

Current findings on the antitumor effects of metformin on esophageal squamous cell carcinoma (Review)

NOBUFUMI SEKINO, MASAYUKI KANO, KENTARO MURAKAMI, TAKESHI TOYOZUMI,
KOICHI HAYANO, GAKU OHIRA and HISAHIRO MATSUBARA

Department of Frontier Surgery, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan

Received July 30, 2023; Accepted May 21, 2024

DOI: 10.3892/mco.2024.2756

Abstract. Esophageal squamous cell carcinoma (ESCC) is an intractable type of cancer that requires novel therapeutic modalities, since the therapeutic outcomes are often inadequate, even in response to multidisciplinary treatment. The antitumor effect of metformin, an antidiabetic drug, has been reported in esophageal cancer; however, its effects are diverse. Since various multidisciplinary therapies are used in ESCC, the antitumor effect of metformin is expected to be synergistic in some treatment strategies. The present review summarizes the antitumor effects of metformin and discusses its use in combination with existing therapies. The present study reviewed relevant studies where the molecular targets of metformin (AMPK and inflammatory system signals) were described, followed by the classification and organization of its antitumor effects, and subsequently summarized the current research on its antitumor effects, especially in ESCC. A number of studies have reported that metformin prevents the development of ESCC and exerts its antitumor effects through various pathways. In addition, metformin has been shown to inhibit tumor growth, induce apoptosis, inhibit cancer cell invasion, migration and angiogenesis into the tumor, and decrease tumor malignancy, such as metastasis. Furthermore, it may modulate host tumor immunity in a tumor-suppressive manner and is expected to improve prognosis following treatment for ESCC. Notably, metformin may be beneficial in combination with chemotherapy, such as cisplatin, and radiation. By contrast, it has been shown to potentially induce resistance to 5-fluorouracil. Finally, the effects of metformin in combination with other therapies are discussed in the present study, and perspectives on the potential benefits of metformin for future ESCC treatment are presented. In conclusion, the present review may be useful in improving the understanding of the wide range

of antitumor effects of metformin. Although some concerning points remain, using metformin in ESCC treatment could be promising. Notably, more knowledge needs to be accumulated regarding the effects of metformin on ESCC.

Contents

1. Introduction
2. Target molecules of metformin
3. ESCC prevention effects
4. Antitumor and anti-inflammatory effects on ESCC progression
5. Combination with current therapies
6. Future treatment strategy
7. Conclusion

1. Introduction

Esophageal cancer, an incurable gastrointestinal cancer, is considered the sixth leading cause of cancer-related deaths and the eighth most common type of cancer globally (1). Esophageal squamous cell carcinoma (ESCC) is the predominant type of esophageal carcinoma worldwide, especially in the Asian belt (2). Esophageal cancer is prone to lymph node metastasis in the early stages of the disease because the lymphatic system is well-developed in the esophagus. Duan *et al* (3) reported that the lymph node metastasis rate was 17.5% (25/143) even in pathological T1 esophageal cancer. Furthermore, poor outcomes in patients with esophageal cancer are also related to the propensity for metastases, even when tumors are superficial (2). Due to the nature of esophageal cancer, a variety of treatment strategies are required, and multidisciplinary treatments, such as surgery, chemotherapy, and radiation therapy, are not sufficiently effective (4). Thus, new treatment methods are required to improve the clinical outcomes.

Recently, the antitumor effect of metformin, an antidiabetic drug, has been reported in many cancers (5). Due to its safety, metformin is widely used to treat diabetes mellitus (DM). Lactic acidosis is thought to be a significant side effect of the drug. However, it has been reported that metformin does not increase the risk of lactic acidosis in patients with type 2 diabetes who do not have heart, renal, or liver failure (6). Since

Correspondence to: Dr Nobufumi Sekino, Department of Frontier Surgery, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan
E-mail: n.sekino@chiba-u.jp

Key words: esophageal squamous cell carcinoma, metformin, antitumor effects, cancer immunity, review

it is a well-established DM management drug, metformin can be anticipated to exhibit beneficial effects as a repurposed cancer drug.

A wide range of therapeutic strategies, including surgery, chemotherapy, radiation therapy, and immunotherapy, have been selected to treat refractory ESCC. These strategies are often used in combination to improve therapeutic efficacy further. Metformin is expected to enhance the therapeutic efficacy of many treatment strategies for ESCC because of its broad antitumor effects and to exert synergistic effects in these combined treatments. Therefore, it is very important to know more about the antitumor effects of metformin; however, owing to the diversity of the impact of metformin, a comprehensive understanding of its mechanism is complex.

In this review, we described the molecular targets of metformin in ESCC and then categorized and organized the antitumor effects of metformin in cancer prevention, cancer suppression, host immunity, and cancer metabolism (Fig. 1). Finally, we discussed the combined effects of metformin and other therapies (Fig. 2), and then we provided a perspective on the potential benefits of metformin in the future treatment of ESCC.

2. Target molecules of metformin

Many target proteins of metformin have been reported, and their associated effects are diverse. The critical target of metformin is the 5'-adenosine monophosphate-activated protein kinase (AMPK) (7,8). A large body of evidence suggests that AMPK functions as a tumor suppressor and that its expression is down-regulated in many cancers (9). Metformin activates AMPK via liver kinase B1 (LKB1) (7). As a result, the expression of the p-mammalian target of rapamycin (mTOR), p-p70S6K, and cyclin D1, as well as the expression of the two mTOR-related genes, 4EBP1 and S6K1, are suppressed, resulting in the suppression of downstream molecular signaling and consequently ESCC carcinogenesis (10,11).

Another molecular target for metformin is the transforming growth factor (TGF)- β signaling pathway. Nakayama *et al* (12) reported that metformin inhibits epithelial-mesenchymal transition (EMT) by suppressing the Smad phosphorylation pathway and part of the non-Smad pathway downstream of TGF- β that induces EMT.

Recently, it was reported that metformin inhibits signal transducer and activator of transcription 3 (STAT3) and nuclear factor-kappa B (NF- κ B) signaling, which are essential proteins in the inflammatory response of ESCC. STAT3 is an inflammation-related molecule often correlated with metformin (8,13,14). Smoking is one of the risk factors for ESCC, and nicotine ingested through smoking interacts with the choline receptor nicotinic alpha7 subunit (CHRNA7), which induces cancer stem cells (CSCs) and cancer-initiating cells (CICs), and subsequently activates the Janus Kinase (JAK2)/STAT3/Sox2 signaling pathway (13). Metformin suppresses CHRNA7 expression via hypermethylation of CHRNA7 promoter DNA (13). This inactivation of the STAT3-Bcl-2 pathway by metformin contributes to the inhibition of ESCC growth through crosstalk between apoptosis and autophagy (14). NF- κ B is also a master regulator of inflammation and immunity. It has been reported that metformin

decreases the nuclear translocation of NF- κ B, thus inhibiting its activation (15) and inhibiting the phosphorylation of AKT, an upstream regulator of NF- κ B (16). Since metformin has a wide range of effects, its antitumor effects are intertwined and diverse.

3. ESCC prevention effects

Metformin effectively reduces the risk of tumor development in many types of cancer, including ESCC (17). A prospective cohort study conducted in Sweden between 2005 and 2015 showed that metformin reduces the risk of ESCC (HR 0.68, 95% CI 0.54-0.85). The incidence of ESCC was 3.5 per 100,000 person-years in metformin users and 5.3 per 100,000 person-years in non-users of metformin. The risk reduction was more pronounced in the new metformin users (HR 0.44, 95% CI 0.28-0.64) and in participants aged 60-69 years (HR 0.45, 95% CI 0.31-0.66) (18).

The preventive effects of metformin on ESCC development have been demonstrated *in vivo*. Fan *et al* (10) used N-nitroso-N-methyl benzylamine (NMBzA), a specific carcinogen that induces and promotes esophageal cancer, to create a model for the ESCC progression in rats. They reported that metformin significantly decreased the incidence of ESCC and the number of precancerous lesions in rats treated with NMBzA (10).

These reports suggest that metformin reduces the risk of ESCC. Thus, metformin has the potential to be an effective prophylactic drug in patients with achalasia and other diseases that are at high risk for the development of ESCC. Therefore, further studies are warranted.

4. Antitumor and anti-inflammatory effects on ESCC progression

Cell proliferation and programmed cell death (apoptosis and pyroptosis). Many studies have shown that metformin affects ESCC cell proliferation and apoptosis both *in vivo* and *in vitro*. In animal studies, metformin did not affect body weight or vital signs (10). It also did not cause significant changes in liver function, kidney function, or blood glucose levels (19). In addition, no noticeable toxic reactions were observed (11). This report provides evidence for the safety of metformin.

Metformin has also been reported to inhibit esophageal inflammation by decreasing the expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and interleukin-6 (IL-6) *in vivo*. Analysis of esophageal epithelial cells showed that proliferating cell number antigen (PCNA), a proliferation marker, was decreased, and cleaved caspase-3, an apoptosis marker, was increased (10). In addition, metformin reduces tumor size in an ESCC cell line xenograft model (11,15,20). Analysis of tumors indicated that growth was inhibited while apoptosis and autophagy were induced (14,15). These mechanisms are proposed to involve increased expression of AMPK, p53, p21CIP1, and p27KIP1, and repression of cyclinD1 (20), and the mTOR-related genes, 4EBP1 and S6K1 (11), in addition to reducing Stat3 activity and Bcl-2 expression (14).

On the level of the cell cycle, metformin induces G0/G1 arrest, inhibits proliferation, increases apoptosis, and inhibits colony and

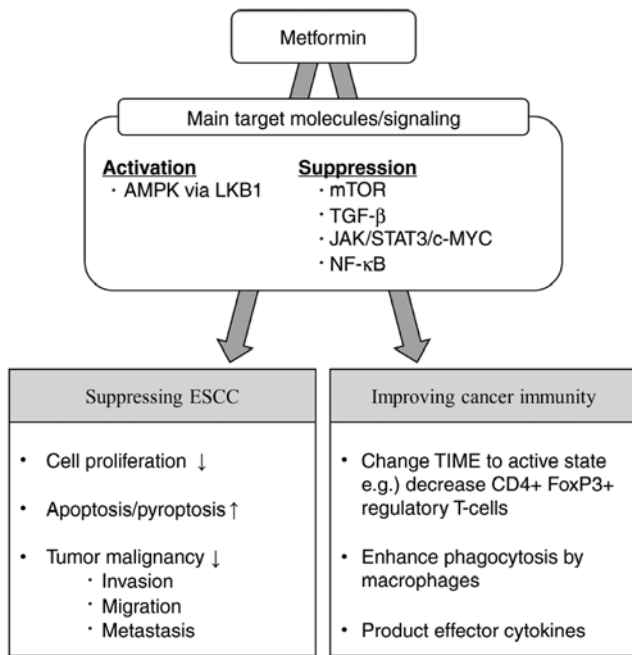


Figure 1. The effects of metformin against ESCC. Metformin exerts its antitumor effects by suppressing tumors and improving host tumor immunity. ESCC, esophageal squamous cell carcinoma; AMPK, 5'-adenosine monophosphate-activated protein kinase; LKB1, liver kinase B1; mTOR, mammalian target of rapamycin; TGF- β , transforming growth factor- β ; JAK, Janus Kinase; STAT3, signal transducer and activator of transcription 3; NF- κ B; nuclear factor- κ B; TIME, tumor immune microenvironment.

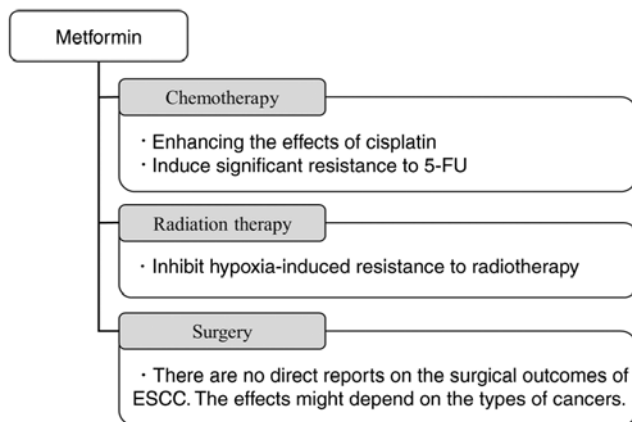


Figure 2. Possible effects of metformin on the effectiveness of ESCC treatment based on basic research. ESCC, esophageal squamous cell carcinoma; 5-FU, 5-fluorouracil.

tumorsphere formation in human ESCC cells *in vitro* (10,11,19). The induction of G0/G1 phase arrest was mediated by increased expression of p21CIP1 and p27KIP1 (20). Concerning apoptosis, STAT3 and its downstream target, Bcl-2, were inactivated, while the expression levels of Bax and caspase-3 were increased (14,19). Furthermore, it has been recently described that metformin induces pyroptosis, a non-apoptotic inflammatory caspase-dependent programmed cell death (PCD), via Gasdermin D (GSDMD) *in vitro* and *in vivo* (21). These results suggest that metformin could be an alternative treatment for ESCCs that are refractory to chemotherapy and radiotherapy, as well as for other cancers with a pyroptotic mechanism.

Surprisingly, the sensitivity of ESCC cells to metformin has been reported to increase under conditions of glucose deprivation (22). Yu *et al* (22) noted that evaluating the effect of metformin under glucose-depleted conditions was more relevant considering the microenvironment of solid tumors (typically lower than 0.5 mM) and that the glucose concentration of the culture medium was influential in the *in vitro* experimental setup. Further research is needed to elucidate the underlying mechanism of the differential observed effects of metformin based on the glucose concentration in the medium.

Interestingly, metformin selectively acts on cancer cells, whereas Peng *et al* (23) reported that metformin acts differently on ESCC cells (EC109) and normal cells (HEEC) because metformin significantly inhibits growth and induces apoptosis in cancer cells. Additionally, metformin suppressed STAT3 phosphorylation and Bcl-2 expression in ESCC cells but not in normal cells (23). Feng *et al* (14) also reported that metformin selectively inhibited the growth of ESCC tumor cells but not immortalized non-cancerous esophageal epithelial cells. These results suggest that metformin acts specifically on cancer cells, which may explain the low observed side effects of metformin administration *in vivo*.

In a human clinical trial, Wang *et al* (8) reported the effects of low-dose metformin (250 mg/day for 7-14 days before surgery) on ESCC and tumor immunity. Low-dose metformin did not affect ESCC tumor growth and apoptosis, as assessed by immunostaining for Ki67 and cleaved caspase-3. As it is possible to use higher doses for DM treatment, future analyses of the effects of metformin at higher doses are also desirable.

Invasion, migration, metastasis. Metformin inhibits cancer cell invasion and migration, which are essential hallmarks for invasion and metastasis. In an *in vitro* study, metformin inhibited the migration and invasion of cancer cells (10,15) and increased the expression of the epithelial marker E-cadherin (15). Furthermore, metformin suppressed the expression of matrix metalloproteinase (MMP)-2, MMP-9, and N-cadherin (16). This expression signature has been suggested to be inhibited in a phosphorylated AKT-dependent manner (16). Metformin also inhibits tumor growth, suppresses lung metastasis, and decreases the expression of MMP-2 and MMP-9 *in vivo* (19). Thus, metformin inhibits the migration and invasion of esophageal cancer cells by regulating the expression of migration- and invasion-related genes.

Nakayama *et al* (12) reported the effects of metformin on EMT induction via ionizing radiation (IR). They first observed that IR irradiation induced the expression of TGF- β , hypoxia-inducible factor (HIF)-1 α , mesenchymal markers (vimentin and N-cadherin), transcription factors (Slug, Snail, and Twist), and MMPs. They observed that metformin suppresses EMT-induced morphology and motility, but the mechanism is not mediated through TGF- β but instead through phosphorylation of its downstream Smad2 and Smad3 (12). Metformin enhanced IR-induced phosphorylation of AMPK. However, mTOR phosphorylation was enhanced by radiation and inhibited by metformin (12).

Yang *et al* (24) reported an experiment to simulate the ESCC microenvironment using a tumor-conditioned medium (TCM) from ESCC cell culture's supernatant or human ESCC tissue homogenate's supernatant. TCM promotes tumor angiogenesis

by transforming normal endothelial cells (NECs) into tumor endothelial cells (TECs). However, metformin inhibited the transition of NECs into TECs in the ESCC microenvironment by inhibiting the JAK/STAT3/c-MYC signaling pathway. Yang *et al* (24) first validated metformin's inhibitory effect on angiogenesis *in vivo* using a human ESCC patient-derived xenograft (PDX) mouse model (24).

These results indicate that metformin inhibits metastasis, invasion, and angiogenesis and exerts antitumor effects. These properties directly relate to patient prognosis, suggesting that metformin may improve prognosis.

Cancer immunity. Metformin improves the tumor immune microenvironment (TIME) in esophageal cancer, as reported in a human clinical trial conducted by Wang *et al* (8). Low-dose metformin (250 mg/day for 7-14 days before surgery) changes the TIME to an anticancer state (8). Metformin reprogrammed TIME to 'infiltration-inflammation' and increased the number of infiltrating CD8+ cytotoxic T lymphocytes and CD20+ B-lymphocytes. Furthermore, an increase in tumor-suppressive macrophages (CD11c+ M1 macrophages) and a decrease in tumor-promoting macrophages (CD163+ M2 macrophages) have been observed (8).

In the ESCC mouse model (4-NQ O-induced orthotopic ESCC mice 16 weeks old, metformin 50 mg/kg/day), short-term metformin treatment reprogrammed TIME as previously observed in humans, while long-term treatment further shifted TIME to an active state (e.g., decreased CD4+ FoxP3+ regulatory T-cells) and suppressed ESCC growth (8). Regarding the mechanism by which metformin regulates CD4+ CD25+ regulatory T-cells in the microenvironment, it has been reported that regulatory T-cells, which proliferate in the tumor mass, are induced to undergo apoptosis, and their number is drastically reduced. Detailed examination revealed that fatty acid-dependent oxidative phosphorylation, the primary source of energy metabolism of regulatory T-cells, was reduced. Instead, the glucose-dependent glycolytic system was enhanced, activating the pathway that leads to cell death (25). Others have reported that metformin enhances the phagocytosis of ESCC cells by macrophages *in vitro* (8) and also alters the production of effector cytokines as tumor necrosis factor- α (TNF- α) and IL-10 in immune cells by inducing AMPK activation and STAT3 inactivation (6).

These results suggested that metformin modulated the immune status of the host in a tumor-suppressive manner (Table I). These findings highlight the promising potential of metformin in combination with treatment focusing on cancer immunity, especially immunotherapy using immune checkpoint inhibitors (ICIs).

Antitumor effect via metabolism, 2-Deoxyglucose (2DG). Since glycolysis is enhanced in tumors even in the presence of oxygen due to the Warburg effect and ATP generation is more dependent on aerobic glycolytic metabolism than on mitochondrial metabolism (26), glucose metabolism is one of the targets of cancer therapy. 2-Deoxyglucose (2DG), a glucose analog, inhibits hexokinase, the first restriction enzyme in the glycolytic system, and acts as an inhibitor of glucose metabolism (27). Both mitochondrial dysfunction and aerobic glycolysis are signs of aggressive cancer growth, and

the glycolytic inhibitor, 2DG, appears to be a promising treatment tool (28). As metformin is known to decrease oxidative phosphorylation in ATP biosynthesis in the mitochondrial inner membrane [27], the combination of metformin and 2-DG is expected to be effective.

The dual combination therapy of metformin and 2-DG synergizes apoptosis induction by decreasing Bcl-2 expression and increasing p53 expression *in vitro* (28). It has been reported that both glycolysis and oxidative phosphorylation are simultaneously induced in ESCC cancer stem cells (CSCs) depending on the Hsp27-AKT-HK2 pathway. In addition, the combination of 2-DG and metformin, which inhibits the glycolytic system and oxidative phosphorylation, led to the suppression of tumor growth, including tumor size and weight, in a xenograft tumor model (29). The combinatorial therapy of metformin and 2DG has already been used for positron emission tomography (PET) scans, seizure disorders, and DM and is expected to allow for rapid evaluation of clinical efficacy (28).

5. Combination with current therapies

Improving treatment outcomes. The use of metformin in ESCC treatment has been reported to enhance the prognosis and prolong overall survival (OS). Van De Voorde *et al* (30) reported that the use of metformin improved OS and distant metastasis-free survival in 196 patients with esophageal cancer treated with trimodality therapy (distant metastasis-free survival, $P=0.040$; overall survival, $P=0.012$). Although this study included a large number of patients with esophageal cancer having adenocarcinoma (adenocarcinoma, $n=137$; squamous cell carcinoma, $n=36$; other, $n=4$) and only a small number of patients taking metformin (non-diabetic, $n=172$; diabetic without metformin, $n=5$; diabetic taking metformin, $n=19$), the study was conducted in a relatively large number of patients and was considered meaningful.

Chemotherapy. Cisplatin and 5-FU, which are used in the treatment of many patients, were also noted. As for cisplatin, there have been a series of reports that its combination with metformin enhances its antitumor effect. Basic experiments suggest that the combination of metformin and chemotherapy is beneficial, as metformin inhibits cell proliferation and promotes cell apoptosis, thereby enhancing the effects of cisplatin (11).

Interestingly, metformin synergistically enhanced the cytotoxicity of cisplatin under glucose-depleted conditions, which is representative of the microenvironment of solid tumors. Possible mechanisms include enhanced cytotoxicity by metformin, markedly reduced intracellular ATP levels, AKT and AMPK signaling pathway abnormalities, and impaired DNA repair (22).

Metformin exerts its antitumor effects by modulating oxidation-reduction homeostasis. It acts as a pro-oxidant by reducing intracellular glutathione, a major intracellular antioxidant against reactive oxygen species (ROS). It has been reported that the concomitant use of metformin suppresses the cisplatin-induced increase in intracellular glutathione and increases sensitivity to cisplatin *in vitro* and *in vivo* (31). However, it was reported that metformin protects

Table I. The effects of metformin on immune cells and cytokines.

A, Immune cells				
Target	Main function	Effect of metformin	Mentioned mechanism	(Refs.)
CD8 ⁺ T cell	Cytotoxic effects	↑	Increase p-AMPK positive cells, TNF-α↑	(8)
CD4 ⁺ Foxp3 ⁺ T cell	Immune suppression (regulatory)	↓		(8)
CD20 ⁺ B cell	Antibody production, T cell activation	↑		(8)
CD11c ⁺ macrophage (M1)	Immune elimination	↑	Increase p-AMPK positive cells, TNF-α↑, IL-10↓	(8)
CD163 ⁺ macrophage (M2)	TAM; angiogenesis promotion	↓		(8)
B, Cytokines				
Target	Main function	Effect of metformin	Mentioned mechanism	(Refs.)
IL-6	Regulation of immune response and inflammation	↓	Activate AMPK and attenuate downstream signaling such as mTOR	(6,10)
IL-10	Suppression of the immune system	↓	Activate AMPK and inactivate STAT3	(6,8)
TNF-α	Elimination of tumor cells	↑	Activate AMPK and inactivate STAT3	(6,8)
TGB-β	Regulates cell proliferation and differentiation, and promotes cell death	↑	Activate AMPK and suppress mTOR signal	(12)

AMPK, 5'-adenosine monophosphate-activated protein kinase; TNF-α, tumor necrosis factor-α; IL, interleukin; mTOR, mammalian target of rapamycin; STAT3, signal transducer and activator of transcription 3; TGB-β, transforming growth factor-β; TAM, tumor-associated macrophage.

cells from the cytotoxicity of cisplatin by inducing a reductive intracellular environment that decreases cisplatin-DNA adduct formation. They also noted that caution should be taken when administering cisplatin to diabetic patients on metformin (32).

Another chemotherapeutic agent used to treat ESCC is 5-fluorouracil (5-FU). Unlike cisplatin, metformin treatment induced significant resistance to 5-FU *in vitro* (33). This resistance is thought to be due to overall changes in nucleotide metabolism, such as increased expression of thymidylate synthase and thymidine kinase 1, which are established mechanisms of 5-FU resistance that increase the intracellular dTTP pool and dilution of 5-FU assimilates (33).

Metformin in combination with cisplatin during chemotherapy has been reported in both directions. However, metformin in combination with 5-FU has the risk of inducing resistance and should be treated with caution. As chemotherapy plays a critical role in the treatment of ESCC, further studies on this combination therapy are required.

Radiation therapy. Radiation therapy (RT) is essential for treating ESCC as chemotherapy and surgery, and the effect of metformin on its efficacy has also been studied. Clinical data

suggest that metformin improves progression-free and overall survival in various cancer patients treated with RT, although the results are not always consistent (34).

Metformin has been reported to inhibit hypoxia-induced resistance to radiotherapy in ESCC. Hypoxia is a critical cause of radioresistance because oxygen is a source of free radicals necessary for ionizing radiation (IR) to kill tumor cells. Hypoxia can induce a series of cellular biological transformations to prevent the harmful effects of IR (35). In ESCC, miR-340-5p, highly expressed in exosomes derived from hypoxic tumor cells, induces radiotherapy resistance by targeting KLF10. However, metformin increases the expression of KLF10 and enhances radiosensitivity (35).

Although there are few reports related to ESCC, reports on other carcinomas suggest that the combination of metformin and RT is promising. RT shows an abscopal effect, which is thought to be induced by tumor immune activation, and metformin enhances tumor immunity, as mentioned earlier. Thus, a synergistic effect is expected in ESCC, and further studies are required to confirm this effect.

Effect on surgery. Since surgery is an essential treatment for ESCC, we would like to discuss the effects of metformin on

surgical outcomes. Although there are no direct reports on the surgical outcomes of ESCC, there are some reports on other cancers as follows, and the impact is controversial. For colorectal cancer, Frangola *et al* (36) showed no association between diabetes or metformin treatment and recurrence-free or disease-free survival after surgery for colorectal cancer. In addition, Kaushik *et al* (37) reported that metformin did not improve prostate cancer outcomes after radical prostatectomy.

In contrast, Chan *et al* (38) found that metformin improved the prognosis of hepatocellular carcinoma (HCC) in patients with DM. The hazard ratio of metformin use in HCC patients with DM was 0.65 ($P < 0.05$, 95% CI=0.60-0.72) for HCC recurrence and 0.79 ($P < 0.05$, 95% CI=0.72-0.88) for OS after liver resection. Interestingly, the risk reduction in hepatocellular carcinoma recurrence after liver resection was significantly associated with a dose/duration dependent on accumulated metformin usage (38). Kaltenmeier *et al* (39) reported that metformin improved outcomes for patients who underwent surgery for colorectal liver metastasis (CRLM). They divided patients into metformin ($n=62$) or no metformin ($n=208$), and patients on metformin had significantly longer Recurrence-free survival (RFS) (HR: 0.44, 95% CI: 0.26-0.75, $P < 0.002$; Median RFS: 49 months vs. 33 months) and OS (HR 0.60, 95% CI 0.31-0.97, $P < 0.048$, Median OS: 72 months vs. 60 months) (39). Similarly, Luo *et al* (40) showed that metformin usage significantly improved OS in hepatitis B virus (HBV)-related HCC patients with DM after radical resection (hazard ratio: 0.558, 95% CI: 0.385-0.810).

These reports suggest that metformin could improve the prognosis after surgery. However, the effects might depend on the types of cancers and patients' conditions. Further studies about the effects of metformin on ESCC after esophagectomy as a radical surgery are required.

6. Future treatment strategy

This chapter discusses how to use metformin in the treatment of ESCC. Of course, further study is needed, but it is beneficial to consider this aspect.

The results of clinical trials should be given particular attention. As mentioned, low doses of metformin administered preoperatively improved TIME, although it did not improve tumor growth or apoptosis (8), so perioperative metformin administration is likely beneficial. Especially in diabetic patients who are not on metformin, it is advisable to change the therapeutic agent to metformin.

In addition, since the prognosis was improved in treatment with trimodality therapy (30), it is desirable to administer metformin after surgery. However, when 5-FU plus cisplatin is used as chemotherapy, it might be necessary to switch to cisplatin alone or different combination drugs, as metformin seems to produce 5-FU resistance. However, the choice of concomitant drug is tough. Docetaxel is under-reported, and 2DG needs further investigation. Nivolumab is most likely useful because it improves the immune status, and this also needs further investigation.

Therefore, even in the absence of diabetes complications, it would make sense to take metformin under careful follow-up, with attention to the above items, to improve treatment outcomes.

7. Conclusion

In this review, we comprehensively searched and summarized studies conducted on the effects of metformin on ESCC. Many studies have reported that metformin exerts antitumor effects; however, its actions are diverse. Hanahan and Weinberg identified important cancer properties as hallmarks of cancer (41), and according to their categories, metformin has been reported to affect cell proliferation, escape from cell death, abnormal glucose metabolism, abnormal immune function, increased inflammation, increased metastatic invasion, and increased angiogenesis. Therefore, metformin is a promising adjunct drug for treating these types of cancer, targeting their specific hallmarks. Many studies have reported the enhanced therapeutic effects of combination therapy, such as improved efficacy of metabolic inhibitors and sensitivity to chemotherapy and radiotherapy, thus nominating metformin as a promising therapeutic approach for ESCC.

Although this paper is only a narrative review based on the literature, metformin is expected to be helpful as an adjunct to the treatment of ESCC, given its clinical effectiveness in preventing ESCC and ultimately improving ESCC treatment outcomes. Metformin is also an appealing therapeutic approach as it is already in use clinically for the treatment of DM and is more likely to be approved as a cancer treatment than a completely novel drug. However, as mentioned earlier, the effects of metformin are diverse and do not necessarily lead to antitumor effects or improved treatment outcomes. Therefore, when treating ESCC, the use of metformin depends on the choice of the treatment approach and its underlying mechanism. In particular, chemotherapy using cisplatin, immunotherapy, and metabolic therapy with 2DG should be further investigated in combination with metformin.

Acknowledgements

Not applicable.

Funding

This study was supported by Surgery II Scholarship Donation from the Department of Frontier Surgery, Graduate School of Medicine, Chiba University (grant no. J09KF00261).

Availability of data and materials

Not applicable.

Authors' contributions

NS and MK conceived, designed and wrote the manuscript. KM, TT, KH and GO critically reviewed and revised the manuscript. HM conceived, designed and supervised the work. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Delman AM, Ammann AM, Turner KM, Vaysburg DM and Van Haren RM: A narrative review of socioeconomic disparities in the treatment of esophageal cancer. *J Thorac Dis* 13: 3801-3808, 2021.
- Pennathur A, Gibson MK, Jobe BA and Luketich JD: Oesophageal carcinoma. *Lancet* 381: 400-412, 2013.
- Duan XF, Tang P, Shang XB, Jiang HJ and Yu ZT: The prevalence of lymph node metastasis for pathological T1 esophageal cancer: A retrospective study of 143 cases. *Surg Oncol* 27: 1-6, 2018.
- Tachimori Y, Ozawa S, Numasaki H, Fujishiro M, Matsubara H, Oyama T, Shinoda M, Toh Y, Udagawa H and Uno T: Registration Committee for Esophageal Cancer of the Japan Esophageal Society: Comprehensive registry of esophageal cancer in Japan, 2009. *Esophagus* 13: 110-137, 2016.
- Kourelis TV and Siegel RD: Metformin and cancer: New applications for an old drug. *Med Oncol* 29: 1314-1327, 2012.
- Tahrani AA, Varughese GI, Scarpello JH and Hanna FWF: Metformin, heart failure, and lactic acidosis: Is metformin absolutely contraindicated? *BMJ* 335: 508-512, 2007.
- Micic D, Cvijovic G, Trajkovic V, Duntas LH and Polovina S: Metformin: Its emerging role in oncology. *Hormones (Athens)* 10: 5-15, 2011.
- Wang S, Lin Y, Xiong X, Wang L, Guo Y, Chen Y, Chen S, Wang G, Lin P, Chen H, *et al*: Low-dose metformin reprograms the tumor immune microenvironment in human esophageal cancer: Results of a phase II clinical trial. *Clin Cancer Res* 26: 4921-4932, 2020.
- Hardie DG: Molecular pathways: Is AMPK a friend or a foe in cancer? *Clin Cancer Res* 21: 3836-3840, 2015.
- Fan H, Yu X, Zou Z, Zheng W, Deng X, Guo L, Jiang W, Zhan Q and Lu SH: Metformin suppresses the esophageal carcinogenesis in rats treated with NMBzA through inhibiting AMPK/mTOR signaling pathway. *Carcinogenesis* 40: 669-679, 2019.
- Wang F, Ding X, Wang T, Shan Z, Wang J, Wu S, Chi Y, Zhang Y, Lv Z, Wang L and Fan Q: Metformin inhibited esophageal squamous cell carcinoma proliferation in vitro and in vivo and enhanced the anti-cancer effect of cisplatin. *PLoS One* 12: e0174276, 2017.
- Nakayama A, Ninomiya I, Harada S, Tsukada T, Okamoto K, Nakanuma S, Sakai S, Makino I, Kinoshita J, Hayashi H, *et al*: Metformin inhibits the radiation-induced invasive phenotype of esophageal squamous cell carcinoma. *Int J Oncol* 49: 1890-1898, 2016.
- Wang L, Du L, Xiong X, Lin Y, Zhu J, Yao Z, Wang S, Guo Y, Chen Y, Geary K, *et al*: Repurposing dextromethorphan and metformin for treating nicotine-induced cancer by directly targeting CHR7 to inhibit JAK2/STAT3/SOX2 signaling. *Oncogene* 40: 1974-1987, 2021.
- Feng Y, Ke C, Tang Q, Dong H, Zheng X, Lin W, Ke J, Huang J, Yeung SCJ and Zhang H: Metformin promotes autophagy and apoptosis in esophageal squamous cell carcinoma by downregulating Stat3 signaling. *Cell Death Dis* 5: e1088, 2014.
- Sekino N, Kano M, Matsumoto Y, Sakata H, Akutsu Y, Hanari N, Murakami K, Toyozumi T, Takahashi M, Otsuka R, *et al*: Antitumor effects of metformin are a result of inhibiting nuclear factor kappa B nuclear translocation in esophageal squamous cell carcinoma. *Cancer Sci* 109: 1066-1074, 2018.
- He Y, Tan X, Hu H, Wang Q, Hu X, Cai X, Guan Y, Chen B and Jing X: Metformin inhibits the migration and invasion of esophageal squamous cell carcinoma cells by downregulating the protein kinase B signaling pathway. *Oncol Lett* 15: 2939-2945, 2018.
- Noto H, Goto A, Tsujimoto T and Noda M: Cancer risk in diabetic patients treated with metformin: A systematic review and meta-analysis. *PLoS One* 7: e33411, 2012.
- Wang QL, Santoni G, Ness-Jensen E, Lagergren J and Xie SH: Association between metformin use and risk of esophageal squamous cell carcinoma in a population-based cohort study. *Am J Gastroenterol* 115: 73-78, 2020.
- Liang F, Wang YG and Wang C: Metformin inhibited growth, invasion and metastasis of esophageal squamous cell carcinoma in vitro and in vivo. *Cell Physiol Biochem* 51: 1276-1286, 2018.
- Cai X, Hu X, Tan X, Cheng W, Wang Q, Chen X, Guan Y, Chen C and Jing X: Metformin induced AMPK activation, G0/G1 phase cell cycle arrest and the inhibition of growth of esophageal squamous cell carcinomas in vitro and in vivo. *PLoS One* 10: e0133349, 2015.
- Wang L, Li K, Lin X, Yao Z, Wang S, Xiong X, Ning Z, Wang J, Xu X, Jiang Y, *et al*: Metformin induces human esophageal carcinoma cell pyroptosis by targeting the miR-497/PELP1 axis. *Cancer Lett* 450: 22-31, 2019.
- Yu H, Bian X, Gu D and He X: Metformin synergistically enhances cisplatin-induced cytotoxicity in esophageal squamous cancer cells under glucose-deprivation conditions. *Biomed Res Int* 2016: 8678634, 2016.
- Peng J, Jing X, Wu J, Hong D, Hu X, Wang Q, Hu H and Cai X: Metformin's effects on apoptosis of esophageal carcinoma cells and normal esophageal epithelial cells: An in vitro comparative study. *Biomed Res Int* 2020: 1068671, 2020.
- Yang Y, Jin G, Liu H, Liu K, Zhao J, Chen X, Wang D, Bai R, Li X, Jiang Y, *et al*: Metformin inhibits esophageal squamous cell carcinoma-induced angiogenesis by suppressing JAK/STAT3 signaling pathway. *Oncotarget* 8: 74673-74687, 2017.
- Kunisada Y, Eikawa S, Tomonobu N, Domae S, Uehara T, Hori S, Furusawa Y, Hase K, Sasaki A and Udono H: Attenuation of CD4⁺CD25⁺ regulatory T cells in the tumor microenvironment by metformin, a type 2 diabetes drug. *EBioMedicine* 25: 154-164, 2017.
- Gatenby RA and Gillies RJ: Glycolysis in cancer: A potential target for therapy. *Int J Biochem Cell Biol* 39: 1358-1366, 2007.
- Zhu Z, Jiang W, McGinley JN and Thompson HJ: 2-Deoxyglucose as an energy restriction mimetic agent: Effects on mammary carcinogenesis and on mammary tumor cell growth in vitro. *Cancer Res* 65: 7023-7030, 2005.
- Shafae A, Pirayesh Islamian J, Zarei D, Mohammadi M, Nejati-Koshki K, Farajollahi A, Aghamiri SMR, Rahmati Yamchi M, Baradaran B and Asghari Jafarabadi M: Induction of apoptosis by a combination of 2-deoxyglucose and metformin in esophageal squamous cell carcinoma by targeting cancer cell metabolism. *Iran J Med Sci* 44: 99-107, 2019.
- Liu CC, Chou KT, Hsu JW, Lin JH, Hsu TW, Yen DHT, Hung SC and Hsu HS: High metabolic rate and stem cell characteristics of esophageal cancer stem-like cells depend on the Hsp27-AKT-HK2 pathway. *Int J Cancer* 145: 2144-2156, 2019.
- Van De Voorde L, Janssen L, Larue R, Houben R, Buijsen J, Sosef M, Vanneste B, Schraepen MC, Berbée M and Lambin P: Can metformin improve 'the tomorrow' of patients treated for oesophageal cancer? *Eur J Surg Oncol* 41: 1333-1339, 2015.
- Li PD, Liu Z, Cheng TT, Luo WG, Yao J, Chen J, Zou ZW, Chen LL, Ma C and Dai XF: Redox-dependent modulation of metformin contributes to enhanced sensitivity of esophageal squamous cell carcinoma to cisplatin. *Oncotarget* 8: 62057-62068, 2017.
- Damelin LH, Jivan R, Veale RB, Rousseau AL and Mavri-Damelin D: Metformin induces an intracellular reductive state that protects oesophageal squamous cell carcinoma cells against cisplatin but not copper-bis(thiosemicarbazones). *BMC Cancer* 14: 314, 2014.
- Mynhardt C, Damelin LH, Jivan R, Peres J, Prince S, Veale RB and Mavri-Damelin D: Metformin-induced alterations in nucleotide metabolism cause 5-fluorouracil resistance but gemcitabine susceptibility in oesophageal squamous cell carcinoma. *J Cell Biochem* 119: 1193-1203, 2018.
- Chevalier B, Pasquier D, Lartigau EF, Chargari C, Schernberg A, Jannin A, Mirabel X, Vantghem MC and Escande A: Metformin: (Future) best friend of the radiation oncologist? *Radiother Oncol* 151: 95-105, 2020.
- Chen F, Xu B, Li J, Yang X, Gu J, Yao X and Sun X: Hypoxic tumour cell-derived exosomal miR-340-5p promotes radioresistance of oesophageal squamous cell carcinoma via KLF10. *J Exp Clin Cancer Res* 40: 38, 2021.
- Fransgaard T, Thygesen LC and Gögenur I: Association between metformin use after surgery for colorectal cancer and oncological outcomes: A nationwide register-based study. *Int J Cancer* 143: 63-72, 2018.
- Kaushik D, Karnes RJ, Eisenberg MS, Rangel LJ, Carlson RE and Bergstrahl EJ: Effect of metformin on prostate cancer outcomes after radical prostatectomy. *Urol Oncol* 32: 43.e1-e7, 2014.

38. Chan KM, Kuo CF, Hsu JT, Chiou MJ, Wang YC, Wu TH, Lee CF, Wu TJ, Chou HS and Lee WC: Metformin confers risk reduction for developing hepatocellular carcinoma recurrence after liver resection. *Liver Int* 37: 434-441, 2017.
39. Kaltenmeier C, Morocco B, Yazdani H, Reitz K, Meyer K, Molinari M, Geller D and Tohme S: Impact of metformin use on survival in patients undergoing liver resection for colorectal cancer metastases. *Am Surg* 87: 1766-1774, 2021.
40. Luo CS, Lin Y, Zhou WP and Shi J: Survival advantage associated with metformin usage in hepatocellular carcinoma patients with diabetes mellitus receiving radical resection: A propensity score matching analysis. *Eur J Gastroenterol Hepatol* 32: 1030-1035, 2020.
41. Hanahan D and Weinberg RA: Hallmarks of cancer: The next generation. *Cell* 144: 646-674, 2011.



Copyright © 2024 Sekino et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.