Minireview

Metabolic Roles of AMPK and Metformin in Cancer Cells

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Metformin is one of the most widely used anti-diabetic agents in the world, and a growing body of evidence suggests that it may also be effective as an anti-cancer drug. Observational studies have shown that metformin reduces cancer incidence and cancer-related mortality in multiple types of cancer. These results have drawn attention to the mechanisms underlying metformin's anti-cancer effects, which may include triggering of the AMP-activated protein kinase (AMPK) pathway, resulting in vulnerability to an energy crisis (leading to cell death under conditions of nutrient deprivation) and a reduction in circulating insulin/IGF-1 levels. Clinical trials are currently underway to determine the benefits, appropriate dosage, and tolerability of metformin in the context of cancer therapy. This review highlights fundamental aspects of the molecular mechanisms underlying metformin's anti-cancer effects, describes the epidemiological evidence and ongoing clinical challenges, and proposes directions for future translational research.

INTRODUCTION

Because cancer cells must generate enough energy and synthesize sufficient levels of biomolecules to support their rapid proliferation, they have a distinct metabolic advantage over normal, non-proliferating cells. One of the primary metabolic changes in cancer cells is the Warburg effect, characterized by increased glycolysis and lactate production regardless of oxygen concentration (Vander Heiden et al., 2009; Warburg, 1956). Unlike oxidative phosphorylation, which is the primary source of ATP in most normal cells, tumor cells have a comparatively greater capacity to use aerobic glycolysis to generate metabolites that are important for cell growth (DeBerardinis et al., 2008). In order to support proliferation, cancer cells require large amounts of nucleotides, amino acids, and lipids, all of which are used in the synthesis of biological macromolecules. Cancer cells make these macromolecular building blocks from intermediates of the glycolytic pathway, such as glucose-6-phosphate (for glycogen) and ribose-5-phosphate, or *via* protein and lipid biosynthesis, which are regulated by several pathways including mTOR signaling. These metabolic alterations, and the vari-

ous differentially activated signaling pathways in cancer cells, have been investigated by many researchers. One particular protein, the highly conserved Ser/Thr protein kinase complex AMP-activated protein kinase (AMPK), is considered to play important roles in cancer metabolism because of its effects on the regulation of cellular energy homeostasis.

In this review, we summarize the existing evidence regarding the relationships between AMPK and tumor metabolism, discuss the pathophysiological implications of this pathway, and propose that the AMPK activator metformin could form the basis of targeted cancer therapy. A greater understanding of the AMPK pathway and its roles in cancer-specific metabolic regulation will open a new therapeutic window for cancer chemotherapy.

ASSOCIATION OF AMPK WITH CANCER METABOLISM

AMPK is a master sensor and regulator of energy homeostasis at both the cellular and whole-body levels, especially under condition of energy stress (Carling, 2004; Hardie, 2007). Under conditions of metabolic stress, activated AMPK switches off ATP-consuming processes while inhibiting cell growth and proliferation in order to save energy (Shackelford and Shaw, 2009). The main upstream kinase of AMPK is LKB1, which phosphorylates AMPK at Thr172 (Carling, 2004). Inactivating mutation of the gene encoding LKB1 causes Peutz-Jeghers syndrome, which is associated with greatly increased risk of malignant tumors in multiple tissues; thus, LKB1 functions as a tumor suppressor (Alessi et al., 2006). Furthermore, recent clinical studies have demonstrated that LKB1 is also the second most commonly mutated tumor suppressor in sporadic human lung cancer, as well as sporadic pancreatic and cervical cancers (specifically, adenocarcinoma and minimal-deviation adenocarcinoma of the uterine cervix) (Sanchez-Cespedes, 2007). Because LKB1 is a crucial upstream regulator of AMPK, many groups have extensively studied the roles of AMPK in tumorigenesis and tumor metabolism (Fig. 1). Under conditions of energy stress, AMPK inhibits mTORC1 by phosphorylating tuberous sclerosis 2 (TSC2) or communicating directly with Raptor (Gwinn et al., 2008), and also reduces lipid biosynthesis by phosphorylating acetyl-CoA carboxylase (Davies et al., 1990). Together, these observations indicate that AMPK activity

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Fig. 1. Interaction of AMPK with cancer metabolism AMPK inhibits mTOR by phosphorylating TSC2 and Raptor, resulting in decreased protein and lipid synthesis, and also inhibits lipid synthesis by phosphorylating ACC. Chronic activation of AMPK may inhibit aerobic glycolysis (Warburg effect), angiogenesis, and invasion via its effect on mTOR. In addition, AMPK opposes IRS action by phosphorylating Ser⁷⁶⁹.

inhibits anabolic processes such as lipid and protein biosynthesis and cell proliferation, thereby providing a tumor suppressor function. Recently, Faubert et al. (2013) provided genetic evidence that AMPK exerts tumor suppressor activity *in vivo*. In their study, inactivation of AMPK in both transformed and non-transformed cells promoted a metabolic shift to aerobic glycolysis and increased biomass accumulation through normoxic stabilization of the hypoxia-inducible factor-1 α (HIF-1 α), a major transcription factor that regulates many key physiological pathways in cancer cells. Activated HIF-1 α increases the transcription of genes involved in glycolytic pathways, angiogenesis, resistance to apoptosis, and metabolic adaptation (Semenza, 2003). In addition, a growing body of evidence supports the idea that activation of mTOR plays a central role in upregulating HIF-1 α protein synthesis (Dekanty et al., 2005), suggesting that AMPK may exert an anti-Warburg effect by inhibiting the mTOR pathway and downregulating HIF-1 α .

EFFECT OF METFORMIN ON ENERGY HOMEOSTASIS IN CANCER CELLS

The anti-diabetic drug metformin was discovered 50 years ago and has become the first-line drug for the treatment of type 2 diabetes. Population-based studies have shown that metformin reduces the risk of cancer and cancer-related mortality in patients with type 2 diabetes (Bowker et al., 2006; Evans et al., 2005). These observational studies led to further laboratory investigations aimed at elucidating the mechanisms underlying the anti-cancer effects of metformin. The first study to demonstrate an antineoplastic activity showed that, in breast cancer

cell lines, metformin acts as a growth inhibitor in a dose-dependent manner through downregulation of the mTOR/S6 kinase pathway (Zakikhani et al., 2006).

Several possible mechanisms related to the anti-cancer effects of metformin could be explained by functional activation of AMPK. Metformin's primary activity is inhibition of complex I of the mitochondrial electron-transport chain, resulting in an increase of the intracellular AMP/ADP ratio and thereby activating AMPK (Owen et al., 2000). This inhibition of oxidative phosphorylation leads to lower ATP levels and reprogramming of cellular energy metabolism in favor of conserving energy and restoring ATP levels, ultimately causing downregulation of energy-consuming processes and an overall cytostatic effect (Pollak, 2012). This cytostatic effect of metformin is mediated by reduction of protein synthesis *via* mTOR inhibition and reduction of fatty-acid synthesis *via* downregulation of fatty-acid synthase expression (Algire et al., 2010; Larsson et al., 2012).

On the other hand, recent experiments have also shown that AMPK activation, which reduces energy consumption, is paradoxically linked to enhanced stress resistance and viability in cancer cells under metabolic stresses such as hypoxia and metastasis (Jeon et al., 2012). AMPK-phosphorylated acetyl-CoA carboxylase (ACC) 1 and ACC2 regenerate NADPH and compensate for the shortage of NADPH produced by the pentose-phosphate pathway under glucose deprivation (Jeon et al., 2012). NADPH, which serves as a reducing agent in many biosynthetic pathways, plays an important role in preventing the formation of reactive oxygen species (ROS) within cells (Barger and Plas, 2010). Therefore, it is possible that AMPK activation could allow cells detached from the extracellular matrix to sur-

Fig. 2. Effect of metformin on energy homeostasis in cancer cells Metformin inhibits complex I of the mitochondrial electron-transport chain, resulting in activation of AMPK. Metformin exerts a cytostatic effect by reducing cellular biosynthesis *via* mTOR and FAS inhibition. Tumor cells that have lost energy-stress control and compensation systems are more sensitive to energetic stress, leading to cell death by metformin treatment. Metformin lowers plasma glucose levels by decreasing gluconeogenesis and glucose uptake, resulting in lower circulating insulin levels. All three mechanisms might function in different types of cancer.

vive, even under conditions of energy stress or glucose deficiency. Furthermore, AMPK may be required for cell survival when the oncogene MYC is deregulated (Liu et al., 2012). These apparently contradictory effects of AMPK on cancer metabolism - energy-saving cytosynthesis vs. improved survival under stress - are probably due to differences in the timing of the loss of AMPK or other cell type-specific differences.

The susceptibility to energy stress induced by metformin also depends on the functional status of AMPK signaling (Fig. 2). Indeed, when mitochondrial respiration is impaired by metformin, cancer cells compensate by boosting glycolysis to improve bioenergetics; this effect is observed in p53^{+/+} cells, but not p53^{-/-} cells (Buzzai et al., 2007). By contrast, cells that have lost these energy stress control and compensation systems are more sensitive to energetic stress, especially under nutrient deprivation, leading to energy crisis and cell death. When mitochondrial ATP production is reduced by metformin, loss of functional AMPK or p53 creates a situation in which the cell has insufficient energy but no compensatory reduction in energy consumption, ultimately resulting in energy crisis and cell death, as well as tumor suppression (Algire et al., 2011; Buzzai et al., 2007). These cytotoxic effects of metformin arise only in the context of a genetic defect, such as loss of p53 and/or LKB1, that is present in the cancer but not in the normal host tissue, providing opportunities for “synthetic lethality” and raising the possibility of a favorable therapeutic index (Pollak, 2012).

BRAF-mutant melanoma can adapt *via* enhanced oxidative phosphorylation after treatment with the BRAF inhibitor vemurafenib, but this resistance (and associated relapse) can be

overcome when the drug is used in conjunction with an inhibitor of oxidative phosphorylation (Haq et al., 2013). This result is consistent with previous studies, suggesting that combination therapy with metformin and a chemotherapeutic agent exerts synergistic effects and delays the development of resistance in cancer (Niehr et al., 2011).

EFFECT OF PHENFORMIN ON CANCER METABOLISM

Phenformin is an anti-diabetic drug of the biguanide class, like metformin, but in the late 1970s it was withdrawn from clinical use for the treatment of type 2 diabetes due to its association with lactic acidosis (Nattrass and Alberti, 1978). Phenformin is a 50-fold more potent inhibitor of mitochondrial complex I, and is more lipophilic than metformin (Owen et al., 2000). In addition, phenformin has broader tissue availability: metformin requires the expression of organic cation transporter1 (OCT1) for tissue uptake, whereas phenformin does not (Shu et al., 2007). Based on these favorable pharmacokinetic characteristics of phenformin, i.e., greater potency and wider tissue distribution, several laboratory studies have proposed it as an antineoplastic agent. Consistent with this idea, phenformin delayed tumor progression in a Pten^{+/+} spontaneous lymphoma mouse model to a much greater extent than metformin (Huang et al., 2008), and it also inhibited development and tumor growth in breast cancer xenografts (Appleyard et al., 2012). Like metformin, phenformin causes more apoptosis and greater depletion of ATP in AMPK-deficient cancer cells; consequently, phenformin has more potent anti-cancer effects under conditions of metabolic

stress. In a recent study, phenformin appeared to be more effective in the treatment of non-small cell lung cancer (NSCLC), via its greater effects on ATP level and apoptosis, in tumors lacking a functional LKB-AMPK pathway (Shackelford et al., 2013). Although phenformin is unlikely to be suitable as a single agent in advanced-stage disease, it may exert greater synergistic effects in combination with other chemotherapeutic agents. Moreover, because up to 30% of NSCLCs and smaller proportions of other cancers exhibit functional loss of the LKB1 pathway (Gill et al., 2011), phenformin has a selective antitumor effect that is not harmful to normal cells with a functionally intact LKB-AMPK pathway. Thus, following clinical investigations aimed at determining the tolerable dosage and duration required for treating cancer, phenformin could be a potent anti-cancer agent.

EFFECTS OF METFORMIN ON CIRCULATING INSULIN/IGF-1 LEVELS

Administration of metformin in patients with type 2 diabetes lowers not only circulating glucose concentration, *via* an increase in muscle glucose uptake and suppression of hepatic gluconeogenesis, but also lowers circulating insulin levels (Bailey and Turner, 1996). Increased circulating levels of insulin and insulin-like growth factor (IGF) have been linked with cancer progression, suggesting that obesity and insulin resistance provide cellular environment to cancer at least in part by activating signaling pathways that drive cell growth and proliferation (Pollak, 2008). Declines in insulin levels during metformin administration suppress tumor growth in the context of hyperinsulinemia, but this cannot explain the clear inhibitory effects on tumor growth in rodent models that are not insulin-resistant (Algire et al., 2011). Although fasting insulin levels decline during metformin treatment in non-diabetic patients with breast cancer, this decline is relatively small, and the clinical benefit related to cancer progression has not been established (Campagnoli et al., 2012). Because it is not yet clear whether declines in fasting and postprandial insulin reduce tumor growth to a clinically significant extent, the long-term effects of metformin on both insulin levels and inhibition of tumor growth in non-diabetic/non-hyperinsulinemic subjects require further investigation.

IGF-1 plays an important anabolic role by increasing protein synthesis, thereby promoting proliferation and metastasis (LeRoith et al., 1995). Although it is not clear how metformin regulates IGF-1 levels, metformin-induced activation of AMPK may increase phosphorylation of IRS1, which inhibits IGF-1-stimulated activation of Akt/Tsc1/mTOR, thereby reducing protein synthesis and proliferation (Ning and Clemmons, 2010). Crosstalk between insulin/IGF-1 receptors and G-protein-coupled receptor signaling, implicated in autocrine-paracrine stimulation of a variety of malignancies, is also disrupted by metformin-induced AMPK activation, leading to attenuated DNA synthesis and proliferation (Rozengurt et al., 2010).

OTHER MECHANISMS RELATED TO METFORMIN'S ANTI-CANCER PROPERTIES

Unfolded protein response

Glucose deprivation, a condition that occurs in solid tumors and ischemic tissues, leads to the accumulation of misfolded or unfolded protein in the endoplasmic reticulum (ER), a condition that activates unfolded protein response (UPR) (Ron and Walter, 2007). The UPR is a complex signaling network that promotes cell survival by limiting the accumulation of unfolded

proteins in the ER. When this protein-folding capacity collapses, cells activate apoptotic pathways, leading to cell death. Metformin inhibits production of the UPR transcriptional activators such as XBP1 and ATF4, thereby disrupting the UPR transcriptional program and inducing massive cell death during glucose deprivation (Saito et al., 2009). These data demonstrate that disruption of the UPR program during glucose deprivation by metformin represents an attractive selective approach for cancer treatment because energetic stress such as glucose deprivation is common features of poorly vascularized solid tumors, but are not observed in normal tissue.

Cell cycle

AMPK plays a crucial role in the regulation of cell division. Whole-genome array-based expression profiling in breast cancer patients has revealed that metformin downregulates the expression of many genes involved in mitosis, including genes encoding kinesins, tubulins, histones, Aurora- and Polo-like kinases, and ribosomal proteins (i.e., protein and macromolecule biosynthesis) (Oliveras-Ferreros et al., 2009). Consistent with this observation, treatment of breast- and lung cancer cell lines with metformin and paclitaxel synergistically increased the number of cells arrested at the G2-M phase of the cell cycle, decreased tumor growth, and increased apoptosis to a greater extent than treatment with either drug alone (Rocha et al., 2011). Metformin can also regulate the DNA-damage response (DDR) via selective activation of the ataxia telangiectasia mutated (ATM) and ATM targets such as the protein kinase Chk2 (Vazquez-Martin et al., 2011). Because the DDR is a major component of tumor suppressor mechanisms, metformin's activation of ATM/Chk2 in response to DNA damage may contribute to its cancer preventive effect.

Immunological effects

Metformin has effects on the whole organism that are related to immunomodulation *via* regulation of cytokines and growth factors (Bonanni et al., 2012). In a previous study, metformin attenuated proliferation of murine T lymphocytes and increased apoptosis and necrosis (Solano et al., 2008). Because the role of anti-inflammatory pathways in the prevention of cancer is well known, this immunomodulatory action of metformin could enhance cancer immunotherapy. Tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF-6) plays a critical role after infection by modulating fatty-acid metabolism in CD8 T-cell, contributing to its development and survival (Pearce et al., 2009). Furthermore, defects in fatty-acid oxidation and CD8 T_M cell generation, defective in the absence of TRAF6, can be restored by metformin administration. Although only a few studies have addressed the immunological effects of metformin on cancer, the experimental data that does exist indicate that this drug can be expected to contribute to improved anti-cancer immunity.

PREVENTIVE EFFECT OF METFORMIN ON CANCER DEVELOPMENT

Recent studies have shown that metformin may also target cancer stem cells. Unlike most cells within a tumor, self-renewing cancer stem cells are resistant to chemotherapy and, following treatment, they can regenerate all types of tumor cells (Polyak and Weinberg, 2009). In mice bearing human breast cancer xenografts (Hirsch et al., 2009), metformin treatment selectively inhibited CD44⁺/24^{-low} cancer stem cells and exerted a synergistic effect with well-established chemotherapeutic agents,

Table 1. Clinical trials investigating the association between metformin and cancer

Cancer type	Study design	Enrolled patient	Outcome	Comparison	Relative risk (95% C.I.)	Reference
Any cancer	Cohort study	10,309 (T2D)	Cancer-related mortality	SU vs MTM	1.3 (1.1-1.6)	Bowker et al. (2006)
Any cancer	Case-control study	2,829 (T2D)	Risk for developing cancer	MTM vs non-MTM	0.77 (0.64-0.92)	Evans et al. (2005)
Any cancer	Cohort study	62,863 (T2D)	Risk for developing cancer	SU vs MTM Insulin vs MTM	1.36 (1.19-1.54) 1.42 (1.27-1.60)	Currie et al. (2009)
Any cancer	Cross-sectional study	7,519 (T2D, non-DM)	Risk for developing cancer Cancer-related mortality	MTM vs non-DM Any treatment excluding MTM vs non-DM	0.92 (0.39-2.20) 1.42 (0.73-2.74)	Baur et al. (2011)
Breast	Nested - case control study	200 (non-DM)	Changes in Ki-67	MTM vs placebo	11.1 (-0.6-24.2) ^a -10.5 (-26.1-8.4) ^b	Bonanni et al. (2012)
Breast	Case-control study	2,529 (T2D, non-DM)	Changes in pathologic complete response (pCR) rates	MTM Non-Metformin Non-DM	24% (13-34) ^c 8% (2.3-14) 16% (15-18)	Jiralerspong et al. (2009)
Colon	Cohort	595 (T2D)	Cancer-related mortality	MTM vs non-MTM	0.66 (0.476-0.923)	Lee et al. (2012)
Liver	Case-control study	2,924 (T2D, non-DM)	Risk of developing cancer	MTM vs SU or Insulin	0.15 (0.04-0.50)	Donadon et al. (2010)
Liver	Case-control study	1,524 (T2D, non-DM)	Risk of developing cancer	MTM vs other SU vs other	0.3 (0.2-0.6) 7.1 (2.9-16.9)	Hassan et al. (2010)
Pancreas	Case-control study	2,763 (T2D)	Risk of developing cancer	MTM vs non-MTM	0.87 (0.59-1.29)	Bodmer et al. (2012)

SU, sulfonylurea; MTM, metformin.

^aProportional change in Ki-67 (%) in women with a HOMA index less than or equal to 2.8.

^bProportional change in Ki-67 (%) in women with a HOMA index greater than 2.8.

^cProportions of pathologic complete response (pCR).

reducing tumor mass and preventing relapse much more effectively than either agent alone. Metformin also regulates breast cancer stem cells by transcriptionally repressing the expression of genes associated with the epithelial-to-mesenchymal transition (EMT) such as *ZEB1*, *TWIST*, and *SNAIL* (Vazquez-Martin et al., 2010). During EMT, epithelial cells lose their differentiated features such as cell-to-cell adhesion and motility defects, and acquire mesenchymal characteristics such as invasion of surrounding tissues and resistance to apoptosis (Polyak and Weinberg, 2009). These transdifferentiation programs promote the growth and survival of cancer cells, and inhibition of EMT by metformin could suppress the ontogeny of cancer stem cells.

CLINICAL TRIAL AND CHALLENGES FOR FUTURE TRANSLATIONAL RESEARCH

On the basis of the aforementioned molecular studies, metformin has been evaluated in several exploratory clinical studies of multiple types of cancers (Table 1). Early reports have shown that the use of metformin in type 2 diabetes patients reduces cancer incidence and cancer-related mortality (Bowker et al., 2006; Evans et al., 2005). One of the largest-scale retrospective cohort studies so far, which enrolled 62,809 diabetic patients, showed that metformin therapy is associated with lower risk than therapy with insulin or insulin secretagogues, especially in colorectal or pancreatic cancer (Currie et al., 2009). Baur et al. (2011) were the first to demonstrate an independent effect

of metformin in lowering cancer prevalence; their large-scale population study adjusted for confounding factors such as age, sex, Hemoglobin A1c, smoking status, and body mass index (BMI). Another recent study investigated whether metformin use before surgery or after chemotherapy is associated with better treatment outcomes in various types of cancer (Bonanni et al., 2012). That study showed that metformin treatment before invasive breast cancer surgery decreased biomarkers of tumor cell proliferation, such as Ki67 staining, in a manner that depended on insulin resistance. Furthermore, diabetic patients with breast cancer receiving metformin and neoadjuvant chemotherapy had an independent higher pathologic complete response (pCR) rate than diabetics not receiving metformin (Jiralerspong et al., 2009). The results of those two clinical studies suggest that metformin may confer a benefit by increasing sensitivity to an established chemotherapy regimen when administered in the context of perioperative care. However, most of these retrospective cohort studies had some limitation in their suitability for evaluating the direct effect of metformin on cancer. First, some previous studies did not consider other confounding effects including BMI, degree of diabetic control, metformin concentration level, and other medications that may affect cancer development. Second, because time-related biases are common in observational studies, the observed relationships may not necessarily be causal. Finally, some clinical studies that focused on the improvement of proliferation index or insulin resistance upon metformin treatment did not directly connect

Table 2. Ongoing clinical trials investigating the association between metformin and cancer

Organ	NCT number (phases)	Title	Completion	Estimated enrollment	Primary outcome measures
Breast	NCT01266486 (II)	Effect of Metformin on Breast Cancer Metabolism	Mar-14	40	Measure metformin-induced effects in phosphorylation of S6K, 4E-BP-1 and AMPK via immunohistochemical analysis.
	NCT01310231 (II)	A Trial of Standard Chemotherapy With Metformin (vs Placebo) in Women With Metastatic Breast Cancer	Sep-15	78	Progression-free survival
	NCT01101438 (III)	Randomized Trial of Metformin vs Placebo in Early Stage Breast Cancer	Jun-16	3,582	Invasive disease-free survival
	NCT01589367 (II)	Neoadjuvant Letrozole Plus Metformin vs Letrozole Plus Placebo for ER-positive Postmenopausal Breast Cancer	Apr-15	208	Primary endpoint: clinical response rate
	NCT01477060 (II)	Modulation of Response to Hormonal Therapy With Lapatinib and/or Metformin in Patients With Metastatic Breast Cancer	Nov-16	168	Rate of patients free from disease progression
	NCT01042379 (II)	I-SPY 2 TRIAL: Neoadjuvant and Personalized Adaptive Novel Agents to Treat Breast Cancer	Nov-14	800	Determine whether adding experimental agents to standard neoadjuvant medications increases the probability of pathologic complete response (pCR) over standard neoadjuvant chemotherapy for each biomarker signature established at trial entry.
Colon	NCT01632020 (II)	Effect of Metformin on Biomarkers of Colorectal Tumor Cell Growth	Jul-14	40	Proliferation status of CRC tumor and adjacent normal tissue following metformin therapy. Mucosal apoptotic status of CRC tumor and adjacent normal tissue following metformin therapy.
	NCT01312467 (II)	A Trial of Metformin for Colorectal Cancer Risk Reduction Among Patients With a History of Colorectal Adenomas and Elevated Body Mass Index	Aug-13	43	Change in activated S6serine235
	NCT01523639 (II)	A Randomized, Placebo-controlled, Double-blind Phase II Study Evaluating if Glucophage Can Avoid Liver Injury Due to Chemotherapy Associated Steatosis	Jul-16	132	Reduction in the chemotherapy-associated steatosis, as assessed by the steatosis subcore of the NAFLD activity score.
Prostate	NCT01433913 (II)	Metformin Hydrochloride in Treating Patients With Prostate Cancer Undergoing Surgery	Nov-13	50	Cell proliferation in the prostatectomy tissue as assessed by Ki67 expression using immunohistochemistry.
	NCT01796028 (II)	Metformin-Docetaxel Association in Metastatic Hormone-refractory Prostate Cancer	Dec-18	100	PSA response rate
	NCT01561482 (II)	Study of Metformin With Simvastatin for Men With Prostate Carcinoma	Mar-15	37	Efficacy, as measured by an improvement in PSA doubling time (PSADT) between baseline and 6 months, of the combination of metformin plus simvastatin in patients with recurrent prostate cancer.
Pancreas	NCT01210911 (II)	Metformin Combined With Chemotherapy for Pancreatic Cancer	Dec-13	120	Survival after 6 months
	NCT01666730 (II)	Metformin Plus Modified FOLFOX 6 in Metastatic Pancreatic Cancer	Mar-14	43	Median overall survival (OS)
	NCT01167738 (II)	Combination Chemotherapy With or Without Metformin Hydrochloride in Treating Patients With Metastatic Pancreatic Cancer	Jan-14	82	Progression-free survival at 6 months

(Continued)

Table 2.

Organ	NCT number (phases)	Title	Completion	Estimated enrollment	Primary outcome measures
Others	NCT01697566 (III)	An Endometrial Cancer Chemoprevention Study of Metformin	May-19	100	Effect of metformin and/or lifestyle intervention on biomarkers
	NCT01333852 (II)	Metformin Plus Paclitaxel for Metastatic or Recurrent Head and Neck Cancer	Feb-11	45	Progression-free survival
	NCT01717482 (II)	Metformin as a Chemoprevention Agent in Non-small Cell Lung Cancer	May-14	24	Number of participants screened
	NCT01750567 (II)	A Pilot Study of Metformin Therapy in Patients With Relapsed Chronic Lymphocytic Leukemia (CLL) and Untreated CLL	Oct-16	53	Time to treatment failure
	NCT01840007 (II)	Pilot Study Evaluating the Efficacy and Safety of Metformin in Melanoma	Feb-14	20	Complete response rate or partial response rate

<http://www.clinicaltrials.gov>

these endpoints with decreased mortality or enhanced chemotherapeutic effects. Nevertheless, the American Diabetes Association and the American Cancer Society consensus stated 'Although still limited, early evidence suggests that metformin is associated with a lower risk of cancer and that exogenous insulin is associated with an increased cancer risk' (Giovannucci et al., 2010).

More than 50 phase II and III clinical trials of metformin in oncology are currently underway (Table 2). Future large-scale long-term follow-up studies should adjust for clinical information that may interfere with interpretation, such as baseline insulin level, BMI, and usage of other medication. Furthermore, further investigations will be required to determine the most suitable candidates for exploitation of the effects of metformin on cancer prevention or treatment because the vulnerability of cancer cells to metformin depends on cancer cell type, nutritional environment around the cancer cells, and coexistence of mutations in functionally related genes (e.g., LKB1 or p53). Therefore, to predict the effectiveness of metformin, it will be important to identify the patients or cancers that possess these characteristics. Finally, future research should seek to determine the optimal and tolerable dosages of metformin and phenformin to maximize their anti-cancer effects without toxicity. Once such information has become available, we predict that the anti-diabetes drug metformin, already proven to be affordable and safe, will be of great value in the treatment of multiple types of cancer (Bodmer et al., 2012; Donadon et al., 2010; Hassan et al., 2010; Lee et al., 2012).

CONCLUSION

Many preclinical, epidemiological, and clinical studies have shown that the first-line anti-diabetic drug metformin reduces overall cancer risk and mortality. Metformin-activated AMPK activity in tumor cells decreases protein and lipid synthesis via downregulation of the mTORC1 signaling pathway, thereby limiting their growth and proliferation. Metformin also inhibits mitochondrial oxidative phosphorylation, leading to reduced ATP levels, and thereby induces energy stress in cancer cells, making them susceptible to energy crisis and cell death in the presence of certain functional mutations. In addition to its effects on energy metabolism and related signaling pathways, metformin also decreases tumor growth *via* several mechanisms including reduction in insulin/IGF-1 level, regulation of the UPR, regula-

tion of the cell cycle, and immunomodulation. Further translational research will be necessary to determine tolerable and optimal dosages of metformin, as well as types of cancer for which it will have the greatest therapeutic benefit. As knowledge about metformin's mechanisms moves from the bench to the clinic, this old drug should be of tremendous value as a new therapeutic agent in oncology.

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