Unknown

(*Continued*)

Title (NCT No.)	Phases	Tumor type	Drug	Status
Study on Low Dose Temozolomide Plus Metformin or Placebo in Patient With Recurrent or Refractory Glioblastoma (NCT03243851)	Phase 2	Glioblastoma	Metformin, temozolomide	Completed
Comparison of Melatonin or Metformin and Dacarbazine Combination Versus Dacarbazine Alone in Disseminated Melanoma (NCT02190838)	Phase 2	Melanoma	Metformin, dacarbazine, melatonin	Terminated
Vincristine, Dexamethasone, Doxorubicin, and PEG-asparaginase (VPLD) and Metformin for Relapsed Childhood Acute Lymphoblastic Leukemia (ALL) (NCT01324180)	Phase 1	Acute lymphoblastic leukemia	Metformin, Vincristine, dexamethasone, asparaginase, doxorubicin	Completed
Temozolomide, Memantine Hydrochloride, Mefloquine, and Metformin Hydrochloride in Treating Patients With Glioblastoma Multiforme After Radiation Therapy (NCT01430351)	Phase 1	Glioblastoma	Metformin, temozolomide	Active, not recruiting
Metformin + Cytarabine for the Treatment of Relapsed/Refractory AML (NCT01849276)	Phase 1	Acute myeloid leukemia	Metformin, cytarabine	Terminated

Table 3 Clinical trials on metformin in combination with molecular target drugs in cancer (data from ClinicalTrails.gov)

Title (NCT No.)	Phases	Tumor type	Drug	Status
Study of Erlotinib and Metformin in Triple Negative Breast Cancer (NCT01650506)	Phase 1	Breast cancer	Metformin, erlotinib	Completed
Randomized Trial of Neo-adjuvant Chemotherapy With or Without Metformin for HER2 Positive Operable Breast Cancer (NCT03238495)	Phase 2	Breast cancer	Metformin, taxotere, carboplatin, herceptin, pertuzumab	Recruiting
Temsirolimus in Combination With Metformin in Patients With Advanced Cancers (NCT01529593)	Phase 1	Advanced cancers	Metformin, temsirolimus	Active, not recruiting
Study of Safety and Efficacy of Dapagliflozin + Metformin XR Versus Metformin XR in Participants With HR + , HER2-, Advanced Breast Cancer While on Treatment With Alpelisib and Fulvestrant (NCT04899349)	Phase 2	Breast cancer	Metformin, alpelisib, fulvestrant	Recruiting
A Study of Liposomal Doxorubicin + Docetaxel + Trastuzumab + Metformin in Operable and Locally Advanced HER2 Positive Breast Cancer (NCT02488564)	Phase 2	Breast cancer	Metformin, doxorubicin, docetaxel, trastuzumab	Completed
Modulation of Response to Hormonal Therapy With Lapatinib and/or Metformin in Patients With Metastatic Breast Cancer (NCT01477060)	Phase 2	Breast cancer	Metformin, lapatinib	Terminated
Lapatinib With Sirolimus or Metformin (NCT01087983)	Phase 1	Advanced cancers	Metformin, lapatinib	Completed
Study to Evaluate the Effect of Metformin in the Prevention of HG in HR[+]/HER2[–] PIK3CA-mut Advanced BC Patients (NCT04300790)	Phase 2	Breast cancer	Metformin, alpelisib, fulvestrant, letrozole, exemestane	Active, not recruiting
A Pharmacokinetic Interaction Study Between Apatinib and Rosuvastatin or Metformin in Solid Tumor Subjects (NCT04428086)	Phase 1	Solid tumor	Metformin, apatinib	Completed
An Efficacy and Safety Study of Erdafitinib (JNJ-42756493) in Participants With Urothelial Cancer (NCT02365597)	Phase 2	Urothelial cancer	Metformin, erdafitinib	Recruiting
Combination of Metformin With Gefitinib to Treat NSCLC (NCT01864681)	Phase 2	Non small cell lung cancer	Metformin, gefitinib	Completed
Metformin And Chloroquine in IDH1/2-mutated Solid Tumors (NCT02496741)	Phases 1 and 2	IDH1/2-mutated solid tumor	Metformin, chloroquine	Completed
Metformin, Nelfinavir, and Bortezomib in Treating Patients With Relapsed and/or Refractory Multiple Myeloma (NCT03829020)	Phase 1	Multiple myeloma	Metformin, bortezomib, nelfinavir	Recruiting
A Phase I/II Trial of Vemurafenib and Metforminto Melanoma Patients (NCT01638676)	Phases 1 and 2	Melanoma	Metformin, vemurafenib	Recruiting
Study of Dabrafenib, Trametinib and Metformin for Melanoma Patients (NCT02143050)	Phases 1 and 2	Melanoma	Metformin, dabrafenib, trametinib	Recruiting
Study of Metformin Plus Paclitaxel/Carboplatin/Bevacizumab in Patients With Adenocarcinoma (NCT01578551)	Phase 2	Lung adenocarcinoma	Metformin, paclitaxel, carboplatin, bevacizumab	Terminated
Paxalisib With a High Fat, Low Carb Diet and Metformin for Glioblastoma (NCT05183204)	Phase 2	Glioblastoma	Metformin, paxalisib	Not yet recruiting
Metformin in Combination With Standard Induction Therapy for Large B cell Lymphoma (NCT02531308)	Phase 2	Diffuse large B cell lymphoma	Metformin, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone, pegfilgrastim	Terminated

Table 4 Clinical trials on metformin in combination with immunotherapy in cancer (data from ClinicalTrails.gov)

Title (NCT No.)	Phases	Tumor type	Drug	Status
Sintilimab Combined With Metformin in First-Line Chemotherapy Refractory Advanced NSCLC Patients (NCT03874000)	Phase 2	Non small cell lung cancer	Metformin, sintilimab	Unknown
Nivolumab and Metformin Hydrochloride in Treating Patients With Stage III-IV Non-small Cell Lung Cancer That Cannot Be Removed by Surgery (NCT03048500)	Phase 2	Non small cell lung cancer	Metformin, nivolumab	Unknown
Nivolumab and Metformin in Patients With Treatment Refractory MSS Colorectal Cancer (NCT03800602)	Phase 2	Colorectal cancer	Metformin, nivolumab	Active, not recruiting
Assessing Safety and Efficacy of Sintilimab and Metformin Combination Therapy in SCLC (NCT03994744)	Phase 2	Small cell lung cancer	Metformin, sintilimab	Unknown
Combining Pembrolizumab and Metformin in Metastatic Head and Neck Cancer Patients (NCT04414540)	Phase 2	Head and neck squamous cell carcinoma	Metformin, pembrolizumab	Recruiting
Anti-PD-1 mAb Plus Metabolic Modulator in Solid Tumor Malignancies (NCT04114136)	Phase 2	Solid tumor	Metformin, nivolumab, pembrolizumab	Recruiting
A Trial of Pembrolizumab and Metformin Versus Pembrolizumab Alone in Advanced Melanoma (NCT03311308)	Phase 1	Melanoma	Metformin, pembrolizumab	Recruiting
Durvalumab With or Without Metformin in Treating Participants With Head and Neck Squamous Cell Carcinoma (NCT03618654)	Phase 1	Head and neck squamous cell carcinoma	Metformin, durvalumab	Active, not recruiting
Metformin Plus Sorafenib for Advanced HCC (NCT02672488)	Phase 2	Hepatocellular carcinoma	Metformin, sorafenib	Unknown

Combination of metformin with chemotherapeutic agents

In combination with antimetabolite drugs

Metabolic reprogramming in cancer cell is described as deregulating cellular metabolism and it is considered one of "hallmarks of cancer" [204]. It is involved in tumor development and proliferation. Cancer cells survive in a state of aerobic glycolysis which produces ATP for emergency, intermediate metabolites for biosynthesis, low pH, and hypoxic microenvironment. Antimetabolite drugs, also known as nucleoside analogs, have been used as primary and effective cancer treatment with the effect to disturb the biosynthesis of DNA and block cancer cell division and proliferation [205]. Antimetabolite agents are widely conducted in a clinical setting with poor pharmacokinetics and common side effects such as poor bioavailability, low penetration in blood-brain barrier, bone marrow suppression, and gastrointestinal toxicity.

Methotrexate

Methotrexate (MTX), a folate analog that interrupts one-carbon transfer reactions and inhibits *de novo* nucleotide synthesis, has been used as a traditional chemotherapeutic agent for treatment of many cancers [206–208]. Unfortunately, hepatorenal toxicity is the underlying adverse effect and has been reported in many cases of high doses or long-term treatments of MTX [209–212]. Previous studies reported that metformin shows a protective effect in MTX-induced hepatorenal toxicity due to its anti-oxidant, anti-inflammatory, and anti-apoptotic properties in rats [212]. Subsequently, combining metformin with MTX has shown to decrease nucleotide metabolism, inhibit cell proliferation, increase sensitivity, and overcome resistance of hepatocarcinoma cell to MTX by transcriptionally inhibiting dihydrofolate reductase (DHFR) [213].

5-Fluorouracil (5-FU)

5-FU is an analog of uracil, that can be converted to 5F-dUMP and 5F-UTP to affect biosynthesis of DNA or disturb the biosynthesis of proteins [214]. It is widely used to treat a range of cancers in clinical settings. Metformin combined with 5-FU synergistically and significantly increases chemosensitivity, inhibits cell proliferation, induces cell cycle arrest and apoptosis in cancer cells both *in vitro* and *in vivo*. Present studies showed that the mechanisms of metformin in combination with 5-FU are complex and unclear in different cancer types. The relevant signaling pathways involved are PI3K/mTOR, YAP, AMPK/mTOR/HIF1α/P-gp, etc. [215–217].

There are some controversial cases that should not be neglected. A study reported that metformin combined with 5-FU did not show anticancer activity *in vitro* and *in vivo* for colorectal cancer [199]. In a clinical trial for refractory metastatic colorectal cancer, metformin had a longer median survival rate when used alone, but it did not show significant difference overall. When combined with 5-FU, metformin showed a modest boost in survival rate compared with either drug used alone [218]. Thus, metformin might show variable effect in different stages of multiple cancers.

Other antimetabolite drugs

Cytarabine (Ara-C) has been used for the first-line treatment of acute myeloid leukemia (AML). Metformin combined with Ara-C significantly enhances chemosensitivity of AML cells through reducing the mitochondrial transfer and oxidative phosphorylation (OXPHOS) [219].

Hydroxycarbamide has been used for treatment of melanoma, resistant chronic myelocytic leukemia, head and neck cancer as well as recurrent or metastatic ovary carcinoma. Interestingly, metformin combines with hydroxyurea to induce fetal hemoglobin (HbF) and reverse the arrest in erythroid maturation caused by hydroxyurea treatment alone [220].

Gemcitabine is a first-line drug used for the treatment of pancreatic cancer. Studies reported that heat shock protein 27 (HSP27) is phosphorylated in gemcitabine-resistant pancreatic cancer cells and metformin reverses chemoresistance through blocking the phosphorylation of HSP27 [221,222], indicating a potential and synergistic effect in pancreatic cancer. Further studies showed that metformin combined with gemcitabine increases chemosensitivity by reducing proportion of $CD133^+$ cells and suppressing ERK/p70S6K signaling pathway [223].

Metformin combines with drugs interfering with microtubules synthesis

Vincristine and its derivatives

Vincristine and its derivatives are alkaloids isolated from *Vinca rosa* Linn., which have been used for the treatment of leukemia due to cell cycle specific cytotoxicity. The drugs bind specifically to tubulin, leading to microtubule depolymerization, cell cycle arrest, and cell apoptosis. Metformin combined with vincristine sensitizes leukemia cells through activation of AMPK [224]. A clinical trial showed that metformin in combination with VPLD (vincristine/dexamethasone/doxorubicin/PEG-asparaginase) can treat relapsed childhood acute lymphoblastic leukemia (ALL) (NCT01324180) with better outcome than patients who received VPLD alone by inducing ER stress, activating AMPK, and inhibiting the unfolded protein response (UPR) [225]. In another study, metformin was used in combination with R-CHOP (rituximab plus cyclophosphamide/doxorubicin/vincristine) to treat diffuse large B cell lymphoma (DLBCL) and grade 3b follicular lymphoma (FL3b) with significantly better overall survival (OS) than those without metformin [226].

Paclitaxel (PTX)

Paclitaxel is a diterpene amide isolated from the Pacific yew. It has been used for multiple cancers by targeting the tubulin, inducing the defects of mitotic spindle assembly, chromosome segregation, and cell division. Metformin in combination with PTX enhances chemosensitivity and inhibits cell proliferation by modulating the AMPK/mTOR pathway in various types of cancers such as endometrial cancer and breast cancer [227,228]. This combination treatment has also induced oxidative stress and activation of mitochondrial-dependent apoptotic pathways in prostate cancer [229]. In addition, metformin and PTX combination was shown to be effective in lung cancer by downregulating the ERCC1 and inhibiting p38-MAPK [230]. Furthermore, this combination has been shown to alter the metabolic target and modulation of mTOR pathway in ovarian cancer [231].

Docetaxel (DCX)

DCX is a chemotherapeutic drug with similar chemical structure and mechanisms to PTX. Although it provides benefits in clinical cases, the side effects are significant due to its effect of enhancing aerobic glycolysis [232]. Previous studies showed metformin combined with DCX reduced cancer cell viability in castration-resistant prostate cancer through decreasing AKT phosphorylation, increasing AMPK phosphorylation, and decreasing acetyl-CoA and HMG-CoAR activity [233,234]. However, metformin addition failed to improve the standard DCX regimen in castration-resistant prostate cancer clinically (NCT01796028). Further research targeting tumor cell metabolism should be performed.

Metformin combines with antibiotics and drugs targeting DNA synthesis

Antibiotics, such as dactinomycin D, daunorubicin, or doxorubicin, can exert anticancer activities, but accompanied with adverse effects such as severe cardiotoxicities. These antibiotics affect the structure of DNA through interrupting the structure of DNA and inhibiting the activity of topoisomerase to exert its anticancer activities. Combining these antibiotics with metformin may be a new strategy worth investigating. The beneficial effects of metformin may help overcome the side effects of these antibiotics.

Doxorubicin (DOX)

DOX exerts anticancer activity accompanied by serious side effects including chemo-resistance and cardiotoxicities. In DOX-resistant breast cancer cells, metformin in combination with DOX shows synergistic activity and overcomes drug resistance by downregulating drug-resistant genes such as P-gp and HIF1α [235,236]. Metformin has been found to prevent DOX-induced cardiotoxicity *in vivo* with multiple mechanisms

including the activation of AMPK pathway, inhibition of oxidative stress, energy starvation, and depletion of intramitochondrial CoA-SH [237–240]. In addition, metformin also enhances the anticancer activity of DOX in breast cancer [241] and reverses drug resistance through inhibiting NF-κB pathway and phosphorylation of STAT3 [242]. Combination treatment of metformin and DOX can prevent cancer relapse and reduce the dosage of chemotherapy [243].

Cisplatin

Cisplatin exerts remarkable anticancer activity, but renal toxicity and drug resistance were discovered in clinical settings. Previous studies reported that metformin enhances anticancer effect in thyroid carcinoma [241] and overcomes resistance of cisplatin by downregulating RAD51 expression in triple-negative breast cancer cells [244] and suppressing IL6/STAT3 pathway in lung cancer [245]. Metformin increases chemo-efficacy of cisplatin via AMPK/mTOR pathway in meningioma [200] and has been found to inhibit Jarid1b in NSCLC in the presence of wild type p53 [246]. Metformin combined with cisplatin was found to strongly inhibit the activity of cancers, promote apoptosis in nasopharyngeal carcinoma, and ovarian cancer [247,248]. This combination treatment can induce cell arrest via phosphorylated YAP1 pathway [249] and attenuate cytotoxicity caused by cisplatin via AMPK/FOXO3α pathway [250]. Moreover, metformin prevents cisplatin-induced cognitive impairment and brain damage in mice [200,251].

Cyclophosphamide (CYP)

CYP exerts powerful and broad-spectrum anticancer activity, while along with obvious side effects such as immunosuppression, special cystitis, and drug resistance. Metformin combination can ameliorate CYP-induced memory impairment and nephrotoxicity [252,253], suggesting the potential for further application.

Other chemotherapeutic agents

Arsenic trioxide (ATO)

ATO is a traditional Chinese medicine that has been used for treating acute promyelocytic leukemia (APL). ATO frequently causes organ toxicity, such as hepatotoxicity, neurotoxicity, and cardiotoxicity. Previous studies showed that metformin ameliorates ATO-induced hepatotoxicity via inhibiting mitochondrial complex I, indicating a possibility for combination drug treatment [254]. Metformin enhances the sensitivity induced by ATO through downregulation of Bcl-2 expression in hepatocellular carcinoma [255]. Metformin also strengthens ATO's effect of suppressing intrahepatic cholangiocarcinoma through activation of AMPK, upregulation of ERK3, and inhibition of mTORC1 [256].

Combination of metformin with molecular targeted drugs

Chemotherapy has long been the primary strategy for cancer treatment, but it is not an eradication method and causes inevitable side effects, such as myelosuppression and gastrointestinal reaction. Compared with traditional chemotherapy drugs, molecular targeted drugs with high efficacy and low toxicity specifically target cancer cells without causing obvious damage to normal cells. Interestingly, metformin not only shows synergistic effects with numerous chemotherapy agents, but also improves the efficacy of targeted therapy in preclinical and clinical cases. The following section summarizes the progress of tumor targeting drugs combined with metformin to treat different cancers. This section is divided into two parts: single-target inhibitors and multi-target inhibitors. According to the different targets, the potential mechanism of action is described in detail, and the development prospect of cancer treatment is discussed.

Metformin combines with single targeted inhibitors

EGFR inhibitors

Gefitinib, the first epidermal growth factor receptor (EGFR) inhibitor, is a reversible inhibitor with quinazoline structure [179]. Gefitinib is highly effective in NSCLC patients with EGFR mutations and provides complete remission of disease progression, but it was found that patients rapidly developed drug resistance after long-term gefitinib treatment. The mechanisms of developing resistance include amino acid mutation, activation of AKT/mTOR pathway, and upregulation of IGF-1R [257]. Therefore, gefitinib combining with metformin can improve efficacy by inhibiting AKT/mTOR or insulin-related pathways, which may be a promising direction for further investigation. Metformin was found to inhibit the expression of anti-apoptosis protein, thus increasing the sensitivity of gefitinib [258]. Previous studies showed that metformin effectively blocks tumor growth and inhibits tumor recurrence in xenografts of gefitinib-resistant cancer cells [259]. The inhibition of IGF-1R was reported to overcome the drug resistance of EGFR tyrosine kinase receptor inhibitors, thus metformin combined with gefitinib showed their synergistic effects in bladder cancer [260]. Overall, combination of metformin and gefitinib would be a promising strategy for tumor drug therapy.

mTOR inhibitors

The combination of mTOR inhibitors and metformin shows potential value in terms of molecular mechanism. Everolimus, an oral mTOR inhibitor, was approved in 2011 for advanced pancreatic neuroendocrine tumors [261]. In breast cancer cells, combination treatment of metformin and everolimus enhanced the inhibitory effects on cell proliferation and colony formation [262]. And the combination shows significant inhibitory effects on obesity-induced tumor growth [263]. Clinical studies have also shown that metformin sensitizes patients with pancreatic neuroendocrine tumors to everolimus [264].

VEGF inhibitors

Bevacizumab is a recombinant humanized monoclonal antibody (mAb) that targets all isoforms of vascular endothelial growth factor A (VEGF-A). It prevents VEGF-A from binding to VEGFR-1 and VEGFR-2, leading to the degeneration of tumor angiogenesis, thereby inhibiting tumor growth [265,266]. One case report showed that bevacizumab combined with metformin improved the performance of patients with recurring endometrial cancer: computed tomography showed reduced radiation density in the lungs and mediastinal lesions in liver disease, suggesting increased tumor necrosis [267]. In addition, bevacizumab combined with metformin specifically targets cancer stem cells and synergistically inhibits angiogenesis in ovarian cancer [268]. Moreover, the clinical trials of bevacizumab combined with metformin in the domestic and overseas are under way.

HER-2 inhibitors

HER2-targeted therapy is effective in breast cancer patients with HER2 overexpression and/or amplification. Trastuzumab, the first anti-HER2 monoclonal antibody developed in 1990, binds to the HER2 receptor in cancer cells and blocks the formation of heterodimers between HER2 and HER1, while also blocks HER3 or HER4, thereby inhibiting signal transduction pathways for cell survival and proliferation (such as PI3K or MAPK) and inducing apoptosis [269]. Trastuzumab has been the standard treatment for stable and early-stage HER2-positive breast cancer for over a decade. However, trastuzumab is also associated with an increased risk of cardiotoxicity and prolonged exposure to trastuzumab can lead to drug resistance [270]. Vazquez-martin *et al.* found that metformin significantly inhibits the self-renewal and proliferation of trastuzumab resistant breast cancer stem cells [271]. In addition, the proliferation of trastuzumab resistant HER2 overexpressing breast cancer cells was also inhibited by metformin. The mechanism of action involves disrupting the HER2/IGF-1R complex, which is found only in the resistant subpopulation [272].

Combination of metformin and multi-target inhibitors

Classical multi-target tumor inhibitors include sorafenib, imatinib, sunitinib, dasatinib, etc. Sorafenib was approved by the FDA in 2007 for the treatment of advanced hepatocellular carcinoma (HCC), but it is often accompanied with serious side-effects and low efficacy in tumor treatment. It has been shown that metformin sensitizes cancer cells to sorafenib. The two drugs work together to inhibit cancer cell proliferation and reduce sphericity, especially in drug-resistant cancer stem cells [273]. The addition of metformin reduced the dose of sorafenib by 25% without loss of its tumor suppressive effect [274]. Metformin can also enhance the anti-metastasis effect of sorafenib in patients with HCC by downregulating the ERK/JNK-mediated NF-κB-dependent pathway and reducing the expression of urokinase-type plasminogen activator (uPA) and MMP-9 [275]. Some preclinical studies have shown that metformin and sorafenib synergistically promote apoptosis and autophagy in HCC cells, showing synergistic antitumor effects [276]. In conclusion, the combination of metformin and sorafenib for the treatment of HCC shows broad application prospects.

Imatinib (IM) is a member of a class of small molecule tyrosine kinase inhibitors that selectivity targets the Abl kinase and launched a new era of tumor-targeted therapy. However, drug resistance frequently occurs, resulting in a significant reduction of therapeutic efficacy in chronic myelocytic leukemia (CML). Inhibition of mTOR and its effectors plays an important role in the generation of the antileukemic effects of BCR-ABL inhibitors [277]. With this aim, Eliza Vakana *et al*. found that metformin inhibited the mTOR signaling cascade in BCR-ABL expressing cells and suppressed the growth of different CML-derived cell lines, as well as primitive progenitors from CML patients. Importantly, metformin was also shown to possess growth inhibitory effects that can specifically target cells expressing the T315I-BCR-ABL mutation eliciting drug resistance [278]. These findings are particularly interesting and can lead the development of metformin as an antineoplastic agent in treatment of CML.

Combination of metformin with immunotherapy

Over the past decade, we have experienced a revolution in cancer treatment, especially in the field of novel cancer immunotherapy represented by immune checkpoint inhibitors (ICIs). ICIs, such as cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) inhibitors, programmed cell death protein (PD-1) inhibitors, and programmed cell

death ligand-1 (PD-L1) inhibitors, have been used in the treatment of a variety of malignant tumors [279]. However, despite great success of current clinical oncology treatments, the low response rate of tumor immunotherapy is a major problem [280,281]. Previous study reported that metformin showed better antitumor effect in immunocompetent mouse models than in immunodeficient models, suggesting that metformin's antitumor effect may be mediated primarily through the immune system [280]. Indeed, metformin could remodel immune cells and immune-related molecules, affecting the tumor microenvironment, and regulating the antitumor immune response [155]. Therefore, we reviewed the clinical benefits of metformin combined with immunotherapy, with emphasis on ICIs therapy, in cancer treatment and the related mechanisms.

Metformin increases the function of CD8$^+$ cells

CD8$^+$ CTLs, a key component for an effective antitumor response, produce effector cytokines and cytotoxic molecules such as granzyme and perforin, which can directly result in tumor cells death [282]. However, some tumor infiltrating CTLs remain in an "exhausted" functional state, marked by increased expression of various cell surface checkpoint proteins (such as PD-1, CTLA4) and decreased development of effector cytokines, due to long-term exposure to tumor antigens. Checkpoint proteins expressed by "exhausted" T cells bind to PD-L1 on the surface of tumor cells, suppressing the CTLs, and leading to tumor immune escape [283]. Eikawa *et al.* and Zhang *et al*. reported that metformin directly increased the number of CD8$^+$ tumor-infiltrating lymphocytes (TILs), stimulated the production of TNFα and IFNγ, and decreased the expression of PD-1, thus saving the cells from exhaustion in an AMPK-dependent manner [284,285]. Not surprisingly, several retrospective studies have reported improved clinical outcomes in patients with NSCLC and melanoma who received ICIs in combination with metformin [285,286].

Metformin can also indirectly alter the function of CD8$^+$ cells in the tumor immune microenvironment (TIME)(Fig. 3). Recently, Cha *et al*. demonstrated that metformin via AMPK disrupts PD-L1/PD1 axis by reducing PD-L1 stability and membrane localization in tumor cells, hence exhibiting stronger cytotoxic effects [280]. Consistent with these findings, the combination of metformin and CTLA4 inhibitor exhibited stronger anti-tumor effect on breast cancer 4T1, B16F10, and CT26 models [280]. Similarly, blocking FOXO3-mediated c-MYC-PD-L1 and STAT3-PD-L1 dual pathways by metformin also promoted CD8$^+$ T cells response and anti-tumor immunity, sensitizing tumors to anti-PD-1 therapy [287]. In addition, metformin could also synergize with

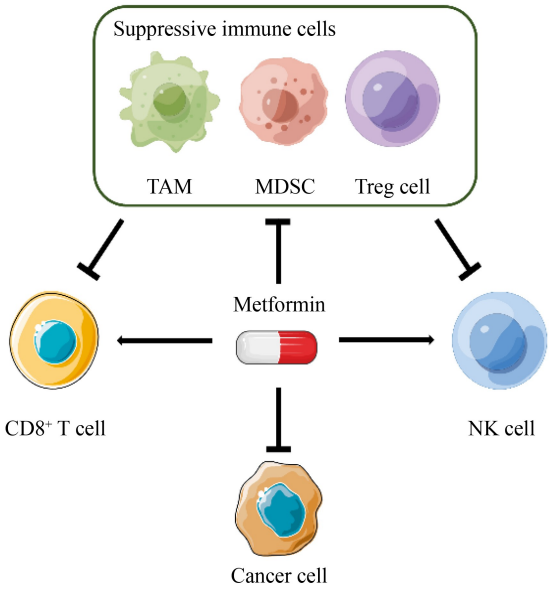

Fig. 3 Modulation of TIME by metformin. Metformin treatment directly augments CD8$^+$ T cell and NK cell antitumor function in the tumor microenvironment and indirectly restores cytotoxic effector cells function by modulating suppressive immune cells, ultimately sensitizing cancer immunotherapy.

tumor vaccine immunotherapy to alter the phenotype and function of CD8$^+$ T cells [288]. Although metformin monotherapy had little therapeutic benefit in highly aggressive tumors, combination of metformin with PD-1 blockades resulting in improved intertumoral T cell function and tumor clearance [289].

Metformin enhances the killing effect of NK cells

While T cells remain a key component of an effective antitumor response, NK cells, as the "first responders," exhibit broad tumor cytotoxicity without the requirement for major histocompatibility complex (MHC) restrictions [290]. The MHC class I polypeptide-related sequence A (MICA), subgroup of MHC class I molecules, serves as a natural ligand of NK cells. Cancer cells with abnormal expression of MICA can escape from anticancer effect of NK cells. Metformin treatment upregulates MICA on the surface of human cervical cancer cells through PI3K/Akt pathway, and downregulates PD-L1 on the surface of breast cancer cells through Foxo3-PD-L1 axis, which indirectly enhances the cytotoxicity of NK cells [287,291]. In addition, low-dose metformin can also activate the AKT/mTOR pathway in a P38 MAPK-dependent manner, triggering the polarization and secretion of cytolytic granules in NK cells [292]. Therefore, the combination of metformin and anti-PD-1 antibodies can augment the cytotoxic function of NK cells and improve the therapeutic efficacy [287,292].

Metformin inhibits suppressive immune cells

Myeloid-derived suppressor cells (MDSCs)

Remodeling or removing suppressive immune cells in TIME is an important way to improve immunotherapy. Myeloid-derived suppressor cells (MDSCs) produce immunosuppressive cytokines that allow tumor immune escape [293]. Several studies have investigated metformin treatment which could prevent PMN-MDSC or M-MDSC orbothaccumulationinTIME,thusprovidingpotentialpreclinical benefits for antitumor immunotherapy [294–296].

Tumor-associated macrophages (TAMs)

In addition to MDSCs, tumor-associated macrophages (TAMs) also contribute to an immunosuppressive tumor microenvironment. In most solid tumors, M1 phenotype macrophages are tumor-resistant whereas M2 leads to tumor promotion. Metformin appears to inhibit M2 polarization of macrophages induced by inflammatory cytokines *in vitro* in an AMPK-dependent manner [297–299]. Furthermore, preclinical, and clinical evidences indicated that M1 macrophages increased and M2 macrophages decreased in the TIME after metformin administration *in vivo* [297,300,301]. In short, metformin may play a beneficial role in combination with tumor immunotherapy by blocking the accumulation of M2-like TAMs in the tumor and affecting TAMs polarization.

Regulatory T cells (Tregs)

Metformin can also target Tregs, which negatively regulates CTL functions needed for tumor elimination. Physiologic concentrations of metformin could inhibit the differentiation of $CD4^+$ naive T cells to inducible Tregs induced by TGF-β *in vitro* and reduce $CD4^+CD25^+$ Tregs in tumors, both appearing to be correlated with reducing Forkhead Box P3 (FOXP3) protein caused by mTORC1 activation and metabolic reprogramming of glycolysis [302]. Unsurprisingly, a preclinical trial showed that metformin combined treatment with anti-PD-1/anti-CTLA4 increased the tumor-infiltrating $CD8^+$/Treg ratio and significantly suppressed tumor growth compared with ICIs monotherapy [303].

Cancer immunotherapy has improved the outcomes of some patients, but its low response rate remains a major problem. In fact, due to the complexity of TIME, it is often necessary to use a cocktail of inhibitors or cytokines to achieve a powerful therapeutic effect, bringing unknown side effects and increase the economic burden of patients. Metformin can target different types of cells in TIME and profoundly reshape the inhibitory microenvironment at multiple levels through various mechanisms, thus it is promising to combine metformin with immunotherapy.

Summary and future perspectives

In the development of antitumor drugs, it has experienced three different stages of change: chemotherapy, targeted therapy, and immunotherapy. Metformin, as one of the most widely recognized metabolic modulators, shows prominent anticancer effects. Interestingly, although it is effective as a standalone drug, metformin is more effective in combination with other antineoplastic drugs and can be incorporated into traditional and emerging strategies. There are two main benefits of using metformin as an effective strategy for cancer treatment, the first is that the combination synergistically and additively enhances efficacy thereby reducing side effects due to lower doses. The second benefit is that when metformin is used in combination with other therapies it can potentially overcome or reverse drug resistance, thereby enhancing therapeutic anticancer benefits. Herein, we summarized and discussed the combination strategy of metformin in cancer, as an impressive and interesting approach for cancer therapy. However, attention should be paid to the potential side effects due to potential drug–drug interactions such as drug amalgamation. In addition, the effectiveness and feasibility of metformin alone or in combination with other treatments may vary between diabetic or non-diabetic patients. The anticancer effects of metformin generally increase with higher doses, which is much higher than the conventional concentration in diabetic patients, thus resulting in intolerable side effects and potential drug toxicity in actual clinical use. Meanwhile, the efficacy of metformin is highly dependent on the concentration of glucose in the tumor microenvironment. Other factors such as the pharmacokinetics of metformin, microenvironment, drug resistance after long-term administration and metabolic environment also should be considered when using metformin alone or in combination with other drugs. In conclusion, although the combination of metformin with clinically available antitumor agents shows strong synergistic effects, its future clinical application requires more sophisticated design and experimental investigation in clinical trials.

Metformin and aging

Aging is a natural process in which multiple function of living organisms gradually decline as people getting older [304]. Aging is considered as a risk factor for a number of age-related chronic diseases, including osteoporosis, cardiovascular disease (CVD), cancer, and neurodegenerative diseases [305]. It is predicted that by 2050, there will be 2.1 billion people aged 60 years or older [306], leading to the growing financial burden of age-related diseases

worldwide. Therefore, there is an urgent need for effective drugs to prevent and delay aging. In this section, we will focus on the effects and mechanisms of metformin alleviating aging and the potential mechanisms inside.

Benefits of metformin in attenuating physiologic aging and age-related diseases

Metformin is also proposed as an "anti-aging" drug. A retrospective analysis of diabetics medicated with metformin showed longer survival than those who were treated with other anti-sugar drugs [32,307]. More importantly, diabetics on long-term metformin therapy survived longer, even when compared with healthy people [307]. Metformin can also effectively extend the healthy life span of different model organisms, including *C. elegans*, *Drosophila melanogaster*, and rodents, despite that some studies used high concentrations of metformin [111,308,309]. Based on these, clinical trials, including MILES (Metformin In Longevity Study), and TAME (Targeting Aging with Metformin), have been designed to determine if metformin can offset aging and extend lifespan (Table 5). Data from the MILES trial suggest that metformin modifies a variety of pathways associated with aging, including metabolic pathways, collagen trimerization and extracellular matrix (ECM) remodeling, adipose tissue and fatty acid metabolism, mitochondrial and DNA mismatch repair which declines with age [310]. This study provides the first evidence that, in older adults, metformin has metabolic and nonmetabolic effects linked to aging. Metformin is now the focus of the first proposed clinical trial of an anti-aging drug—TAME program [311,312]. The goals of TAME are to measure the clinical outcomes by the emergence of new age-related chronic diseases and functional outcomes, such as athletic ability and cognitive impairment. In addition, the biomarkers of aging, such as inflammation and aging, are also considered. TAME trial results will provide more insight into whether metformin can reduce the risk of age-dependent diseases (excluding diabetes) in non-diabetic individuals, and may provide tools to target aging itself rather than related diseases.

In addition to biological aging, epidemiological studies have shown that the use of metformin may reduce the incidence of age-related diseases, such as cancers and neurodegenerative diseases [313]. Metformin was found to induce favorable metabolic and nonmetabolic pathways in skeletal muscle and subcutaneous adipose tissues of older adults with impaired glucose tolerance, the most common form of glucose dysregulation in this age group [310]. Clinical trial results have shown that metformin can slow the development of age-related cardiovascular disease and reduce the incidence of cardiovascular disease in diabetics [314,315]. This result is believed to be a result of metformin's pleiotropic

Table 5 Clinical trials studying effects of metformin on human aging (Data from ClinicalTrails.gov)

Title (NCT No.)	Phase	Recruiting conditions	Status
A Double-Blind, Placebo-Controlled Trial of Anti-Aging, Pro-Autophagy Effects of Metformin in Adults with Prediabetes (NCT03309007)	Phase 3	Prediabetes, aging	Completed
Metformin in Longevity Study (MILES) (NCT02432287)	Phase 4	Aging	Completed
Metformin to Augment Strength Training Effective Response in Seniors (MASTERS) (NCT02308228)	Early phase 1	Aging	Completed
Effect of Metformin on Frailty in 12 Subjects (NCT03451006)	Phase 2	Aging, inflammation, frailty	Terminated
REMAP Trial for Optimizing Surgical Outcomes at UPMC (NCT03861767)	Phase 3	Aging	Completed
Impact of Metformin on Immunity (NCT03713801)	Phase 1	Aging, vaccine response impaired	Active, not recruiting
Phase 1 Study of the Effects of Combining Topical FDA-approved Drugs on Age-related Pathways on the Skin of Healthy Volunteers (NCT03072485)	Phase 1	Aging	Completed
Vaccination Efficacy with Metformin in Older Adults (NCT03996538)	Phase 1	Aging, age-related immunodeficiency, vaccine response impaired	Completed
Antecedent Metabolic Health and Metformin Aging Study (NCT04264897)	Phase 3	Aging, insulin sensitivity, chronic diseases	Recruiting
Metformin and Longevity Genes in Prediabetes (NCT01765946)	Phase 4	Insulin resistance, prediabetes, aging, inflammation	Completed
Diet and Exercise Plus Metformin to Treat Frailty in Obese Seniors (NCT04221750)	Phase 3	Frailty, sarcopenic obesity, aging	Recruiting
VIAging Deceleration Trial Using Metformin, Dasatinib, Rapamycin and Nutritional Supplements (NCT04994561)	Phase 1	Aging	Withdrawn
Metformin for Preventing Frailty in High-risk Older Adults (NCT02570672)	Phase 2	Frailty	Recruiting
Role of Metformin on Muscle Health of Older Adults (NCT03107884)	Early phase 1	Muscle atrophy, insulin resistance	Recruiting
Targeting Aging with Metformin (TAME)	Unknown	Aging, chronic diseases, etc.	Not yet started

effects, which extend beyond glycemic management [32]. Indeed, a recent meta-analysis showed significant reductions in cardiovascular mortality, all-cause mortality, and cardiovascular events in patients with coronary artery disease treated with metformin [316]. Age is the single biggest risk factor for many cancers [317]. Metformin has been widely studied as a promising anticancer drug, which can be used alone or in combination with other anticancer drugs for the treatment of malignant tumors [318], as mentioned in the above part. Aging people become highly prone to neurodegenerative diseases [319], including Alzheimer's disease. The experimental and clinical data showed that metformin can help prevent AD, including lowering risk of AD and enhancing cognitive function [320–322]. These clinically relevant data suggest that metformin can provide a strong protective effect in age-related diseases.

The primary mechanisms of metformin action in attenuating aging

Despite clinical use for metformin as an anti-diabetic even anti-aging drug, the exact cellular mechanisms by which metformin exerts its actions remain unclear. In the following part, we will focus on the main mechanisms of anti-aging action of metformin (Fig. 4).

Metformin improves the deregulated nutrient-sensing

With age increasing, the organism is characterized by deregulated nutrient-sensing and metabolic disorder, further leading to the aging phenotype [323]. These highly conserved nutrient sensing and signal regulation pathways mainly include: (1) the insulin/IGF-1 signaling (IIS) pathway; (2) other nutrient-sensing systems: mTOR, AMPK, and sirtuins. These trophic pathways were mentioned in the 2013 review and supported by omics data [324]. Metformin exerts beneficial effects on energy metabolism and aging by directly regulating key energy sensors. Metformin activates AMPK through a LKB1-dependent mechanism, and long-term administration of low concentration metformin (0.1% w/w in diet) can improve health span in male mice [309]. Long-term treatment of female isolated SHR mice with metformin (100 mg/kg drinking water) also increased their average life expectancy by 37.8% [325]. In summary, the researchers suggest that metformin therapy mimics similar benefits of calorie restriction, such as improved physical performance, increased insulin sensitivity, and reduced LDL and cholesterol levels without reducing caloric intake [326].

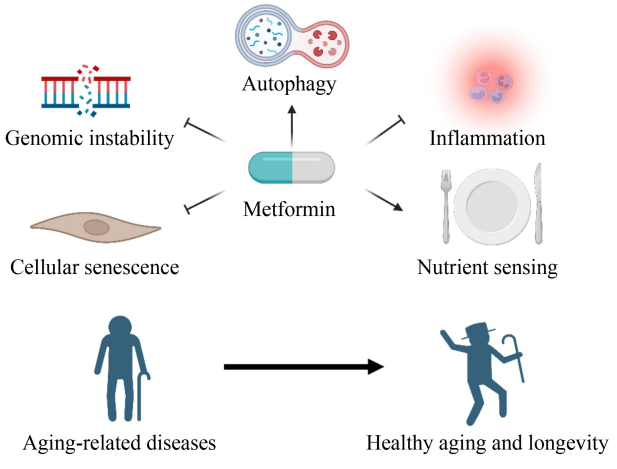

Fig. 4 Primary molecular mechanisms of metformin in aging. The anti-aging effect of metformin is probably a combination of multiple mechanisms, including the improvement of the deregulated nutrient-sensing and genomic instability, the suppression of cell senescence and pro-inflammation responses and enhancing autophagy.

Metformin alleviates genomic instability

Accumulation of genetic damage is a commonly accepted cause of aging, which may disrupt cellular homeostasis and lead to genomic instability [327]. As we age, defects in DNA repair mechanisms affect the expression and transcription of essential genes, leading to cell dysfunction. A large number of preclinical studies have shown that impaired DNA repair ability would lead to premature aging syndromes, such as Werner syndrome and Bloom syndrome [328]. In addition, mitochondrial DNA mutations and nuclear lamina defects during aging can also lead to genomic instability [329]. There are several mechanisms by which metformin alleviates the response to genomic instability in the context of aging and various types of cancer. Studies have shown that metformin exerts the genomic protective effect by reducing oxidative stress, DNA damage, regulating ataxia telangiectasia mutated (ATM) protein, and epigenetic effects [330]. Our team used to report that AMPK-mediated phosphorylation on 53BP1 promotes classic non-homologous end joining (c-NHEJ), suggesting that metformin as a putative activator of AMPK may also regulate this process [331]. A recent clinical trial [332] in Kazakhstan was the first to investigate the changes in lymphocyte DNA damage before and after the use of metformin in obese people. It was found that the use of metformin 850 mg/day for 3 months in the treatment of obesity (BMI greater than 30 kg/m^2) reduced the DNA breakage of lymphocytes. Previous clinical studies have also shown that short-term administration of metformin in the elderly can induce BRCA-mediated DNA damage response and DNA repair in skeletal muscle [310]. The antioxidant properties of metformin are currently controversial. 25 mg/kg metformin can significantly

improve the concentrations of antioxidant enzymes (SOD, CAT) and GSH in the treatment of alloxacil-induced diabetic rats, indicating that metformin can help to protect oxidative stress-induced damage in diabetic complications [333]. In an elderly rat model of another study by Allard *et al*. [334], long-term administration of metformin decreased the transcription and activation of the antioxidant regulator Nrf2. The authors explain that the dose and timing of metformin are important factors to consider in rodent studies, and they combined long-term use of metformin with a relatively high daily dose. Anisimov and colleagues [335] reported that the life-prolonging ability of metformin decreased with increasing age of metformin treatment. Although there is no consensus on the mechanism of how metformin regulates oxidative damage or attenuates genomic instability, more evidences indicate that metformin prevents or reverses age-induced disorders via regulating nuclear and mitochondrial genome stability and chromosome structure.

Metformin downregulates SASP and reduces the burden of senescent cells

Cellular senescence can be defined as a stable arrest of the cell cycle and the accumulation of senescent cells aggravates aging. Senescent cells exhibit dramatic changes in their secretory group, some of which are enriched with pro-inflammatory cytokines and matrix metalloproteinases, known as the "senescence-associated secretory phenotype" (SASP). Although metformin could not work as senolytics, it can effectively inhibit cell senescence and SASP in many age-related diseases. Chronic low-dose metformin delays cellular senescence through NRF2-mediated upregulation of glutathione peroxidase 7 (Gpx7), characterized by the reduced percentage of senescence-associated β-galactosidase (SA-β-Gal)-positive cells [336]. In addition, metformin inhibits the SASP by preventing NF-κB translocation into the nucleus, thereby interfering with IKK/NF-κB activation [337]. In addition to this, metformin can reduce the RNA levels of SASP markers including IL-6 and IL-8 and the protein expression levels of p16 and p21 in human fibroblasts through a DICER1-dependent mechanism [338]. Thus, metformin can function as senomorphics to regulate SASP via varied mechanisms. In the future, drug combinations of metformin can be considered to alleviate aging and extend lifespan.

Metformin downregulates pro-inflammation responses

Aging is associated with a chronic, low-grade inflammatory state called "inflamm-aging," which is associated with age-related diseases [339]. The definition of "inflamm-aging" is based on cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [340]. Despite the fact that there is no clear understanding about the causes of "inflamm-aging," it is a highly significant risk factor for morbidity and mortality in the elderly [341,342].

Multiple studies in patients with T2DM provide evidence of the anti-inflammatory effects of metformin. In T2DM patients, metformin monotherapy was associated with significantly lower levels of inflammatory molecules, including TNF-α, soluble TNF receptor 1 (sTNFRI), and sTNFRII compared with other anti-diabetic monotherapy [343]. Chen *et al*. found that metformin reduced blood IL-6 and urine MCP-1 levels in patients with T2DM in a time- and dose-dependent manner [344]. In another T2D study, metformin treatment significantly reduced levels of NF-κB, IL-1β, and niacin receptor GPR109A in peripheral blood leukocytes [345]. Notably, in patients with atherosclerotics, most of whom were free from diabetes, plasma levels of inflammatory factors (e.g., IL-6 and TNF-α) also reduced after metformin treatment [346]. Together, these clinically relevant data suggest a promising role of metformin in targeting age-related chronic inflammation, but whether the effect varies between diabetics and non-diabetics remain further study.

The mechanism by which metformin alleviates inflammation has been extensively studied in animal models. The anti-inflammatory effects of metformin initiated with AMPK activation followed by downstream inhibition of the mTOR and NF-κB signaling cascades [337,347,348]. In addition to AMPK-mediated effects, several effects have been described that appear to be unrelated to AMPK. Metformin upregulated miR-146a and miR-155 to improve endothelial cell inflammation [349]. Metformin increased the expression of miR-34a-5p and miR-125b-5p, which in turn inhibits the production of pro-inflammatory cytokines from macrophages [350]. In addition, metformin treatment did not change the phosphorylation of AMPK, but it did enhance the expression of PFKFB3/iPFK2 axis in adipocytes, which is associated with the anti-inflammatory effects of metformin [91].

Metformin enhances autophagy

Autophagy-mediated protein degradation is deteriorated with aging, leading to an imbalance in protein abundance and loss of proteostasis [351]. Dysregulation of the autophagy pathway hinders the removal of dysfunctional, damaged, or extra organelles, has a major impact on cellular health, and can lead to the development of metabolic and aging-related diseases [352,353].

Evidence from different model species supports the role of autophagy in the regulation of aging and age-related diseases. For example, the autophagy-related gene (ATG)

was needed to extend longevity in long-lived *C. elegans* with daf-2 mutation [354]. By preventing Beclin-1 from interacting with the negative regulator Bcl-2, Becn1F121A/F121A mutant mice exhibited higher levels of basal autophagic flux, and thus lived longer and experienced fewer age-related symptoms *in vivo* [355]. These findings suggest that individuals with impaired autophagy are susceptible to age-related diseases and shortened lifespan.

Clinically, limited paper found that metformin upregulated mitochondrial autophagy of mononuclear cells in T2DM patients, and subsequently improved mitochondrial morphology and function [356]. Metformin enhanced autophagy in $CD4^+$ T cells from older subjects and improved mitochondrial bioenergetics and T cell inflammation [357]. One study also showed that metformin improves polycystic ovary syndrome (PCOS) by activating autophagy and reducing testosterone levels [358]. Experimental data from animal models fully compensate for the lack of clinical data. Depending on AMPK-autophagy axis, metformin improved age-related pathological phenotypes, such as hepatic steatosis [359], atherosclerosis [360] and neurodegeneration [361]. In summary, these studies suggest that autophagy plays a role in metformin prolonging lifespan and improving age-related diseases.

Additional potential mechanisms of metformin against aging

Progressive telomere attrition and shortening is associated with biological aging and aging-related diseases [362]. Metformin was reported to reduce telomere attrition in leukocytes of patients with mild aging-related diabetes, showing a significant anti-aging effect [363]. Similar studies were also processed and found that metformin prevented shortening of leukocyte telomere length in diabetic individuals [364,365]. However, the mechanisms by which metformin reduces telomere attrition remain elusive. Further studies are needed to illustrate how metformin prevents telomere attrition and maintains telomere integrity.

Mitochondrial dysfunction caused by various pathological factors affects cell metabolism and impairs body function and health [366]. It is reported that metformin improved the physiologic function of the elderly by eliminating exercise mediated increase in mitochondrial respiration of skeletal muscle [367]. ROS accumulation and metabolic disorder resulted from mitochondrial dysfunction during aging are both the potential targets of metformin [368]. Metformin treatment of *in vitro*-aged adipose-derived stromal cells (ASCs) from older women decreased oxidative stress and mitochondrial dysfunction, via activation of AMPK, which resulted in decreased senescence, implying that metformin might be a useful drug to target age-related adipose tissue dysfunction [369]. Metformin was reported to regulate mitochondrial biogenesis and senescence through AMPK mediated H3K79 methylation, thus alleviating age-associated vascular dysfunction [370]. Metformin also inhibits mitochondrial ETC complex I, thereby directly affecting mitochondrial induced oxidative stress by reducing ROS or other indirect mechanisms to scavenge ROS [371]. However, there is no consensus conclusion on whether improving mitochondrial dysfunction by metformin is tissue specific and the appropriate treatment, metformin monotherapy or in combination with other interventions.

With aging, there is a systemic decline the regenerative capacity of tissues, represented by the disruption of homeostasis of stem and progenitor cells [372]. Metformin was found to restore CNS remyelination capacity by rejuvenating aged stem cells via AMPK [373]. Furthermore, in *Drosophila melanogaster* midgut stem cells, metformin was demonstrated to attenuate aging-related phenotypes by regulating autophagy-related genes and AKT/mTOR signaling pathway [374]. Ma *et al*. recently discovered a novel target of low-dose metformin [81]. These findings revealed that metformin binds PEN2 and initiates a signaling route that intersects, through ATP6AP1, the lysosomal glucose-sensing pathway for AMPK activation. The signaling pathway ensures the beneficial effect of metformin, including lifespan extension in nematodes. This ensures that metformin exerts its therapeutic benefits in patients without substantial adverse effects.

Metformin was found to induce hormesis and improve resilience under stress conditions in various organs, cells, and endpoints, possibly via AMPK-Nrf2 axis, thus preventing numerous aging-related health conditions. But the detailed mechanisms remain further illustrated. These distinct biomedical effects of metformin have important implications for future studies, showing the potential to influence development of study design, patient dose optimization, and dose interval [375].

The microbiome is widely recognized as a core regulator of host health [376]. A high-throughput method was applied to investigate host-microbe-drug-nutrient interactions, where metformin host effects are identified to be regulated by a bacterial nutrient signaling pathway. Metabolic modeling of human gut microbiomes links metformin to microbial agmatine and metformin-bacterial interactions engage host lipid metabolism to extend lifespan [111].

Summary and future perspectives

Most clinical data on the health advantages of metformin came from studies of people with diabetes, insulin resistance, and obesity who were also frequently taking

numerous drugs to treat co-occurring conditions. Metformin therapy appears to be relatively safe over the long-term, enables effective glycemic control, and provides additional health benefits not directly related to glycemia management. Metformin affects neuroendocrine control, mediates weight loss, enhances insulin sensitivity, and favorably alters lipid profiles, which are all beneficial for ameliorating aging.

However, there also emerged some negative or contradictory results. Complementary findings from UK Biobank and meta-analyses revealed that the neurocognitive impact of T2DM suggests marked acceleration of normal brain aging, but metformin treatment did not improve neurocognitive outcomes [377]. A series of sometimes controversial data triggered a debate, that is, whether the beneficial effect of metformin extends people's lifespan or enhances health span via its anti-hyperglycemic effect remains elusive. Despite the data in support of anti-aging benefits, whether metformin has a protective effect in those subjects without disease also remains controversial. It is also worth noting that anti-aging effects of metformin on various species appear to be age-dependent [335,378]. Metformin even was reported to shorten lifespan when provided in late life, contrary to its positive effects in young organisms in *C. elegans* and human primary cells, since metformin exacerbated aging-associated mitochondrial dysfunction, causing respiratory failure [379]. Another clinical trial, MASTERS study, also did not support the use of metformin to enhance the benefits of physical activity in healthy elderly people [380]. This data implied that metformin induces deleterious changes of conserved metabolic pathways in late life, which could bring into question its benefits for older individuals without diabetes. Several other clinical trials (NCT04264897, NCT02570672, NCT03107884) on the anti-aging effect of metformin are currently underway, which may address some of the concerns and the results of these studies will better assess the benefits of metformin on healthy longevity. Metformin's safety and effectiveness data in young, healthy people are still lacking. It is necessary to conduct well-designed randomized controlled trials to determine whether metformin medication at a younger age is risk-free and indeed promotes longevity and optimal health maintenance. Again, we must also consider that aging is a heterogeneous phenomenon and that different individuals in the same population may respond differently to metformin. The use of metformin in clinical settings has certain limitations due to its short half-life and relatively low bioavailability. We also can not ignore the side effects of long-term using metformin, including vitamin B12 deficiency, gastrointestinal complaints, lactic acidosis and even nervous system disorders mentioned below. Despite the issues with side effects addressed in many ways for translation, more research is needed to calibrate the balance between safety and anti-aging efficacy of metformin [381].

Metformin and neurological disorder

Metformin has many applications and is one of the most prescribed drugs in the world [154]. Recent studies of this drug have found that metformin can affect neurons in the brain through influencing the activation of AMPK and activate different pathways to enhance mitochondrial functions including activation of transcriptional coactivator peroxisome proliferator-activated receptor-λ coactivator 1-α (PGC-1α) [382–384]. Metformin induces phosphorylation and activation of AMPK in neurons can also inhibit downstream effectors such as acetyl-CoA carboxylases (ACCs) 1 and 2 which suppress fatty acid synthesis and promote fatty acid oxidation [385]. Administration of metformin was found to inactivate downstream effector ACC in human stem cells and dorsal root ganglia (DRG) [386,387]. Although metformin has been a well-known activator of AMPK, there was a controversial study that showed a lowering of AMPK activation after metformin administration in the hypothalamus of rats [125]. This implies that metformin can have various effects on neurons in different brain regions. Although there are different mechanisms in play regarding AMPK, administration of metformin has been found to reduce neuronal damage and promote neuroblast proliferation [388].

Metformin is a drug that has been approved for use in clinical settings. It has potential to modulate and protect neurons under certain conditions and has been used in reprogramming neurons [389,390]. It has been suggested that dysregulation of insulin function contributes greatly to aging and the development of neurological diseases such as Alzheimer's disease (AD), Parkinson's disease, and many others [391,392]. There have been many studies linking diabetes and insulin resistance to development of neurological disorders, however the mechanism is currently unknown [393–395]. There have been many studies regarding metformin and its effect on neurodegenerative disease in both humans and animals (Fig. 5), however, the studies have yielded conflicting results, therefore metformin has not yet been recognized for therapeutic use [391].

Metformin and neurodegenerative diseases

Alzheimer's disease

AD is one of the most common types of dementia in the world, effecting 45 million people worldwide [391]. Dementia is a common neurological disorder characterized by the loss of two or more cognitive abilities and has a drastic negative impact on those afflicted [396]. The patients suffering from AD possess

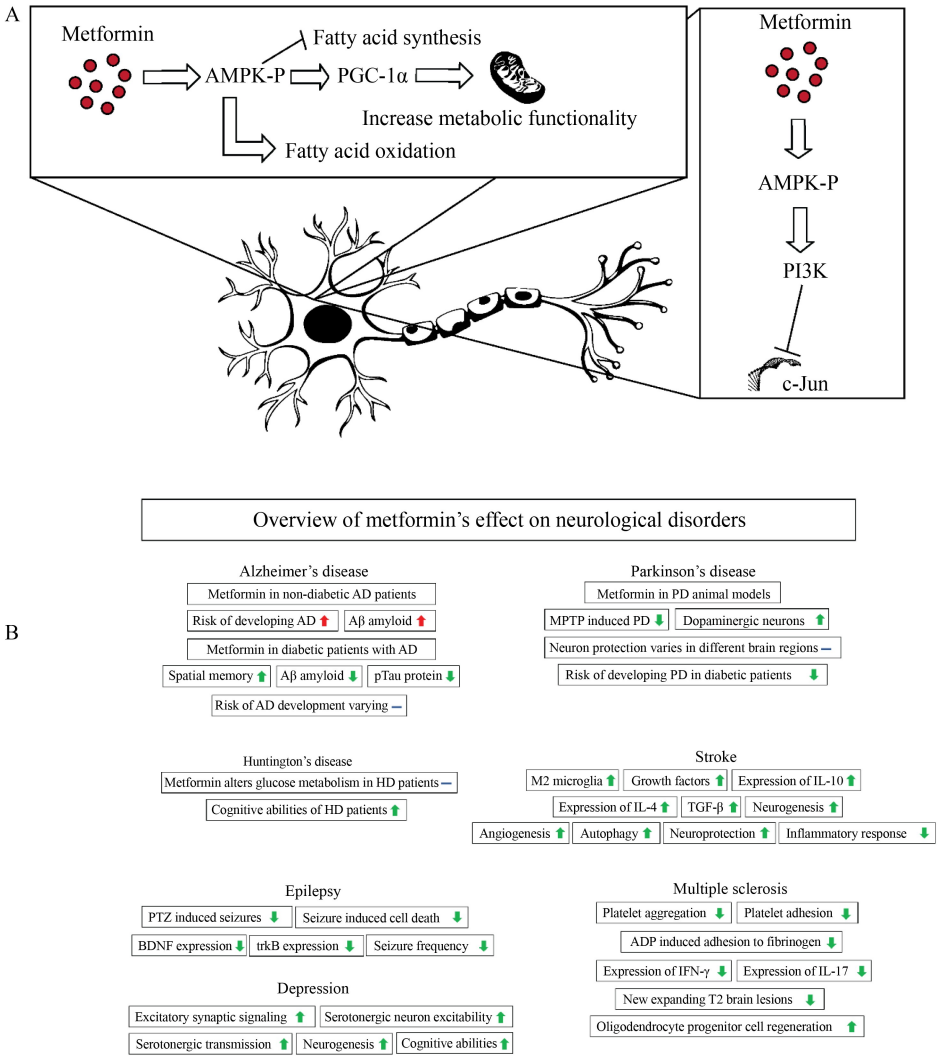

Fig. 5 Overview of metformin's effect on neurological disorders. (A) Metformin's effect in neurons through activating AMPK and its pathways that lead to neuronal protection. (B) Metformin's effect on neurological disorders; the arrows represent increase or (activated) and decrease or (inhibited), while the "–" sign represents uncertainty or contradictory research. Red is negative effect, green is positive effect, and blue is contradictory results.

signs of progressive memory loss and decline of cognitive function with neurofibrillary tangles (NFT) and amyloid-β plaques (Aβ) expressing throughout the brain [397]. The tau protein is associated with microtubules and is responsible for their stabilization [398]. Abnormal tau hyperphosphorylation is associated with NFTs in AD [399]. The Aβ plaques are thought to be due to β-secretase β-site amyloid precursor protein cleaving enzyme 1 (BACE1) and γ-secretase complex cleaving the membrane of the amyloid precursor protein (APP) [400]. Dysregulation and buildup of these proteins in the brain are believed to be one of the underlying causes of AD. In recent research, metformin has been used to counteract AD and improve spatial memory in diabetic mice through preventing p-tau and amyloid plaque load, preventing neuronal death [401]. However, another research has found that metformin may cause an increased risk of AD through increasing production of both intracellular and extracellular Aβ production [402]. The increase of Aβ production may be due to AMPK effecting the APP metabolism in the lipid rafts through altering cholesterol and sphingomyelin homeostasis [403].

In clinical settings, metformin was mostly found to reduce diabetes associated cognitive impairment and AD in type 2 diabetes mellitus (T2DM) patients [38,404,405]. Incidence involving dementia was lowered in T2DM patients receiving metformin or a combination of metformin and sulfonylurea than patients receiving sulfonylurea or thiazolidinediones alone in two studies [406–408]. Although most studies showed the benefit of metformin on lowering AD, one long-term study of using metformin alone for treatment of T2DM found that it had higher risk of developing AD [409]. This signified that T2DM patients that receive metformin may have varying

effects. Later research supported this theory through use of latent class analysis which was able to group different T2DM patients who have received metformin into various risk-profiles of developing AD [410]. This varying effect may be due to metformin's effect on T2DM alone which has been known to cause memory impairments [411]. Therefore, after recovery, patients with T2DM should abstain from further treatment of metformin. Furthermore, AD patients with T2DM should not take metformin in the long term, as it may exacerbate AD progression through increasing Aβ production, although this still need further research. Due to the varying effects of metformin, there is still much research that needs to be conducted to ensure its efficacy in treatment of AD and T2DM associated AD.

Metformin and Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease that exists in the elderly population [412]. It effects about 1% of the elderly population over the age of 60 years and 4% in those population over the age of 80 years [413]. Patients suffering from PD suffer from progressive loss of dopaminergic neurons in the sub-nigra (SN) region of the brain [412,414]. Insoluble proteins known as Lewy bodies were found in various brain regions, consisting of aggregated α synuclein (aSyn) [415,416]. These Lewy bodies can spread throughout several brain region along with disease progression [417,418]. Metformin has been shown to reduce mean platelet volume (MPV) in patients with PD that have platelet dysfunctions demonstrating antiatherogenic effect [419,420]. Recent research using metformin in 1-methyl-4-fenyl-1,2,3,6-tetrahydropyridin (MPTP) induced acute PD mice models showed many varied degrees of success despite similar experimental design [421–425]. In most studies, metformin was found to reduce the effect of MPTP on dopaminergic neurons in the SN [421–424]. There were two studies that contradicted earlier studies, reporting that metformin had no protective effects against MPTP induced neuronal cell loss and the loss of dopaminergic neurons in the SN, however, in one study, protection of dopaminergic neurons in the striatum was found [423,425]. These contradicting studies show that metformin may have very different functions in different brain regions of MPTP induced PD model or the fact that there may be different AMPK effects in dopaminergic neurons in certain regions of the brain. Furthermore, the metformin may have varying effects that are dependent on the different stages of the disease, such that it may prevent or inhibit neuronal loss in early stages of PD, however, the effects are negligible in the later stages of the disease.

In clinical setting, metformin is often used in combination with other drugs instead of its standalone to treat PD [391]. A clinical study to evaluate the effects of metformin on type 2 diabetes and its relation with PD, was used to determine the efficacy of sulfonylurea, metformin, and in combination [426]. The test found that the patients who received sulfonylurea had increased risk of PD compared to those who received anti-hyperglycemic agents, metformin, or a combination of metformin and sulfonylurea [391]. This suggests that metformin may have been able to counteract the adverse effects of sulfonylurea [426]. Metformin has also shown to aggravate PD when taken alone, signifying the potential usefulness as a supplementary drug. Incorporation of metformin in treatments for PD may have potential due to its neuron protection properties which may help eliminate potential adverse effects elicited by other medications. Although there is potential in combination drug use of metformin with PD medications, extensive studies are still required to determine the overall effects it will have on patients.

Metformin and Huntington's disease

Huntington's disease (HD) is a neurodegenerative disease that causes involuntary jerky, irregular, and spasmodic movement. This disease causes progressive cognitive decline and is often diagnosed around 30–40 years of age. HD is a dominantly inherited genetic disorder caused by CAG trinucleotide repeat expansions in the HTT gene which encodes the Huntingtin protein [427]. The defect in the HTT gene often causes production of abnormally long versions of the Huntingtin protein which are broken down by the cell into toxic fragments that aggregate and accumulate in neurons causing neurodegeneration [391]. Reports of metabolic changes in patients with HD have been contradictory [391]. Clinical studies of patients with HD have been reported to have altered glucose metabolism and increased T2DM [428,429]. However, other studies found no changes in metabolism through comparing the glucose tolerance test and examining the changes in pancreatic tissues between patients with HD and control [430,431]. Metformin has been used in clinical settings to treat HD patients and showed that HD patients with T2DM showed improved cognitive results compared to HD patients that do not have T2DM [391]. In contrast, non-HD T2DM patients that took metformin performed worse on cognitive tests compared with non-diabetic controls [432]. The improvement of cognitive functionality may only be due to recovery of T2DM and not solely HD itself. Furthermore, the mutation in the HTT gene in those with HD has been found to cause abnormal activation of AMPKα1 under oxidative stress causing neurotoxicity to surrounding neurons [433]. This would explain why taking metformin worsened cognitive functionality of HD patients, contributing to increased neurotoxicity and neurodegeneration. Taken together,

metformin has shown poor influence in restoring cognitive functionality in patients with HD and may even worsen this condition. Thus, metformin should be used with caution and best be avoided when dealing with HD. This signifies that metformin has limited influence on restoring cognitive functionality in patients with HD.

Metformin and multiple sclerosis

Multiple sclerosis (MS) is a chronic demyelination of the neurons in the central nervous system (CNS) caused by the immune system [434]. Progression of MS has been shown to affect the normal functionality of B and T cells through the dysfunction of regulatory T cells (TR) [435–439]. Th17 cells secrete pro-inflammatory cytokines such as IL-17A, IL-17F, IL-21 and tumor necrosis factor α (TNF-α) and through infiltration of the CNS can disrupt the blood brain barrier (BBB) [440,441]. Metformin has shown therapeutic effect on animal model of systemic lupus erythematosus (SLE), an autoimmune disease of the skin, through inhibition of oxidative phosphorylation (OXPHOS) and lowering the $CD4^+$ T cell activation [442]. Metformin has shown to have a positive effect on fibrinolysis and thus regulate neuroinflammation [443–445]. Research has also found that metformin reduces platelet aggregation, lowers platelet adhesion, and decreases adenosine diphosphate (ADP) induced adhesion to fibrinogen [434,446]. A study has shown that elevating AMPK expression can affect the expression of IFN-γ and IL-17 [447]. Furthermore, TR has shown increased expression in metformin supplemented patients [447]. Increased TR can suppress inflammation in those with MS which can reduce swelling and help reduce damage to adjacent undamaged neurons thereby reducing size of the brain lesions. This was reflected in clinical research where patients given 6–24 months of treatment using metformin showed significant reduction in the number of new or expanding T2 brain lesions [434]. Metformin activating AMPK can also affect neurogenesis and may be able to repair damaged neurons caused by MS [448]. In recent research metformin was found to restore regenerative ability of oligodendrocyte progenitor cells which play important roles in remyelination processes which become impaired in MS [373,449]. Taken together, metformin has shown to have many benefits in treating patients with MS with little adverse effects. Treatment of MS using metformin may also be considered for combination drug therapy as it has shown to have potential in this aspect.

Metformin and other neurological disorders

Metformin and epilepsy

Epilepsy is a neurological disorder that causes uncontrolled involuntary spasms. It is characterized by hyper-excitatory seizures caused by imbalance of excitatory and inhibitory neurons [450]. Progression of epilepsy has been shown to cause neuronal loss in the brain due to excitotoxicity [451,452]. The overall mechanism of epilepsy is still poorly understood; however, it is accepted that the spontaneous discharge of epilepsy is caused by either the increased excitability of excitatory neurons or decreased excitability of inhibitory neurons [453]. In animal models, metformin was found anti-seizure properties. In one experiment metformin was able to attenuate pentylenetetrazol (PTZ) induced seizures in mice and increase the latency to seizure onset while decreasing seizure duration, suggesting that metformin may modulate epileptogenesis through ameliorating the expression of C/EBP homologous protein and suppress the endoplasmic reticulum (ER) stress through AMPK-PI3K-c-Jun kinase pathway and thereby protect against BBB disruption and reduce neuronal apoptosis [454]. Furthermore, administration of metformin was able to lower the seizure stage, suppress kindle seizure progression, and protect against learning memory impairment in PTZ kindled mice model [455]. Metformin has also demonstrated anti-seizure effect on kainic acid (KA) induced chronic seizures, shortening epilepsy duration, suggesting that AMPK activation may play an important role in seizure regulation [456]. A study showed that AMPK activation can upregulate Bcl-2-modifying factor (Bmf), which can alleviate seizure-induced cell death in the hippocampus, suggesting that metformin may also impact seizure progression through this pathway [457]. Evaluation of metformin's effect on epilepsy has also found that seizure progression was reduced in response to metformin administration 45 days after induction of status epilepticus (SE) in rats [458]. Furthermore, another study found that treatment with metformin decreased levels of phosphorylated mTOR protein and elevated levels of phosphorylated AMPK protein in pilocarpine induced epileptic mice model [458]. Metformin treatment has also been found to suppress the expression of BDNF and its receptor, trkB, which has been reported to be elevated in brains of epileptic mice [458,459]. In clinical studies, 500 mg of metformin was given orally to patients with tuberous sclerosis, and the seizure frequency was found to decrease by 44% as opposed to the placebo group [460]. In patients with Lafora disease, the patients who were given metformin showed a reduction in seizure frequency, however, there was lack of clinical response in late stages of the disease [461]. This is probably due to the increased threshold required to mitigate the late-stage seizures. The use of metformin in treating seizures may be applied in early stages of the disease to prevent seizure progression. In late-stage seizures, metformin may be combined with

other seizure medication to help alleviate different adverse effect while maintaining anti-seizure effects. Taken together, metformin has shown great potential in treating epilepsy and diseases that may result in epilepsy.

Metformin and depression

Major depressive disorder (MDD) is a complex neurological disorder that is characterized by lack of energy, dysfunction of cognition and memory [462]. The pathology of MDD is still unclear, it is often associated with chronic stress resulting from either environmental or physiologic factors [463]. Imbalance of excitatory and inhibitory (EI) inputs were found in the brain of depressed mice model [464]. Furthermore, MDD were found to have altered synaptic signaling due to structural alterations in both hippocampus and the prefrontal cortex (PFC) and decrease of both glutamate and GABA neurotransmitters [465]. Disruption of glutamate excitatory projection neurons which are responsible for circuit level transmissions in the brain region may cause structural changes in the brain [465]. GABAergic functionality is also disrupted in MDD resulting in alteration in synaptic transmission [466–469]. Alterations to serotonergic neuron transmissions are present in those afflicted with MDD [470]. Metformin is able to increase the frequency of excitatory postsynaptic current (EPSC) which may alleviate disruption of depression caused by MDD [471]. Furthermore, metformin can also stimulate serotoninergic neurons excitability and modulate serotonergic transmission [472,473]. Regulation of serotonergic neurons signifies that metformin may be able to regulate mood in those afflicted by MDD. In evaluating metformin's effect on T2DM and its associated mood disorders, administration of metformin in T2DM rats showed decreased depressive-like behaviors [474]. Furthermore, it was found that metformin was able to promote neurogenesis in mice and humans [475]. In clinical settings, metformin was found to have antidepressant effects in diabetic patients through improving their cognitive functions [476]. Metformin's activation of AMPK can increase neuronal activity, which shows anti-depression effect similar with physical exercise ameliorating depressive-like behaviors via AMPK, although this mechanism remains elusive [477]. In a case study, metformin was found to lower the diabetic patient's risk of developing MDD [478]. This showed that metformin can counteract diabetic related depression through increasing metabolism. Furthermore, metformin's increase of neuronal activity and generation of ATP may be a contributing factor. Metformin has demonstrated potential in treating depression in T2DM, however, whether metformin is able to treat patients that do not have T2DM remains to be seen.

Metformin and stroke

Stroke is a common neurological disease that is caused by cardiac dysfunction that can lead to cerebral ischemia [479]. Ischemia results in mitochondrial deficits, oxidative stress, disruption of the BBB, increased neuron apoptosis, and neuroinflammation [479]. Metformin may be able to activate M2 microglia to promote clearance of cellular debris and heighten the expression of growth factors [479–481]. It is also able to further activate anti-inflammatory molecules such as interleukin-4, 10, and TGF-β [479–481]. These activated M2 microglia are neuroprotective in response to ischemic injury, however, they are temporary, and will revert back to M1 phenotype [481]. In an earlier study, metformin upregulated AMK and released elevated levels of interleukin-10 and anti-inflammatory cytokine [482]. Administration of metformin for 30 days showed improvement in neurogenesis, angiogenesis, and functional recovery in mice [482]. This was further supported in another study, where rats receiving metformin prior to stroke induction showed that quantity of viable neurons was significantly higher in metformin pre-treatment group compared with the control group [483]. Metformin treated group also showed increased autophagic response through AMPK activation which can help reduce cellular protein accumulation and thereby improve recovery [483,484]. Although there are animal studies of using metformin in treating, it is still in its infancy. Although pretreatment with metformin may reduce the risk of stroke, its clinical data are still limited. A follow-up clinical study in diabetic patients prescribed with metformin show that metformin can lower patient's risk of stroke by almost 50% [485]. In light of this, there is still a lot of clinical research that needs to be done for evaluating the efficacy of metformin in stroke. Overall, metformin's effect of neurogenesis, neuronal protection, and anti-inflammatory effects are undeniable. Metformin's activation of AMPK may also provide energy to neurons to help negate the negative effect induced by the lack of energy due to oxygen deprivation. Therefore, metformin may be a promising candidate in treatment of ischemic stroke.

Future prospects

Metformin is an oral anti-diabetic drug that has shown potential in treating many neurological disorders. It has shown to have potential to have an effect on strokes, seizures, and MS. However, many more clinical tests are needed to ensure their efficacy. The current findings of metformin, mainly show its benefits in alleviating symptoms caused by diabetes, however, its neuroprotection and its effect on neuron recovery are undoable. There are many controversial findings of metformin that suggest that not all of metformin and

AMPK functionalities have been identified. Although there are many findings that metformin may cause the worsening neuro-degenerative diseases through alternative pathways, it has definitely demonstrated its effect in neuron protection and protecting against adverse effects in other medication when used in conjunction making metformin a promising candidate for clinical consideration. As such, more pre-clinical experiments of metformin in treating other neurological disorders such as AD, PD, MDD, and HD should be conducted to ensure its efficacy and its long-term safety to debunk the controversies before clinical trials. Furthermore, these tests should also involve those who possesses these neurological disorders, but not as a result of T2DM. Overall, metformin has shown to have a wide range of beneficial effects on neurological disorders.

Discussion

In this review, we systematically summarize and discuss the development history of metformin, its protective role and molecular mechanism in various diseases, including metabolism and metabolic related diseases, cancer, and the combination strategy, aging and aging related diseases, neurodegenerative diseases, and other neurological disorders. But in view of this, we can not help asking this question: is metformin a drug for all diseases? It is unclear whether all these putative beneficial effects are secondary to its role as an antihyperglycemic and insulin-sensitizing agent, or stem from other cellular effects. Whether metformin can improve these diseases in normal patients instead of diabetics, remains to be further studied. Moreover, approximate 30% of individuals may experience metformin adverse effects, necessitating careful monitoring along with dose and formulation (extended-release versions) adjustments [486]. It is important to pay attention to the concentration and dose of metformin used in preclinical studies, with many of that have employed concentrations in excess of 10 to 1000-fold of maximal plasma levels observed in humans [487]. Therefore, more studies including clinical trials, are required to determine the impact of metformin on multiple diseases and illustrate the indicated mechanisms.

Acknowledgements

This work is founded by China Postdoctoral Science Foundation (No. 2021M703345), the National Natural Science Foundation of China (Nos. 82130099, 81971265, and 81821005), Shanghai Science and Technology Development Funds (Nos. 22YF1457100 and 22ZR1415200), National Key R&D Program of China (No. 2022YFA1303802), the National Natural Science Foundation of China for Innovation Research Group (No. 81821005), the Lingang Laboratory (Nos. LG202103-03-04, LG202103-03-01, and LG202103-03-05).

Compliance with ethics guidelines

Ying Dong, Yingbei Qi, Haowen Jiang, Tian Mi, Yunkai Zhang, Chang Peng, Wanchen Li, Yongmei Zhang, Yubo Zhou, Yi Zang, and Jia Li declare no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee

References

1. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond) 2012; 122(6): 253–270
2. Salpeter SR, Buckley NS, Kahn JA, Salpeter EE. Meta-analysis: metformin treatment in persons at risk for diabetes mellitus. Am J Med 2008; 121(2): 149–157.e2
3. Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B. Metformin: from mechanisms of action to therapies. Cell Metab 2014; 20(6): 953–966
4. Carling D. AMPK signalling in health and disease. Curr Opin Cell Biol 2017; 45: 31–37
5. Bailey CJ. Metformin: historical overview. Diabetologia 2017; 60(9): 1566–1576
6. Bailey CJ, Day C. Metformin: its botanical background. Pract Diabetes Int 2004; 21(3): 115–117
7. Hill J. The Vegetable System, or, The Internal Structure and The Life of Plants. London: the author, 1761–1775
8. Watanabe CK. Studies in the metabolic changes induced by administration of guanidine bases. J Biol Chem 1918; 34(1): 51–63
9. Ríos JL, Francini F, Schinella GR. Natural products for the treatment of type 2 diabetes mellitus. Planta Med 2015; 81(12–13): 975–994
10. Pineda CT, Ramanathan S, Fon Tacer K, Weon JL, Potts MB, Ou YH, White MA, Potts PR. Degradation of AMPK by a cancer-specific ubiquitin ligase. Cell 2015; 160(4): 715–728
11. Wang GS, Hoyte C. Review of biguanide (metformin) toxicity. J Intensive Care Med 2019; 34(11–12): 863–876
12. Rabinowitch IM. Observations on the use of synthalin in the treatment of diabetes mellitus. Can Med Assoc J 1927; 17(8): 901–904
13. Slotta KH. Tschesche RJEJoIC. Über Biguanide, II: Die blutzucker-senkende Wirkung der Biguanide. 1929; 62: 1398–1405
14. Hesse E. Taubmann GJN-SAfePuP. Die Wirkung des Biguanids und seiner Derivate auf den Zuckerstoffwechsel. 1929; 142: 290–308
15. Bailey CJ, Turner RC. Metformin. N Engl J Med 1996; 334(9): 574–579
16. Werner EA, Bell J. CCXIV—The preparation of methylguanidine, and of ββ-dimethylguanidine by the interaction of dicyanodiamide, and methylammonium and dimethylammonium

chlorides respectively. J Chem Soc Trans 1922; 121(0): 1790–1794
17. Sylow L, Kleinert M, Richter EA, Jensen TE. Exercise-stimulated glucose uptake—regulation and implications for glycaemic control. Nat Rev Endocrinol 2017; 13(3): 133–148
18. Samson SL, Garber AJ. Metformin and other biguanides: pharmacology and therapeutic usage. International Textbook of Diabetes Mellitus. 2015. 641–656
19. Meinert CL. Clinical Trials: Design, Conduct and Analysis. Oxford University Press, 1986
20. Schäfer G. Biguanides. A review of history, pharmacodynamics and therapy. Diabete Metab 1983; 9(2): 148–163
21. LaMoia TE, Shulman GI. Cellular and molecular mechanisms of metformin action. Endocr Rev 2021; 42(1): 77–96
22. GARCIA EY. Flumamine, a new synthetic analgesic and anti-flu drug. J Philipp Med Assoc 1950; 26(7): 287–293
23. Curd FHS, Davey DG, Rose FL. Studies on synthetic antimalarial drugs; some biguanide derivatives as new types of antimalarial substances with both therapeutic and causal prophylactic activity. Ann Trop Med Parasitol 1945; 39(3–4): 208–216
24. Sterne JJMM. Du nouveau dans les antidiabetiques. La NN dimethylamine guanyl guanide (NNDG). 1957; 36: 1295–1296
25. Sterne J. Blood sugar-lowering effect of 1,1-dimethylbiguanide. Therapie 1958; 13(4): 650–659 (in French)
26. Beringer A. Treatment of diabetes mellitus with biguanides. Wien Med Wochenschr 1958; 108(43): 880–882
27. Woods A, Vertommen D, Neumann D, Turk R, Bayliss J, Schlattner U, Wallimann T, Carling D, Rider MH. Identification of phosphorylation sites in AMP-activated protein kinase (AMPK) for upstream AMPK kinases and study of their roles by site-directed mutagenesis. J Biol Chem 2003; 278(31): 28434–28442
28. McKENDRY JB, Kuwayti K, Rado PP. Clinical experience with DBI (phenformin) in the management of diabetes. Can Med Assoc J 1959; 80(10): 773–778
29. Ungar G, Freedman L, Shapiro SL. Pharmacological studies of a new oral hypoglycemic drug. Proc Soc Exp Biol Med 1957; 95(1): 190–192
30. King P, Peacock I, Donnelly R. The UK prospective diabetes study (UKPDS): clinical and therapeutic implications for type 2 diabetes. Br J Clin Pharmacol 1999; 48(5): 643–648
31. Turner RC. The U. K. prospective diabetes study. A review. Diabetes Care 1998; 21(Suppl 3): C35–C38
32. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008; 359(15): 1577–1589
33. Lund SS, Rossing P, Vaag AA. Follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2009; 360(4): 416–418
34. Kumar P, Khan K. Effects of metformin use in pregnant patients with polycystic ovary syndrome. J Hum Reprod Sci 2012; 5(2): 166–169
35. Ghazeeri GS, Nassar AH, Younes Z, Awwad JT. Pregnancy outcomes and the effect of metformin treatment in women with polycystic ovary syndrome: an overview. Acta Obstet Gynecol Scand 2012; 91(6): 658–678
36. Nicholson W, Bolen S, Witkop CT, Neale D, Wilson L, Bass E. Benefits and risks of oral diabetes agents compared with insulin in women with gestational diabetes: a systematic review. Obstet Gynecol 2009; 113(1): 193–205
37. Choi YJ. Efficacy of adjunctive treatments added to olanzapine or clozapine for weight control in patients with schizophrenia: a systematic review and meta-analysis. ScientificWorldJournal 2015; 2015: 970730
38. Campbell JM, Stephenson MD, de Courten B, Chapman I, Bellman SM, Aromataris E. Metformin use associated with reduced risk of dementia in patients with diabetes: a systematic review and meta-analysis. J Alzheimers Dis 2018; 65(4): 1225–1236
39. Yang Y. Metformin for cancer prevention. Front Med 2011; 5(2): 115–117
40. Bailey CJ, Day C. Traditional plant medicines as treatments for diabetes. Diabetes Care 1989; 12(8): 553–564
41. Kato T, Kondo T, Mizuno K. Occurrence of guanidino compounds in several plants. Soil Sci Plant Nutr 1986; 32(3): 487–491
42. Rathke B. Ueber biguanid. Ber Dtsch Chem Ges 1879; 12(1): 776–784
43. Metformin (Glucophage(R)). Mother To Baby | Fact Sheets. Brentwood: Organization of Teratology Information Specialists (OTIS). Copyright by OTIS. January 2022
44. DeFronzo RA, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. N Engl J Med 1995; 333(9): 541–549
45. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002; 346(6): 393–403
46. The Selection and Use of Essential Medicines. World Health Organ Tech Rep Ser 2015; vii-xv: 1–546
47. Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, Furlong TJ, Greenfield JR, Greenup LC, Kirkpatrick CM, Ray JE, Timmins P, Williams KM. Clinical pharmacokinetics of metformin. Clin Pharmacokinet 2011; 50(2): 81–98
48. Pentikäinen PJ, Neuvonen PJ, Penttilä A. Pharmacokinetics of metformin after intravenous and oral administration to man. Eur J Clin Pharmacol 1979; 16(3): 195–202
49. Idkaidek N, Arafat T. Metformin IR versus XR pharmacokinetics in humans. J Bioequiv Availab 2011; 3: 233–235
50. Harahap Y, Purnasari S, Hayun H, Dianpratami K, Wulandari M. Bioequivalence Study of Metformin HCl XR Caplet Formulations in Healthy Indonesian Volunteers. J Bioequiv Availab 2011; 3: 16–19
51. Oefelein MG, Tong W, Kerr S, Bhasi K, Patel RK, Yu D. Effect of concomitant administration of trospium chloride extended release on the steady-state pharmacokinetics of metformin in healthy adults. Clin Drug Investig 2013; 33(2): 123–131
52. Timmins P, Donahue S, Meeker J, Marathe P. Steady-state pharmacokinetics of a novel extended-release metformin formulation. Clin Pharmacokinet 2005; 44(7): 721–729
53. Rhee SJ, Lee S, Yoon SH, Cho JY, Jang IJ, Yu KS. Pharmacokinetics of the evogliptin/metformin extended-release (5/1,000 mg) fixed-dose combination formulation compared to the corresponding loose combination, and food effect in healthy subjects. Drug Des Devel Ther 2016; 10: 1411–1418
54. Zhou M, Xia L, Wang J. Metformin transport by a newly cloned

proton-stimulated organic cation transporter (plasma membrane monoamine transporter) expressed in human intestine. Drug Metab Dispos 2007; 35(10): 1956–1962
55. Kawoosa F, Shah ZA, Masoodi SR, Amin A, Rasool R, Fazili KM, Dar AH, Lone A, Ul Bashir S. Role of human organic cation transporter-1 (OCT-1/SLC22A1) in modulating the response to metformin in patients with type 2 diabetes. BMC Endocr Disord 2022; 22(1): 140
56. Shu Y, Sheardown SA, Brown C, Owen RP, Zhang S, Castro RA, Ianculescu AG, Yue L, Lo JC, Burchard EG, Brett CM, Giacomini KM. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. J Clin Invest 2007; 117(5): 1422–1431
57. Christensen MMH, Højlund K, Hother-Nielsen O, Stage TB, Damkier P, Beck-Nielsen H, Brøsen K. Steady-state pharmacokinetics of metformin is independent of the OCT1 genotype in healthy volunteers. Eur J Clin Pharmacol 2015; 71(6): 691–697
58. Chen EC, Liang X, Yee SW, Geier EG, Stocker SL, Chen L, Giacomini KM. Targeted disruption of organic cation transporter 3 attenuates the pharmacologic response to metformin. Mol Pharmacol 2015; 88(1): 75–83
59. Lee N, Hebert MF, Wagner DJ, Easterling TR, Liang CJ, Rice K, Wang J. Organic cation transporter 3 facilitates fetal exposure to metformin during pregnancy. Mol Pharmacol 2018; 94(4): 1125–1131
60. Chen L, Shu Y, Liang X, Chen EC, Yee SW, Zur AA, Li S, Xu L, Keshari KR, Lin MJ, Chien HC, Zhang Y, Morrissey KM, Liu J, Ostrem J, Younger NS, Kurhanewicz J, Shokat KM, Ashrafi K, Giacomini KM. OCT1 is a high-capacity thiamine transporter that regulates hepatic steatosis and is a target of metformin. Proc Natl Acad Sci USA 2014; 111(27): 9983–9988
61. Müller J, Lips KS, Metzner L, Neubert RH, Koepsell H, Brandsch M. Drug specificity and intestinal membrane localization of human organic cation transporters (OCT). Biochem Pharmacol 2005; 70(12): 1851–1860
62. Nakamichi N, Shima H, Asano S, Ishimoto T, Sugiura T, Matsubara K, Kusuhara H, Sugiyama Y, Sai Y, Miyamoto K, Tsuji A, Kato Y. Involvement of carnitine/organic cation transporter OCTN1/SLC22A4 in gastrointestinal absorption of metformin. J Pharm Sci 2013; 102(9): 3407–3417
63. Takane H, Shikata E, Otsubo K, Higuchi S, Ieiri I. Polymorphism in human organic cation transporters and metformin action. Pharmacogenomics 2008; 9(4): 415–422
64. Yoon H, Cho HY, Yoo HD, Kim SM, Lee YB. Influences of organic cation transporter polymorphisms on the population pharmacokinetics of metformin in healthy subjects. AAPS J 2013; 15(2): 571–580
65. Liang X, Chien HC, Yee SW, Giacomini MM, Chen EC, Piao M, Hao J, Twelves J, Lepist EI, Ray AS, Giacomini KM. Metformin is a substrate and inhibitor of the human thiamine transporter, THTR-2 (SLC19A3). Mol Pharm 2015; 12(12): 4301–4310
66. Han TK, Proctor WR, Costales CL, Cai H, Everett RS, Thakker DR. Four cation-selective transporters contribute to apical uptake and accumulation of metformin in Caco-2 cell monolayers. J Pharmacol Exp Ther 2015; 352(3): 519–528
67. Kurlovics J, Zake DM, Zaharenko L, Berzins K, Klovins J, Stalidzans E. Metformin transport rates between plasma and red blood cells in humans. Clin Pharmacokinet 2022; 61(1): 133–142
68. Markowicz-Piasecka M, Huttunen KM, Mateusiak L, Mikiciuk-Olasik E, Sikora J. Is metformin a perfect drug? Updates in pharmacokinetics and pharmacodynamics Curr Pharm Des 2017; 23(17): 2532–2550
69. Scheen AJ. Clinical pharmacokinetics of metformin. Clin Pharmacokinet 1996; 30(5): 359–371
70. Song R. Mechanism of metformin: a tale of two sites. Diabetes Care 2016; 39(2): 187–189
71. Lee N, Duan H, Hebert MF, Liang CJ, Rice KM, Wang J. Taste of a pill: organic cation transporter-3 (OCT3) mediates metformin accumulation and secretion in salivary glands. J Biol Chem 2014; 289(39): 27055–27064
72. Hibma JE, Zur AA, Castro RA, Wittwer MB, Keizer RJ, Yee SW, Goswami S, Stocker SL, Zhang X, Huang Y, Brett CM, Savic RM, Giacomini KM. The effect of famotidine, a MATE1-selective inhibitor, on the pharmacokinetics and pharmacodynamics of metformin. Clin Pharmacokinet 2016; 55(6): 711–721
73. Posma RA, Venema LH, Huijink TM, Westerkamp AC, Wessels AMA, De Vries NJ, Doesburg F, Roggeveld J, Ottens PJ, Touw DJ, Nijsten MW, Leuvenink HGD. Increasing metformin concentrations and its excretion in both rat and porcine *ex vivo* normothermic kidney perfusion model. BMJ Open Diabetes Res Care 2020; 8: e000816
74. Ma YR, Zhou Y, Huang J, Qin HY, Wang P, Wu XA. The urinary excretion of metformin, ceftizoxime and ofloxacin in high serum creatinine rats: can creatinine predict renal tubular elimination? Life Sci 2018; 196: 110–117
75. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics 2012; 22(11): 820–827
76. McCreight LJ, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract. Diabetologia 2016; 59(3): 426–435
77. Sirtori CR, Franceschini G, Galli-Kienle M, Cighetti G, Galli G, Bondioli A, Conti F. Disposition of metformin (N,N-dimethylbiguanide) in man. Clin Pharmacol Ther 1978; 24(6): 683–693
78. Tucker GT, Casey C, Phillips PJ, Connor H, Ward JD, Woods HF. Metformin kinetics in healthy subjects and in patients with diabetes mellitus. Br J Clin Pharmacol 1981; 12(2): 235–246
79. Szymczak-Pajor I, Wenclewska S, Śliwińska A. Metabolic action of metformin. Pharmaceuticals (Basel) 2022; 15(7): 810
80. He L, Wondisford FE. Metformin action: concentrations matter. Cell Metab 2015; 21(2): 159–162
81. Ma T, Tian X, Zhang B, Li M, Wang Y, Yang C, Wu J, Wei X, Qu Q, Yu Y, Long S, Feng JW, Li C, Zhang C, Xie C, Wu Y, Xu Z, Chen J, Yu Y, Huang X, He Y, Yao L, Zhang L, Zhu M, Wang W, Wang ZC, Zhang M, Bao Y, Jia W, Lin SY, Ye Z, Piao HL, Deng X, Zhang CS, Lin SC. Low-dose metformin targets the lysosomal AMPK pathway through PEN2. Nature 2022; 603(7899): 159–165
82. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia 2017; 60(9): 1577–1585
83. Saely CH, Geiger K, Drexel H. Brown versus white adipose tissue: a mini-review. Gerontology 2012; 58(1): 15–23
84. Abdullahi A, Jeschke MG. Taming the flames: targeting white adipose tissue browning in hypermetabolic conditions. Endocr

Rev 2017; 38(6): 538–549
85. Breining P, Jensen JB, Sundelin EI, Gormsen LC, Jakobsen S, Busk M, Rolighed L, Bross P, Fernandez-Guerra P, Markussen LK, Rasmussen NE, Hansen JB, Pedersen SB, Richelsen B, Jessen N. Metformin targets brown adipose tissue *in vivo* and reduces oxygen consumption *in vitro*. Diabetes Obes Metab 2018; 20(9): 2264–2273
86. Virtanen KA, Hällsten K, Parkkola R, Janatuinen T, Lönnqvist F, Viljanen T, Rönnemaa T, Knuuti J, Huupponen R, Lönnroth P, Nuutila P. Differential effects of rosiglitazone and metformin on adipose tissue distribution and glucose uptake in type 2 diabetic subjects. Diabetes 2003; 52(2): 283–290
87. Karise I, Bargut TC, Del Sol M, Aguila MB, Mandarim-de-Lacerda CA. Metformin enhances mitochondrial biogenesis and thermogenesis in brown adipocytes of mice. Biomed Pharmacother 2019; 111: 1156–1165
88. Çakır I, Hadley CK, Pan PL, Bagchi RA, Ghamari-Langroudi M, Porter DT, Wang Q, Litt MJ, Jana S, Hagen S, Lee P, White A, Lin JD, McKinsey TA, Cone RD. Histone deacetylase 6 inhibition restores leptin sensitivity and reduces obesity. Nat Metab 2022; 4(1): 44–59
89. Tokubuchi I, Tajiri Y, Iwata S, Hara K, Wada N, Hashinaga T, Nakayama H, Mifune H, Yamada K. Beneficial effects of metformin on energy metabolism and visceral fat volume through a possible mechanism of fatty acid oxidation in human subjects and rats. PLoS One 2017; 12(2): e0171293
90. Hu Y, Young AJ, Ehli EA, Nowotny D, Davies PS, Droke EA, Soundy TJ, Davies GE. Metformin and berberine prevent olanzapine-induced weight gain in rats. PLoS One 2014; 9(3): e93310
91. Qi T, Chen Y, Li H, Pei Y, Woo SL, Guo X, Zhao J, Qian X, Awika J, Huo Y, Wu C. A role for *PFKFB3*/iPFK2 in metformin suppression of adipocyte inflammatory responses. J Mol Endocrinol 2017; 59(1): 49–59
92. Jing Y, Wu F, Li D, Yang L, Li Q, Li R. Metformin improves obesity-associated inflammation by altering macrophages polarization. Mol Cell Endocrinol 2018; 461: 256–264
93. Marcelin G, Gautier EL, Clément K. Adipose tissue fibrosis in obesity: etiology and challenges. Annu Rev Physiol 2022; 84(1): 135–155
94. Luo T, Nocon A, Fry J, Sherban A, Rui X, Jiang B, Xu XJ, Han J, Yan Y, Yang Q, Li Q, Zang M. AMPK activation by metformin suppresses abnormal extracellular matrix remodeling in adipose tissue and ameliorates insulin resistance in obesity. Diabetes 2016; 65(8): 2295–2310
95. Waki H, Tontonoz P. Endocrine functions of adipose tissue. Annu Rev Pathol 2007; 2(1): 31–56
96. Naghiaee Y, Didehdar R, Pourrajab F, Rahmanian M, Heiranizadeh N, Mohiti A, Mohiti-Ardakani J. Metformin downregulates miR223 expression in insulin-resistant 3T3L1 cells and human diabetic adipose tissue. Endocrine 2020; 70(3): 498–508
97. Cruciani S, Garroni G, Balzano F, Pala R, Bellu E, Cossu ML, Ginesu GC, Ventura C, Maioli M. Tuning adipogenic differentiation in ADSCs by metformin and vitamin D: involvement of miRNAs. Int J Mol Sci 2020; 21(17): 6181
98. 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(8): 1167–1174
99. 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(5754): 1642–1646
100. Fullerton MD, Galic S, Marcinko K, Sikkema S, Pulinilkunnil T, Chen ZP, O'Neill HM, Ford RJ, Palanivel R, O'Brien M, Hardie DG, Macaulay SL, Schertzer JD, Dyck JR, van Denderen BJ, Kemp BE, Steinberg GR. Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. Nat Med 2013; 19(12): 1649–1654
101. Bonora E, Cigolini M, Bosello O, Zancanaro C, Capretti L, Zavaroni I, Coscelli C, Butturini U. Lack of effect of intravenous metformin on plasma concentrations of glucose, insulin, C-peptide, glucagon and growth hormone in non-diabetic subjects. Curr Med Res Opin 1984; 9(1): 47–51
102. Buse JB, DeFronzo RA, Rosenstock J, Kim T, Burns C, Skare S, Baron A, Fineman M. The primary glucose-lowering effect of metformin resides in the gut, not the circulation: results from short-term pharmacokinetic and 12-week dose-ranging studies. Diabetes Care 2016; 39(2): 198–205
103. Lee H, Ko G. Effect of metformin on metabolic improvement and gut microbiota. Appl Environ Microbiol 2014; 80(19): 5935–5943
104. Shin NR, Lee JC, Lee HY, Kim MS, Whon TW, Lee MS, Bae JW. An increase in the *Akkermansia* spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. Gut 2014; 63(5): 727–735
105. Fu X, Wang X, Duan Z, Zhang C, Fu X, Yang J, Liu X, He J. Histone H3k9 and H3k27 acetylation regulates IL-4/STAT6-mediated Igε transcription in B lymphocytes. Anat Rec (Hoboken) 2015; 298(8): 1431–1439
106. Karlsson FH, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, Nielsen J, Bäckhed F. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 2013; 498(7452): 99–103
107. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S, Prifti E, Vieira-Silva S, Gudmundsdottir V, Pedersen HK, Arumugam M, Kristiansen K, Voigt AY, Vestergaard H, Hercog R, Costea PI, Kultima JR, Li J, Jørgensen T, Levenez F, Dore J; MetaHIT consortium; Nielsen HB, Brunak S, Raes J, Hansen T, Wang J, Ehrlich SD, Bork P, Pedersen O. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature 2015; 528(7581): 262–266
108. Mueller NT, Differding MK, Zhang M, Maruthur NM, Juraschek SP, Miller ER 3rd, Appel LJ, Yeh HC. Metformin affects gut microbiome composition and function and circulating short-chain fatty acids: a randomized trial. Diabetes Care 2021; 44(7): 1462–1471
109. Cabreiro F, Au C, Leung KY, Vergara-Irigaray N, Cochemé HM, Noori T, Weinkove D, Schuster E, Greene ND, Gems D. Metformin retards aging in *C. elegans* by altering microbial folate and methionine metabolism. Cell 2013; 153(1): 228–239
110. Bauer PV, Duca FA, Waise TMZ, Rasmussen BA, Abraham MA, Dranse HJ, Puri A, O'Brien CA, Lam TKT. Metformin alters upper small intestinal microbiota that impact a glucose-SGLT1-

sensing glucoregulatory pathway. Cell Metab 2018; 27(1): 101–117.e5

111. Pryor R, Norvaisas P, Marinos G, Best L, Thingholm LB, Quintaneiro LM, De Haes W, Esser D, Waschina S, Lujan C, Smith RL, Scott TA, Martinez-Martinez D, Woodward O, Bryson K, Laudes M, Lieb W, Houtkooper RH, Franke A, Temmerman L, Bjedov I, Cochemé HM, Kaleta C, Cabreiro F. Host-microbe-drug-nutrient screen identifies bacterial effectors of metformin therapy. Cell 2019; 178(6): 1299–1312.e29
112. Kitabchi AE, Temprosa M, Knowler WC, Kahn SE, Fowler SE, Haffner SM, Andres R, Saudek C, Edelstein SL, Arakaki R, Murphy MB, Shamoon H; Diabetes Prevention Program Research Group. Role of insulin secretion and sensitivity in the evolution of type 2 diabetes in the diabetes prevention program: effects of lifestyle intervention and metformin. Diabetes 2005; 54(8): 2404–2414
113. Hashemitabar M, Bahramzadeh S, Saremy S, Nejaddehbashi F. Glucose plus metformin compared with glucose alone on β-cell function in mouse pancreatic islets. Biomed Rep 2015; 3(5): 721–725
114. Lupi R, Del Guerra S, Tellini C, Giannarelli R, Coppelli A, Lorenzetti M, Carmellini M, Mosca F, Navalesi R, Marchetti P. The biguanide compound metformin prevents desensitization of human pancreatic islets induced by high glucose. Eur J Pharmacol 1999; 364(2–3): 205–209
115. Patanè G, Piro S, Rabuazzo AM, Anello M, Vigneri R, Purrello F. Metformin restores insulin secretion altered by chronic exposure to free fatty acids or high glucose: a direct metformin effect on pancreatic beta-cells. Diabetes 2000; 49(5): 735–740
116. Cen J, Sargsyan E, Forslund A, Bergsten P. Mechanisms of beneficial effects of metformin on fatty acid-treated human islets. J Mol Endocrinol 2018; 61(3): 91–99
117. Moon JS, Karunakaran U, Elumalai S, Lee IK, Lee HW, Kim YW, Won KC. Metformin prevents glucotoxicity by alleviating oxidative and ER stress-induced CD36 expression in pancreatic beta cells. J Diabetes Complications 2017; 31(1): 21–30
118. Liu SN, Liu Q, Sun SJ, Hou SC, Wang Y, Shen ZF. Metformin ameliorates β-cell dysfunction by regulating inflammation production, ion and hormone homeostasis of pancreas in diabetic KKAy mice. Acta Pharmaceutica Sinica (Yao Xue Xue Bao) 2014; 49(11): 1554–1562 (in Chinese)
119. Jiang Y, Huang W, Wang J, Xu Z, He J, Lin X, Zhou Z, Zhang J. Metformin plays a dual role in MIN6 pancreatic β cell function through AMPK-dependent autophagy. Int J Biol Sci 2014; 10(3): 268–277
120. Lablanche S, Cottet-Rousselle C, Lamarche F, Benhamou PY, Halimi S, Leverve X, Fontaine E. Protection of pancreatic INS-1 β-cells from glucose- and fructose-induced cell death by inhibiting mitochondrial permeability transition with cyclosporin A or metformin. Cell Death Dis 2011; 2(3): e134
121. Jung TW, Lee MW, Lee YJ, Kim SM. Metformin prevents endoplasmic reticulum stress-induced apoptosis through AMPK-PI3K-c-Jun NH_2 pathway. Biochem Biophys Res Commun 2012; 417(1): 147–152
122. Lee A, Morley JE. Metformin decreases food consumption and induces weight loss in subjects with obesity with type II non-insulin-dependent diabetes. Obes Res 1998; 6(1): 47–53
123. Paolisso G, Amato L, Eccellente R, Gambardella A, Tagliamonte MR, Varricchio G, Carella C, Giugliano D, D'Onofrio F. Effect of metformin on food intake in obese subjects. Eur J Clin Invest 1998; 28(6): 441–446
124. Glueck CJ, Fontaine RN, Wang P, Subbiah MT, Weber K, Illig E, Streicher P, Sieve-Smith L, Tracy TM, Lang JE, McCullough P. Metformin reduces weight, centripetal obesity, insulin, leptin, and low-density lipoprotein cholesterol in nondiabetic, morbidly obese subjects with body mass index greater than 30. Metabolism 2001; 50(7): 856–861
125. Chau-Van C, Gamba M, Salvi R, Gaillard RC, Pralong FP. Metformin inhibits adenosine 5′-monophosphate-activated kinase activation and prevents increases in neuropeptide Y expression in cultured hypothalamic neurons. Endocrinology 2007; 148(2): 507–511
126. Stevanovic D, Janjetovic K, Misirkic M, Vucicevic L, Sumarac-Dumanovic M, Micic D, Starcevic V, Trajkovic V. Intracerebroventricular administration of metformin inhibits ghrelin-induced hypothalamic AMP-kinase signalling and food intake. Neuroendocrinology 2012; 96(1): 24–31
127. Kim YW, Kim JY, Park YH, Park SY, Won KC, Choi KH, Huh JY, Moon KH. Metformin restores leptin sensitivity in high-fat-fed obese rats with leptin resistance. Diabetes 2006; 55(3): 716–724
128. Aubert G, Mansuy V, Voirol MJ, Pellerin L, Pralong FP. The anorexigenic effects of metformin involve increases in hypothalamic leptin receptor expression. Metabolism 2011; 60(3): 327–334
129. Mullican SE, Lin-Schmidt X, Chin CN, Chavez JA, Furman JL, Armstrong AA, Beck SC, South VJ, Dinh TQ, Cash-Mason TD, Cavanaugh CR, Nelson S, Huang C, Hunter MJ, Rangwala SM. GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates. Nat Med 2017; 23(10): 1150–1157
130. Emmerson PJ, Wang F, Du Y, Liu Q, Pickard RT, Gonciarz MD, Coskun T, Hamang MJ, Sindelar DK, Ballman KK, Foltz LA, Muppidi A, Alsina-Fernandez J, Barnard GC, Tang JX, Liu X, Mao X, Siegel R, Sloan JH, Mitchell PJ, Zhang BB, Gimeno RE, Shan B, Wu X. The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. Nat Med 2017; 23(10): 1215–1219
131. Borner T, Shaulson ED, Ghidewon MY, Barnett AB, Horn CC, Doyle RP, Grill HJ, Hayes MR, De Jonghe BC. GDF15 induces anorexia through nausea and emesis. Cell Metab 2020; 31(2): 351–362.e5
132. Coll AP, Chen M, Taskar P, Rimmington D, Patel S, Tadross JA, Cimino I, Yang M, Welsh P, Virtue S, Goldspink DA, Miedzybrodzka EL, Konopka AR, Esponda RR, Huang JT, Tung YCL, Rodriguez-Cuenca S, Tomaz RA, Harding HP, Melvin A, Yeo GSH, Preiss D, Vidal-Puig A, Vallier L, Nair KS, Wareham NJ, Ron D, Gribble FM, Reimann F, Sattar N, Savage DB, Allan BB, O'Rahilly S. GDF15 mediates the effects of metformin on body weight and energy balance. Nature 2020; 578(7795): 444–448
133. Klein AB, Nicolaisen TS, Johann K, Fritzen AM, Mathiesen CV, Gil C, Pilmark NS, Karstoft K, Blond MB, Quist JS, Seeley RJ, Færch K, Lund J, Kleinert M, Clemmensen C. The GDF15-GFRAL pathway is dispensable for the effects of metformin on energy balance. Cell Rep 2022; 40(8): 111258
134. Day EA, Ford RJ, Smith BK, Mohammadi-Shemirani P, Morrow

MR, Gutgesell RM, Lu R, Raphenya AR, Kabiri M, McArthur AG, McInnes N, Hess S, Paré G, Gerstein HC, Steinberg GR. Metformin-induced increases in GDF15 are important for suppressing appetite and promoting weight loss. Nat Metab 2019; 1(12): 1202–1208

135. American Diabetes Association Professional Practice Committee. 9. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2022. Diabetes Care 2022; 45(Suppl 1): S125–S143
136. Stumvoll M, Häring HU, Matthaei S. Metformin. Endocr Res 2007; 32(1–2): 39–57
137. Wang C, Liu F, Yuan Y, Wu J, Wang H, Zhang L, Hu P, Li Z, Li Q, Ye J. Metformin suppresses lipid accumulation in skeletal muscle by promoting fatty acid oxidation. Clin Lab 2014; 60(6): 887–896
138. Zabielski P, Chacinska M, Charkiewicz K, Baranowski M, Gorski J, Blachnio-Zabielska AU. Effect of metformin on bioactive lipid metabolism in insulin-resistant muscle. J Endocrinol 2017; 233(3): 329–340
139. Pavlovic K, Krako Jakovljevic N, Isakovic AM, Ivanovic T, Markovic I, Lalic NM. Therapeutic vs. supratherapeutical metformin concentrations: different effects on energy metabolism and mitochondrial function in skeletal muscle cells *in vitro*. Front Pharmacol 2022; 13: 930308
140. Malin SK, Stewart NR. Metformin may contribute to inter-individual variability for glycemic responses to exercise. Front Endocrinol (Lausanne) 2020; 11: 519
141. Natali A, Ferrannini E. Effects of metformin and thiazolidinediones on suppression of hepatic glucose production and stimulation of glucose uptake in type 2 diabetes: a systematic review. Diabetologia 2006; 49(3): 434–441
142. Zhou Z, Tang Y, Jin X, Chen C, Lu Y, Liu L, Shen C. Metformin inhibits advanced glycation end products-induced inflammatory response in murine macrophages partly through AMPK activation and RAGE/NFκB pathway suppression. J Diabetes Res 2016; 2016: 4847812
143. He L, Sabet A, Djedjos S, Miller R, Sun X, Hussain MA, Radovick S, Wondisford FE. Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. Cell 2009; 137(4): 635–646
144. Lee JM, Seo WY, Song KH, Chanda D, Kim YD, Kim DK, Lee MW, Ryu D, Kim YH, Noh JR, Lee CH, Chiang JY, Koo SH, Choi HS. AMPK-dependent repression of hepatic gluconeogenesis via disruption of CREB. CRTC2 complex by orphan nuclear receptor small heterodimer partner. J Biol Chem 2010; 285(42): 32182–32191
145. Caton PW, Nayuni NK, Kieswich J, Khan NQ, Yaqoob MM, Corder R. Metformin suppresses hepatic gluconeogenesis through induction of SIRT1 and GCN5. J Endocrinol 2010; 205(1): 97–106
146. Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA, Prigaro BJ, Wood JL, Bhanot S, MacDonald MJ, Jurczak MJ, Camporez JP, Lee HY, Cline GW, Samuel VT, Kibbey RG, Shulman GI. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature 2014; 510(7506): 542–546
147. Lin HZ, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, Diehl AM. Metformin reverses fatty liver disease in obese, leptin-deficient mice. Nat Med 2000; 6(9): 998–1003
148. Woo SL, Xu H, Li H, Zhao Y, Hu X, Zhao J, Guo X, Guo T, Botchlett R, Qi T, Pei Y, Zheng J, Xu Y, An X, Chen L, Chen L, Li Q, Xiao X, Huo Y, Wu C. Metformin ameliorates hepatic steatosis and inflammation without altering adipose phenotype in diet-induced obesity. PLoS One 2014; 9(3): e91111
149. El-Mir MY, Nogueira V, Fontaine E, Avéret N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. J Biol Chem 2000; 275(1): 223–228
150. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J 2000; 348(3): 607–614
151. Stephenne X, Foretz M, Taleux N, van der Zon GC, Sokal E, Hue L, Viollet B, Guigas B. Metformin activates AMP-activated protein kinase in primary human hepatocytes by decreasing cellular energy status. Diabetologia 2011; 54(12): 3101–3110
152. Batandier C, Guigas B, Detaille D, El-Mir MY, Fontaine E, Rigoulet M, Leverve XM. The ROS production induced by a reverse-electron flux at respiratory-chain complex 1 is hampered by metformin. J Bioenerg Biomembr 2006; 38(1): 33–42
153. Fontaine E. Metformin-induced mitochondrial complex I inhibition: facts, uncertainties, and consequences. Front Endocrinol (Lausanne) 2018; 9: 753
154. Pernicova I, Korbonits M. Metformin—mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol 2014; 10(3): 143–156
155. Vancura A, Bu P, Bhagwat M, Zeng J, Vancurova I. Metformin as an anticancer agent. Trends Pharmacol Sci 2018; 39(10): 867–878
156. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. BMJ 2005; 330(7503): 1304–1305
157. Monami M, Colombi C, Balzi D, Dicembrini I, Giannini S, Melani C, Vitale V, Romano D, Barchielli A, Marchionni N, Rotella CM, Mannucci E. Metformin and cancer occurrence in insulin-treated type 2 diabetic patients. Diabetes Care 2011; 34(1): 129–131
158. Kasznicki J, Sliwinska A, Drzewoski J. Metformin in cancer prevention and therapy. Ann Transl Med 2014; 2(6): 57
159. Peng M, Darko KO, Tao T, Huang Y, Su Q, He C, Yin T, Liu Z, Yang X. Combination of metformin with chemotherapeutic drugs via different molecular mechanisms. Cancer Treat Rev 2017; 54: 24–33
160. Wen KC, Sung PL, Wu ATH, Chou PC, Lin JH, Huang CF, Yeung SJ, Lee MH. Neoadjuvant metformin added to conventional chemotherapy synergizes anti-proliferative effects in ovarian cancer. J Ovarian Res 2020; 13(1): 95
161. Zhang HH, Guo XL. Combinational strategies of metformin and chemotherapy in cancers. Cancer Chemother Pharmacol 2016; 78(1): 13–26
162. Mallik R, Chowdhury TA. Metformin in cancer. Diabetes Res Clin Pract 2018; 143: 409–419
163. Skuli SJ, Alomari S, Gaitsch H, Bakayoko A, Skuli N, Tyler BM. Metformin and cancer, an ambiguanidous relationship. Pharmaceuticals (Basel) 2022; 15(5): 626
164. Heckman-Stoddard BM, DeCensi A, Sahasrabuddhe VV, Ford

LG. Repurposing metformin for the prevention of cancer and cancer recurrence. Diabetologia 2017; 60(9): 1639–1647
165. Long YC, Zierath JR. AMP-activated protein kinase signaling in metabolic regulation. J Clin Invest 2006; 116(7): 1776–1783
166. Huang X, Wullschleger S, Shpiro N, McGuire VA, Sakamoto K, Woods YL, McBurnie W, Fleming S, Alessi DR. Important role of the LKB1-AMPK pathway in suppressing tumorigenesis in PTEN-deficient mice. Biochem J 2008; 412(2): 211–221
167. Zakikhani M, Dowling R, Fantus IG, Sonenberg N, Pollak M. Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. Cancer Res 2006; 66(21): 10269–10273
168. Gao C, Fang L, Zhang H, Zhang WS, Li XO, Du SY. Metformin induces autophagy via the AMPK-mTOR signaling pathway in human hepatocellular carcinoma cells. Cancer Manag Res 2020; 12: 5803–5811
169. Dowling RJ, Zakikhani M, Fantus IG, Pollak M, Sonenberg N. Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. Cancer Res 2007; 67(22): 10804–10812
170. Shen P, Reineke LC, Knutsen E, Chen M, Pichler M, Ling H, Calin GA. Metformin blocks MYC protein synthesis in colorectal cancer via mTOR-4EBP-eIF4E and MNK1-eIF4G-eIF4E signaling. Mol Oncol 2018; 12(11): 1856–1870
171. Wang Y, Xu W, Yan Z, Zhao W, Mi J, Li J, Yan H. Metformin induces autophagy and G0/G1 phase cell cycle arrest in myeloma by targeting the AMPK/mTORC1 and mTORC2 pathways. J Exp Clin Cancer Res 2018; 37(1): 63
172. Lu CC, Chiang JH, Tsai FJ, Hsu YM, Juan YN, Yang JS, Chiu HY. Metformin triggers the intrinsic apoptotic response in human AGS gastric adenocarcinoma cells by activating AMPK and suppressing mTOR/AKT signaling. Int J Oncol 2019; 54(4): 1271–1281
173. Chen YH, Yang SF, Yang CK, Tsai HD, Chen TH, Chou MC, Hsiao YH. Metformin induces apoptosis and inhibits migration by activating the AMPK/p53 axis and suppressing PI3K/AKT signaling in human cervical cancer cells. Mol Med Rep 2021; 23(1): 88
174. Sun Y, Tao C, Huang X, He H, Shi H, Zhang Q, Wu H. Metformin induces apoptosis of human hepatocellular carcinoma HepG2 cells by activating an AMPK/p53/miR-23a/FOXA1 pathway. Onco Targets Ther 2016; 9: 2845–2853
175. Kim HG, Hien TT, Han EH, Hwang YP, Choi JH, Kang KW, Kwon KI, Kim BH, Kim SK, Song GY, Jeong TC, Jeong HG. Metformin inhibits P-glycoprotein expression via the NF-κB pathway and CRE transcriptional activity through AMPK activation. Br J Pharmacol 2011; 162(5): 1096–1108
176. Zheng L, Yang W, Wu F, Wang C, Yu L, Tang L, Qiu B, Li Y, Guo L, Wu M, Feng G, Zou D, Wang H. Prognostic significance of AMPK activation and therapeutic effects of metformin in hepatocellular carcinoma. Clin Cancer Res 2013; 19(19): 5372–5380
177. Zheng Z, Bian Y, Zhang Y, Ren G, Li G. Metformin activates AMPK/SIRT1/NF-κB pathway and induces mitochondrial dysfunction to drive caspase3/GSDME-mediated cancer cell pyroptosis. Cell Cycle 2020; 19(10): 1089–1104
178. Dong Y, Hu H, Zhang X, Zhang Y, Sun X, Wang H, Kan W, Tan MJ, Shi H, Zang Y, Li J. Phosphorylation of PHF2 by AMPK releases the repressive H3K9me2 and inhibits cancer metastasis. Signal Transduct Target Ther 2023; 8(1): 95
179. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004; 350(21): 2129–2139
180. Isoda K, Young JL, Zirlik A, MacFarlane LA, Tsuboi N, Gerdes N, Schönbeck U, Libby P. Metformin inhibits proinflammatory responses and nuclear factor-kappaB in human vascular wall cells. Arterioscler Thromb Vasc Biol 2006; 26(3): 611–617
181. Hattori Y, Suzuki K, Hattori S, Kasai K. Metformin inhibits cytokine-induced nuclear factor kappaB activation via AMP-activated protein kinase activation in vascular endothelial cells. Hypertension 2006; 47(6): 1183–1188
182. Guo Q, Liu Z, Jiang L, Liu M, Ma J, Yang C, Han L, Nan K, Liang X. Metformin inhibits growth of human non-small cell lung cancer cells via liver kinase B-1-independent activation of adenosine monophosphate-activated protein kinase. Mol Med Rep 2016; 13(3): 2590–2596
183. Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z, Dupuy F, Chambers C, Fuerth BJ, Viollet B, Mamer OA, Avizonis D, DeBerardinis RJ, Siegel PM, Jones RG. AMPK is a negative regulator of the Warburg effect and suppresses tumor growth *in vivo*. Cell Metab 2013; 17(1): 113–124
184. Algire C, Amrein L, Zakikhani M, Panasci L, Pollak M. Metformin blocks the stimulative effect of a high-energy diet on colon carcinoma growth *in vivo* and is associated with reduced expression of fatty acid synthase. Endocr Relat Cancer 2010; 17(2): 351–360
185. Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, Zhao F, Viollet B, Thompson CB. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer Res 2007; 67(14): 6745–6752
186. Xie Y, Wang JL, Ji M, Yuan ZF, Peng Z, Zhang Y, Wen JG, Shi HR. Regulation of insulin-like growth factor signaling by metformin in endometrial cancer cells. Oncol Lett 2014; 8(5): 1993–1999
187. Lee J, Hong EM, Kim JH, Jung JH, Park SW, Koh DH, Choi MH, Jang HJ, Kae SH. Metformin induces apoptosis and inhibits proliferation through the AMP-activated protein kinase and insulin-like growth factor 1 receptor pathways in the bile duct cancer cells. J Cancer 2019; 10(7): 1734–1744
188. Karnevi E, Said K, Andersson R, Rosendahl AH. Metformin-mediated growth inhibition involves suppression of the IGF-I receptor signalling pathway in human pancreatic cancer cells. BMC Cancer 2013; 13(1): 235
189. Birzniece V, Lam T, McLean M, Reddy N, Shahidipour H, Hayden A, Gurney H, Stone G, Hjortebjerg R, Frystyk J. Insulin-like growth factor role in determining the anti-cancer effect of metformin: RCT in prostate cancer patients. Endocr Connect 2022; 11(4): e210375
190. Zakikhani M, Blouin MJ, Piura E, Pollak MN. Metformin and rapamycin have distinct effects on the AKT pathway and proliferation in breast cancer cells. Breast Cancer Res Treat 2010; 123(1): 271–279
191. Chaudhary SC, Kurundkar D, Elmets CA, Kopelovich L, Athar M. Metformin, an antidiabetic agent reduces growth of cutaneous

squamous cell carcinoma by targeting mTOR signaling pathway. Photochem Photobiol 2012; 88(5): 1149–1156
192. Würth R, Pattarozzi A, Gatti M, Bajetto A, Corsaro A, Parodi A, Sirito R, Massollo M, Marini C, Zona G, Fenoglio D, Sambuceti G, Filaci G, Daga A, Barbieri F, Florio T. Metformin selectively affects human glioblastoma tumor-initiating cell viability: a role for metformin-induced inhibition of Akt. Cell Cycle 2013; 12(1): 145–156
193. 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(13): 4366–4372
194. Jang SK, Hong SE, Lee DH, Kim JY, Kim JY, Ye SK, Hong J, Park IC, Jin HO. Inhibition of mTORC1 through ATF4-induced REDD1 and Sestrin2 expression by metformin. BMC Cancer 2021; 21(1): 803
195. Pierotti MA, Berrino F, Gariboldi M, Melani C, Mogavero A, Negri T, Pasanisi P, Pilotti S. Targeting metabolism for cancer treatment and prevention: metformin, an old drug with multi-faceted effects. Oncogene 2013; 32(12): 1475–1487
196. Yenmis G, Yaprak Sarac E, Besli N, Soydas T, Tastan C, Dilek Kancagi D, Yilanci M, Senol K, Karagulle OO, Ekmekci CG, Ovali E, Tuncdemir M, Ulutin T, Kanigur Sultuybek G. Anti-cancer effect of metformin on the metastasis and invasion of primary breast cancer cells through mediating NF-kB activity. Acta Histochem 2021; 123(4): 151709
197. Wheaton WW, Weinberg SE, Hamanaka RB, Soberanes S, Sullivan LB, Anso E, Glasauer A, Dufour E, Mutlu GM, Budigner GS, Chandel NS. Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. eLife 2014; 3: e02242
198. Soranna D, Scotti L, Zambon A, Bosetti C, Grassi G, Catapano A, La Vecchia C, Mancia G, Corrao G. Cancer risk associated with use of metformin and sulfonylurea in type 2 diabetes: a meta-analysis. Oncologist 2012; 17(6): 813–822
199. Sui X, Xu Y, Yang J, Fang Y, Lou H, Han W, Zhang M, Chen W, Wang K, Li D, Jin W, Lou F, Zheng Y, Hu H, Gong L, Zhou X, Pan Q, Pan H, Wang X, He C. Use of metformin alone is not associated with survival outcomes of colorectal cancer cell but AMPK activator AICAR sensitizes anticancer effect of 5-fluorouracil through AMPK activation. PLoS One 2014; 9(5): e97781
200. Guo L, Cui J, Wang H, Medina R, Zhang S, Zhang X, Zhuang Z, Lin Y. Metformin enhances anti-cancer effects of cisplatin in meningioma through AMPK-mTOR signaling pathways. Mol Ther Oncolytics 2021; 20: 119–131
201. Deng J, Peng M, Wang Z, Zhou S, Xiao D, Deng J, Yang X, Peng J, Yang X. Novel application of metformin combined with targeted drugs on anticancer treatment. Cancer Sci 2019; 110(1): 23–30
202. Saengboonmee C, Sanlung T, Wongkham S. Repurposing metformin for cancer treatment: a great challenge of a promising drug. Anticancer Res 2021; 41(12): 5913–5918
203. Morale MG, Tamura RE, Rubio IGS. Metformin and cancer hallmarks: molecular mechanisms in thyroid, prostate and head and neck cancer models. Biomolecules 2022; 12(3): 357
204. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144(5): 646–674
205. Luengo A, Gui DY, Vander Heiden MG. Targeting metabolism for cancer therapy. Cell Chem Biol 2017; 24(9): 1161–1180
206. Ranganathan P, McLeod HL. Methotrexate pharmacogenetics: the first step toward individualized therapy in rheumatoid arthritis. Arthritis Rheum 2006; 54(5): 1366–1377
207. Pålsson-McDermott EM, O'Neill LAJ. Targeting immunometabolism as an anti-inflammatory strategy. Cell Res 2020; 30(4): 300–314
208. Di Martino L, Tosello V, Peroni E, Piovan E. Insights on metabolic reprogramming and its therapeutic potential in acute leukemia. Int J Mol Sci 2021; 22(16): 8738
209. Ogawa M, Matsuda T, Ogata A, Hamasaki T, Kumanogoh A, Toyofuku T, Tanaka T. DNA damage in rheumatoid arthritis: an age-dependent increase in the lipid peroxidation-derived DNA adduct, heptanone-etheno-2′-deoxycytidine. Autoimmune Dis 2013; 2013: 183487
210. El-Sheikh AA, Morsy MA, Abdalla AM, Hamouda AH, Alhaider IA. Mechanisms of thymoquinone hepatorenal protection in methotrexate-induced toxicity in rats. Mediators Inflamm 2015; 2015: 859383
211. Rizk FH, Saadany AAE, Dawood L, Elkaliny HH, Sarhan NI, Badawi R, Abd-Elsalam S. Metformin ameliorated methotrexate-induced hepatorenal toxicity in rats in addition to its antitumor activity: two birds with one stone. J Inflamm Res 2018; 11: 421–429
212. Owumi SE, Ajijola IJ, Agbeti OM. Hepatorenal protective effects of protocatechuic acid in rats administered with anticancer drug methotrexate. Hum Exp Toxicol 2019; 38(11): 1254–1265
213. Wang Y, Lu H, Sun L, Chen X, Wei H, Suo C, Feng J, Yuan M, Shen S, Jia W, Wang Y, Zhang H, Li Z, Zhong X, Gao P. Metformin sensitises hepatocarcinoma cells to methotrexate by targeting dihydrofolate reductase. Cell Death Dis 2021; 12(10): 902
214. Poulsen KL, Olivero-Verbel J, Beggs KM, Ganey PE, Roth RA. Trovafloxacin enhances lipopolysaccharide-stimulated production of tumor necrosis factor-α by macrophages: role of the DNA damage response. J Pharmacol Exp Ther 2014; 350(1): 164–170
215. Harada K, Ferdous T, Harada T, Ueyama Y. Metformin in combination with 5-fluorouracil suppresses tumor growth by inhibiting the Warburg effect in human oral squamous cell carcinoma. Int J Oncol 2016; 49(1): 276–284
216. Honjo S, Ajani JA, Scott AW, Chen Q, Skinner HD, Stroehlein J, Johnson RL, Song S. Metformin sensitizes chemotherapy by targeting cancer stem cells and the mTOR pathway in esophageal cancer. Int J Oncol 2014; 45(2): 567–574
217. Tian Y, Tang B, Wang C, Sun D, Zhang R, Luo N, Han Z, Liang R, Gao Z, Wang L. Metformin mediates resensitivity to 5-fluorouracil in hepatocellular carcinoma via the suppression of YAP. Oncotarget 2016; 7(29): 46230–46241
218. Miranda VC, Braghiroli MI, Faria LD, Bariani G, Alex A, Bezerra Neto JE, Capareli FC, Sabbaga J, Lobo Dos Santos JF, Hoff PM, Riechelmann RP. Phase 2 trial of metformin combined with 5-fluorouracil in patients with refractory metastatic colorectal cancer. Clin Colorectal Cancer 2016; 15(4): 321–328.e1
219. You R, Wang B, Chen P, Zheng X, Hou D, Wang X, Zhang B, Chen L, Li D, Lin X, Huang H. Metformin sensitizes AML cells

to chemotherapy through blocking mitochondrial transfer from stromal cells to AML cells. Cancer Lett 2022; 532: 215582

220. Zhang Y, Paikari A, Sumazin P, Ginter Summarell CC, Crosby JR, Boerwinkle E, Weiss MJ, Sheehan VA. Metformin induces FOXO3-dependent fetal hemoglobin production in human primary erythroid cells. Blood 2018; 132(3): 321–333
221. Taba K, Kuramitsu Y, Ryozawa S, Yoshida K, Tanaka T, Maehara S, Maehara Y, Sakaida I, Nakamura K. Heat-shock protein 27 is phosphorylated in gemcitabine-resistant pancreatic cancer cells. Anticancer Res 2010; 30(7): 2539–2543
222. Baron B, Wang Y, Maehara S, Maehara Y, Kuramitsu Y, Nakamura K. Resistance to gemcitabine in the pancreatic cancer cell line KLM1-R reversed by metformin action. Anticancer Res 2015; 35(4): 1941–1949
223. Chai X, Chu H, Yang X, Meng Y, Shi P, Gou S. Metformin increases sensitivity of pancreatic cancer cells to gemcitabine by reducing $CD133^+$ cell populations and suppressing ERK/P70S6K signaling. Sci Rep 2015; 5(1): 14404
224. Yi Y, Gao L, Wu M, Ao J, Zhang C, Wang X, Lin M, Bergholz J, Zhang Y, Xiao ZJ. Metformin sensitizes leukemia cells to vincristine via activation of AMP-activated protein kinase. J Cancer 2017; 8(13): 2636–2642
225. Trucco M, Barredo JC, Goldberg J, Leclerc GM, Hale GA, Gill J, Setty B, Smith T, Lush R, Lee JK, Reed DR. A phase I window, dose escalating and safety trial of metformin in combination with induction chemotherapy in relapsed refractory acute lymphoblastic leukemia: Metformin with induction chemotherapy of vincristine, dexamethasone, PEG-asparaginase, and doxorubicin. Pediatr Blood Cancer 2018; 65(9): e27224
226. Fan X, Zhong HJ, Zhao BB, Ou Yang BS, Zhao Y, Ye J, Lu YM, Wang CF, Xiong H, Chen SJ, Janin A, Wang L, Zhao WL. Metformin prolonged the survival of diffuse large B-cell lymphoma and grade 3b follicular lymphoma patients responding to first-line treatment with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone: a prospective phase II clinical trial. Transl Cancer Res 2018; 7(4): 1044–1053
227. Hanna RK, Zhou C, Malloy KM, Sun L, Zhong Y, Gehrig PA, Bae-Jump VL. Metformin potentiates the effects of paclitaxel in endometrial cancer cells through inhibition of cell proliferation and modulation of the mTOR pathway. Gynecol Oncol 2012; 125(2): 458–469
228. Rocha GZ, Dias MM, Ropelle ER, Osório-Costa F, Rossato FA, Vercesi AE, Saad MJ, Carvalheira JB. Metformin amplifies chemotherapy-induced AMPK activation and antitumoral growth. Clin Cancer Res 2011; 17(12): 3993–4005
229. Zhao Y, Zeng X, Tang H, Ye D, Liu J. Combination of metformin and paclitaxel suppresses proliferation and induces apoptosis of human prostate cancer cells via oxidative stress and targeting the mitochondria-dependent pathway. Oncol Lett 2019; 17(5): 4277–4284
230. Tseng SC, Huang YC, Chen HJ, Chiu HC, Huang YJ, Wo TY, Weng SH, Lin YW. Metformin-mediated downregulation of p38 mitogen-activated protein kinase-dependent excision repair cross-complementing 1 decreases DNA repair capacity and sensitizes human lung cancer cells to paclitaxel. Biochem Pharmacol 2013; 85(4): 583–594
231. Lengyel E, Litchfield LM, Mitra AK, Nieman KM, Mukherjee A, Zhang Y, Johnson A, Bradaric M, Lee W, Romero IL. Metformin inhibits ovarian cancer growth and increases sensitivity to paclitaxel in mouse models. Am J Obstet Gynecol. 2015; 212(4): 479.e1–479.e10
232. Mayer MJ, Klotz LH, Venkateswaran V. The effect of metformin use during docetaxel chemotherapy on prostate cancer specific and overall survival of diabetic patients with castration resistant prostate cancer. J Urol 2017; 197(4): 1068–1075
233. Mayer MJ, Klotz LH, Venkateswaran V. Evaluating metformin as a potential chemosensitizing agent when combined with docetaxel chemotherapy in castration-resistant prostate cancer cells. Anticancer Res 2017; 37(12): 6601–6607
234. Babcook MA, Shukla S, Fu P, Vazquez EJ, Puchowicz MA, Molter JP, Oak CZ, MacLennan GT, Flask CA, Lindner DJ, Parker Y, Daneshgari F, Gupta S. Synergistic simvastatin and metformin combination chemotherapy for osseous metastatic castration-resistant prostate cancer. Mol Cancer Ther 2014; 13(10): 2288–2302
235. Fontebasso AM, Schwartzentruber J, Khuong-Quang DA, Liu XY, Sturm D, Korshunov A, Jones DT, Witt H, Kool M, Albrecht S, Fleming A, Hadjadj D, Busche S, Lepage P, Montpetit A, Staffa A, Gerges N, Zakrzewska M, Zakrzewski K, Liberski PP, Hauser P, Garami M, Klekner A, Bognar L, Zadeh G, Faury D, Pfister SM, Jabado N, Majewski J. Mutations in SETD2 and genes affecting histone H3K36 methylation target hemispheric high-grade gliomas. Acta Neuropathol 2013; 125(5): 659–669
236. Li Y, Luo J, Lin MT, Zhi P, Guo WW, Han M, You J, Gao JQ. Co-delivery of metformin enhances the antimultidrug resistant tumor effect of doxorubicin by improving hypoxic tumor microenvironment. Mol Pharm 2019; 16(7): 2966–2979
237. Ashour AE, Sayed-Ahmed MM, Abd-Allah AR, Korashy HM, Maayah ZH, Alkhalidi H, Mubarak M, Alhaider A. Metformin rescues the myocardium from doxorubicin-induced energy starvation and mitochondrial damage in rats. Oxid Med Cell Longev 2012; 2012: 434195
238. Ajzashokouhi AH, Bostan HB, Jomezadeh V, Hayes AW, Karimi G. A review on the cardioprotective mechanisms of metformin against doxorubicin. Hum Exp Toxicol 2020; 39(3): 237–248
239. Kobashigawa LC, Xu YC, Padbury JF, Tseng YT, Yano N. Metformin protects cardiomyocyte from doxorubicin induced cytotoxicity through an AMP-activated protein kinase dependent signaling pathway: an *in vitro* study. PLoS One 2014; 9(8): e104888
240. Shafiei-Irannejad V, Samadi N, Yousefi B, Salehi R, Velaei K, Zarghami N. Metformin enhances doxorubicin sensitivity via inhibition of doxorubicin efflux in P-gp-overexpressing MCF-7 cells. Chem Biol Drug Des 2018; 91(1): 269–276
241. Chen G, Xu S, Renko K, Derwahl M. Metformin inhibits growth of thyroid carcinoma cells, suppresses self-renewal of derived cancer stem cells, and potentiates the effect of chemotherapeutic agents. J Clin Endocrinol Metab 2012; 97(4): E510–E520
242. Hirsch HA, Iliopoulos D, Struhl K. Metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth. Proc Natl Acad Sci USA 2013; 110(3): 972–977
243. Iliopoulos D, Hirsch HA, Struhl K. Metformin decreases the dose of chemotherapy for prolonging tumor remission in mouse xenografts involving multiple cancer cell types. Cancer Res 2011; 71(9): 3196–3201

244. Lee JO, Kang MJ, Byun WS, Kim SA, Seo IH, Han JA, Moon JW, Kim JH, Kim SJ, Lee EJ, In Park S, Park SH, Kim HS. Metformin overcomes resistance to cisplatin in triple-negative breast cancer (TNBC) cells by targeting RAD51. Breast Cancer Res 2019; 21(1): 115
245. Lin CC, Yeh HH, Huang WL, Yan JJ, Lai WW, Su WP, Chen HH, Su WC. Metformin enhances cisplatin cytotoxicity by suppressing signal transducer and activator of transcription-3 activity independently of the liver kinase B1-AMP-activated protein kinase pathway. Am J Respir Cell Mol Biol 2013; 49(2): 241–250
246. Tortelli TC Jr, Tamura RE, de Souza Junqueira M, da Silva Mororó J, Bustos SO, Natalino RJM, Russell S, Désaubry L, Strauss BE, Chammas R. Metformin-induced chemosensitization to cisplatin depends on P53 status and is inhibited by Jarid1b overexpression in non-small cell lung cancer cells. Aging (Albany NY) 2021; 13(18): 21914–21940
247. Shi L, Mei Y, Duan X, Wang B. Effects of cisplatin combined with metformin on proliferation and apoptosis of nasopharyngeal carcinoma cells. Comput Math Methods Med 2022; 2022: 2056247
248. Yasmeen A, Beauchamp MC, Piura E, Segal E, Pollak M, Gotlieb WH. Induction of apoptosis by metformin in epithelial ovarian cancer: involvement of the Bcl-2 family proteins. Gynecol Oncol 2011; 121(3): 492–498
249. He K, Li Z, Ye K, Zhou Y, Yan M, Qi H, Hu H, Dai Y, Tang Y. Novel sequential therapy with metformin enhances the effects of cisplatin in testicular germ cell tumours via YAP1 signalling. Cancer Cell Int 2022; 22(1): 113
250. Liang Z, Zhang T, Zhan T, Cheng G, Zhang W, Jia H, Yang H. Metformin alleviates cisplatin-induced ototoxicity by autophagy induction possibly via the AMPK/FOXO3a pathway. J Neurophysiol 2021; 125(4): 1202–1212
251. Zhou W, Kavelaars A, Heijnen CJ. Metformin prevents cisplatin-induced cognitive impairment and brain damage in mice. PLoS One 2016; 11(3): e0151890
252. Haas CS, Creighton CJ, Pi X, Maine I, Koch AE, Haines GK, Ling S, Chinnaiyan AM, Holoshitz J. Identification of genes modulated in rheumatoid arthritis using complementary DNA microarray analysis of lymphoblastoid B cell lines from disease-discordant monozygotic twins. Arthritis Rheum 2006; 54(7): 2047–2060
253. Tohamy AF, Hussein S, Moussa IM, Rizk H, Daghash S, Alsubki RA, Mubarak AS, Alshammari HO, Al-Maary KS, Hemeg HA. Lucrative antioxidant effect of metformin against cyclophosphamide induced nephrotoxicity. Saudi J Biol Sci 2021; 28(5): 2755–2761
254. Ling S, Shan Q, Liu P, Feng T, Zhang X, Xiang P, Chen K, Xie H, Song P, Zhou L, Liu J, Zheng S, Xu X. Metformin ameliorates arsenic trioxide hepatotoxicity via inhibiting mitochondrial complex I. Cell Death Dis 2017; 8(11): e3159
255. Yang X, Sun D, Tian Y, Ling S, Wang L. Metformin sensitizes hepatocellular carcinoma to arsenic trioxide-induced apoptosis by downregulating Bcl2 expression. Tumour Biol 2015; 36(4): 2957–2964
256. Ling S, Xie H, Yang F, Shan Q, Dai H, Zhuo J, Wei X, Song P, Zhou L, Xu X, Zheng S. Metformin potentiates the effect of arsenic trioxide suppressing intrahepatic cholangiocarcinoma: roles of p38 MAPK, ERK3, and mTORC1. J Hematol Oncol 2017; 10(1): 59
257. Wheeler DL, Dunn EF, Harari PM. Understanding resistance to EGFR inhibitors-impact on future treatment strategies. Nat Rev Clin Oncol 2010; 7(9): 493–507
258. Chen H, Wang Y, Lin C, Lu C, Han R, Jiao L, Li L, He Y. Vorinostat and metformin sensitize EGFR-TKI resistant NSCLC cells via BIM-dependent apoptosis induction. Oncotarget 2017; 8(55): 93825–93838
259. Li L, Han R, Xiao H, Lin C, Wang Y, Liu H, Li K, Chen H, Sun F, Yang Z, Jiang J, He Y. Metformin sensitizes EGFR-TKI-resistant human lung cancer cells *in vitro* and *in vivo* through inhibition of IL-6 signaling and EMT reversal. Clin Cancer Res 2014; 20(10): 2714–2726
260. Pan YH, Jiao L, Lin CY, Lu CH, Li L, Chen HY, Wang YB, He Y. Combined treatment with metformin and gefitinib overcomes primary resistance to EGFR-TKIs with EGFR mutation via targeting IGF-1R signaling pathway. Biologics 2018; 12: 75–86
261. Saif MW. Pancreatic neoplasm in 2011: an update. JOP 2011; 12(4): 316–321
262. Ariaans G, Jalving M, Vries EG, Jong S. Anti-tumor effects of everolimus and metformin are complementary and glucose-dependent in breast cancer cells. BMC Cancer 2017; 17(1): 232
263. Fuentes-Mattei E, Velazquez-Torres G, Phan L, Zhang F, Chou PC, Shin JH, Choi HH, Chen JS, Zhao R, Chen J, Gully C, Carlock C, Qi Y, Zhang Y, Wu Y, Esteva FJ, Luo Y, McKeehan WL, Ensor J, Hortobagyi GN, Pusztai L, Fraser Symmans W, Lee MH, Yeung SC. Effects of obesity on transcriptomic changes and cancer hallmarks in estrogen receptor-positive breast cancer. J Natl Cancer Inst 2014; 106(7): dju158
264. Pusceddu S, Vernieri C, Di Maio M, Marconcini R, Spada F, Massironi S, Ibrahim T, Brizzi MP, Campana D, Faggiano A, Giuffrida D, Rinzivillo M, Cingarlini S, Aroldi F, Antonuzzo L, Berardi R, Catena L, De Divitiis C, Ermacora P, Perfetti V, Fontana A, Razzore P, Carnaghi C, Davì MV, Cauchi C, Duro M, Ricci S, Fazio N, Cavalcoli F, Bongiovanni A, La Salvia A, Brighi N, Colao A, Puliafito I, Panzuto F, Ortolani S, Zaniboni A, Di Costanzo F, Torniai M, Bajetta E, Tafuto S, Garattini SK, Femia D, Prinzi N, Concas L, Lo Russo G, Milione M, Giacomelli L, Buzzoni R, Delle Fave G, Mazzaferro V, de Braud F. Metformin use is associated with longer progression-free survival of patients with diabetes and pancreatic neuroendocrine tumors receiving everolimus and/or somatostatin analogues. Gastroenterology 2018; 155(2): 479–489.e7
265. Gerber HP, Ferrara N. Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. Cancer Res 2005; 65(3): 671–680
266. Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat Rev Drug Discov 2004; 3(5): 391–400
267. Indraccolo S, Randon G, Zulato E, Nardin M, Aliberti C, Pomerri F, Casarin A, Nicoletto MO. Metformin: a modulator of bevacizumab activity in cancer? A case report Cancer Biol Ther 2015; 16(2): 210–214
268. Markowska A, Sajdak S, Markowska J, Huczyński A. Angiogenesis and cancer stem cells: new perspectives on therapy of ovarian cancer. Eur J Med Chem 2017; 142: 87–94
269. Klapper LN, Waterman H, Sela M, Yarden Y. Tumor-inhibitory

antibodies to HER-2/ErbB-2 may act by recruiting c-Cbl and enhancing ubiquitination of HER-2. Cancer Res 2000; 60(13): 3384–3388

270. Zeglinski M, Ludke A, Jassal DS, Singal PK. Trastuzumab-induced cardiac dysfunction: a 'dual-hit'. Exp Clin Cardiol 2011; 16(3): 70–74
271. 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(19): 7507–7511
272. Liu B, Fan Z, Edgerton SM, Yang X, Lind SE, Thor AD. Potent anti-proliferative effects of metformin on trastuzumab-resistant breast cancer cells via inhibition of erbB2/IGF-1 receptor interactions. Cell Cycle 2011; 10(17): 2959–2966
273. Groenendijk FH, Mellema WW, van der Burg E, Schut E, Hauptmann M, Horlings HM, Willems SM, van den Heuvel MM, Jonkers J, Smit EF, Bernards R. Sorafenib synergizes with metformin in NSCLC through AMPK pathway activation. Int J Cancer 2015; 136(6): 1434–1444
274. Chen G, Nicula D, Renko K, Derwahl M. Synergistic anti-proliferative effect of metformin and sorafenib on growth of anaplastic thyroid cancer cells and their stem cells. Oncol Rep 2015; 33(4): 1994–2000
275. Hsieh SC, Tsai JP, Yang SF, Tang MJ, Hsieh YH. Metformin inhibits the invasion of human hepatocellular carcinoma cells and enhances the chemosensitivity to sorafenib through a downregulation of the ERK/JNK-mediated NF-κB-dependent pathway that reduces uPA and MMP-9 expression. Amino Acids 2014; 46(12): 2809–2822
276. Lai HY, Tsai HH, Yen CJ, Hung LY, Yang CC, Ho CH, Liang HY, Chen FW, Li CF, Wang JM. Metformin resensitizes sorafenib-resistant HCC cells through AMPK-dependent autophagy activation. Front Cell Dev Biol 2021; 8: 596655
277. Mitchell R, Hopcroft LEM, Baquero P, Allan EK, Hewit K, James D, Hamilton G, Mukhopadhyay A, O'Prey J, Hair A, Melo JV, Chan E, Ryan KM, Maguer-Satta V, Druker BJ, Clark RE, Mitra S, Herzyk P, Nicolini FE, Salomoni P, Shanks E, Calabretta B, Holyoake TL, Helgason GV. Targeting BCR-ABL-independent TKI resistance in chronic myeloid leukemia by mTOR and autophagy inhibition. J Natl Cancer Inst 2018; 110(5): 467–478
278. Vakana E, Altman JK, Glaser H, Donato NJ, Platanias LC. Antileukemic effects of AMPK activators on BCR-ABL-expressing cells. Blood 2011; 118(24): 6399–6402
279. Bagchi S, Yuan R, Engleman EG. Immune checkpoint inhibitors for the treatment of cancer: clinical impact and mechanisms of response and resistance. Annu Rev Pathol 2021; 16(1): 223–249
280. Cha JH, Yang WH, Xia W, Wei Y, Chan LC, Lim SO, Li CW, Kim T, Chang SS, Lee HH, Hsu JL, Wang HL, Kuo CW, Chang WC, Hadad S, Purdie CA, McCoy AM, Cai S, Tu Y, Litton JK, Mittendorf EA, Moulder SL, Symmans WF, Thompson AM, Piwnica-Worms H, Chen CH, Khoo KH, Hung MC. Metformin promotes antitumor immunity via endoplasmic-reticulum-associated degradation of PD-L1. Mol Cell 2018; 71(4): 606–620.e7
281. Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med 2018; 50(12): 1–11
282. Philip M, Schietinger A. $CD8^+$ T cell differentiation and dysfunction in cancer. Nat Rev Immunol 2022; 22(4): 209–223
283. Yi JS, Cox MA, Zajac AJ. T-cell exhaustion: characteristics, causes and conversion. Immunology 2010; 129(4): 474–481
284. Eikawa S, Nishida M, Mizukami S, Yamazaki C, Nakayama E, Udono H. Immune-mediated antitumor effect by type 2 diabetes drug, metformin. Proc Natl Acad Sci USA 2015; 112(6): 1809–1814
285. Zhang Z, Li F, Tian Y, Cao L, Gao Q, Zhang C, Zhang K, Shen C, Ping Y, Maimela NR, Wang L, Zhang B, Zhang Y. Metformin enhances the antitumor activity of $CD8^+$ T lymphocytes via the AMPK-miR-107-Eomes-PD-1 Pathway. J Immunol 2020; 204(9): 2575–2588
286. Afzal MZ, Mercado RR, Shirai K. Efficacy of metformin in combination with immune checkpoint inhibitors (anti-PD-1/anti-CTLA-4) in metastatic malignant melanoma. J Immunother Cancer 2018; 6(1): 64
287. Chung YM, Khan PP, Wang H, Tsai WB, Qiao Y, Yu B, Larrick JW, Hu MC. Sensitizing tumors to anti-PD-1 therapy by promoting NK and $CD8^+$ T cells via pharmacological activation of FOXO3. J Immunother Cancer 2021; 9(12): e002772
288. Munoz LE, Huang L, Bommireddy R, Sharma R, Monterroza L, Guin RN, Samaranayake SG, Pack CD, Ramachandiran S, Reddy SJC, Shanmugam M, Selvaraj P. Metformin reduces PD-L1 on tumor cells and enhances the anti-tumor immune response generated by vaccine immunotherapy. J Immunother Cancer 2021; 9(11): e002614
289. Scharping NE, Menk AV, Whetstone RD, Zeng X, Delgoffe GM. Efficacy of PD-1 blockade is potentiated by metformin-induced reduction of tumor hypoxia. Cancer Immunol Res 2017; 5(1): 9–16
290. Wu SY, Fu T, Jiang YZ, Shao ZM. Natural killer cells in cancer biology and therapy. Mol Cancer 2020; 19(1): 120
291. Xia C, Liu C, He Z, Cai Y, Chen J. Metformin inhibits cervical cancer cell proliferation by modulating PI3K/Akt-induced major histocompatibility complex class I-related chain A gene expression. J Exp Clin Cancer Res 2020; 39(1): 127
292. Xia W, Qi X, Li M, Wu Y, Sun L, Fan X, Yuan Y, Li J. Metformin promotes anticancer activity of NK cells in a p38 MAPK dependent manner. OncoImmunology 2021; 10(1): 1995999
293. Tesi RJ. MDSC; the most important cell you have never heard of. Trends Pharmacol Sci 2019; 40(1): 4–7
294. Xu P, Yin K, Tang X, Tian J, Zhang Y, Ma J, Xu H, Xu Q, Wang S. Metformin inhibits the function of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. Biomed Pharmacother 2019; 120: 109458
295. Qin G, Lian J, Huang L, Zhao Q, Liu S, Zhang Z, Chen X, Yue D, Li L, Li F, Wang L, Umansky V, Zhang B, Yang S, Zhang Y. Metformin blocks myeloid-derived suppressor cell accumulation through AMPK-DACH1-CXCL1 axis. OncoImmunology 2018; 7(7): e1442167
296. Li L, Wang L, Li J, Fan Z, Yang L, Zhang Z, Zhang C, Yue D, Qin G, Zhang T, Li F, Chen X, Ping Y, Wang D, Gao Q, He Q, Huang L, Li H, Huang J, Zhao X, Xue W, Sun Z, Lu J, Yu JJ, Zhao J, Zhang B, Zhang Y. Metformin-induced reduction of CD39 and CD73 blocks myeloid-derived suppressor cell activity in patients with ovarian cancer. Cancer Res 2018; 78(7):

1779–1791
297. Ding L, Liang G, Yao Z, Zhang J, Liu R, Chen H, Zhou Y, Wu H, Yang B, He Q. Metformin prevents cancer metastasis by inhibiting M2-like polarization of tumor associated macrophages. Oncotarget 2015; 6(34): 36441–36455
298. Chiang CF, Chao TT, Su YF, Hsu CC, Chien CY, Chiu KC, Shiah SG, Lee CH, Liu SY, Shieh YS. Metformin-treated cancer cells modulate macrophage polarization through AMPK-NF-κB signaling. Oncotarget 2017; 8(13): 20706–20718
299. Wang JC, Sun X, Ma Q, Fu GF, Cong LL, Zhang H, Fan DF, Feng J, Lu SY, Liu JL, Li GY, Liu PJ. Metformin's antitumour and anti-angiogenic activities are mediated by skewing macrophage polarization. J Cell Mol Med 2018; 22(8): 3825–3836
300. Wang S, Lin Y, Xiong X, Wang L, Guo Y, Chen Y, Chen S, Wang G, Lin P, Chen H, Yeung SJ, Bremer E, Zhang H. Low-dose metformin reprograms the tumor immune microenvironment in human esophageal cancer: results of a phase II clinical trial. Clin Cancer Res 2020; 26(18): 4921–4932
301. Saito A, Kitayama J, Horie H, Koinuma K, Ohzawa H, Yamaguchi H, Kawahira H, Mimura T, Lefor AK, Sata N. Metformin changes the immune microenvironment of colorectal cancer in patients with type 2 diabetes mellitus. Cancer Sci 2020; 111(11): 4012–4020
302. Kunisada Y, Eikawa S, Tomonobu N, Domae S, Uehara T, Hori S, Furusawa Y, Hase K, Sasaki A, Udono H. Attenuation of $CD4^+CD25^+$ regulatory T cells in the tumor microenvironment by metformin, a type 2 diabetes drug. EBioMedicine 2017; 25: 154–164
303. Veeramachaneni R, Yu W, Newton JM, Kemnade JO, Skinner HD, Sikora AG, Sandulache VC. Metformin generates profound alterations in systemic and tumor immunity with associated antitumor effects. J Immunother Cancer 2021; 9(7): e002773
304. da Costa JP, Vitorino R, Silva GM, Vogel C, Duarte AC, Rocha-Santos T. A synopsis on aging—theories, mechanisms and future prospects. Ageing Res Rev 2016; 29: 90–112
305. Childs BG, Durik M, Baker DJ, van Deursen JM. Cellular senescence in aging and age-related disease: from mechanisms to therapy. Nat Med 2015; 21(12): 1424–1435
306. Rudnicka E, Napierała P, Podfigurna A, Męczekalski B, Smolarczyk R, Grymowicz M. The World Health Organization (WHO) approach to healthy ageing. Maturitas 2020; 139: 6–11
307. Bannister CA, Holden SE, Jenkins-Jones S, Morgan CL, Halcox JP, Schernthaner G, Mukherjee J, Currie CJ. Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls Diabetes Obes Metab 2014; 16(11): 1165–1173
308. Chen J, Ou Y, Li Y, Hu S, Shao LW, Liu Y. Metformin extends *C. elegans* lifespan through lysosomal pathway. eLife 2017; 6: e31268
309. Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen M, Gomes AP, Ward TM, Minor RK, Blouin MJ, Schwab M, Pollak M, Zhang Y, Yu Y, Becker KG, Bohr VA, Ingram DK, Sinclair DA, Wolf NS, Spindler SR, Bernier M, de Cabo R. Metformin improves healthspan and lifespan in mice. Nat Commun 2013; 4(1): 2192
310. Kulkarni AS, Brutsaert EF, Anghel V, Zhang K, Bloomgarden N, Pollak M, Mar JC, Hawkins M, Crandall JP, Barzilai N. Metformin regulates metabolic and nonmetabolic pathways in skeletal muscle and subcutaneous adipose tissues of older adults. Aging Cell 2018; 17(2): e12723
311. Justice JN, Niedernhofer L, Robbins PD, Aroda VR, Espeland MA, Kritchevsky SB, Kuchel GA, Barzilai N. Development of clinical trials to extend healthy lifespan. Cardiovasc Endocrinol Metab 2018; 7(4): 80–83
312. Barzilai N, Crandall JP, Kritchevsky SB, Espeland MA. Metformin as a tool to target aging. Cell Metab 2016; 23(6): 1060–1065
313. Blitzer AL, Ham SA, Colby KA, Skondra D. Association of metformin use with age-related macular degeneration: a case-control study. JAMA Ophthalmol 2021; 139(3): 302–309
314. Goldberg RB, Aroda VR, Bluemke DA, Barrett-Connor E, Budoff M, Crandall JP, Dabelea D, Horton ES, Mather KJ, Orchard TJ, Schade D, Watson K, Temprosa M; Diabetes Prevention Program Research Group. Effect of long-term metformin and lifestyle in the diabetes prevention program and its outcome study on coronary artery calcium. Circulation 2017; 136(1): 52–64
315. Zilov AV, Abdelaziz SI, AlShammary A, Al Zahrani A, Amir A, Assaad Khalil SH, Brand K, Elkafrawy N, Hassoun AAK, Jahed A, Jarrah N, Mrabeti S, Paruk I. Mechanisms of action of metformin with special reference to cardiovascular protection. Diabetes Metab Res Rev 2019; 35(7): e3173
316. Han Y, Xie H, Liu Y, Gao P, Yang X, Shen Z. Effect of metformin on all-cause and cardiovascular mortality in patients with coronary artery diseases: a systematic review and an updated meta-analysis. Cardiovasc Diabetol 2019; 18(1): 96
317. Havas A, Yin S, Adams PD. The role of aging in cancer. Mol Oncol 2022; 16(18): 3213–3219
318. Morales DR, Morris AD. Metformin in cancer treatment and prevention. Annu Rev Med 2015; 66(1): 17–29
319. Coyle C, Cafferty FH, Vale C, Langley RE. Metformin as an adjuvant treatment for cancer: a systematic review and meta-analysis. Ann Oncol 2016; 27(12): 2184–2195
320. Farr SA, Roesler E, Niehoff ML, Roby DA, McKee A, Morley JE. Metformin improves learning and memory in the SAMP8 mouse model of Alzheimer's disease. J Alzheimers Dis 2019; 68(4): 1699–1710
321. Samaras K, Makkar S, Crawford JD, Kochan NA, Wen W, Draper B, Trollor JN, Brodaty H, Sachdev PS. Metformin use is associated with slowed cognitive decline and reduced incident dementia in older adults with type 2 diabetes: the Sydney Memory and Ageing Study. Diabetes Care 2020; 43(11): 2691–2701
322. Zhou JB, Tang X, Han M, Yang J, Simó R. Impact of antidiabetic agents on dementia risk: a Bayesian network meta-analysis. Metabolism 2020; 109: 154265
323. Bettedi L, Foukas LC. Growth factor, energy and nutrient sensing signalling pathways in metabolic ageing. Biogerontology 2017; 18(6): 913–929
324. Admasu TD, Chaithanya Batchu K, Barardo D, Ng LF, Lam VYM, Xiao L, Cazenave-Gassiot A, Wenk MR, Tolwinski NS, Gruber J. Drug synergy slows aging and improves healthspan through IGF and SREBP lipid signaling. Dev Cell 2018; 47(1): 67–79.e5

325. Anisimov VN, Berstein LM, Egormin PA, Piskunova TS, Popovich IG, Zabezhinski MA, Tyndyk ML, Yurova MV, Kovalenko IG, Poroshina TE, Semenchenko AV. Metformin slows down aging and extends life span of female SHR mice. Cell Cycle 2008; 7(17): 2769–2773
326. Sunjaya AP, Sunjaya AF. Targeting ageing and preventing organ degeneration with metformin. Diabetes Metab 2021; 47(1): 101203
327. Kubben N, Misteli T. Shared molecular and cellular mechanisms of premature ageing and ageing-associated diseases. Nat Rev Mol Cell Biol 2017; 18(10): 595–609
328. Foo MXR, Ong PF, Dreesen O. Premature aging syndromes: from patients to mechanism. J Dermatol Sci 2019; 96(2): 58–65
329. Kauppila TES, Bratic A, Jensen MB, Baggio F, Partridge L, Jasper H, Grönke S, Larsson NG. Mutations of mitochondrial DNA are not major contributors to aging of fruit flies. Proc Natl Acad Sci USA 2018; 115(41): E9620–E9629
330. Kulkarni AS, Gubbi S, Barzilai N. Benefits of metformin in attenuating the hallmarks of aging. Cell Metab 2020; 32(1): 15–30
331. Jiang Y, Dong Y, Luo Y, Jiang S, Meng FL, Tan M, Li J, Zang Y. AMPK-mediated phosphorylation on 53BP1 promotes c-NHEJ. Cell Rep 2021; 34(7): 108713
332. Kudabayeva K, Kosmuratova R, Bazargaliyev Y, Sartayeva A, Kereyeva N. Effects of metformin on lymphocyte DNA damage in obese individuals among Kazakh population. Diabetes Metab Syndr 2022; 16(8): 102569
333. Chukwunonso Obi B, Chinwuba Okoye T, Okpashi VE, Nonye Igwe C, Olisah Alumanah E. Comparative study of the antioxidant effects of metformin, glibenclamide, and repaglinide in alloxan-induced diabetic rats. J Diabetes Res 2016; 2016: 1635361
334. Allard JS, Perez EJ, Fukui K, Carpenter P, Ingram DK, de Cabo R. Prolonged metformin treatment leads to reduced transcription of Nrf2 and neurotrophic factors without cognitive impairment in older C57BL/6J mice. Behav Brain Res 2016; 301: 1–9
335. Anisimov VN, Berstein LM, Popovich IG, Zabezhinski MA, Egormin PA, Piskunova TS, Semenchenko AV, Tyndyk ML, Yurova MN, Kovalenko IG, Poroshina TE. If started early in life, metformin treatment increases life span and postpones tumors in female SHR mice. Aging (Albany NY) 2011; 3(2): 148–157
336. Fang J, Yang J, Wu X, Zhang G, Li T, Wang X, Zhang H, Wang CC, Liu GH, Wang L. Metformin alleviates human cellular aging by upregulating the endoplasmic reticulum glutathione peroxidase 7. Aging Cell 2018; 17(4): e12765
337. Moiseeva O, Deschênes-Simard X, St-Germain E, Igelmann S, Huot G, Cadar AE, Bourdeau V, Pollak MN, Ferbeyre G. Metformin inhibits the senescence-associated secretory phenotype by interfering with IKK/NF-κB activation. Aging Cell 2013; 12(3): 489–498
338. Noren Hooten N, Martin-Montalvo A, Dluzen DF, Zhang Y, Bernier M, Zonderman AB, Becker KG, Gorospe M, de Cabo R, Evans MK. Metformin-mediated increase in DICER1 regulates microRNA expression and cellular senescence. Aging Cell 2016; 15(3): 572–581
339. Bektas A, Schurman SH, Sen R, Ferrucci L. Aging, inflammation and the environment. Exp Gerontol 2018; 105: 10–18
340. Piber D, Olmstead R, Cho JH, Witarama T, Perez C, Dietz N, Seeman TE, Breen EC, Cole SW, Irwin MR. Inflammaging: age and systemic, cellular, and nuclear inflammatory biology in older adults. J Gerontol A Biol Sci Med Sci 2019; 74(11): 1716–1724
341. Rea IM, Gibson DS, McGilligan V, McNerlan SE, Alexander HD, Ross OA. Age and age-related diseases: role of inflammation triggers and cytokines. Front Immunol 2018; 9: 586
342. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci 2014; 69(Suppl 1): S4–S9
343. Tizazu AM, Nyunt MSZ, Cexus O, Suku K, Mok E, Xian CH, Chong J, Tan C, How W, Hubert S, Combet E, Fulop T, Ng TP, Larbi A. Metformin monotherapy downregulates diabetes-associated inflammatory status and impacts on mortality. Front Physiol 2019; 10: 572
344. Chen W, Liu X, Ye S. Effects of metformin on blood and urine pro-inflammatory mediators in patients with type 2 diabetes. J Inflamm (Lond) 2016; 13(1): 34
345. Xu X, Lin S, Chen Y, Li X, Ma S, Fu Y, Wei C, Wang C, Xu W. The effect of metformin on the expression of GPR109A, NF-κB and IL-1β in peripheral blood leukocytes from patients with type 2 diabetes mellitus. Ann Clin Lab Sci 2017; 47(5): 556–562
346. Xu W, Deng YY, Yang L, Zhao S, Liu J, Zhao Z, Wang L, Maharjan P, Gao S, Tian Y, Zhuo X, Zhao Y, Zhou J, Yuan Z, Wu Y. Metformin ameliorates the proinflammatory state in patients with carotid artery atherosclerosis through sirtuin 1 induction. Transl Res 2015; 166(5): 451–458
347. Saisho Y. Metformin and inflammation: its potential beyond glucose-lowering effect. Endocr Metab Immune Disord Drug Targets 2015; 15(3): 196–205
348. Kristófi R, Eriksson JW. Metformin as an anti-inflammatory agent: a short review. J Endocrinol 2021; 251(2): R11–R22
349. Gou L, Liu G, Ma R, Regmi A, Zeng T, Zheng J, Zhong X, Chen L. High fat-induced inflammation in vascular endothelium can be improved by *Abelmoschus esculentus* and metformin via increasing the expressions of miR-146a and miR-155. Nutr Metab (Lond) 2020; 17(1): 35
350. Luo X, Hu R, Zheng Y, Liu S, Zhou Z. Metformin shows anti-inflammatory effects in murine macrophages through Dicer/microribonucleic acid-34a-5p and microribonucleic acid-125b-5p. J Diabetes Investig 2020; 11(1): 101–109
351. Hipp MS, Kasturi P, Hartl FU. The proteostasis network and its decline in ageing. Nat Rev Mol Cell Biol 2019; 20(7): 421–435
352. Kitada M, Koya D. Autophagy in metabolic disease and ageing. Nat Rev Endocrinol 2021; 17(11): 647–661
353. Leidal AM, Levine B, Debnath J. Autophagy and the cell biology of age-related disease. Nat Cell Biol 2018; 20(12): 1338–1348
354. Meléndez A, Tallóczy Z, Seaman M, Eskelinen EL, Hall DH, Levine B. Autophagy genes are essential for dauer development and life-span extension in *C. elegans*. Science 2003; 301(5638): 1387–1391
355. Fernández ÁF, Sebti S, Wei Y, Zou Z, Shi M, McMillan KL, He C, Ting T, Liu Y, Chiang WC, Marciano DK, Schiattarella GG, Bhagat G, Moe OW, Hu MC, Levine B. Disruption of the beclin 1-BCL2 autophagy regulatory complex promotes longevity in mice. Nature 2018; 558(7708): 136–140
356. Bhansali S, Bhansali A, Dutta P, Walia R, Dhawan V. Metformin upregulates mitophagy in patients with T2DM: a randomized placebo-controlled study. J Cell Mol Med 2020; 24(5):

2832–2846
357. Bharath LP, Agrawal M, McCambridge G, Nicholas DA, Hasturk H, Liu J, Jiang K, Liu R, Guo Z, Deeney J, Apovian CM, Snyder-Cappione J, Hawk GS, Fleeman RM, Pihl RMF, Thompson K, Belkina AC, Cui L, Proctor EA, Kern PA, Nikolajczyk BS. Metformin enhances autophagy and normalizes mitochondrial function to alleviate aging-associated inflammation. Cell Metab 2020; 32(1): 44–55.e6
358. Xu B, Dai W, Liu L, Han H, Zhang J, Du X, Pei X, Fu X. Metformin ameliorates polycystic ovary syndrome in a rat model by decreasing excessive autophagy in ovarian granulosa cells via the PI3K/AKT/mTOR pathway. Endocr J 2022; 69(7): 863–875
359. Li M, Sharma A, Yin C, Tan X, Xiao Y. Metformin ameliorates hepatic steatosis and improves the induction of autophagy in HFD-induced obese mice. Mol Med Rep 2017; 16(1): 680–686
360. You G, Long X, Song F, Huang J, Tian M, Xiao Y, Deng S, Wu Q. Metformin activates the AMPK-mTOR pathway by modulating lncRNA *TUG1* to induce autophagy and inhibit atherosclerosis. Drug Des Devel Ther 2020; 14: 457–468
361. Kodali M, Attaluri S, Madhu LN, Shuai B, Upadhya R, Gonzalez JJ, Rao X, Shetty AK. Metformin treatment in late middle age improves cognitive function with alleviation of microglial activation and enhancement of autophagy in the hippocampus. Aging Cell 2021; 20(2): e13277
362. Whittemore K, Vera E, Martínez-Nevado E, Sanpera C, Blasco MA. Telomere shortening rate predicts species life span. Proc Natl Acad Sci USA 2019; 116(30): 15122–15127
363. Huang J, Peng X, Dong K, Tao J, Yang Y. The association between antidiabetic agents and leukocyte telomere length in the novel classification of type 2 diabetes mellitus. Gerontology 2021; 67(1): 60–68
364. Liu J, Ge Y, Wu S, Ma D, Xu W, Zhang Y, Yang Y. Association between antidiabetic agents use and leukocyte telomere shortening rates in patients with type 2 diabetes. Aging (Albany NY) 2019; 11(2): 741–755
365. Rosa ECCC, Dos Santos RRC, Fernandes LFA, Neves FAR, Coelho MS, Amato AA. Leukocyte telomere length correlates with glucose control in adults with recently diagnosed type 2 diabetes. Diabetes Res Clin Pract 2018; 135: 30–36
366. Sun N, Youle RJ, Finkel T. The mitochondrial basis of aging. Mol Cell 2016; 61(5): 654–666
367. Konopka AR, Laurin JL, Schoenberg HM, Reid JJ, Castor WM, Wolff CA, Musci RV, Safairad OD, Linden MA, Biela LM, Bailey SM, Hamilton KL, Miller BF. Metformin inhibits mitochondrial adaptations to aerobic exercise training in older adults. Aging Cell 2019; 18(1): e12880
368. Jang JY, Blum A, Liu J, Finkel T. The role of mitochondria in aging. J Clin Invest 2018; 128(9): 3662–3670
369. Starling S. Metformin reduces ageing adipose senescence. Nat Rev Endocrinol 2021; 17(12): 708
370. Karnewar S, Neeli PK, Panuganti D, Kotagiri S, Mallappa S, Jain N, Jerald MK, Kotamraju S. Metformin regulates mitochondrial biogenesis and senescence through AMPK mediated H3K79 methylation: relevance in age-associated vascular dysfunction. Biochim Biophys Acta Mol Basis Dis 2018; 186(4 Pt A): 1115–1128
371. Vial G, Detaille D, Guigas B. Role of mitochondria in the mechanism(s) of action of metformin. Front Endocrinol (Lausanne) 2019; 10: 294
372. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013; 153(6): 1194–1217
373. Neumann B, Baror R, Zhao C, Segel M, Dietmann S, Rawji KS, Foerster S, McClain CR, Chalut K, van Wijngaarden P, Franklin RJM. Metformin restores CNS remyelination capacity by rejuvenating aged stem cells. Cell Stem Cell 2019; 25(4): 473–485.e8
374. Na HJ, Park JS, Pyo JH, Jeon HJ, Kim YS, Arking R, Yoo MA. Metformin inhibits age-related centrosome amplification in *Drosophila* midgut stem cells through AKT/TOR pathway. Mech Ageing Dev 2015; 149: 8–18
375. Calabrese EJ, Agathokleous E, Kapoor R, Dhawan G, Kozumbo WJ, Calabrese V. Metformin-enhances resilience via hormesis. Ageing Res Rev 2021; 71: 101418
376. Schmidt TSB, Raes J, Bork P. The human gut microbiome: from association to modulation. Cell 2018; 172(6): 1198–1215
377. Antal B, McMahon LP, Sultan SF, Lithen A, Wexler DJ, Dickerson B, Ratai EM, Mujica-Parodi LR. Type 2 diabetes mellitus accelerates brain aging and cognitive decline: complementary findings from UK Biobank and meta-analyses. eLife 2022; 11: e73138
378. Smith DL Jr, Elam CF Jr, Mattison JA, Lane MA, Roth GS, Ingram DK, Allison DB. Metformin supplementation and life span in Fischer-344 rats. J Gerontol A Biol Sci Med Sci 2010; 65(5): 468–474
379. Espada L, Dakhovnik A, Chaudhari P, Martirosyan A, Miek L, Poliezhaieva T, Schaub Y, Nair A, Döring N, Rahnis N, Werz O, Koeberle A, Kirkpatrick J, Ori A, Ermolaeva MA. Loss of metabolic plasticity underlies metformin toxicity in aged *Caenorhabditis elegans*. Nat Metab 2020; 2(11): 1316–1331
380. Walton RG, Dungan CM, Long DE, Tuggle SC, Kosmac K, Peck BD, Bush HM, Villasante Tezanos AG, McGwin G, Windham ST, Ovalle F, Bamman MM, Kern PA, Peterson CA. Metformin blunts muscle hypertrophy in response to progressive resistance exercise training in older adults: a randomized, double-blind, placebo-controlled, multicenter trial: the MASTERS trial. Aging Cell 2019; 18(6): e13039
381. Xenos D, Mecocci P, Boccardi V. A blast from the past: to tame time with metformin. Mech Ageing Dev 2022; 208: 111743
382. Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol 2012; 13(4): 251–262
383. Cantó C, Auwerx J. AMP-activated protein kinase and its downstream transcriptional pathways. Cell Mol Life Sci 2010; 67(20): 3407–3423
384. Feige JN, Auwerx J. Transcriptional coregulators in the control of energy homeostasis. Trends Cell Biol 2007; 17(6): 292–301
385. Garcia D, Shaw RJ. AMPK: mechanisms of cellular energy sensing and restoration of metabolic balance. Mol Cell 2017; 66(6): 789–800
386. Chung MM, Nicol CJ, Cheng YC, Lin KH, Chen YL, Pei D, Lin CH, Shih YN, Yen CH, Chen SJ, Huang RN, Chiang MC. Metformin activation of AMPK suppresses AGE-induced inflammatory response in hNSCs. Exp Cell Res 2017; 352(1): 75–83
387. Wang S, Kobayashi K, Kogure Y, Yamanaka H, Yamamoto S, Yagi H, Noguchi K, Dai Y. Negative regulation of TRPA1 by

AMPK in primary sensory neurons as a potential mechanism of painful diabetic neuropathy. Diabetes 2018; 67(1): 98–109
388. Yuan R, Wang Y, Li Q, Zhen F, Li X, Lai Q, Hu P, Wang X, Zhu Y, Fan H, Yao R. Metformin reduces neuronal damage and promotes neuroblast proliferation and differentiation in a cerebral ischemia/reperfusion rat model. Neuroreport 2019; 30(3): 232–240
389. Mertens J, Paquola ACM, Ku M, Hatch E, Böhnke L, Ladjevardi S, McGrath S, Campbell B, Lee H, Herdy JR, Gonçalves JT, Toda T, Kim Y, Winkler J, Yao J, Hetzer MW, Gage FH. Directly reprogrammed human neurons retain aging-associated transcriptomic signatures and reveal age-related nucleocytoplasmic defects. Cell Stem Cell 2015; 17(6): 705–718
390. Kim Y, Zheng X, Ansari Z, Bunnell MC, Herdy JR, Traxler L, Lee H, Paquola ACM, Blithikioti C, Ku M, Schlachetzki JCM, Winkler J, Edenhofer F, Glass CK, Paucar AA, Jaeger BN, Pham S, Boyer L, Campbell BC, Hunter T, Mertens J, Gage FH. Mitochondrial aging defects emerge in directly reprogrammed human neurons due to their metabolic profile. Cell Rep 2018; 23(9): 2550–2558
391. Rotermund C, Machetanz G, Fitzgerald JC. The therapeutic potential of metformin in neurodegenerative diseases. Front Endocrinol (Lausanne) 2018; 9: 400
392. Craft S, Watson GS. Insulin and neurodegenerative disease: shared and specific mechanisms. Lancet Neurol 2004; 3(3): 169–178
393. Ninomiya T. Diabetes mellitus and dementia. Curr Diab Rep 2014; 14(5): 487
394. Neumann KF, Rojo L, Navarrete LP, Farías G, Reyes P, Maccioni RB. Insulin resistance and Alzheimer's disease: molecular links & clinical implications. Curr Alzheimer Res 2008; 5(5): 438–447
395. Verdile G, Fuller SJ, Martins RN. The role of type 2 diabetes in neurodegeneration. Neurobiol Dis 2015; 84: 22–38
396. Arvanitakis Z, Shah RC, Bennett DA. Diagnosis and management of dementia: review. JAMA 2019; 322(16): 1589–1599
397. Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT. Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology 1992; 42(3): 631–639
398. Johnson GV, Stoothoff WH. Tau phosphorylation in neuronal cell function and dysfunction. J Cell Sci 2004; 117(24): 5721–5729
399. Gu JL, Liu F. Tau in Alzheimer's disease: pathological alterations and an attractive therapeutic target. Curr Med Sci 2020; 40(6): 1009–1021
400. Sun X, Bromley-Brits K, Song W. Regulation of β-site APP-cleaving enzyme 1 gene expression and its role in Alzheimer's disease. J Neurochem 2012; 120(Suppl 1): 62–70
401. Oliveira WH, Braga CF, Lós DB, Araújo SMR, França MR, Duarte-Silva E, Rodrigues GB, Rocha SWS, Peixoto CA. Metformin prevents p-tau and amyloid plaque deposition and memory impairment in diabetic mice. Exp Brain Res 2021; 239(9): 2821–2839
402. Chen Y, Zhou K, Wang R, Liu Y, Kwak YD, Ma T, Thompson RC, Zhao Y, Smith L, Gasparini L, Luo Z, Xu H, Liao FF. Antidiabetic drug metformin (GlucophageR) increases biogenesis of Alzheimer's amyloid peptides via up-regulating BACE1 transcription. Proc Natl Acad Sci USA 2009; 106(10): 3907–3912
403. Won JS, Im YB, Kim J, Singh AK, Singh I. Involvement of AMP-activated-protein-kinase (AMPK) in neuronal amyloidogenesis. Biochem Biophys Res Commun 2010; 399(4): 487–491
404. Ng TP, Feng L, Yap KB, Lee TS, Tan CH, Winblad B. Long-term metformin usage and cognitive function among older adults with diabetes. J Alzheimers Dis 2014; 41(1): 61–68
405. Yokoyama H, Ogawa M, Honjo J, Okizaki S, Yamada D, Shudo R, Shimizu H, Sone H, Haneda M. Risk factors associated with abnormal cognition in Japanese outpatients with diabetes, hypertension or dyslipidemia. Diabetol Int 2015; 6(4): 268–274
406. Hsu CC, Wahlqvist ML, Lee MS, Tsai HN. Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin. J Alzheimers Dis 2011; 24(3): 485–493
407. Cheng C, Lin CH, Tsai YW, Tsai CJ, Chou PH, Lan TH. Type 2 diabetes and antidiabetic medications in relation to dementia diagnosis. J Gerontol A Biol Sci Med Sci 2014; 69(10): 1299–1305
408. Orkaby AR, Cho K, Cormack J, Gagnon DR, Driver JA. Metformin vs sulfonylurea use and risk of dementia in US veterans aged ⩾65 years with diabetes. Neurology 2017; 89(18): 1877–1885
409. Imfeld P, Bodmer M, Jick SS, Meier CR. Metformin, other antidiabetic drugs, and risk of Alzheimer's disease: a population-based case-control study. J Am Geriatr Soc 2012; 60(5): 916–921
410. Wang CP, Lorenzo C, Habib SL, Jo B, Espinoza SE. Differential effects of metformin on age related comorbidities in older men with type 2 diabetes. J Diabetes Complications 2017; 31(4): 679–686
411. Arvanitakis Z, Tatavarthy M, Bennett DA. The relation of diabetes to memory function. Curr Neurol Neurosci Rep 2020; 20(12): 64
412. Emamzadeh FN, Surguchov A. Parkinson's disease: biomarkers, treatment, and risk factors. Front Neurosci 2018; 12: 612
413. Marino BLB, de Souza LR, Sousa KPA, Ferreira JV, Padilha EC, da Silva CHTP, Taft CA, Hage-Melim LIS. Parkinson's disease: a review from pathophysiology to treatment. Mini Rev Med Chem 2020; 20(9): 754–767
414. Damier P, Hirsch EC, Agid Y, Graybiel AM. The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. Brain 1999; 122(8): 1437–1448
415. Zhao X, He H, Xiong X, Ye Q, Feng F, Zhou S, Chen W, Xia K, Qian S, Yang Y, Xie C. Lewy body-associated proteins A-synuclein (a-syn) as a plasma-based biomarker for Parkinson's disease. Front Aging Neurosci 2022; 14: 869797
416. Tan JM, Wong ES, Lim KL. Protein misfolding and aggregation in Parkinson's disease. Antioxid Redox Signal 2009; 11(9): 2119–2134
417. Braak H, Del Tredici K, Bratzke H, Hamm-Clement J, Sandmann-Keil D, Rüb U. Staging of the intracerebral inclusion body pathology associated with idiopathic Parkinson's disease (preclinical and clinical stages). J Neurol 2002; 249 Suppl 3: III/1–5
418. Beach TG, Adler CH, Lue L, Sue LI, Bachalakuri J, Henry-Watson J, Sasse J, Boyer S, Shirohi S, Brooks R, Eschbacher J, White CL 3rd, Akiyama H, Caviness J, Shill HA, Connor DJ, Sabbagh MN, Walker DG; Arizona Parkinson's Disease Consortium. Unified staging system for Lewy body disorders:

correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. Acta Neuropathol 2009; 117(6): 613–634
419. Dolasık I, Sener SY, Celebı K, Aydın ZM, Korkmaz U, Canturk Z. The effect of metformin on mean platelet volume in dıabetıc patients. Platelets 2013; 24(2): 118–121
420. Koçer A, Yaman A, Niftaliyev E, Dürüyen H, Eryılmaz M, Koçer E. Assessment of platelet indices in patients with neurodegenerative diseases: mean platelet volume was increased in patients with Parkinson's disease. Curr Gerontol Geriatr Res 2013; 2013: 986254
421. Lu M, Su C, Qiao C, Bian Y, Ding J, Hu G. Metformin prevents dopaminergic neuron death in MPTP/P-induced mouse model of Parkinson's disease via autophagy and mitochondrial ROS clearance. Int J Neuropsychopharmacol 2016; 19(9): pyw047
422. Patil SP, Jain PD, Ghumatkar PJ, Tambe R, Sathaye S. Neuroprotective effect of metformin in MPTP-induced Parkinson's disease in mice. Neuroscience 2014; 277: 747–754
423. Bayliss JA, Lemus MB, Santos VV, Deo M, Davies JS, Kemp BE, Elsworth JD, Andrews ZB. Metformin prevents nigrostriatal dopamine degeneration independent of AMPK activation in dopamine neurons. PLoS One 2016; 11(7): e0159381
424. Katila N, Bhurtel S, Shadfar S, Srivastav S, Neupane S, Ojha U, Jeong GS, Choi DY. Metformin lowers α-synuclein phosphorylation and upregulates neurotrophic factor in the MPTP mouse model of Parkinson's disease. Neuropharmacology 2017; 125: 396–407
425. Ismaiel AA, Espinosa-Oliva AM, Santiago M, García-Quintanilla A, Oliva-Martín MJ, Herrera AJ, Venero JL, de Pablos RM. Metformin, besides exhibiting strong *in vivo* anti-inflammatory properties, increases mptp-induced damage to the nigrostriatal dopaminergic system. Toxicol Appl Pharmacol 2016; 298: 19–30
426. Wahlqvist ML, Lee MS, Hsu CC, Chuang SY, Lee JT, Tsai HN. Metformin-inclusive sulfonylurea therapy reduces the risk of Parkinson's disease occurring with type 2 diabetes in a Taiwanese population cohort. Parkinsonism Relat Disord 2012; 18(6): 753–758
427. McColgan P, Tabrizi SJ. Huntington's disease: a clinical review. Eur J Neurol 2018; 25(1): 24–34
428. Montojo MT, Aganzo M, González N. Huntington's disease and diabetes: chronological sequence of its association. J Huntingtons Dis 2017; 6(3): 179–188
429. Lalić NM, Marić J, Svetel M, Jotić A, Stefanova E, Lalić K, Dragasević N, Milicić T, Lukić L, Kostić VS. Glucose homeostasis in Huntington disease: abnormalities in insulin sensitivity and early-phase insulin secretion. Arch Neurol 2008; 65(4): 476–480
430. Boesgaard TW, Nielsen TT, Josefsen K, Hansen T, Jørgensen T, Pedersen O, Nørremølle A, Nielsen JE, Hasholt L. Huntington's disease does not appear to increase the risk of diabetes mellitus. J Neuroendocrinol 2009; 21(9): 770–776
431. Russo CV, Salvatore E, Saccà F, Tucci T, Rinaldi C, Sorrentino P, Massarelli M, Rossi F, Savastano S, Di Maio L, Filla A, Colao A, De Michele G. Insulin sensitivity and early-phase insulin secretion in normoglycemic Huntington's disease patients. J Huntingtons Dis 2013; 2(4): 501–507
432. Hervás D, Fornés-Ferrer V, Gómez-Escribano AP, Sequedo MD, Peiró C, Millán JM, Vázquez-Manrique RP. Metformin intake associates with better cognitive function in patients with Huntington's disease. PLoS One 2017; 12(6): e0179283
433. Ju TC, Chen HM, Chen YC, Chang CP, Chang C, Chern Y. AMPK-α1 functions downstream of oxidative stress to mediate neuronal atrophy in Huntington's disease. Biochim Biophys Acta 2014; 1842(9): 1668–1680
434. Dziedzic A, Saluk-Bijak J, Miller E, Bijak M. Metformin as a potential agent in the treatment of multiple sclerosis. Int J Mol Sci 2020; 21(17): 5957
435. Dos Passos GR, Sato DK, Becker J, Fujihara K. Th17 cells pathways in multiple sclerosis and neuromyelitis optica spectrum disorders: pathophysiological and therapeutic implications. Mediators Inflamm 2016; 2016: 5314541
436. Kalra S, Lowndes C, Durant L, Strange RC, Al-Araji A, Hawkins CP, Curnow SJ. Th17 cells increase in RRMS as well as in SPMS, whereas various other phenotypes of Th17 increase in RRMS only. Mult Scler J Exp Transl Clin 2020; 6(1): 2055217319899695
437. Álvarez-Sánchez N, Cruz-Chamorro I, Díaz-Sánchez M, Lardone PJ, Guerrero JM, Carrillo-Vico A. Peripheral CD39-expressing T regulatory cells are increased and associated with relapsing-remitting multiple sclerosis in relapsing patients. Sci Rep 2019; 9(1): 2302
438. Li YF, Zhang SX, Ma XW, Xue YL, Gao C, Li XY, Xu AD. The proportion of peripheral regulatory T cells in patients with multiple sclerosis: a meta-analysis. Mult Scler Relat Disord 2019; 28: 75–80
439. Hofstetter H, Gold R, Hartung HP. Th17 cells in MS and experimental autoimmune encephalomyelitis. Int MS J 2009; 16(1): 12–18
440. Wei L, Laurence A, Elias KM, O'Shea JJ. IL-21 is produced by Th17 cells and drives IL-17 production in a STAT3-dependent manner. J Biol Chem 2007; 282(48): 34605–34610
441. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, Giuliani F, Arbour N, Becher B, Prat A. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nat Med 2007; 13(10): 1173–1175
442. Mehta MM, Chandel NS. Targeting metabolism for lupus therapy. Sci Transl Med 2015; 7(274): 274fs5
443. Krysiak R, Okopien B. Haemostatic effects of metformin in simvastatin-treated volunteers with impaired fasting glucose. Basic Clin Pharmacol Toxicol 2012; 111(6): 380–384
444. Krysiak R, Gdula-Dymek A, Okopień B. Effect of metformin on selected parameters of hemostasis in fenofibrate-treated patients with impaired glucose tolerance. Pharmacol Rep 2013; 65(1): 208–213
445. Serdyńska-Szuster M, Banaszewska B, Spaczyński R, Pawelczyk L. Effects of metformin therapy on markers of coagulation disorders in hyperinsulinemic women with polycystic ovary syndrome. Ginekol Pol 2011; 82(4): 259–264
446. Markowicz-Piasecka M, Huttunen KM, Sadkowska A, Sikora J. Pleiotropic activity of metformin and its sulfonamide derivatives on vascular and platelet haemostasis. Molecules 2019; 25(1): 125
447. Negrotto L, Farez MF, Correale J. Immunologic effects of metformin and pioglitazone treatment on metabolic syndrome and multiple sclerosis. JAMA Neurol 2016; 73(5): 520–528
448. Jang S, Kim H, Jeong J, Lee SK, Kim EW, Park M, Kim CH, Lee JE, Namkoong K, Kim E. Blunted response of hippocampal

AMPK associated with reduced neurogenesis in older versus younger mice. Prog Neuropsychopharmacol Biol Psychiatry 2016; 71: 57–65
449. Dulamea AO. The contribution of oligodendrocytes and oligodendrocyte progenitor cells to central nervous system repair in multiple sclerosis: perspectives for remyelination therapeutic strategies. Neural Regen Res 2017; 12(12): 1939–1944
450. Qi Y, Cheng H, Lou Q, Wang X, Lai N, Gao C, Wu S, Xu C, Ruan Y, Chen Z, Wang Y. Paradoxical effects of posterior intralaminar thalamic calretinin neurons on hippocampal seizure via distinct downstream circuits. iScience 2022; 25(5): 104218
451. Tóth K, Eross L, Vajda J, Halász P, Freund TF, Maglóczky Z. Loss and reorganization of calretinin-containing interneurons in the epileptic human hippocampus. Brain 2010; 133(9): 2763–2777
452. Meldrum BS. Excitotoxicity and selective neuronal loss in epilepsy. Brain Pathol 1993; 3(4): 405–412
453. Qi Y, Cheng H, Wang Y, Chen Z. Revealing the precise role of calretinin neurons in epilepsy: we are on the way. Neurosci Bull 2022; 38(2): 209–222
454. Hussein AM, Eldosoky M, El-Shafey M, El-Mesery M, Ali AN, Abbas KM, Abulseoud OA. Effects of metformin on apoptosis and α-synuclein in a rat model of pentylenetetrazole-induced epilepsy. Can J Physiol Pharmacol 2019; 97(1): 37–46
455. Zhao RR, Xu XC, Xu F, Zhang WL, Zhang WL, Liu LM, Wang WP. Metformin protects against seizures, learning and memory impairments and oxidative damage induced by pentylenetetrazole-induced kindling in mice. Biochem Biophys Res Commun 2014; 448(4): 414–417
456. Yang Y, Zhu B, Zheng F, Li Y, Zhang Y, Hu Y, Wang X. Chronic metformin treatment facilitates seizure termination. Biochem Biophys Res Commun 2017; 484(2): 450–455
457. Moran C, Sanz-Rodriguez A, Jimenez-Pacheco A, Martinez-Villareal J, McKiernan RC, Jimenez-Mateos EM, Mooney C, Woods I, Prehn JH, Henshall DC, Engel T. Bmf upregulation through the AMP-activated protein kinase pathway may protect the brain from seizure-induced cell death. Cell Death Dis 2013; 4(4): e606
458. Mehrabi S, Sanadgol N, Barati M, Shahbazi A, Vahabzadeh G, Barzroudi M, Seifi M, Gholipourmalekabadi M, Golab F. Evaluation of metformin effects in the chronic phase of spontaneous seizures in pilocarpine model of temporal lobe epilepsy. Metab Brain Dis 2018; 33(1): 107–114
459. Heinrich C, Lähteinen S, Suzuki F, Anne-Marie L, Huber S, Häussler U, Haas C, Larmet Y, Castren E, Depaulis A. Increase in BDNF-mediated TrkB signaling promotes epileptogenesis in a mouse model of mesial temporal lobe epilepsy. Neurobiol Dis 2011; 42(1): 35–47
460. Amin S, Mallick AA, Edwards H, Cortina-Borja M, Laugharne M, Likeman M, O'Callaghan FJK. The metformin in tuberous sclerosis (MiTS) study: a randomised double-blind placebo-controlled trial. EClinicalMedicine 2021; 32: 100715
461. Bisulli F, Muccioli L, d'Orsi G, Canafoglia L, Freri E, Licchetta L, Mostacci B, Riguzzi P, Pondrelli F, Avolio C, Martino T, Michelucci R, Tinuper P. Treatment with metformin in twelve patients with Lafora disease. Orphanet J Rare Dis 2019; 14(1): 149
462. Zhang YM, Ye LY, Li TY, Guo F, Guo F, Li Y, Li YF. New monoamine antidepressant, hypidone hydrochloride (YL-0919), enhances the excitability of medial prefrontal cortex in mice via a neural disinhibition mechanism. Acta Pharmacol Sin 2022; 43(7): 1699–1709
463. Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M, Mohr DC, Schatzberg AF. Major depressive disorder. Nat Rev Dis Primers 2016; 2(1): 16065
464. Fogaça MV, Duman RS. Cortical GABAergic dysfunction in stress and depression: new insights for therapeutic interventions. Front Cell Neurosci 2019; 13: 87
465. Duman RS, Sanacora G, Krystal JH. Altered connectivity in depression: GABA and glutamate neurotransmitter deficits and reversal by novel treatments. Neuron 2019; 102(1): 75–90
466. Fee C, Banasr M, Sibille E. Somatostatin-positive gamma-aminobutyric acid interneuron deficits in depression: cortical microcircuit and therapeutic perspectives. Biol Psychiatry 2017; 82(8): 549–559
467. Ghosal S, Hare B, Duman RS. Prefrontal cortex GABAergic deficits and circuit dysfunction in the pathophysiology and treatment of chronic stress and depression. Curr Opin Behav Sci 2017; 14: 1–8
468. Krystal JH, Sanacora G, Blumberg H, Anand A, Charney DS, Marek G, Epperson CN, Goddard A, Mason GF. Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. Mol Psychiatry 2002; 7(S1 Suppl 1): S71–S80
469. Luscher B, Shen Q, Sahir N. The GABAergic deficit hypothesis of major depressive disorder. Mol Psychiatry 2011; 16(4): 383–406
470. Vahid-Ansari F, Albert PR. Rewiring of the serotonin system in major depression. Front Psychiatry 2021; 12: 802581
471. Chen WB, Chen J, Liu ZY, Luo B, Zhou T, Fei EK. Metformin enhances excitatory synaptic transmission onto hippocampal CA1 pyramidal neurons. Brain Sci 2020; 10(10): 706
472. Zemdegs J, Martin H, Pintana H, Bullich S, Manta S, Marqués MA, Moro C, Layé S, Ducrocq F, Chattipakorn N, Chattipakorn SC, Rampon C, Pénicaud L, Fioramonti X, Guiard BP. Metformin promotes anxiolytic and antidepressant-like responses in insulin-resistant mice by decreasing circulating branched-chain amino acids. J Neurosci 2019; 39(30): 5935–5948
473. Duval F, Mokrani MC, Bailey P, Corrêa H, Crocq MA, Son Diep T, Macher JP. Serotonergic and noradrenergic function in depression: clinical correlates. Dialogues Clin Neurosci 2000; 2(3): 299–308
474. Shivavedi N, Kumar M, Tej GNVC, Nayak PK. Metformin and ascorbic acid combination therapy ameliorates type 2 diabetes mellitus and comorbid depression in rats. Brain Res 2017; 1674: 1–9
475. Wang J, Gallagher D, DeVito LM, Cancino GI, Tsui D, He L, Keller GM, Frankland PW, Kaplan DR, Miller FD. Metformin activates an atypical PKC-CBP pathway to promote neurogenesis and enhance spatial memory formation. Cell Stem Cell 2012; 11(1): 23–35
476. Guo M, Mi J, Jiang QM, Xu JM, Tang YY, Tian G, Wang B. Metformin may produce antidepressant effects through improvement of cognitive function among depressed patients with diabetes mellitus. Clin Exp Pharmacol Physiol 2014; 41(9): 650–656

477. Odaira T, Nakagawasai O, Takahashi K, Nemoto W, Sakuma W, Lin JR, Tan-No K. Mechanisms underpinning AMP-activated protein kinase-related effects on behavior and hippocampal neurogenesis in an animal model of depression. Neuropharmacology 2019; 150: 121–133
478. Wium-Andersen IK, Osler M, Jørgensen MB, Rungby J, Wium-Andersen MK. Diabetes, antidiabetic medications and risk of depression — a population-based cohort and nested case-control study. Psychoneuroendocrinology 2022; 140: 105715
479. Leech T, Chattipakorn N, Chattipakorn SC. The beneficial roles of metformin on the brain with cerebral ischaemia/reperfusion injury. Pharmacol Res 2019; 146: 104261
480. Paintlia AS, Paintlia MK, Mohan S, Singh AK, Singh I. AMP-activated protein kinase signaling protects oligodendrocytes that restore central nervous system functions in an experimental autoimmune encephalomyelitis model. Am J Pathol 2013; 183(2): 526–541
481. Xia CY, Zhang S, Gao Y, Wang ZZ, Chen NH. Selective modulation of microglia polarization to M2 phenotype for stroke treatment. Int Immunopharmacol 2015; 25(2): 377–382
482. Jin Q, Cheng J, Liu Y, Wu J, Wang X, Wei S, Zhou X, Qin Z, Jia J, Zhen X. Improvement of functional recovery by chronic metformin treatment is associated with enhanced alternative activation of microglia/macrophages and increased angiogenesis and neurogenesis following experimental stroke. Brain Behav Immun 2014; 40: 131–142
483. Zhu J, Liu K, Huang K, Gu Y, Hu Y, Pan S, Ji Z. Metformin improves neurologic outcome via AMP-activated protein kinase-mediated autophagy activation in a rat model of cardiac arrest and resuscitation. J Am Heart Assoc 2018; 7(12): e008389
484. Demaré S, Kothari A, Calcutt NA, Fernyhough P. Metformin as a potential therapeutic for neurological disease: mobilizing AMPK to repair the nervous system. Expert Rev Neurother 2021; 21(1): 45–63
485. Cheng YY, Leu HB, Chen TJ, Chen CL, Kuo CH, Lee SD, Kao CL. Metformin-inclusive therapy reduces the risk of stroke in patients with diabetes: a 4-year follow-up study. J Stroke Cerebrovasc Dis 2014; 23(2): e99–e105
486. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. Ann Intern Med 2002; 137(1): 25–33
487. Badrick E, Renehan AG. Diabetes and cancer: 5 years into the recent controversy. Eur J Cancer 2014; 50(12): 2119–2125