Effects of metformin on cancers in experimental and clinical studies: Focusing on autophagy and AMPK/mTOR Signaling Pathways

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Abstract

Metformin (MET) is a preferred drug for the treatment of type 2 diabetes mellitus. Recent studies show that apart from its blood glucose-lowering effects, it also inhibits the development of various tumours, by inducing autophagy. Further, various studies have confirmed the inhibitory effects of MET on cancer cell lines' propagation, migration, and invasion. However, despite the many publications on the anticancer potential of MET, there is no existing comprehensive review and account of its autophagy-associated anticancer activity, this review. This review noted that MET exerts its anticancer effects by regulating key signalling pathways such as phosphoinositide 3-kinase (PI3K), LC3-I and LC3-II, Beclin-1, p53, and the autophagy-related gene (ATG), inhibiting the mTOR protein, downregulating the expression of p62/SQSTM1, and blockage of the cell cycle at the G0/G1. Moreover, MET can stimulate autophagy through pathways associated with the 5' adenosine monophosphate-activated protein kinase (AMPK), thereby inhibiting he development and progression of various human cancers, including hepatocellular carcinoma, prostate cancer, pancreatic cancer, osteosarcoma, myeloma, and non-small cell lung cancer. Therefore, this detailed review provides a framework for further investigations that may appraise the autophagy-induced anticancer potential of MET and its repurposing for cancer treatment.
Metformin (MET) is a preferred drug for the treatment of type 2 diabetes mellitus. Recent studies show that apart from its blood glucose-lowering effects, it also inhibits the development of various tumours, by inducing autophagy. Further, various studies have confirmed the inhibitory effects of MET on cancer cell lines' propagation, migration, and invasion. However, despite the many publications on the anticancer potential of MET, there is no existing comprehensive review and account of its autophagy-associated anticancer activity, this review. This review noted that MET exerts its anticancer effects by regulating key signalling pathways such as phosphoinositide 3-kinase (PI3K), LC3-I and LC3-II, Beclin-1, p53, and the autophagy-related gene (ATG), inhibiting the mTOR protein, downregulating the expression of p62/SQSTM1, and blockage of the cell cycle at the G0/G1. Moreover, MET can stimulate autophagy through pathways associated with the 5' adenosine monophosphate-activated protein kinase (AMPK), thereby inhibiting he development and progression of various human cancers, including hepatocellular carcinoma, prostate cancer, pancreatic cancer, osteosarcoma, myeloma, and non-small cell lung cancer. Therefore, this detailed review provides a framework for further investigations that may appraise the autophagy-induced anticancer potential of MET and its repurposing for cancer treatment.

**Keywords:** AMPK; Autophagy; Autophagy Markers; Cancer; Metformin; mTOR pathway

**Running Head:** Metformin and Cancer

**Graphical Abstract:** Metformin inhibits tumour growth. Metformin’s inhibitory impact on cancer cell lines involves autophagy and cell cycle arrest by regulation of key signalling pathways and gene expressions such as the mammalian target of rapamycin (mTOR) protein, light chain 3-II (LC3-II), p62/SQSTM1 and autophagy-related gene (ATG).

**Abbreviations:**
AKT: Protein kinase B
AMPK: 5’ Adenosine Monophosphate-Activated Protein Kinase
ATGs: Autophagy Related Genes
CRISPR/Cas9: Clustered regularly interspaced short palindromic repeats-associated protein 9
CEBP: CCAAT/Enhancer-Binding Protein Delta
EGFR: Epidermal growth factor receptor
ERK1/2: Extracellular Signal-Regulated Kinase 1/2
ERα-positive: Estrogen Receptor Alpha-Positive
ESCC: Esophageal Squamous Cell Carcinoma
GC: Gastrointestinal Cancer
HbA1c: Glycated Hemoglobin
HCC: Hepatocellular Carcinoma
HER2: Human Epidermal Growth Factor Receptor 2
LC3-II: light chain 3 II
MAP1LC3A-I: Microtubule Associated Protein 1 Light Chain 3 Alpha
MET: Metformin
mTOR: Mammalian Target of Rapamycin
NSCLC: Non-Small Cell Lung Cancer
PI3K: Phosphoinositide 3-kinase
TKIs: Tyrosine kinase inhibitors

Introduction

Metformin (1,1-dimethylbiguanide hydrochloride, MET) was first approved by the Food and Drug Administration in 1994 as an antidiabetic drug. Since then, it has been used as a first-choice medication to treat hyperglycemia and insulin resistance [1-4]. MET enhances tissue sensitivity to insulin and decreases glycated hemoglobin (HbA1c) levels. This is facilitated to some extent by the stimulation of the 5’ adenosine monophosphate-activated protein kinase (AMPK) signalling pathway [5], and other metabolic and cellular physiological processes, including inflammation, proliferation, autophagy, cell apoptosis, oxidative damage, and senescence [6-9].

Various studies showed that the MET is not only capable of decreasing the risk of cancer in patients suffering from diabetes but could also prevent and treat non-diabetic patients suffering from pancreatic, bladder, lung, breast, thyroid, and prostate cancers [10-13]. Besides, MET showed promising results in averting colorectal cancer, squamous cell carcinoma, oral ductal carcinoma in situ, human cholangiocarcinoma, and endometrial cancer [14-18]. Research demonstrates that MET exerts its anticancer effects by activating AMPK, disrupting the crosstalk between the insulin receptor and the G protein-coupled receptor signalling system [19, 20]. Also, the direct insulin-independent action of MET by the AMPK activation leads to downregulating of the mammalian target of rapamycin (mTOR) signalling and protein synthesis in tumour cells [21].

AMPK, a serine/threonine-protein kinase, controls autophagy and mitochondrial homeostasis by restricting mTOR activity [22, 23]. Autophagy refers to an evolutionarily conserved process in which cellular materials are sent to lysosomes for degradation, which causes the breakdown and ultimately the turnover of the generated macromolecules [24]. This is accomplished by participating in cell proliferation, apoptosis, and ridding of damaged cellular components [25]. There are several physiological and pathophysiological roles
of autophagy in normal and cancerous cells [26]. Autophagy acts as a double-edged sword for tumour cells: the proliferation and survival of tumour cells are facilitated by abnormal autophagy of tumour cells, whereas moderate autophagy has an anti-tumour effect [27, 28]. Compared to normal cells, cancer cells have a high dependency on autophagy, whose level of activity increases significantly due to cellular damage and extensive energy demands [29], especially when exposed to chemotherapy and nutrient deprivation [30].

Autophagy suppresses the initial stages of carcinogenesis and its increased activity inhibits tumor cells and advancing cancer cells [31]. Its main goal is to eliminate potentially hazardous cellular components, maintaining genomic stability, and stimulating immune response processes to avert malignant transformation [32]. Research shows that autophagy may be influenced by MET [33]. Also, it has been found that MET is an activator of AMPK through the phosphorylation of AMPK at Thr172, triggering mitophagy and macroautophagy [34]. Elsewhere, it has been deduced that MET can directly promote autophagy by inhibiting the mTOR restriction or indirectly by modulating the AMPK pathway [35-37]. The effect of autophagy on cancer cells may vary depending on cell type, genetic background, cell cycle phase, and microenvironment [38, 39].

Recent evidence indicates that MET induces autophagy in different cancers, including colon cancer, melanoma, and in Ishikawa endometrial cancer cells [40]. Besides, MET prevents gastric cancer by triggering beclin1-dependent autophagy via the AMPK-mTOR signaling pathway [33]. Moreover, an inhibitory role is exhibited by MET in the treatment of androgen receptor (AR)-negative prostate cancer by activating autophagy through the AMPK/autophagy pathway [28]. Moon et al. reported that the antitumor effects of MET are driven by autophagy inhibition in ovarian cancer cells [41]. Elsewhere, Chen et al. demonstrated that autophagy is restrained by MET in breast cancer [42]. These findings underscore that based on the environmental conditions or the cell type, autophagy may either support cell survival or induce cell death [43]. Despite the many reports on the anticancer activity of MET through autophagy, there is a paucity of nuanced and comprehensive review of MET’s potential in preventing or treating cancer by inducing autophagy and the precise mechanisms of action. Therefore, this review presents a comprehensive of the autophagy-induced anticancer activity of MET to provide crucial information for appraising its anticancer efficacy and shape future studies that may validate its repurposing for cancer therapy.

**Autophagy process**

There are three main types of autophagy: macro-autophagy, chaperone-mediated autophagy, and micro-autophagy. The degradation of cytosolic constituents in the lysosome is supported by all these types, but through different pathways [44]. In macro-autophagy, referred to as ‘autophagy’ from now on, a phagophore covers some part of the cytoplasm, including organelles, to produce an autophagosome and its outer membrane consequently integrates with lysosomes to become autolysosomes [45]. Autophagy is used by cells to avert tumorigenesis; however, in certain cases, it is also used to support tumour cell survival. Autophagy plays a dynamic and complex role in cancer, which also depends on time and context [46].

Previous research has found the pivotal activity of Beclin-1 in autophagy [33, 47]. Specifically, Beclin-1 is a critical member of a Class III PI3K complex that is involved in the generation of autophagosomes. It is an essential component for other autophagy proteins to be localized on the pre-autophagosomal membrane, thereby promoting the initiation and progression of the autophagy process [48].

Autophagy and unfolded protein response signalling are critical for cellular homeostasis during metabolic stresses like nutrient depletion and hypoxia. Subsequently, autophagy is stimulated to remove impaired organelles and aggregated protein as well as energy sources are provided to cells that are lacking in nutrients. During the early stages of autophagy, several key events occur to promote the formation and elongation of autophagosomes. These events include the conversion of LC3 I to LC3 II, the conjugation of ATG12 to ATG5, and the activation of Beclin-1, which work together to facilitate the formation of the double-membraned vesicle known as an autophagosome. During the later stages of autophagy, the protein P62 plays a crucial role in the selective targeting and degradation of ubiquitinated protein aggregates. P62 binds to these aggregates and facilitates their transport to the autophagosome, where they are enclosed
and delivered to the lysosome for degradation. There is an integration of autophagosomes with lysosomes, and the acidic conditions cause the degradation of the P62-containing autophagic vesicles [39, 49]. It was confirmed by Moon et al. that MET triggered autophagy through the higher conversion of LC3B and expression of ATG12-ATG5, as well as lower P62 activity [41].

**MET and AMPK/mTOR Signaling Pathways**

Met has accepted anti-cancer activities, which operate through diverse pathways. These pathways involve both AMPK-dependent and —independent inhibition of mTOR, a critical player in tumour growth and preventing apoptosis and autophagy. Metformin’s indirect suppression of mTOR, which occurs independently of AMPK, is achieved by reducing systemic insulin levels. [50-52]. AMPK as a protein kinase is highly present in all eukaryotic cells. Phosphorylation of AMPK is primarily facilitated by liver kinase B1 (LKB1), which is frequently mutated in various types of cancers [53]. Metformin’s anti-tumour effects that are dependent on AMPK are initiated by the activation of AMPK through LKB1. This activation results in the suppression of the downstream mTOR signalling axis [54]. In addition, metformin can also suppress complex I of the mitochondrial respiratory chain, which decreases ATP production and consequently activates AMPK. [55]. AMPK has two signalling pathways that inhibit mTOR activity. The first pathway involves tuberous sclerosis complex 2 (TSC2), which is activated by AMPK. TSC2 is a GTPase-activating protein that binds to TSC1 to form a complex. This complex work together to inhibit the Ras homolog enriched in the brain (Rheb) and mTOR, effectively preventing mTORC1 [56, 57]. The second pathway involves the direct phosphorylation and suppression of RAPTOR, which is a scaffold protein of the mTORC1 complex. AMPK directly phosphorylates the regulatory-associated protein of mTOR (RAPTOR), leading to its inhibition and subsequent downregulation of mTORC1 activity [58]. Activation of AMPK by MET results in the suppression of mTORC1, leading to decreased phosphorylation of the major downstream effectors, eukaryotic initiation factor 4E-binding proteins (4E-BP) and ribosomal protein S6 kinases (S6Ks). This prevention is related to the presence of tuberous sclerosis complex 1/2 [54, 59]. The indirect mechanism of action of metformin is through insulin, type-1 insulin-like growth factor (IGF-1), and its receptor. The IGF-1 receptor belongs to the family of tyrosine protein kinase receptors. It has an important activity in promoting malignant cell phenotype, facilitating cell metastasis, protecting cells from apoptosis, and enhancing cell proliferation [60, 61]. When insulin levels decrease, the signalling axis involving phosphoinositide 3-kinase (PI3K) and protein kinase B (AKT) is also reduced. As a result, the activity of TSC2 is increased, leading to the suppression of mTOR [62]. In addition to its role in activating AMPK, MET could also independently inhibit mTOR through the stimulation of DNA-damage-inducible transcript 4 (REDD1). REDD1, in turn, activates TSC2, leading to mTOR inhibition. Alternatively, MET can inhibit mTOR by directly inhibiting the activity of Rag GTPases [63, 64]. Previous studies have investigated that the levels of amino acid can modulate the activity of Rag GTPases and downstream mTOR signalling. However, metformin’s suppression of Rag GTPases appears to be independent of changes in the levels of amino acid. This suggests that metformin may affect mTOR signalling through a unique and alternative mechanism that does not rely on the regulation of Rag GTPases by amino acid availability [64, 65].

The AMPK/mTOR signalling axis is widely recognized for its association with autophagy, as it is important for promoting the initiation of this cellular process [66]. In their study, Fan et al. demonstrated that MET had a suppressive effect on the progression of carcinogenesis in a rat model treated with N-nitrosomethylbenzylamine (NMBzA) by modulating the AMPK/mTOR signalling pathway [67]. In a separate investigation, Gao et al. observed that MET treatment induced autophagy and autophagic flux while activating the AMPK/mTOR in human hepatocellular carcinoma (HCC) cells. The researchers suggested that a combination therapy involving MET and molecular markers of the AMPK/mTOR pathway could potentially serve as a therapeutic approach for HCC [68].

p53, a tumour suppressor protein, has a pivotal function in various cellular processes like apoptosis, cell cycle arrest, metabolism, and differentiation [69] Current evidence depicted the role of p53 in autophagy induction and demonstrated that p53 could trigger autophagy through both transcription-dependent and transcription-independent mechanisms [70, 71]. Figure 1 shows a summary of the effects of Met.
Figure 1. Mechanism action of MET against tumour cells. MET exerts a direct inhibitory effect on complex I of the mitochondrial electron transport chain, resulting in a reduction in the ATP/AMP ratio. This, in turn, activates AMPK, which serves to inhibit mTOR and activate P53, thereby impacting various cellular processes downstream. In addition, Metformin’s systemic effects cause a reduction in insulin availability and subsequently modulate the proliferative pathway, PI3K/AKT, indirectly.

Role of MET in different cancers

Gastrointestinal cancer (GC)

MET is a first-choice drug for type 2 diabetes treatment with high safety, tolerability, and efficacy, and it has been shown a notable relationship between the duration of use of MET and a lower risk of gastrointestinal cancer in patients with type 2 diabetes [72, 73]. A significant pathway in MET effect on glucose metabolism and also on anti-tumor processes is the AMPK/mTOR pathway [74]. Liu et al. found that MET (300 mg/kg) restricts the spread and invasion of GC cells. MET is also capable of supporting beclin1-dependent autophagy in GC cells. Beclin1 acts as a tumour suppressor and restricts the malignant phenotypes of GC cells, both in vitro and in vivo. In addition, MET is capable of upregulating beclin1-mediated autophagy to prevent GC cells from moving into the AMPK-mTOR signalling system [33]. Although some studies depicted that autophagy could restrict tumours, others are of the view that autophagy is capable of stimulating malignant tumours [75, 76]. The principal signalling system of upstream autophagy regulation is AMPK/mTOR [77]. It was shown in prior studies that wild-type P53 brings about the stimulation of AMPK signalling and restricts the mTOR to trigger autophagy, whereas mutant P53 is capable of suppressing autophagy through various pathways, such as by blocking AMPK signalling [78, 79]. Chen et al. depicted that acute and chronic radiation-induced intestinal toxicity was decreased by MET administration (500 mg/kg for mice), which could also increase the radiosensitivity of colorectal tumours with P53 mutation through the optimization of autophagy. This study used mouse intestines with acute and chronic histological damage to analyze radioprotection, and it used the IEC-6 cell line to examine the processes in vitro [80].

Moreover, MET could have a protective role for the liver against viral or chemical hepatotoxicants [81]. MET decreased the risk of HCC and appeared to curtail HCC development in type II diabetes mellitus patients [82]. Also, MET administration (0.2 mM) triggered apoptosis as well as prevented autophagy in H4IIE rat HCC cells through AMPK and P38 mitogen-activated protein kinase signalling pathways. MET also reduced the activity of various autophagy-related proteins, such as LC3B and beclin-1, in the AMPK-dependent pathway [83]. Gao et al. determined that the use of MET (10 mM) could trigger autophagy, and autophagic flux, giving rise to higher levels of protein 1 light chain 2 II (LC3-II) and reduced levels of P62. In addition, it could stimulate the AMPK expression and suppress mTOR and P70 S6 kinase phosphorylation in human HCC cells [68]. Another research investigated that MET exerts its anticancer role by restricting the proliferation and invasion as well as triggering autophagy in HCC cell lines in vitro (HepG2 and Bel-7402) through the PI3K/AKT/mTOR pathway. They showed that MET stimulated autophagy in HepG2 and Bel-7402 cells and tumours. It was also found that MET treatment (400 μmol/L, for cells) was able to reduce proliferation and give rise to autophagy of HCC cells by targeting mTOR. A substantial reduction in the xenograft tumour weight in BALB/c athymic nude mice was also caused by MET (200mg/kg). It also suppressed Beclin-1 and LC3II expression, as well as PI3K, AKT phosphorylation, and mTOR [84]. G1 phase arrest and apoptosis in HCC are also caused by MET [85]. Protein LC3-II takes part in the development of autophagosomes and is one of the autophagic markers [86, 87]. Autolysosomes can cause the degradation of p62, which is a substrate of autophagy [86]. Tsai et al. discovered that MET (25mM) can cause autophagy by increasing the transcription factor of CCAAT/enhancer-binding protein delta (CEBPD). In addition, MET treatment also caused a simultaneous increase in the growth arrest and apoptosis of Huh7 cells. The authors showed that combination therapy with MET and rapamycin could increase autophagic cell death via the AMPK-dependent and the AMPK-independent pathways, respectively. It is implied by the findings that a pro-apoptotic role is performed by autophagy in the MET-regulated anticancer activity in Huh7 cells. The findings (Figure 2) also show that LC3B and ATG3 gene transcription is triggered and LC3B puncta formation increases in a transcription-dependent manner by the CEBPD. In addition, after the decrease in
CEBPD due to MET treatment, there was a downregulation of the CEBPD-responsive gene P27. The ratio of LC3B-II/LC3B-I increased in this study, while the P62 expression decreased in MET-treated Huh7 cells. The increase in CEBPD is also able to improve the LC3B-II/LC3B-I ratio and decrease the expression of P62 in Huh7 cells. These results together provide novel insight and therapeutic methods by targeting autophagy when treating HCC [88].

**Figure 2. A schematic model of the molecular mechanism underlying the ability of MET to enhance anticancer effects.**

According to Ling et al., a combination of MET (10 mmol/l) and sorafenib (a protein kinase inhibitor) prevents proliferation, supports apoptosis, and stimulates autophagy of HCC by targeting the mTOR pathway *in vitro* (Bel-7402 cells and HepG2 cells) as well as in vivo (mice). The authors noted that MET induced AMPK and this would consequently prevent the activation of ERK1/2 [89].

The third most prevalent cancer of the digestive tract and the sixth main cause of cancer-related mortality worldwide is esophageal squamous cell carcinoma (ESCC) [90]. Feng et al. found that ESCC cells were exposed to MET-induced apoptotic cell death due to the pharmacological or genetic suppression of autophagy. MET treatment mechanistically inactivated signal transducer and activator of transcription 3 (Stat3) and Bcl-2. Consequently, MET-induced autophagy and apoptosis were increased by small interfering RNA (siRNA)-mediated Stat3 knockdown, while simultaneously increasing the preventive impact of MET on cell viability. In the same way, MET suppresses the Bcl-2, which suppresses apoptosis as well as autophagy. MET downregulated Stat3 and Bcl-expression which triggered apoptosis and autophagy and restricted tumour development *in vivo* (nude mice). The inhibition of the Stat3-Bcl-2 signalling axis promotes MET-induced growth inhibition of ESCC by allowing crosstalk between apoptosis and autophagy. MET was found to increase p-AMPK and decrease p-mTOR in this study. The reduced conversion of LC3-I to LC3-II shows that AMPK knowledge restricted MET-regulated autophagy to some extent. MET-triggered autophagy was also decreased by the overexpression of Bcl-2, as shown by the decreased conversion of LC3 and insufficient upregulation of Beclin-1 and ATG5 (two vital autophagic regulators) (Figure 3) [91].

**Figure 3. Proposed mechanisms to explain the effect of MET in ESCC**

**Ovarian cancer**

Ovarian cancer is the most dangerous gynecological cancer in developed countries and the second-leading cause of mortality due to gynecological malignancies all over the world [92]. Late diagnosis and chemoresistance are the main factors contributing to the high lethality of ovarian cancer, making it one of the most challenging gynecologic malignancies to treat effectively. These obstacles significantly weaken the chemotherapeutic efficacy in patients with ovarian cancer. [93, 94]. Notably, studies have shown that MET can inhibit cell viability, invasion, and autophagy while promoting apoptosis in paclitaxel-resistant ovarian cancer cells such as SKOV3/PR and A2780/PR. Yu et al. found that MET treatment resulted in a considerable reduction in the expression of lncRNA small nucleolar RNA host gene 7 (SNHG7), a gene that triggers paclitaxel resistance in both cell lines. The researchers were able to provide evidence that MET treatment could effectively reverse the paclitaxel resistance and autophagy induced by SNHG7 in ovarian cancer cells. Additionally, they demonstrated that MET treatment stimulated miR-3127-5p in paclitaxel-resistant cells by preventing the expression of SNHG7 [95]. Furthermore, it was shown by Zou et al. that MET (10 mmol/l) restricted the spread and invasion of SKOV3 cells as well as triggered cellular apoptosis and autophagy they showed that microtubule-associated protein 1 light chain 3 alpha-II (MAP1LC3A-II) served as a biomarker for examining cellular autophagy. With a higher dose of MET treatment, MAP1LC3A-I expression was downregulated in the SKOV3 cells, whereas MAP1LC3A-II expression was upregulated. This showed that MET increased the cellular autophagy of SKOV3 cells [96]. Additionally, Moon et al. illustrated that the antitumor activities of MET in ovarian cancer cells were improved by autophagy inhibition. This means that growth inhibition and apoptosis are increased by the repression of autophagy by inhibitors like 3-MA and BafA1 [41].

**Pancreatic cancer**
The prevalence and fatality rates of pancreatic cancer, a solid aggressive tumour, are both rising [97]. As demonstrated by Chen et al., co-treatment of the human pancreatic cancer cell lines ASPC-1 and PANC-1 with MET (30 mM) and pitavastatin (10 mM) could more effectively decrease cell proliferation and migration, support cell cycle arrest, stimulate AMPK, and suppress PI3K/mTOR while trigger cell apoptosis and autophagy. The scientists discovered that MET raised the AMPK phosphorylation in ASPC-1 cells. These findings showed that using a combination of MET and pitavastatin could be effective for human pancreatic cancer in the future [2]. Moreover, in mouse insulinoma (MIN6) cells, Jiang et al. found that MET (2mM) stimulated autophagy via AMPK signalling. They found that MET restricted the proliferation of MIN6 pancreatic β cells and facilitated cell death in normal cell culture conditions. In contrast, MIN6 cells were protected against palmitic acid-induced cell apoptosis by MET. Therefore, a dual part was performed by MET in controlling MIN6 pancreatic β cell survival [98].

Breast cancer

The most common malignancy among women is breast cancer, which is responsible for the second-greatest number of cancer-related deaths [99]. It has been found in various studies that MET may play a part in the prevention, neoadjuvant, extended adjuvant, and advanced disease treatment for breast cancer [100, 101]. Patients with breast cancer who had MET therapy presented notably higher survival rates than those who did not receive MET [102]. It has been demonstrated in studies that the overexpression of H19 supports the tumorigenic characteristics of breast cancer cells in vivo [103]. It should be noted that H19 is expressed to a large extent in nearly all tumour tissues and performs a critical part in the growth of tumours [104, 105]. Several factors regulate the expression level of H19 like MET [106]. Chen et al. proved that MET can induce ferroptosis by restricting autophagy through H19, which depicted the part played by H19 in the antitumor impact of MET and offered a novel understanding of MET [42].

Also, breast cancer often leads to dysregulation of PI3K/AKT/mTOR activities [107, 108]. As a reaction to signals from growth factors and nutrients, it has been discovered that mTOR limits autophagy; however, the downstream mTOR effectors that control autophagy have not yet been discovered. Hence, by limiting mTOR, AMPK promotes autophagy [109]. Liu et al. tested various human breast cancer cell lines for cell proliferation and autophagy after using MET (1 M) singly and also in combination with RAD001 and/or chemotherapeutic agents. On its own, MET restricted cell proliferation and triggered apoptosis in various breast cancer cell lines (ERα-positive, HER2-positive, and triple-negative). There was a more profound cytotoxic impact of MET in triple-negative breast cancer cell lines in comparison to other cell lines. Caspases and apoptosis-inducing factors are somewhat necessary for the cell apoptosis that MET induced. An interesting finding made by the authors was that the MET stimulated cell autophagy. They determined that a promising method for treating breast cancer was using MET alone or in combination with chemotherapeutic agents and/or the mTOR inhibitor RAD001. Hence, a treatment strategy that may be used is targeting AMPK simultaneously through MET and the PI3K/AKT/mTOR pathway by an mTOR inhibitor [110]. Tan et al. found that MET (2.5, 5, and 10 mM) triggered autophagy, which was depicted by the increase in LC3II protein expression (an autophagosomal marker in mammals) and the decrease in P62/ SQSTM1 protein expression (an autophagy adaptor protein). The findings also showed that the impact of MET on apoptosis may be increased by the autophagy inhibitors 3-MA or ATG5-siRNA, which suggested that MET-induced autophagy is a pro-survival technique. Furthermore, by preventing autophagy, MCF-7 might function more effectively [111]. Besides, according to Huang et al. study, an upsurge has been found in the expression of ATG3, ATG5, and LC3-II in the MET-treated cells (human breast cancer cells MCF7), which showed that the anti-tumor effect of MET included autophagy. In addition, for MET-treated cells, there was a gradual decrease in the expression of p62/SQSTM-1, which is an autophagy receptor involved in proteasomal or autophagosomal protein degradation, from 0 to 20 mmol/L. This also depicted that autophagy was stimulated by MET in a dose-dependent manner [112]. Nevertheless, it has been shown in various studies that a dual part is played autophagy in cancer [113, 114].

In addition, the cell cycle at the G0/G1 phase was blocked by MET, which was consistent with a few earlier studies where it has been shown to cause cell cycle stoppage at the G0/G1 phase in breast cancer and
Prostate cancer

Prostate cancer is a malignant tumour that occurs in the male urological system [118]. One study showed that MET was capable of preventing tumorigenesis of prostate cancer; however, it is yet to be examined whether MET can also be effective in prostate cancer patients not suffering from diabetes [119]. Chen et al. showed that MET inhibited the progression of AR-negative prostate cancer. According to this study, overexpression of p-AMPK and LC3II demonstrated that Metformin’s inhibitory effect on prostate cancer required AMPK activation by inducing autophagy. Another important discovery was that MET therapy for prostate cancer may utilize the AMPK/autophagy pathway after the delivery of AMPK siRNA or 3-MA. MET was administered to DU145 and PC3 cells independently in the study for 6, 12, and 24 hours at a dosage of 20 mM. Western blot results showed that LC3II expression was upregulated, which was an indication of autophagy (Figure 4). MET was also discovered to be capable of lowering the ratio of p-ERK/ERK in the MAPK pathway [28].

Figure 4. A simple schema to illustrate how MET impacts the growth of prostate cancer cells that lack AR expression

Sahra et al. showed that there is a shift from autophagy to apoptosis following the inclusion of MET to 2-deoxyglucose (2DG)-treated cells. Increasing the concentration of 2DG brings about the augmentation of LC3-II and the production of autophagosomes in prostate cancer cells. When MET is added to 2DG-treated cells, there is a complete disappearance of LC3-I and LC3-II, as well as a reduction in the expression of Beclin-1. This study found that using MET along with 2DG restricted autophagy and triggered AMPK-dependent apoptosis in prostate cancer cells [120].

Myeloma

A recent study found that treating diabetes patients with MET for at least 4 years reduced the likelihood that their monoclonal gammopathy of undetermined significance would progress to multiple myeloma. It was further determined by Wu et al. that MET was linked to enhanced outcomes in myeloma patients suffering from diabetes mellitus [121]. The mTOR pathway is vital for the proliferation and survival of tumour cells and there are two mTOR complexes such as mTORC1 and mTORC2 [122]. The findings of Wang et al. demonstrated that MET (20mM) inhibited both mTORC1 and mTORC2 signaling systems via AMPK activation in myeloma cells, which also triggered autophagy and cell-cycle stoppage. In this study, transmission electron microscopy observations demonstrated that autophagosomes were accumulated in MET-treated myeloma cells, with a simultaneous increase in ATG1/ULK1 complex expression, the downstream target of mTORC1, and the main regulator of autophagy. To sum up, it was reported by the researchers that MET restricted the growth of myeloma through autophagy induction and G0/G1 phase cell cycle arrest through a technique that may cause inhibition of both the mTORC1 and mTORC2 pathways regulated by AMPK activation in human multiple myeloma cell lines (RPMI8226 and U266). It was further deduced by the xenograft mouse model that MET (250 mg/Kg) upregulated AMPK and downregulated mTOR to restrict tumour growth [123].

Based on Jagannathan et al. study cytosolic LC3B-I is transformed by lipidation into LC3B-II, which is the active, autophagosome membrane-bound form. Cell treatment was carried out with bortezomib, MET, or a combination of both, and a western blot was used to monitor LC3B-I conversion. It was reported that MET inhibited glucose-mediated protein 78 (GRP78)-dependent autophagy, which was a critical driver of bortezomib-induced autophagy, and also promoted the pharmacological repositioning of MET so that the anti-myeloma effect of bortezomib could be improved [116].

Lung cancer

Investigations have indicated that the stimulation of AMPK by MET, enhanced chemotherapy results and overall survival of patients suffering from Stage I-IV non-small cell lung cancer (NSCLC). In addition, it restricts neoplastic cell proliferation via a PI3K/AKT/mTOR pathway in cancer cells [124-126].
et al. depicted that osimertinib, a drug used for NSCLC treatment, triggered pro-survival autophagy in NSCLC cells (H1975 and PC-9GR), while MET (5 mM) additionally sensitized H1975 and PC-9GR cells to osimertinib by restricting autophagy. The mechanism that was possibly followed was that autophagy may be suppressed in a time-dependent manner by the consistent activation of AMPK triggered by MET [127]. Furthermore, Xiao reported that by inducing autophagy, MET altered programmed cell death in erlotinib or chemotherapeutic agent-treated NSCLC cells in vitro. MET could simultaneously act as a dual mTOR activator and inhibitor in heterogeneous cancer cell populations that had undergone treatment with chemotherapeutic drugs. The autophagy inhibitor mTOR, an important nutritional sensor and growth activator, is inactivated after MET therapy, which can cause low ATP levels to become more sensitive. MET has been discovered to control the balance between death and survival in NSCLC cell lines H1650 and A549. MET exerted this effect by simultaneously enhancing autophagy marker LC3B expression and severely limiting drug-induced multi-nuclei generation [128].

Osteosarcoma stem cells

Osteosarcoma is a primary malignant tumor that is diagnosed most commonly in children and adolescents with a poor prognosis [129]. According to Zhao et al., treatment with MET (0, 6.4, 12.8, 25.6, and 51.2 mM) led to the activation of AMPK phosphorylation (p-AMPK) in the K7M2 OSCs as well as the MG63 osteosarcoma stem cells (OSCs) in a dose-dependent manner. This study also confirmed that MET can prevent mTOR phosphorylation which extended the inactivation of mTOR and ultimately triggered autophagy. Therefore, an AMPK signaling pathway may be involved in MET-induced autophagy in K7M2 and MG63 OSCs. Since AMPK inhibition led to the promotion of p-mTOR protein expression and the inhibition of LC3 expression, the outcomes were distinct from those of MET inhibition. The outcomes revealed that the autophagy markers LC3, ATG5, and ATG7 significantly elevated in OSCs that had received treatment with MET, suggesting that MET can induce autophagy. The authors proved that AMPK activation restricted the mTOR pathway, where there seems to be an increase in autophagy. Furthermore, it was determined by our mouse xenograft model that MET can potentially be used to target OSCs [130]. It was demonstrated by Li et al. that MET (5-40 mM) was capable of significantly avoiding tumor development by triggering cell cycle arrest and programmed cell death in human osteosarcoma. In addition, MET induced the ROS-dependent JNK/c-Jun signaling pathway, which in turn activated two different types of programmed cell death. The inhibition of autophagy with CQ increased MET-induced apoptosis, which means that a protective part was performed by MET-induced autophagy in OSCs [131].

Other cancers

Cell autophagy is controlled by the mTOR pathway in stressful conditions, particularly in reaction to anticancer agents [132]. Shi et al. found that MET therapy improved tumor cell responsiveness to doxorubicin in murine lymphoma models, which was consistent with higher autophagy in those tumors. This work found that AMPK might target the dysfunction of the mTOR pathway in lymphoma, offering an effective metabolism-mediated model for suppressing cell development [115]. Also, greater dosages of MET (2–5 mM) can cause apoptosis in endometrial cancer cell lines [133]. According to Takahashi et al., MET caused the Ishikawa endometrial cancer cells to undergo autophagic cell death. MET-treated cells had noticeably lower viability and proliferation as well as noticeably increased apoptosis through intrinsic as well as extrinsic routes. Furthermore, the MET administration boosted autophagy. The suppression of autophagy, either through Beclin1 knockdown or through 3-methyladenine-mediated restriction of caspase-3/7, decreased Metformin’s anti-proliferative impact on endometrial cancer cells [40]. Pusceddu et al. investigated the relationship between glycemia and progression-free survival (PFS) in patients suffering from pancreatic neuroendocrine tumors (pNETs). They observed that PFS was notably higher in diabetes patients treated with MET [134]. However, another study by Supabphol et al. illustrated that in patients with biliary tract cancer and pre-existing diabetes mellitus, the use of metformin did not show a significant association with decreased risk or improved survival outcomes [135]. The Effect of MET on different molecular pathways in different cancers is listed in Table 1.

Table 1. Effects of MET on molecular pathways targeting autophagy in different cancers
MET as an anticancer agent in clinical trials

Clinical trials have provided evidence of the benefits of MET in treating various types of tumors, including those that have shown high response rates when used in combination with other chemotherapy medications. These findings implied that MET could potentially perform as a strategy for cancer therapy. However, more extensive clinical research is necessary to fully explore its potential applications. Here, we provide a brief overview of some relevant clinical studies [136]. The total daily dosage of MET used in studies ranges from 500 to 3000 mg, reflecting the dosing strategy commonly employed in the management of type 2 diabetes. However, GI toxicity has been identified as a limiting factor for doses exceeding 2500 mg per day [137]. In future clinical trials, researchers recommend aiming to reach the maximum tolerated dose of 2500 mg per day to attain the desired anti-cancer effects. Previous preclinical studies have reported that high concentrations of MET were necessary to achieve effective anti-cancer activity, hence the importance of optimizing the dosage in clinical trials [138].

Strong evidence exists regarding the relationship between the administration of MET and a decreased incidence of pancreatic cancer, as well as improved overall survival in patients with colorectal cancer [139-141]. Based on a meta-analysis of 21 studies, it was investigated that treatment with MET in patients with both diabetes mellitus and pancreatic cancer provided a survival benefit, particularly in those with early and intermediate stages of the disease. These findings suggest that MET could potentially serve as an adjuvant chemotherapeutic option [142]. Bever et al. carried out a study on 22 patients with metastatic pancreatic adenocarcinoma and they underwent therapy with either oral MET alone (850 mg every 12 hours) or in combination with rapamycin (4 mg daily). Based on the results, the intervention was found to be well-tolerated by the patients, with some individuals showing signs of stable disease. This stabilization of the disease was found to be associated with better survival outcomes, indicating that the intervention may be an effective treatment option for these patients [143]. Miranda et al. conducted a study on patients with colorectal cancer, they illustrated that a combination of 850 mg of MET, 425 mg/m2 5-fluorouracil, and 50 mg leucovorin taken every 12 hours was linked to longer survival outcomes in obese individuals [144]. Another trial called METAL (MET in advanced lung cancer) was conducted to assess the clinical usefulness of combining MET with erlotinib as a second-line therapy for patients with stage IV non-small-cell lung cancer. Twelve patients were enrolled and administered 1500 mg of MET with 150 mg of erlotinib. The results indicated that this combination therapy led to better prognoses, suggesting that it could potentially enhance survival rates and overall treatment outcomes [145]. Interim analyses of ongoing studies investigating the use of neoadjuvant MET therapy in newly diagnosed breast cancer patients have shown that this approach is safe. MET could directly impact primary breast cancer, including the downregulation of phosphodiesterase 3B (PDE3B), a crucial regulator of cAMP synthesis. In combination with AMPK activation, this approach could potentially serve as an adjuvant therapy for breast cancer [146]. Goodwin et al. conducted a clinical trial that found that the use of 850 mg of MET resulted in weight loss and an improvement in metabolic

<table>
<thead>
<tr>
<th>Reference</th>
<th>Dosage of MET</th>
<th>Effect/Mechanism</th>
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<tbody>
<tr>
<td>(Park, 2015)</td>
<td>0.2 mM</td>
<td>reduced different autophagy-related proteins like LC3B and beclin-1 in an AMPK-dependent manner</td>
</tr>
<tr>
<td>(Liu et al., 2020)</td>
<td>300 mg/kg</td>
<td>Upregulated beclin1-mediated autophagy to inhibit GC cells through the AMPK-mTOR pathway</td>
</tr>
<tr>
<td>(Gao et al., 2020)</td>
<td>10 mM</td>
<td>Induced autophagy, and autophagic flux, resulted in elevated levels of protein 1 light chain 3</td>
</tr>
<tr>
<td>(Sun et al., 2020)</td>
<td>400 μmol/L</td>
<td>Reduced the expression of Beclin-1 and LC3II as well as the phosphorylation levels of mTOR</td>
</tr>
<tr>
<td>(Tsai et al., 2017)</td>
<td>5 mM</td>
<td>Enhanced autophagic cell death through AMPK</td>
</tr>
<tr>
<td>(Ling et al., 2017)</td>
<td>10 mM/l</td>
<td>Activated AMPK, which would in turn suppress ERK1/2 activation</td>
</tr>
<tr>
<td>(Chen et al., 2021)</td>
<td>30 mM</td>
<td>Increased AMPK phosphorylation levels in ASPC-1 cells</td>
</tr>
<tr>
<td>(Jiang et al., 2014)</td>
<td>2 mM</td>
<td>Activated AMPK through AMPK signaling</td>
</tr>
<tr>
<td>(Liu et al., 2012)</td>
<td>1 M</td>
<td>Simultaneously targeted AMPK and PI3K/AKT/mTOR pathway</td>
</tr>
<tr>
<td>(Wang et al., 2018)</td>
<td>20 mM</td>
<td>Inhibited myeloma proliferation by autophagy induction and G0/G1 phase cell cycle arrest</td>
</tr>
<tr>
<td>(Chen et al., 2019)</td>
<td>5 mM</td>
<td>Activated AMPK which could inhibit autophagy in a time-dependent manner</td>
</tr>
<tr>
<td>(Xiao et al., 2017)</td>
<td>2 and 5 mM</td>
<td>Simultaneously acted as an mTOR activator as well as an inhibitor in heterogeneous cancer cell lines</td>
</tr>
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These findings implied that MET could potentially form as a strategy for cancer therapy. However, more extensive clinical research is necessary to fully explore its potential applications. Here, we provide a brief overview of some relevant clinical studies [136]. The total daily dosage of MET used in studies ranges from 500 to 3000 mg, reflecting the dosing strategy commonly employed in the management of type 2 diabetes. However, GI toxicity has been identified as a limiting factor for doses exceeding 2500 mg per day [137]. In future clinical trials, researchers recommend aiming to reach the maximum tolerated dose of 2500 mg per day to attain the desired anti-cancer effects. Previous preclinical studies have reported that high concentrations of MET were necessary to achieve effective anti-cancer activity, hence the importance of optimizing the dosage in clinical trials [138].

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health in patients with early-stage breast cancer. The study also provided evidence supporting the potential activity of MET in breast cancer treatment, as it was found to reduce levels of various metabolic markers, including insulin, leptin, and high-sensitivity C-reactive protein (hs-CRP) in breast cancer patients [147]. Despite the good tolerability of therapy with aromatase-inhibitor in combination with 500 mg MET in postmenopausal patients with hormone receptor-positive metastatic breast cancer, the study did not yield positive results [148]. According to retrospective research involving patients with metastatic renal cell carcinoma (mRCC), the use of MET in conjunction with the tyrosine kinase inhibitor (TKI) sunitinib was linked to improved overall survival (OS) in patients with diabetes mellitus, compared to the use of other diabetic agents [149]. In another retrospective research, it was investigated that the addition of metformin to sunitinib or an alternative TKI, pazopanib, in patients with mRCC led to better PFS and OS outcomes, irrespective of diabetic status [150].

**Metformin and Chemotherapy and Limitations**

Research has demonstrated that MET, a medication commonly used to treat diabetes, can positively alter molecular and cellular pathways that influence the clinical presentation of various diseases, including cancer [151]. Studies in the field of oncology have provided evidence of metformin’s antitumor properties, including its potential to reduce the risk of metastasis, enhance the effectiveness of chemotherapy, and improve patient survival rates, as well as clinical and pathological outcomes [152-155]. In both in vitro and in vivo studies, the use of MET in conjunction with chemotherapy has shown promising results in inhibiting tumor growth and prolonging remission in different cancers, including breast, pancreatic, prostate, lung, and ovarian cancers. These findings are supported by multiple studies [156, 157]. In addition to its individual antitumor properties, MET has been shown to improve the efficacy of chemotherapy in a synergistic manner. For example, when used in conjunction with chemotherapy drugs like paclitaxel, carboplatin, and doxorubicin, MET allows for a four-fold decrease in the required dose of doxorubicin while maintaining the same level of efficacy. This not only improves the overall effectiveness of chemotherapy but also reduces the associated toxicity [157].

MET exerts a direct effect on cells through a variety of mechanisms, both AMPK-dependent and independent [64, 158]. Most of the current knowledge regarding the direct effect of MET has been gathered from in vitro studies, where the concentration of the drug is typically between 5 to 10 mM. However, this concentration is significantly higher than the steady-state levels of MET found in the plasma of patients with type 2 diabetes mellitus, which typically range between 10 to 40 μM with standard prescribed doses. Despite this difference, studies have shown that even at these lower concentrations, MET is still capable of exerting its therapeutic effects in vivo [159]. Studies conducted in vitro have indicated that at low concentrations, MET is not potent enough to induce AMPK activation, although it can still bring about metabolic changes. These findings suggest that the therapeutic effects of MET at lower concentrations may be mediated by AMPK-independent mechanisms [160]. The effectiveness of MET in inhibiting the malignant growth of cancer cells in humans at plasma concentrations similar to those found in patients with T2DM is still a matter of debate, as the inhibitory effect of MET is primarily mediated by AMPK activation. However, this limitation could potentially be overcome by combining MET with other activators that operate through different mechanisms. Studies in mice have shown that administering MET at 50 mg/kg per day results in a maximal concentration of 50 to 60 μM in the hepatic portal vein. These findings suggest that higher doses of MET may be necessary to achieve therapeutic effects in humans [161].

MET is associated with several common side effects like GI symptoms such as nausea, vomiting, and diarrhea, as well as abdominal discomfort, headache, and dizziness [162]. While these side effects are generally mild and manageable, they can still have a notable effect on patient quality of life. In rare cases, MET can lead to a serious condition known as lactic acidosis, which results from the buildup of lactic acid in the blood [162]. The risk of lactic acidosis is higher in patients with kidney or liver disease, alcoholism, or heart failure, as well as in those taking certain medications. Therefore, it is essential to monitor patients carefully for signs of lactic acidosis, particularly those with underlying medical conditions or taking medications that may increase the risk [162, 163].

**Overcome the limitations of the therapeutic effects of metformin**

While MET shows promise as
a cancer therapy, it also has some limitations that need to be addressed to improve its effectiveness. To enhance the therapeutic potential of MET, several strategies have been proposed and are currently being investigated. These approaches aim to overcome the limitations of MET and maximize its benefits in cancer therapy. To enhance the efficacy of MET in cancer therapy, researchers have been exploring various combination therapies. For example, a recent study has illustrated that combining MET with nanostructured poly lactic-coglycolic acid (PLGA) can improve its effectiveness in treating breast cancer [164]. Additionally, Han et al. have shown that concurrent administration of MET with EGFR-TKIs can lead to better clinical results and delay the onset of EGFR-TKI resistance in non-small cell lung cancer patients. In their study, they explored the effects of MET combined with EGFR-TKI on the prognosis of non-small cell lung cancer patients with type 2 diabetes. The results indicated that patients who received EGFR-TKI plus metformin had notably longer median PFS and median OS compared to those who received EGFR-TKI plus other hypoglycemic agents besides MET. These findings suggest that combining MET with other drugs can be an advantageous approach to overcome its limitations and improve cancer treatment outcomes [165].

Moreover, a new extended-release formulation of MET (MET XR) has been developed to address the limitations of the older immediate-release formulation. This new formulation offers a more convenient dosing regimen and improved tolerability, making it easier for patients to adhere to their treatment regimen. With its extended-release mechanism, MET XR delivers the medication over a longer period, providing a more consistent and sustained therapeutic effect. This innovation has the potential to improve patient outcomes and quality of life by minimizing the side effects associated with the older formulation and promoting better compliance with treatment. As a result, the development of MET XR represents an important step forward in the treatment of various conditions where MET is indicated [166]. CRISPR/Cas9 is a groundbreaking gene editing technology that holds great promise in overcoming the limitations of MET in cancer therapy. This innovative approach allows scientists to precisely target and modify specific genes implicated in cancer development and progression. By using CRISPR/Cas9 to selectively edit cancer-related genes, researchers hope to develop more personalized and effective cancer treatments. Clinical trials are currently underway to assess the safety and efficacy of CRISPR-based cancer therapeutics, and early results are promising. The potential of CRISPR/Cas9 to revolutionize cancer therapy is an exciting development that could lead to more precise and effective treatments for patients in the future [167].

Additionally, Autophagy is a resistance mechanism that limits the effectiveness of enzalutamide, a small molecule used to treat castration-resistant prostate cancer. To overcome this limitation, researchers have been investigating the potential of MET as an autophagy inhibitor. Preclinical studies have shown that combining MET with enzalutamide enhances therapeutic responses and improves treatment outcomes. By inhibiting autophagy, MET can help to overcome the resistance to enzalutamide and enhance its efficacy in treating prostate cancer. These results implied that the combination of enzalutamide and MET could be a promising therapeutic strategy for patients with castration-resistant prostate cancer [168].

In summary, while MET (mesenchymal-epithelial transition factor) has shown potential therapeutic benefits in cancer treatment, it also has some limitations that need to be addressed. Fortunately, researchers have been exploring various strategies to enhance the efficacy of MET and overcome these limitations. These approaches include combination therapy, new formulations, gene editing, and autophagy inhibition. By using these strategies, researchers hope to improve the effectiveness of MET in treating various types of cancer. However, further research is needed to fully understand the potential of MET in cancer therapy, and to determine the optimal approaches for using this promising therapeutic agent.

Conclusion

This review article examined the effect of MET on autophagy, particularly the AMPK-mTOR signaling pathway in various forms of cancer. MET, being a glucose-lowering drug, can induce autophagy to treat different types of cancer. MET has been found to regulate beclin1-mediated autophagy, which can prevent the migration of GC cells into the AMPK-mTOR signaling axis. In vitro studies using HCC lines (HepG2 and Bel-7402) have demonstrated that MET enhances the anticancer effects by restricting growth and invasion, promoting apoptosis and autophagy, and regulating the PI3K/AKT/mTOR pathway. MET has been found
to reverse taxol resistance and SNHG7-mediated autophagy in ovarian cancer cells. In pancreatic cancer cells, metformin has been observed to effectively reduce cell proliferation and migration, induce cell cycle arrest, stimulate AMPK, and suppress PI3K/mTOR while promoting apoptosis and autophagy. The anticancer properties of MET in prostate cancer require the activation of AMPK through autophagy induction. MET has been illustrated in myeloma cells to suppress both the mTORC1 and mTORC2 signaling pathways by activating AMPK, which induces autophagy and cell cycle arrest.

Persistent activation of AMPK induced by MET has been depicted to suppress autophagy in a time-dependent manner in lung cancer cells. MET is known to inhibit the phosphorylation of mTOR (p-mTOR), leading to mTOR inactivation and the induction of autophagy. These findings suggest that the AMPK signaling pathway might have an important impact on MET-induced autophagy in K7M2 and MG63 osteosarcoma cells. The addition of MET to therapeutic protocols may extend the period of cancer non-recurrence and decrease the side effects of chemotherapy, making it a promising addition to cancer treatments. Multiple signaling pathways that affect the effectiveness of MET on autophagy have been reviewed, and the pharmacological effects of MET in combination with its actions on autophagy have been discussed. Our study provides evidence of the inhibitory effect of MET on the migration and invasion of cancer cells. These findings imply that MET may have potential therapeutic benefits in preventing metastasis and improving long-term patient outcomes. These findings may help to improve the application of MET in the treatment of diseases by mediating autophagy. However, further studies are needed to investigate these potential effects in more detail.

Authors’ contributions
G.M & P.K.Y; investigation, A.Y, A.G & N.Sh ; resources, A.H, E.R.A & M.A.T; data curation, M.AA, M.G. & M.Y.Z; writing—original draft preparation, M.G & M.Y.Z.; writing—review and editing, G.M & M.Y.Z.; visualization, M.Y.Z. & G.M.; supervision, M.Y.Z. and M.G; project administration. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

Data availability
Not applicable

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