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REVIEW ARTICLE

THERAPEUTIC USE OF METFORMIN IN THYROID CANCER

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Summary

Thyroid cancer is a rare type of malignancy. However, thyroid cancer constitutes more than 90 % of endocrine tumors. Metformin (N', N'-dimethybiguanide) is the most commonly prescribed drug in the world, and the annual number of prescriptions for this drug exceeds 120 million. Metformin is the first-line oral treatment for patients with type II diabetes. Metformin has recently been investigated for potential anti-cancer activity in patients with thyroid cancer by stimulating the Adenosine Mono-Phosphate-Activated Protein Kinase (AMPK) pathway in some types of tumors. In general, the anti-cancer mechanism of metformin acts directly by blocking mitochondrial oxidative phosphorylation through down-regulation of mitochondrial complex I and mitochondrial glycerophosphate dehydrogenase. This leads to a state of metabolic stress that in turn stimulates the AMPK pathway due to ATP reduction, and leads to inhibition of the mechanical (mammalian) target of the rapamycin (mTOR) pathway, which subsequently inhibits cancer cell proliferation and stimulates apoptosis and autophagy with cell cycle perturbation. Metformin also acts in an independent manner, in addition to its indirect actions that target insulin resistance. In this review, we reviewed 21 studies on the use of metformin in thyroid cancer, which showed that administration of metformin in diabetic patients is associated with a reduced incidence of thyroid cancer. On the other hand, the use of metformin enhances the response to anticancer drugs in thyroid cancer. Overall, we need further prospective studies to elucidate the synergistic mechanism of metformin when it is used to treat thyroid cancer as adjuvant therapy with anticancer drugs.

Key words: AMPK; Cancer; Metformin; mTOR; Thyroid

Introduction

Metformin, the biguanide class, is anti-diabetic drug. Over the past years, metformin has been known to have many beneficial effects that include body weight reduction, complications of neurological and psychiatric disorders, cardiovascular disorders, and additional effects in the treatment of cancer, metabolic disorder and nonalcoholic fatty liver disease (1-3). The anti-tumor effect of metformin was determined in patients with diabetes mellitus (DM) on metformin therapy, who showed a reduced incidence of cancer compared to diabetic patients treated with other anti-hyperglycemic drugs (4-7). Understanding the antitumor effect of metformin has encouraged a series

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of preclinical and clinical studies to reveal the anticancer activity of metformin for the prevention and treatment of different types of cancer (8). Several findings indicated the anticancer activity of metformin, as a single drug or as an adjuvant therapy, in different types of cancer (9-15). A meta-analysis study was conducted on 13,008 diabetic type II patients with cancer, and it was noted that there was an improvement in survival in patients receiving metformin compared to patients receiving other antidiabetic drugs (16). Another meta-analysis study of 65,540 type II diabetic patients with cancer reported an association between metformin use and a lower incidence of cancer with an improvement in overall survival in cancer patients (17). However, there are insufficient data on the activity of metformin in the treatment of endocrine tumors (18). Endocrine tumors (e.g. pituitary, parathyroid and thyroid gland) may be benign or develop into cancer (19). However, the incidence of endocrine tumor is increasing annually (18). During the past years, the growth-inhibiting effects of metformin have been demonstrated in patients with endocrine cancer. Although the available information is insufficient, evidence from several studies has shown that the anticancer effects of metformin are exerted through direct or indirect mechanisms (8). Metformin targets mitochondrial respiratory chain complex I, Adenosine Mono-Phosphate-Activated Protein Kinase (AMPK) and the Mammalian Target of Rapamycin (mTOR) (20). These targets are components of important pathways that relate to metabolism, generation, cells lifespan and apoptosis. In addition, several other molecular targets of metformin have been discovered in cellular carcinomas including cyclins, miRNAs and mitochondrial genes (8). In the present work, we review recent studies that support the link between metformin use and its therapeutic effects in treatment of some types of cancers.

Incidence of Thyroid Cancer in Metformin-Treated Diabetic Patients

Thyroid cancer accounts for approximately 90 % of endocrine malignancies (21). The incidence of thyroid cancer grows rapidly than other types of cancer. There were > 56000 patients in the United States, during year 2012, with thyroid cancer (22). Adiposity and Type 2 diabetes mellitus (T2DM) are risk factors associated with a higher incidence of thyroid cancer (23). A retrospective study in Korean subjects showed that people who received metformin for a long time or had accumulated doses had a lower rate of thyroid cancer (24). The second study was conducted in Taiwanese and included 1.4 million type II diabetic patients who were receiving the antidiabetic drug, metformin, and had a lower incidence of thyroid cancer compared to those who did not receive metformin (25). In contrast to the above two studies, a UK clinical study of 98 patients with type II diabetes and 416 healthy subjects demonstrated that there was no association between metformin intake and the risk of thyroid cancer (26).

In addition to reducing the risk of thyroid cancer, intake of metformin is associated with a long disease-free life in type II diabetic patients with thyroid cancer (27). A retrospective study including 240 diabetic patients with thyroid cancer were receiving metformin reported a small size of thyroid cancer, high remission rate and prolonged complication free life when compared with diabetic patients who not intake of metformin and patients with only thyroid cancer (22).

Mechanism of metformin's anticancer action

Beyond its effect on glucose level, metformin has been reported to reduce the risk of cancer and tumorigenesis. It generally acts through inhibition of ATP generation and the electron transport chain (ETC). However, new evidence shows that the mechanistic target of rapamycin complex 1 (mTORC1) and AMP-activated protein kinase (AMPK) are both regulated by metformin through various processes which are not dependent on ETC inhibition and cellular ATP levels (8).

Metformin has been found to achieve its anticancer effect through two mechanisms involving (AMPK- and mTORC1) independent and (AMPK-, and mTORC1)-dependent (Figure 1). The independent process is associated with a decrease in the level of insulin and glucose in the blood, as well as decreased biosynthesis of precursors resulting from the tricarboxylic acid (TCA) cycle. Metformin also lowers the generation of reactive oxygen species (ROS), oxidative stress, and DNA damage by blocking the ETC (28).

In addition, metformin suppresses the production of specificity protein (Sp)1, Sp3, and Sp4, as well as pro-oncogenic, Sp-regulated genes. The most widely expressed transcription factors specificity protein are (Sp)1, Sp3, and Sp4 in a variety of malignancies, such as pancreatic cancer, and have been identified as potential targets for anticancer medicines (29).

The AMPK- and mTORC1-dependent pathway involves direct inhibition of mTORC1 subunits phosphorylation, lipid synthesis suppression, inhibition of the NFB pathway, and enhanced protein acetylation. The direct action of metformin and the AMPK-independent suppression of mTORC1 is responsible for mTORC1-dependent processes. Metformin can be employed in combined therapies for many diseases such as cancer, metabolic, neurodegenerative, and aging-related disorders due to its effect on AMPK and mTORC1 which have an essential role in the physiology and pathology of the human body (30). Metformin has systemic effects in addition to cellular effects, which contribute to its anticancer therapeutic potential. The reduction of insulin/insulin-like growth factor (IGF)-1 signaling, the reduction of proinflammatory cytokine levels, the reduction of expression of cell adhesion molecules, the suppression of the Warburg effect, and the release of lactate by tumors are all examples of systemic effects (28).

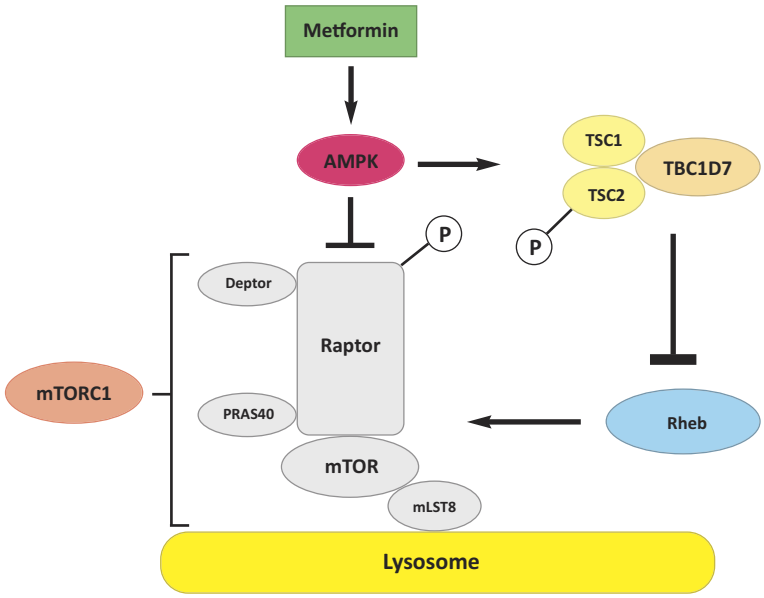

Figure 1. Metformin Blocks mTORC1 in an AMPK-Dependent pathway. AMPK is stimulated by metformin which inhibits mTORC1 signaling in at least two ways. First, mTORC1 is inhibited by AMPK following phosphorylation of the mTORC1 raptor subunit. Second, the tuberous sclerosis complex (TSC), which consists of TSC1, TSC2, and TBC1D7, is activated by AMPK through TSC2 subunit phosphorylation. As a result, Rheb is inhibited. Abbreviations: AMPK, AMP-activated protein kinase; mLST8, mammalian lethal with SEC13 protein 8; mTORC1, mechanistic target of rapamycin complex 1; Rheb, Ras homolog enriched in the brain.

Inhibition of Complex I of the ETC by metformin

At the molecular level, complex I of the mitochondrial ETC is metformin's specific target. Molecular modeling of a metformin analog targeted to mitochondria indicated that it possibly restricts electron transport from complex I's catalytic site to the binding site of ubiquinone; metformin is probably to do the same (31). However, metformin's activity causes a decline in decreased nicotinamide adenine dinucleotide (NADH) oxidation and ATP generation. In vitro, this impact has shown to be more compelling than in vivo (32). In vitro, metformin inhibits ETC complex I at higher concentration than in in vivo (33).This seeming conflict may be explained because metformin positive charge and the mitochondrial inner membrane polarization which are made metformin concentration in the specific organs, like the liver, is much greater than in the serum (34). Inhibitory action of metformin on ETC in the hepatocytes requires more time because it requires extra period to attain the high concentration in mitochondria when there is a low metformin concentration in the blood (34). Tagging of metformin with lipophilic cationic groups, which increasing hydrophobicity and the positive charge of metformin, increase metformin targeting to mitochondria (35). Metformin targeting mitochondria is particularly efficient in killing cancer cells of pancreas, in addition to increasing inhibition of ETC (36). Metformin at high concentration inhibits ETC, mTORC1 through dependent and independent mechanisms and increase ratio of AMP/ATP which reduces gluconeogenesis (37).When ETC is inhibited, it is

unable to oxidize NADH and suppression of TCA cycle. Metformin reduces oxidation of glucose and enhances reliance on metabolism of reductive glutamine (38).Also, ETC inhibition activates glycolysis as an alternate mechanism for generation of ATP and enhances generation of lactate, allowing oxidation of NADH into nicotinamide adenine dinucleotide (NAD+). Metformin reduces intermediates of TCA cycle through blocking both the oxidation of NADH to NAD+ and generation of ribonucleotide and deoxyribonucleotide triphosphates (39).when ETC is inhibited by metformin ,ratio of the NADH/NAD+ rises, pyruvate act as an electron acceptor instead of oxygen to aids in cells growth in culture that lack a functioning ETC (40). When metformin is used to block the ETC, causing a decrease in mitochondrial NAD+, increase in NADH and change in way of the malate-aspartate shuttle, malate is exported to cytosol. In cytosol, malate transforms to oxaloacetate, and then the cytosolic aspartate aminotransferase (cAST) transaminates oxaloacetate to aspartate. In addition, cells rely on cAST to synthesis of aspartate, which permits cells with inhibited ETC to grow when aspartate is provided (41). Since Aspartate used as a precursor to synthesis of purine and pyrimidine therefor it is essential for synthesis of protein (42). It found that reduction of TCA cycle intermediates and accumulation of NADH in tumor tissues of patients with ovarian cancer treated by metformin against those not treated by metformin. Also, aspartate was not decreased according to metabolomics analysis (43). These results showed that the low micromolar concentration of metformin is adequate to target complex I of the ETC in hepatic tissues. This agrees with idea that metformin targets complex I of the ETC in non-hepatic tissues as well (44).

Metformin-activated AMPK's anticancer effects

They are at least somewhat independent of AMPK's involvement in gluconeogenesis control. Action of AMPK is required for appropriate regulation of cell proliferation; lack of liver kinase B1 (LKB1) is common in tumor, and a germline mutation in LKB1 causes Peutz-Jeghers syndrome, which is a tumor-predisposing factor [69]. AMPK also phosphorylates p53, however the significance of metformin in p53 activation remains unknown (45). Because fast development of cancer cells necessitates an enhanced fatty acid synthesis rate to accommodate construction of cellular membranes, activation of AMPK limits synthesis of fatty acid synthesis and growth of tumor (46). Inhibitory phosphorylation of acetyl-CoA carboxylase (ACC) by AMPK is a corresponding mechanism. Citrate lyase acts on citrate to produce nucleocytosolic acetyl-CoA, which is a crucial precursor for de novo synthesis of fatty acid. Acetyl-CoA is carboxylated to malonyl-CoA, the initial and rate-limiting step in de novo synthesis of fatty acid, is catalyzed by ACC. HMG-CoA reductase, that catalyzing the rate-initial reaction in production of cholesterol, is similarly phosphorylated and inhibited by AMPK (47). Metformin activates AMPK, which raises acetylation of histone and non-histone proteins in addition to blocking formation of lipid. Acetylation of histones needs a substrate (acetyl-coA) for histone acetyltransferases in the nucleocytosolic compartment. Stimulating effect of metformin on AMPK reduces acetyl-CoA to malonyl-CoA conversion, this leads to an increasing in nucleocytosolic acetyl-CoA, increasing histone and non-histone proteins acetylation, and alteration expression of gene (47). Also, metformin has other epigenetic effects, such as affecting DNA and methylation of histone (48). AMPK's control of mTORC1 may be the significant anticancer impact of metformin-activated AMPK, as mTORC1 suppression reduces synthesis of protein and proliferation of cell.

Differentiated Thyroid Cancer

***In vitro* studies**

Differentiated Thyroid Cancer (29) accounting for about 95% of thyroid cancer. Inhibitory growth effect of metformin exerts on cell lines of thyroid cancer (22, 49). Metformin enhances arrest of cell cycle in G1 phase and apoptosis, results in decreasing cellular viability (49). Activation of AMPK by metformin causes to inhibit expression of p70S6K/pS6 which is essential protein for tumor growth (22). In cases of decreasing cellular levels of ATP, proliferation of cells is blocked (50). Inhibition of mitochondrial complex 1 by metformin leading to raise cellular ratio of AMP/ADP thus leading to activate AMPK pathway, then begins a chain of steps to conserve cellular energy (51). Activated AMPK pathway activates tuberous sclerosis complex 2 (TSC2), thus leading to inhibit mTOR route (52). Inhibitory growth effect of metformin on malignancy cell is due to downstream mTOR pathway, resulting in decreasing synthesis of protein (p70S6K) that is essential for growing of cancer cells (53). A preclinical study showed that metformin decreased synthesis of protein (p70S6K) in thyroid cancer cell line treated by metformin (22). Metformin activate AMPK pathway and reduce Protein kinase (AKT) signaling pathway

by blocking insulin receptor substrate-1 (IRS-1) phosphorylation that results in inhibition of phosphatidylinositide 3-kinase (PI3K)/AKT signaling pathway. These anticancer effects blocked expansion of papillary thyroid cancer and enhanced necrosis of cancers in mice (54). Moreover, Metformin inhibits mTOR, which is AKT route aim, through blocking transmission to AKT route (55). Also, Inhibitory action of metformin on insulin-like growth factor 1 receptor (IGF-1R) inhibits growth of cell cancer (56). Mitochondrial glycerophosphate dehydrogenase (mGPDH), an enzyme exists on the inner membrane of mitochondria and participates in the glycerol-3-phosphate shuttle, was detected as a target of metformin in cancer of thyroid cells (57). Primarily, mGPDH has been determined to be the target of metformin, which acts by inhibiting gluconeogenesis in the liver of rats (58). mGPDH transports the generating electrons in the cytosol from breakdown of glucose to the mitochondrial electron transport chain. mGPDH and its counterpart cGPDH constitute a significant connection between oxidative phosphorylation and glycolysis (59). Many studies showed that the inhibitory effect of metformin and expression of mGPDH result in a decrease the oxidative phosphorylation that is negatively affecting on the development of thyroid cancer cells (57). As mGPDH is a metformin target, preclinical studies demonstrated that thyroid cancer with high mGPDH expression is more vulnerable to impacts of metformin than that with low expression of mGPDH. Furthermore, thyroid cancer cell lines showed high expression mGPDH than normal cell lines (57). Metformin is a mitochondrial function inhibitor that influences on respiration of mitochondria by inhibiting both mitochondrial complex 1 and mGPDH, according to these finding. Metformin therapy blocked uptake of glucose by cells of Papillary Thyroid Cancer (PTC) in preclinical study. This decrease in uptake of glucose was linked to low expression genes of HexoKinase 2 (HK2) and glucose transporter (GLUT) (60).

Impacts of metformin on excess production of thyroid hormones

Although Differentiated Thyroid Cancer (29) is not usually leading to excess thyroid hormones secretion, metastatic Follicular Thyroid Cancer (FTC) may cause overproduction of thyroid hormones resulting in clinical signs of thyrotoxicosis (61). There is no evidence that metformin plays a part in control of thyroid hormone output in thyroid tumor. A clinical study was conducted on patients with thyroid cancer and insulin resistance, it was founded that thyroid hormones remain within acceptable limits. However, the levels of free triiodothyronine were increased significantly in metformin-treated patients (62). It was established that by an animal model study with thyroid cancer, metformin treatment caused an increase in free thyroxine and free triiodothyronine, with a small decrease in thyrotropin levels (63). Metformin also has an important role in indirect regulating insulin signaling, a growth stimulator for thyroid cancer. The presence of elevated insulin level, usually seen in insulin resistance, is associated with higher risk of thyroid cancer (64). Furthermore, insulin may boost the stimulatory effect of Thyroid Stimulating Hormone (TSH). The high levels of TSH have been linked with the advanced of thyroid cancer, especially when it presents with growth factors (insulin and IGF) (65). Metformin therapy is linked to a decrease in levels of insulin. Therefore it causing in blocking of thyroid cancer by counteracting stimulatory action of insulin (66).

Retrospective Clinical Studies

Many clinical studies conducted to evaluate the therapeutic effects of metformin on thyroid cancer. One retrospective study revealed that metformin can attenuate the myelosuppressive effect of radioactive iodine when added as an adjuvant therapy (67). However, another study evaluated the effect of metformin on levels of TSH in patients with thyroid cancer. The results of this study showed that the use of low-dose metformin (500 mg once daily) did not reduce TSH levels in levothyroxine-treated patients (68).

Medullary thyroid cancer (MTC)

The incidence of MTC is between 3-5% of thyroid cancer. Treatment is routinely by tyrosine kinase inhibitors (TKIs) (69). However, it does not result in full recovery but prolongs the time to progression of the disease. At this time, limited data is present about using of metformin in the treatment of MTC because rareness of MTC occurring.

***In vitro* studies**

Limited preclinical trials are presented to determine anti-tumor effect for metformin on MTC. *In vitro* study was conducted on thyroid cancer cell lines (variant origins) and normal cell lines. It was shown that metformin

at therapeutic concentration could reduce the growth of cancer cells (70). Metformin anti-cancer activity was investigated in two cell lines of MTC, growth inhibitory effect, decreasing capacity of spheroids formation, blocking of mTOR pathway, inhibition of Extracellular signal-regulated kinase (p-ERK)activity and reduced cyclin-D1 expression were detected in this trial (71). Also, it was shown that inhibition activity of metformin is not associated with the activation of AMPK.

PI3K/AKT is the most important pathway to activate mTOR. Interestingly, one study revealed that metformin cannot block the activated AKT according to the levels of p-AKT in MTC cell lines. Accordingly, mTOR inhibition can be achieved by another mechanism. Therefore, it has been showed that mTOR can be activated by ERK (p-ERK) by blocking tuberous sclerosis complex 2 (TSC2) or by (regulatory-associated protein of mTOR (RAPTOR) phosphorylation. Hence, the inhibitory effect of metformin on the p-ERK pathway resulting inhibition of the mTOR pathway in MTC and DTC cell lines (72).

Impacts of metformin on excess production of thyroid hormones in MTC

Calcitonin, biomarker of tumor, is produced in MTC. High levels of calcitonin may cause disturbance of calcium levels. There is no clinical data about metformin activity on overproduction of calcitonin and Adrenocorticotropic hormone (ACTH) in patients with (MTC).

Anaplastic thyroid cancer (ATC)

Incidence of ATC

ATC is a rarely occur cancer of all thyroid cancers, having a high rate of mortality with bad prediction (73). Patients with ATC are usually managed by surgery, radioactive and chemotherapeutic agents (74). There is currently no clinical data present considering metformin activities in the treatment of ATC.

***In vitro* trials**

Preclinical trials reported the effect of metformin on ATC cell lines in inhibiting the growth of cancer cells and enhancing apoptosis (49, 75). Also, Metformin blocks migration of cancer cells and impacting on ATC cancer cell morphology (75). In a preclinical trial, metformin can potentiate the anti-cancer effects of sorafenib (multi-kinase inhibitor), enhancing apoptosis and cell cycle arrest in cell lines with ATC. Downregulation of ERK pathway may lead to blocking the growth of cancer cells (76). In another research, conducted on ATC and PTC cell lines, metformin and BRAFV600E inhibitor resulted in a decrease in cellular viability of cancer cells and enhancing apoptosis (77).

Furthermore, we need more studies to elucidate the metformin effects on ATC.

***In vivo* studies**

At present, there are no in vivo studies were conducted on the metformin activity in ATC.

Efficacy of Metformin in other types of cancer

Many studies showed the anticancer action of metformin, through either genes or pathway, on other cancer types. Metformin appeared to exerts its anticancer action in breast cancer through activation of AMPK, cell cycle arrest enhancing and down- regulation of cyclin D1 (78). In bladder cancer, anticancer actions of metformin are showed by *in vitro* study, metformin inhibit cyclooxygenase-2 (COX2) and also signal transducer and activator of transcription 3 (STAT3) pathway that resulting in reducing expression B-cell lymphoma 2 (Bcl-2) and cyclin D1. Subsequently proliferation of cell is inhibited and apoptosis is induced (79). Furthermore, Metformin has been demonstrated to have anti-cancer actions in ovarian cancer cells by lowering the expression of genes involved in apoptosis control, such as vascular endothelial growth factor (VEGF), caspase-3, Bcl-2-associated X and Bcl-2 (44). Metformin also suppressed cell growth and promoted apoptosis by activating AMPK and decreasing the expression

of mTOR, P53 and protein kinase B (Akt) (46). In stomach cancer, *in vitro* study was conducted on gastric cancer cell line, Metformin decreased proliferation of cell and stopped the G0-G1 phase of the cell cycle, which was linked to a large decrease in G1 cyclins (72). Reducing proliferation of cell is a time- and concentration-dependent way, according to the results of the aforementioned in vitro investigations. Metformin concentrations utilized were substantially greater than those seen in normal human plasma (48). As a result, extrapolating these findings to clinical practice is limited. Furthermore, it is unclear if the cells were seeded in varied concentrations to assess the impact of Metformin on parameters such as confluence in these investigations. However, there are limited investigations on the efficacy of Metformin in thyroid cancer (129). Also it is necessary to assess the efficacy of metformin in thyroid cancer and other cancer types at lower concentration and longer time metformin exposure.

Combination therapies of Metformin and other drugs

Metformin, either alone or combined with other drugs, switches metabolism from anabolism to catabolism by concurrently targeting AMPK, mTORC1and other pathways, which may explain its positive impacts in control metabolism and growth. Rapamycin, first generation of mTOR inhibitors, is combined with metformin to treat patients with cancer and organ transplant. Temsirolimus and everolimus, rapamycin derivatives were licensed by Pfizer (2007) and Novartis (2009) respectively, are used to treat renal cancer. Rapalogs, allosteric inhibitors, have limited activity to treat cancer because they only block phosphorylation of a subset of mTORC1 (80). ATP-competitive inhibitors, second generation of mTOR inhibitors are now being tested in clinical studies, decrease catalytic activity of mTORC1 and seem to have higher activity than rapalogs. RapaLink, third generation of mTOR inhibitors, is newly disclosed and combined with the ATP-competitive inhibitor (49). Further researches are required to show anticancer activity of metformin-based combination therapy. The success in determining whether metformin mechanism in activation of AMPK and inhibition of mTORC1 depends on the cellular level of ATP or independent on the state of cellular energy, as well as if the two pathways coexist in various types of cell, will be critical in developing future treatment methods. The development of innovative, highly active and selective metformin analogues, particularly mitochondrially-targeted ones, will aid these efforts. There are many preclinical and clinical trials that have demonstrated the anticancer activity of Metformin alone or in combination with other chemotherapeutic agents in different types of cancer as shown in Table 1.

Table 1. shows the effects of metformin on thyroid cancer and other types of cancer as reported in some preclinical and clinical trials.

Type of trial	Type of Tumor	Therapy regimen	Result
Preclinical *in vitro* (81)	Thyroid Cancer	Variable dose of metformin	Inhibited cell proliferation, colony formation and increased the percentage of apoptotic cells upon conc. of metformin. Metformin also induced cell cycle arrest in G0/G1 phase
Preclinical *in vitro* (82)	Thyroid cancer	Variable dose of metformin	Decreasing cellular proliferation enhancing of apoptosis upon conc. of metformin
Preclinical *in vitro* (22)	Thyroid cancer	Dose of metformin range from 0.5 to 5mM	Decreasing cellular proliferation upon conc. and period of treatment
Preclinical *in vitro* (49)	Thyroid cancer	Dose of metformin range from 0.1 to 40 mM	Blocking cellular proliferation, cell cycle and enhancing apoptosis upon conc. of metformin
Preclinical *in vitro* (54)	PTC	Dose of metformin range from 0.1 to 20 mM	Blocking cell viability and enhancing apoptosis upon conc. of metformin
in vivo		Dose of metformin 10, 50 and 100 mg/ml in water for 1 month	Decreasing in size of malignancy upon conc. of metformin
Preclinical *in vitro* (57)	DTC	Dose of metformin 5 mM	Blocking proliferation of cell and mitochondrial respiratory chain
in vivo		12.5 mg of metformin in water for 28 days	Blocking tumor progression

Type of trial	Type of Tumor	Therapy regimen	Result
Preclinical *in vitro* (60)	PTC	Dose of metformin range from 2.5 to 10 mM	Decreasing in viability of cell and uptake of glucose
in vivo		Dose of metformin 300 mg/kg/day for 7 days	Decreasing uptake of glucose
Preclinical *in vitro* (70)	Thyroid cancer	Dose of metformin range from 0.03 to 20 mM	Blocking proliferation, migration and enhancing of apoptosis upon conc. of metformin
Preclinical *in vitro* (71)	MTC	Dose of metformin range from 0.5 to 5 mM	Blocking growth of cell upon conc. of metformin and period of treatment
Preclinical *in vitro* (75)	ATC	Dose of metformin range from 2.5 to 60 mM	Decreasing in cellular viability, apoptosis and migration upon conc. of metformin
Preclinical *in vitro* (76)	ATC	Combination therapy of metformin (dose 5 mM) and sorafenib	Enhancing apoptosis and arrest of cellular cycle using combined therapy of metformin and anticancer (sorafenib)
Preclinical *in vitro* (77)	ATC	Combination therapy of metformin (dose 2mM) and vemurafenib	Decreasing in cellular viability and enhancing apoptosis using combination therapy of metformin and Anticancer drug (vemurafenib)
Clinical (24)	Thyroid cancer	Patients with Thyroid cancer and diabetes mellitus (receiving 531 mg per day as average dose of metformin) against group not receiving metformin	Decreasing complication of thyroid cancer
Clinical (25)	Thyroid cancer	Patients with Thyroid cancer and diabetes mellitus (receiving variable dose of metformin)against group not receiving metformin	Decreasing complication of thyroid cancer
Clinical (26)	Thyroid cancer	Patients with Thyroid cancer and diabetes mellitus (receiving variable dose of metformin) against group not receiving metformin	No role of metformin in decreasing complication of thyroid cancer
Clinical (27)	DTC	Patients with Thyroid cancer and diabetes mellitus (receiving 979 mg per day as average dose of metformin) against group not receiving metformin	Decreasing complication of thyroid cancer
Clinical (22)	Thyroid cancer	Patients with Thyroid cancer and diabetes mellitus (receiving variable dose of metformin) against thyroid cancer group	Small size of cancer in patients with thyroid cancer and diabetes mellitus
Clinical (83)	Thyroid cancer	Patients with Thyroid cancer and diabetes mellitus (receiving variable dose of metformin) against group not receiving metformin	Decreasing size and growth rate of cancer. Improved compliance to anti-cancer therapy
Preclinical *in vitro* (84)	Colorectal cancer	Combination therapy of metformin and Oxaliplatin (OXA)	When coupled with OXA, metformin is more effective on the two HCT116 cell lines and has a synergistic effect
Preclinical *in vitro* (84)	Colorectal cancer	Combination therapy of Metformin and Fluorouracil (5-FU)	The addition of 5-FU does not improve cell viability decrease
Preclinical *in vitro* (84)	Colorectal cancer	Combination therapy of Metformin and irinotecan (IRI)	IRI alone reduced cell viability significantly, whereas IRI combined with Metformin lowered its efficacy (IRI versus IRI-MET in HT29 cells). This was especially true for the sequential treatments in which MET was given first, followed by IRI.

Type of trial	Type of Tumor	Therapy regimen	Result
Preclinical (85) *in vitro*	Neuroendocrine tumor (NET)	Dose of Metformin range from 0.1 to 10 mM	Reducing in cellular viability upon conc. of metformin and period of treatment
Preclinical (86) *in vitro*	Pancreatic NET (pNET)	Dose of Metformin 10mM	Reducing in cellular viability upon period of treatment
Preclinical (87) *in vitro*	Breast cancer (BC)	Combination therapy of Metformin and Tamoxifen	Metformin is efficacious in reducing the proliferation of ER-positive BC cells when combined with tamoxifen

Conclusion

It is suggested from all trials and studies that metformin has anticancer activity in diabetic type II patients with thyroid cancer. Metformin may act through multiple mechanisms either directly by activation of AMPK pathway or indirectly by antagonizing insulin action. Metformin has many targets that have been detected in endocrine tumors like mGPDH, cyclins and GSK3. The precise mechanism of metformin to regulate activity of these targets is unknown and needs to be discovered. Also, metformin can exert an inhibitory effect on growth of cancer cell through inhibiting insulin and IGF-1. Furthermore, antitumor effect of metformin could be exerted through downregulation of growth factors like TSH, insulin and IGF. It is important to note that the anti-stimulating growth activities of metformin are largely due to block of growth factors that lead to decreased proliferation of cancer cells more than enhancing apoptosis. Therefore, using metformin as an adjuvant therapy with other anticancer agents may lead to a high compliance rate rather than using metformin as alone therapy. Further studies are required to demonstrate the synergistic mechanism of metformin in thyroid cancer. Therefore, using metformin as aiding agent to another therapy may lead to high compliance rate rather than using metformin as alone therapy.

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Conflict of Interest

The authors declare no competing interests.

Adherence to Ethical Standards

Not applicable.

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