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Current Clinical Pharmacology, 2018, *13*, 000-000

REVIEW ARTICLE

Metformin as a Radiation Modifier; Implications to Normal Tissue Protection and Tumor Sensitization

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ARTICLE HISTORY

Received: September 20, 2018
Revised: October 19, 2018
Accepted: October 22, 2018

DOI:
10.2174/1574884713666181025141559

Abstract: ***Background*:** Nowadays, ionizing radiation is used for several applications in medicine, industry, agriculture, and nuclear power generation. Besides the beneficial roles of ionizing radiation, there are some concerns about accidental exposure to radioactive sources. The threat posed by its use in terrorism is of global concern. Furthermore, there are several side effects to normal organs for patients who had undergone radiation treatment for cancer. Hence, the modulation of radiation response in normal tissues was one of the most important aims of radiobiology. Although, so far, several agents have been investigated for protection and mitigation of radiation injury. Agents such as amifostine may lead to severe toxicity, while others may interfere with radiation therapy outcomes as a result of tumor protection. Metformin is a natural agent that is well known as an antidiabetic drug. It has shown some antioxidant effects and enhances DNA repair capacity, thereby ameliorating cell death following exposure to radiation. Moreover, through targeting endogenous ROS production within cells, it can mitigate radiation injury. This could potentially make it an effective radiation countermeasure. In contrast to other radioprotectors, metformin has shown modulatory effects through induction of several genes such as AMPK, which suppresses reduction/oxidation (redox) reactions, protects cells from accumulation of unrepaired DNA, and attenuates initiation of inflammation as well as fibrotic pathways. Interestingly, these properties of metformin can sensitize cancer cells to radiotherapy.

***Conclusion*:** In this article, we aimed to review the interesting properties of metformin such as radioprotection, radiomitigation and radiosensitization, which could make it an interesting adjuvant for clinical radiotherapy, as well as an interesting candidate for mitigation of radiation injury after a radiation disaster.

Keywords: Radiation, metformin, radioprotection, mitigation, radiosensitization, inflammation, redox, fibrosis, tumor hypoxia, DNA repair, mitochondria, AMPK, tumor resistance, cell cycle.

INTRODUCTION

Nowadays, ionizing radiation is an inseparable agent in human life. Radiation is useful for several applications such as industrial, agricultural, nuclear power generation, diagnostic and therapeutic medicinal procedures [1]. In contrast to these useful applications, there are some concerns about accidental exposure to radiation workers [2-4]. Incidents such as World War 2, Chernobyl nuclear weapon explosion, as well as some terrorist activities in recent years, are major concerns related to the use of radioactive agents [5]. Nowadays, the most important and widest application of ionizing radiation is related to its clinical applications for diagnosis of diseases as well as its therapeutic applications for cancers [6]. Usually, diagnostic applications of ionizing radiation have not led to any remarkable toxicity, however, high doses of radia-

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1574-8847/18 $58.00+.00

tion for therapeutic purposes could lead to significant side effects in normal tissues [7, 8].

Normal tissue toxicity is a major limiting factor in the delivery of an effective radiation dose to tumor cells [9]. This issue is even more critical when a radiosensitive organ is located within the radiation field [10, 11]. Exposure of bone marrow cells leads to massive apoptosis, thereby causing lymphopenia and reduction of other blood cells, which affect the immune system potently [12-16]. The gastrointestinal system is another sensitive organ to radiation. Exposure to a heavy dose of ionizing radiation during radiotherapy may lead to severe damages to the epithelial cells of gastrointestinal system, as well as some late effects such as chronic inflammation, ulcer and bleeding [17-20]. Some of these late effects could lead to death, some months to years after exposure to high doses of ionizing radiation. Chronic inflammation in the lung may lead to pneumonitis, which occurs some months after radiotherapy or exposure to an accidental radiation event (more than 7-8Gy) [21-24]. In higher doses of ionizing radiation, the incidence of fibrosis is possible [23, 25-27]. Cardiovascular and cerebral disorders, as well as skin reactions are other important side effects to normal tissues that may appear following radiotherapy or a radiation disaster [28, 29].

So far, several chemical or natural agents have been proposed for the management of normal tissue toxicity during radiotherapy [30, 31]. Amifostine is the most famous agent that has been proposed for the amelioration of xerostomia in head and neck patients. However, as a result of its high toxicity, it has not been able to protect most organs [32-35]. Hence, there is a need for the continuous search for low toxic and effective radiation modifiers. However, the possible protection of tumor cells is of utmost concern while administering several radioprotectors such as classic antioxidants [36-39]. In the present review, we aimed to propose metformin as a radioprotector with potent anti-cancer effects. Furthermore, we proposed the potent redox modulatory effect of metformin as an appropriate radiation mitigator for applications in radiation disasters.

MECHANISMS OF RADIATION-INDUCED TOXICITY

Radiation-induced DNA Damage and Cell Death; Early Effects

DNA is the most critical macromolecule within cells while ionizing radiation is the most important clastogenic agents that can be used in medicine or some other applications. For a typical dose of ionizing radiation in conventional radiotherapy (2Gy per each fraction), more than 1000 single strand breaks (SSBs), more than 1000 base mutation and more than 20 double-strand breaks (DSBs) can occur within each exposed cell [29]. In some new radiotherapy techniques such as stereotactic radiotherapy, using higher doses of ionizing radiation could possibly lead to severe DNA damage in irradiated cells [40, 41]. The response of cells to DNA damage is highly dependent on DNA damage response (DDR) by DNA repair enzymes [42-44]. Moreover, the expression of pro-apoptosis factors such as p53 and BAX affect cell death induction through apoptosis in some radiosensitive organs such as bone marrow and intestine [45-49].

If cells are not able to complete the repair process of damaged DNA, cells may undergo death through different pathways such as mitotic catastrophe, apoptosis, necrosis, autophagy, and senescence [50, 51]. If the numbers of cell deaths are very high, it may lead to loss of organ function [52, 53]. For example, high apoptosis in bone marrow cells affects the immune system activities in patients that undergo radiotherapy or people that were exposed to an accidental radiation event [54-56]. Apoptosis and necrosis of stem cells in the tongue and intestine could lead to some problems in the digestion of food, which is a common side effect for patients with head and neck, as well as abdominal cancers [57-59].

RADIATION-INDUCED INFLAMMATION AND FIBROSIS; LATE EFFECTS

Although DNA damage and cell death occur some hours to days after irradiation [60, 61], evidences have shown that both oxidized DNA and dead cells trigger several signaling cascades that lead to severe toxicity in irradiated organs. The products of dead cells including apoptotic bodies and intracellular contents that are released following necrosis, senescence or autophagy are able to trigger tolerogenesis or inflammation by immune cells [41]. Apoptotic bodies are digested by macrophages, thereby preventing inflammatory responses by lymphocytes. However, digestion of apoptotic bodies leads to the release of tolerogenic cytokines such as IL-10 and TGF-β [62]. On the other hand, disruption of cell membrane by necrosis leads to the release of some danger alarms such as HMGB1, uric acid, and oxidized nucleus or mitochondrial DNA (mtDNA), which trigger lymphocytes, macrophages and dendritic cells to release inflammatory cytokines [63, 64].

Toll-like receptors (TLRs) are the most important receptors for danger alarms that are released by dying cells or oxidized DNA. It has been proposed that TLR2, TLR4, TLR5 and TLR9 are the main receptors for releasing danger alarms by ionizing radiation [65-68]. Binding of danger alarms to TLRs leads to the upregulation of inflammatory mediators such as nuclear factor-kB (NF-kB), signal transducers and activators of transcription (STATs) as well as mitogen-activated protein kinases (MAPKs), leading to the release of inflammatory cytokines by lymphocytes and macrophages [69-71]. Increased levels of IL-1, IL-2, IL-4, IL-6, IL-8, IL-13, IL-18, IL-33, TNF-α and IFN-γ are the most important changes in the level of inflammatory cytokines following exposure to a high dose of ionizing radiation [72].

Evidences have shown that pro-fibrotic cytokines such as IL-4, IL-13 and TGF-β through upregulation of pro-oxidant enzymes, induce continuous production of ROS, which mediate an increase in the level of fibrotic mediators such as alpha-smooth muscle actin (α-SMA) [73, 74]. These changes lead to the accumulation of collagen and fibronectin in intercellular spaces, leading to tissue stiffness and appearance of fibrosis [75]. On the other hand, inflammatory cytokines stimulate the production of prostaglandins, nitric oxide (NO) and ROS, leading to symptoms of pain and inflammation in

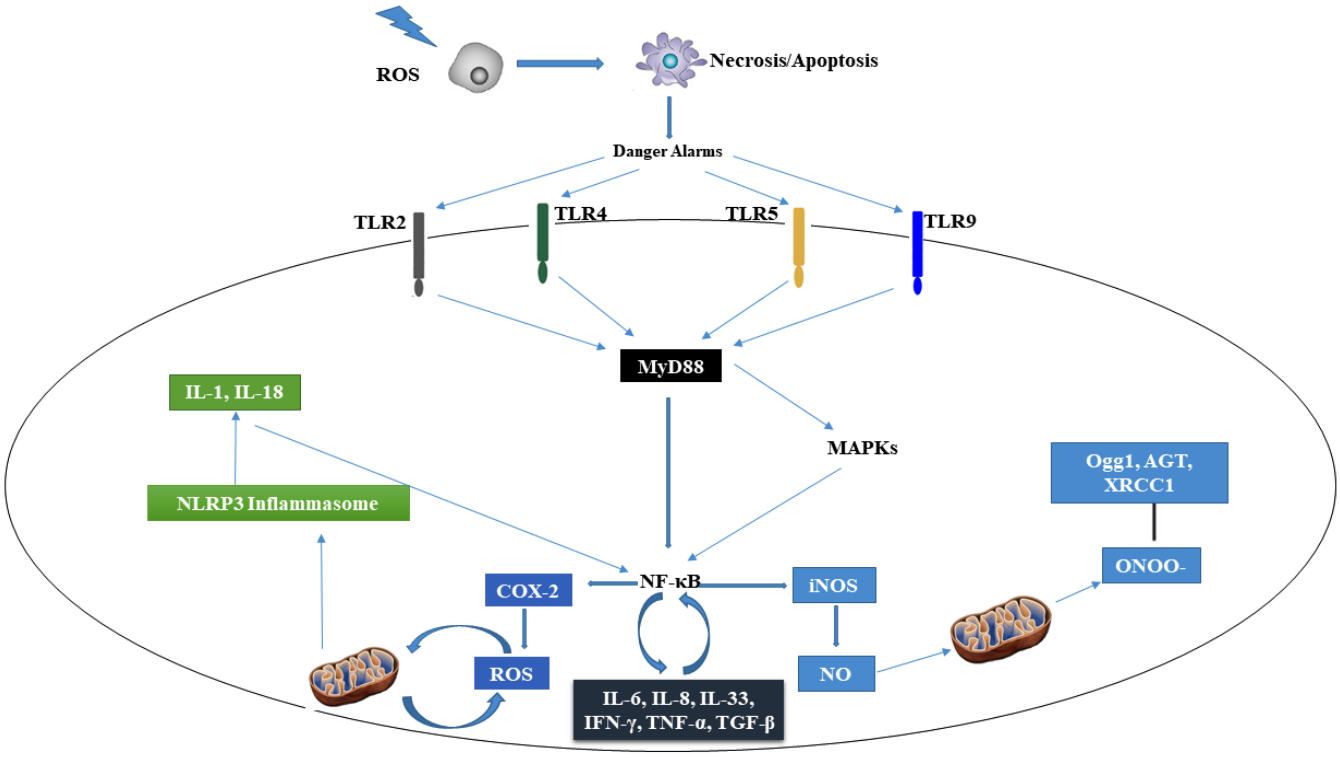

Fig. (1). Molecular mechanisms of normal tissue toxicity following exposure to ionizing radiation.

irradiated tissues [76]. Both pro-inflammatory and pro-fibrotic cytokines are able to induce reduction/oxidation (redox) reactions through stimulation of pro-oxidant enzymes and mitochondria, leading to further DNA oxidation and cell death [77]. This triggers upregulation of inflammation and fibrosis mediators in a positive feedback loop [78]. Chronic oxidative injury following exposure to radiation plays a key role in the development of fibrosis and inflammation in several organs. This may lead to disruption of organ function and some signs such as ulcer and bleeding, which can potentially affect the quality of life of patients who had undergone radiotherapy [79].

METFORMIN

Metformin originates from Galega Officinalis (French lilac) and was first identified in 1922 by Emil Werner and James Bell. This herb is rich in guanidine, a blood glucose lowering agent (a substantial agent in metformin). Some studies conducted in 1950 and later, revealed some other properties of metformin such as antiviral, polyuria, bacteriostatic, halitosis *etc*. Metformin is approved by the U.S. Food and Drug Administration (FDA) for type 2 diabetes and is the most used anti-diabetic drug worldwide [80].

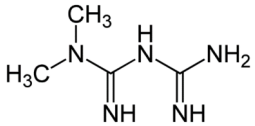

Fig. (2). Chemical structure of metformin.

In addition to the anti-diabetic effect of metformin, it has been found to possess some interesting properties such as antioxidant, anticancer, genomic stability, anti-inflammation and anti-fibrosis, which are interesting for its use as an adjuvant in oncology [81]. These properties propose its use for protecting normal tissues against the toxic effects of chemotherapy and radiotherapy, as well as tumor suppression.

POTENTIAL ANTIOXIDANT EFFECTS OF METFORMIN

Some experimental studies have confirmed the antioxidant effect of metformin. As shown in Fig. **1**, metformin is a hydrogen-rich agent. It is well known that hydrogen is able to interact with free radicals and neutralize their reactivity [82, 83]. In addition to the direct antioxidant effect of metformin, studies have revealed that it can scavenge free radicals through stimulation of antioxidant defense in cells [84,85]. A study by Obi *et al.* showed that treatment of diabetic rats with metformin alleviates oxidative injury through upregulation of superoxide dismutase (SOD), glutathione (glutathione), and catalase (CAT) [86]. Human studies have also shown that the use of metformin for diabetic patients reduces the level of oxidative stress markers remarkably [87-89].

It seems that the most potent effect of metformin against oxidative stress is related to the modulation of cellular metabolisms, following exposure to an oxidant agent. Metformin is a potent inhibitor of redox reactions through suppression of mitochondria electron chain 1 (ETC1). In response to oxidant stimulus, mitochondria amplify the production of superoxide, leading to mutation in mtDNA, and mitochondrial malfunction, as a consequence. Inhibition of ETC1 by metformin can inhibit increased superoxide production and attenuate redox activities within cells [90, 91].

In addition to mitochondria, metformin has a potent inhibitory effect on regulation of NADPH oxidase enzymes. NADPH oxidase is composed of 7 subfamilies; NOX1-5 and dual oxidase 1 and 2 (duox1&2). These enzymes are upregulated following exposure of cells to stress conditions such as oxidative stress and inflammation. All of these enzymes are able to generate superoxide and hydrogen peroxidase in response to inflammation and oxidative stress. NOX1-5 genes are stimulated by some cytokines such as IL-1 and TGF-β, while duox1&2 may be upregulated by IL-4, IL-13 and IFN-γ. Metformin has shown ability to attenuate the upregulation of NOX family enzymes, leading to protection against oxidative injury and subsequent consequences such as fibrosis [85]. It is possible that metformin *via* reduction of macrophage activity decreases the release of cytokines and upregulation of NOX genes [92]. Metformin also has shown ability to inhibit the upregulation of duox1&2 in rat's lung and heart tissues. This was associated with amelioration of inflammatory cells' infiltration as well as reduction of IL-4 level [93, 94].

IMMUNE SYSTEM REGULATION BY METFORMIN

Immune system responses play a central role in the response of both normal tissues and tumor cells to therapeutic strategies like radiotherapy, chemotherapy and immunotherapy [29]. Metformin has shown interesting immune system modulation properties that can improve the response of tumor cells and amelioration of normal tissue toxicity. As mentioned in the previous section, the mitochondria are main target for metformin. Continuous ROS production by the mitochondria further amplifies oxidized DNA and cell death that activate redox reaction through stimulation of inflammatory mediators [77, 95]. It has been confirmed that the mitochondria have a close link with other pro-oxidant enzymes such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), NADPH oxidase *etc* [96]. It has also been shown that metformin stimulates the activities of cytotoxic lymphocytes against cancer cells [97, 98]. It has been revealed that suppression of NF-κB and STAT-3 by metformin are involved in the inhibition of inflammatory cytokines as well as cellular transformation [99, 100]. These properties of metformin make it a candidate for the prevention and treatment of some immune-based diseases [101, 102].

DNA REPAIR BOOSTING BY METFORMIN

Enhancing DNA repair response to genotoxic agents is an interesting effect of metformin. In addition to the antioxidant and anti-inflammatory effects of metformin that prevent DNA damage and cell death, it can also trigger enzymes involved in DNA repair process to further ameliorate genomic instability [103, 104]. It seems that AMPK plays a central role in boosting DNA repair response to genotoxic agents. AMPK is a stimulator for ataxia telangiectasia mutated (ATM), a central regulatory gene in DNA repair process. ATM induces phosphorylation of γH2AX in response to DNA damage, by initiating the activation of enzymes involved in DNA damage response [105]. Also, AMPK phosphorylate p53 leads to the arrest of cell cycle [106, 107].

Turacli *et al*. evaluated the potential modulatory effect of metformin on base excision repair (BER) pathway genes in diabetic patients. Patients received 2000 mg metformin per day (2×1000 mg) for 6 months. Afterwards, blood samples were collected to detect the protein level of DNA polymerase beta as well as X-ray repair cross-complementing Gene1 (XRCC1), two important proteins that are involved in BER pathway of DNA repair. Their results showed no significant change in the level of DNA polymerase beta for diabetic patients with or without metformin. Although the level of XRCC1 and p53 were reduced in diabetic patients without metformin, patients who received metformin showed a remarkable increase for both of them [108].

METFORMIN AS RADIOPROTECTOR

The antioxidant and anti-apoptosis activity of metformin, as well as enhancing DNA repair responses are interesting properties that can help attenuate the initiation of inflammation and fibrosis following exposure to radiation. As earlier mentioned, interactions between pro-inflammatory and pro-fibrotic cytokines with redox mediators play central role in chronic oxidative stress. Preventing DNA damage and cell death, as well as inhibition of redox activity are interesting effects of metformin that make it a potential candidate for the alleviation of early and late effects of radiotherapy. In recent years, some studies have been conducted to evaluate the potential radioprotective effect of metformin in different cells [81]. Xu *et al*. evaluated the potential radioprotective effect of metformin on mice bone marrow stem cells. Mice were treated with 250 mg/kg metformin 1 day before and 1 week after whole-body irradiation with 4Gy. Results showed that while irradiation led to chronic ROS production and oxidative DNA damage in hematopoietic stem cells (HPSc) for some weeks, treatment with metformin potently reduced ROS production and subsequent DNA damage. Further analyses showed that the upregulation of NADPH oxidase 4 (NOX4) is mainly responsible for chronic oxidative stress. Metformin could suppress the upregulation of NOX4. Moreover, as NOX4 plays a key role in the senescence of stem cells, metformin administration reduced senescence, however, it could not mitigate apoptosis in HPSc. Furthermore, metformin showed increased activity of antioxidant enzymes such as SOD, GPx and CAT [109].

A study by Cheki *et al*. revealed the radioprotective effect of metformin on human lymphocyte cells before irradiation with different doses of 1, 2, 3 and 4Gy as well as treatment with two different metformin concentrations (10 and 50 μM). This study showed that treatment of lymphocytes with 50 μM metformin did not cause any genotoxicity such as micronuclei formation, and increase in other DNA damage markers like dicentrics, acentrics, and rings. Their results also showed that treatment with metformin before irradiation ameliorates micronuclei formation and induction of nucleoplasmic bridges, as well as increased proliferation index of irradiated cells. Moreover, analysis of metaphase showed that the numbers of dicentrics, acentrics, and rings were reduced significantly. In contrast to another study by Xu *et al*., this study showed that metformin attenuates apoptosis induction in irradiated cells. The protective effect of metformin for all parameters was more remarkable for 50 μM compared to 10 μM [110]. It has been found that metformin treatment reduces BAX to Bcl-2 ratio, thus reducing apoptosis induction in human lymphocytes [111]. In a human study, it has

been shown that metformin treatment can improve the decreased numbers of peripheral blood cells such as complete blood count (CBC), absolute neutrophil count (ANC), absolute lymphocyte count (ALC) and platelet numbers following ^{131}I therapy for thyroid cancer therapy [112]. By contrast to these studies, a study by Mota *et al*. showed no protective effect of metformin for radiation sensorineural hearing loss in *in vitro* pig models [113]. In addition to this study, metformin has shown that through stimulation of AMPK, it ameliorates oxidative injury and elevates antioxidant defense against toxicity of radiation plus cisplatin [114].

METFORMIN AS A RADIATION MITIGATOR

Mitigation of radiation injury is essential for the treatment of exposed people to an accidental radiological or nuclear disaster. In this situation, exposed people may undergo hematopoietic or gastrointestinal system syndrome, which could lead to death after some days to weeks. Also, exposure of other organs such as skin, heart, kidney or lung to a high dose of radiation may lead to organ failure, months to years after exposure [115-117]. So far, some studies have shown that treatment with some antioxidants or redox modulatory agents after exposure can mitigate radiation injury in these organs [3, 118]. Metformin, a potent inhibitor of redox system has shown promising properties for mitigation of radiation toxicity. For the first time, in 2014, Miller *et al*. proposed the potential mitigatory effect of metformin in both *in vitro* and *in vivo* models. They evaluated spleen clonogenic assay for irradiated mice with or without metformin. For evaluating clonogenic assay, the irradiated bone marrow cells were injected into other mice. The stem cells whose clonogenic abilities were preserved were able to divide and develop new colonies in the spleen. They injected 400 mg/kg metformin 30 min before irradiation with 7Gy, gave a 1.8-fold increase for spleen clonogenic assay. Similar results were obtained when metformin was administered 24 h after irradiation. Interestingly, when metformin in combination with captopril, MESNA or N-acetyl-cysteine was administered to mice, clonogenic assay showed an increase by more than 2.5 fold. *In vitro* studies also showed that post-exposure treatment with metformin or in combinational forms increases survival of SA-NH murine sarcoma, human microvascular endothelial cells (HMEC) and mouse embryo fibroblasts (MEF) after irradiation with 4Gy [119].

Lung pneumonitis and fibrosis is a threat following nuclear disasters as well as radiological terrorism. Mitigation of lung injury following exposure to an acute dose of radiation has been examined by several studies [4, 120-122]. Wang *et al*., revealed the protective effect of metformin against radiation-induced fibrosis in rat's lung tissues. Rats were irradiated with 20Gy X-rays followed by daily treatment with 200 mg/kg metformin (starting from 4 h after irradiation to 2 weeks). Their results showed that while exposure to radiation led to an increase in the rate of respiration, treatment with metformin reversed it significantly. Moreover, metformin treatment caused the amelioration of interstitial septal thickening, fibrosis and inflammatory markers. It has been proposed that metformin, through modulation of TGF-β–Smad1/2 pathway alleviates differentiation of fibroblasts and accumulation of collagen in irradiated lung tissues [123].

Skin injury is a common and serious side effect that can be observed after radiotherapy or a nuclear event. Evidences from Chernobyl nuclear explosion showed occurrence of skin toxicity in exposed people [124]. Mitigation of radia-

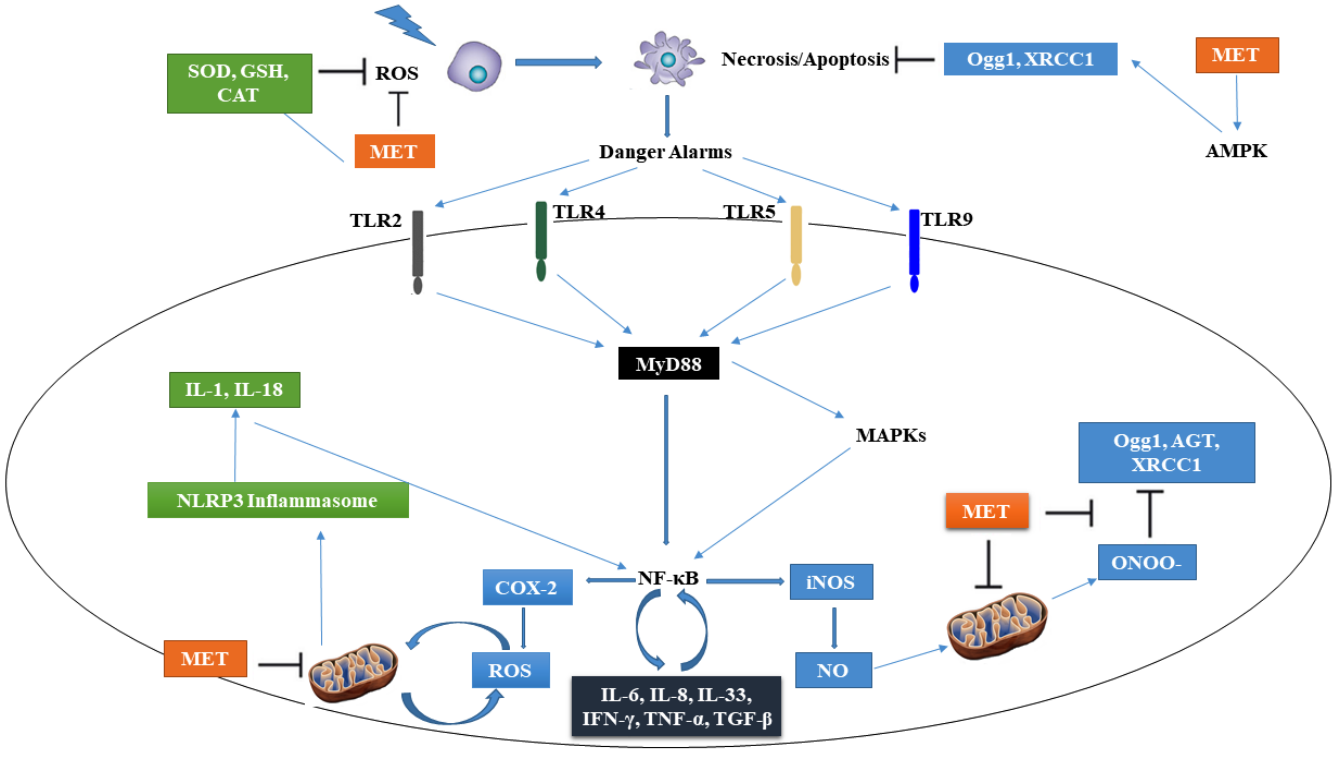

Fig. (3). Mechanisms of radioprotection and radiomitigation by metformin on normal cells/tissues.

tion-induced skin injury is an interesting issue in radiation biology. Treatment with metformin has shown ability to mitigate radiation-induced skin injury. For confirmation of this issue, a 1 x 1 cm^2 mice skin was irradiated with 30 or 50Gy orthovoltage X-rays followed by treatment with metformin (with a dose of 50 or 100 mg/kg administered thrice a day for 1 week after irradiation). Results showed that metformin has ability to prevent deposition of collagen and attenuates the increased level of pro-inflammatory and pro-fibrosis cytokines such as TGF-β, IL-6, C-C motif chemokine 11 (CCL11), STAT-3 and oxidative injury. Results showed that suppression of FOXO-3 by metformin plays a key role in amelioration of skin fibrosis [125].

METFORMIN AS RADIOSENSITIZER

Mechanisms of Tumor Resistance to Radiotherapy

Experimental and pre-clinical studies have shown that radiotherapy leads to several changes in the tumor microenvironment which stimulates tumor adaptation to radiation, leading to a reduction in therapeutic efficiency [126-128]. This may also lead to invasion and spread of tumor cells which facilitate metastasis in patients. For better management of tumor response to radiotherapy, we need to understand the mechanisms of tumor resistance to radiotherapy. Tumor microenvironment includes various types of cells such as tumor cells, macrophages, lymphocytes, dendritic cells, endothelial cells, vascular, *etc*. [129, 130]. Amongst the different cells within the tumor microenvironment, endothelial cells are highly sensitive to ionizing radiation [131]. Interaction of these cells with ionizing radiation leads to apoptosis. Also, therapeutic doses of radiotherapy can cause massive injury to microvascular, leading to disruption of blood supply. This is associated with hypoxia and necrosis of some tumor cells. Apoptosis and necrosis lead to release of inflammatory and pro-fibrotic cytokines. On the other hand, vascular injury and hypoxia lead to upregulation of angiogenesis genes such as hypoxia-inducible factor-1 (HIF-1), vascular endothelial growth factor (VEGF), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) [132, 133].

HIF-1 and VEGF are the most potent stimulators of angiogenesis in tumor cells. The expression of these genes has a direct relationship with radioresistance of tumor [134, 135]. Also, these factors are involved in invasion and metastasis of cancer cells [136]. On the other hand, increased level of both pro-fibrosis and pro-inflammatory cytokines lead to the increased expression of several inflammatory mediators such as NF-kB, which increases cell resistance to apoptosis [137, 138]. Moreover, increased TGF-β and IL-10 which occur following irradiation, lead to attenuation of the activities of lymphocytes and dendritic cells against cancer cells [139, 140]. Hence, inhibition of inflammation and angiogenesis factors in tumor cells is an interesting idea for sensitization of cancer cells to radiotherapy [141, 142]. Some clinical trial studies have shown promising results following inhibition of COX-2, NF-kB, TGF-β and *etc* [30]. However, clinical studies have shown high toxicity following selective inhibition of VEGF [143]. Metformin, a natural and low toxic agent has shown promising tumor suppressive effects that could make it a potential adjuvant for several cancers. It has also been confirmed that metformin, through activation of AMPK, induces regulation of several antitumor signaling pathways [143, 144].

THE PLEIOTROPIC ACTIONS OF METFORMIN IN NORMAL/CANCER CELLS

An interesting property of metformin is its differential effects on normal and cancer cells. One of the interesting effects of metformin is the changes in ROS levels of both normal and cancer cells. In cancers, ROS can promote differentiation and division in lower levels, while high production of it leads to cell death [145]. Metformin has shown the ability to disrupt redox balance in some cancer cells, leading to more ROS production, DNA damage and cell death [146, 147]. However, metformin reduces oxidative injury in normal cells through inhibition of endogenous production of ROS. Inhibition of NADPH oxidase and mitochondria are known protective effects of metformin against oxidative stress [148]. One of the important properties of metformin is the inhibition of inflammation in both normal and tumor cells [149-151]. As earlier mentioned, inflammation leads to chronic oxidative stress and is responsible for the appearance of severe side effects of radiotherapy such as pneumonitis, fibrosis, dermatitis, mucositis, vascular injury, and others. On the other hand, upregulation of inflammatory mediators such as COX-2, HIF-1, NF-kB, mTOR, and STATs induces proliferation, angiogenesis and more survival. Thus, they are responsible for the radioresistance of most cancer cells [152]. Targeting of these inflammatory mediators has been proposed for radioprotection of normal tissues as well as radiosensitization of tumors. Metformin through suppression of these mediators induces apoptosis and autophagy in cancer cells [153, 154]. It has been reported that metformin suppresses mTOR *via* activation of AMPK, leading to autophagy of pancreatic cancer cells [155].

Mitochondria targeting is an interesting strategy for cancer sensitization. The Mitochondria produce ROS in response to radiation and most chemotherapy drugs, leading to oxidative injury in normal tissues. On the other hand, cancer cells need more ATP production by mitochondria, because of high proliferation rate [156-158]. Inhibition of MTC1 in mitochondria is a strategy for sensitization of tumor cells [159]. It has been shown that treatment of MCF-7 cells with metformin leads to depletion of ATP and sensitization of cells to doxorubicin [160]. Andrzejewski *et al*. showed that metformin can inhibit mitochondrial respiration and citric acid cycle, leading to insufficient energy supply in tumor cells [161]. It is widely known that that tumor cells need glucose for proliferation, metformin *via* inhibition of cell respiration reduces the growth of tumor cells [162].

EVIDENCES ON RADIOSENSITIVE EFFECTS OF METFORMIN ON CANCER CELLS

So far, several *in vitro* and *in vivo* studies have confirmed the radiosensitive effect of metformin on different cancer cells. Metformin has shown the ability to target cancer cells without any toxicity to normal cells [163]. Studies have shown some mechanisms for radiosensitization of metformin such as targeting mitochondria, changes in tumor metabolism, increasing ROS production, targeting DNA repair,

change in cell cycle distribution, and oxygenation of hypoxic cells.

Disruption of Tumor Cells Metabolism

An interesting effect of metformin is targeting of mitochondria and other metabolic pathways within cells that are necessary for tumor growth. The mammalian target of rapamycin (mTOR) is one of the most important genes that can shift metabolism of cells from catabolic to anabolic and plays a key role in resistance of tumor cells. Some studies proposed that by contrast to normal tissues, metformin is able to increase metabolism of ROS in tumor cells, leading to increased apoptosis of tumor cells [164]. Metformin has shown ability to reduce mitochondria activity through suppression of MTC1, leading to ATP deprivation and inhibition of growth in hepatoma cancer cells [165]. A study by Song *et al*. revealed the radiosensitive activity of metformin in both *in vitro* and xenograft models. They showed that treatment of mouse fibrosarcoma and MCF-7 cells with metformin can inhibit proliferation of these cells. Also, when cells were incubated with 1 or 5 μM metformin before irradiation with 0-8Gy X-rays, the cells' survival reduced significantly compared to cells irradiated without metformin treatment. The suppressing effect of metformin showed a dose-dependent manner. Molecular analysis showed that metformin, through activation of AMPK, suppresses mTOR and its downstream genes such as S6K1 and 4EBP1. Metformin showed that through direct killing of cancer stem cells, it inhibits the growth of both mouse fibrosarcoma and MCF-7 cells [164].

Mitochondria targeting by metformin is an interesting effect of metformin that can sensitize cells to ionizing radiation. A study by Cheng *et al*., evaluated the role of mitochondria in sensitization of pancreatic tumor cells to radiation. Human pancreatic cancer cells were treated with 0.1 to 2 μM metformin or 0.1 to 1 μM meto-Met_{10} (an analogue of metformin) and irradiated with up to 6Gy X-rays. Results showed that both metformin and meto-Met_{10} inhibit the proliferation of pancreatic cancer cells, while meto-Met_{10} was most effective. Moreover, both metformin and meto-Met_{10} could attenuate the respiration of mitochondria in a dose-dependent manner. This study showed that metformin and meto-Met_{10}, through inhibition of ETC1 in mitochondria, increased the production of superoxide in pancreatic cancer cells, while it does not cause ROS production in normal cells. Meto-Met_{10} is more effective for mitochondria and cell growth targeting compared to metformin [166].

TARGETING OF DNA REPAIR AND CELL CYCLE

Metformin and its analogues have shown ability to inhibit cell growth through cell cycle arrest. Moreover, by contrast to its effect on normal cells, metformin has been shown to increase the level of ROS and suppresses DNA repair enzymes in cancer cells, thus induces delay of cell proliferation and tumor growth, as well as increased induction of cell death *via* accumulation of unrepaired damaged DNA [167]. Cheng *et al*. showed the inhibitory effect of metformin and meto-Met_{10} on the growth of pancreatic cancer cells. They showed that metformin as well as meto-Met_{10}, through activation of AMPK, reduces the activity of cyclin D, leading to cells' arrest in G_1 and inhibition of cell division [166]. Arrest in G_1 phase of cell cycle has been revealed for hepatocellular carcinoma (HCC) cells when cells were treated with metformin before exposure to gamma or carbon radiations. Irradiation of HCC cells leads to stopping of cell cycle in G_2, while metformin reduces arrest in G_2 and increases the number of G_1 cells. It is supposed that metformin, through induction of DSBs in HCC cells, causes a delay in cell cycle progression. Also, metformin *via* targeting mTOR may inhibit proliferation of these cancer cells [168].

Cancer cell cycle arrest in G_2 by metformin has been observed in several studies. Fasih *et al*. revealed the radiosensitization effect of metformin on pancreatic cancer cells. Their study showed that radiation causes a G_2/M arrest as well as a decrease in G_0/G_1-phase cells. However, metformin treatment did not change the distribution of cells compared to radiation alone. Meanwhile, metformin treatment before irradiation leads to more induction of DSBs compared to radiation alone. Authors suggested that activation of AMPK is necessary for sensitization of cancer cells to radiation [169]. It has been revealed that metformin, through suppression of RAD51 in these cells, attenuates homologous recombination (HR) pathway, leading to accumulation of unrepaired DSBs. Moreover, metformin abrogates kinases of the G_2 point, leading to increasing mitotic catastrophe [170]. Similar results were obtained by Jeong *et al*. on colorectal cancer cells and xenografts. They also showed that metformin treatment attenuated the expression of HR DNA repair pathway genes such as Rad50, RAD51, ERCC1, MRE11, BRCA1 and BRCA2 [171]. Accumulation of cells in G_2/M phase of cell cycle following metformin treatment and irradiation has also been revealed for hepatoma cells. This was associated with remarkable induction of DSBs as well as 80% reduction in cell viability [165].

In contrast to these studies, Muaddi *et al*., in an *in vitro* study showed that treatment of A549 and HCT116 cells with metformin may lead to a low protective effect against radiation. They showed that AMPK and p53 activation are involved in this protective effect. Inhibition of AMPK and p53 in metformin-treated cancer cells led to significant decreases in survival for both A549 and HCT116 cells [172].

TUMOR OXYGENATION

From several years ago, it has been well known that hypoxia is one of the most important players for tumor resistance to radiotherapy. Hypoxia increases survival of irradiated cells and also triggers several signaling pathways which are involved in tumor angiogenesis, growth and metastasis. Therefore, inhibition of hypoxia can play a key role in cancer therapy, especially through radiotherapy. It has been reported that some metformin targets such as mTOR are involved in tumor hypoxia [173]. Some studies have shown that metformin facilitates tumor cell killing through oxygenation of hypoxic cells within the tumor [174, 175]. Pra *et al*., showed that metformin has ability to sensitize xenograft prostate cancer cells by augmenting the oxygenation of hypoxic cancer cells. Metformin can reduce the oxygen consumption by cells with high concentration of oxygen, thereby increasing the diffusion of oxygen to more distant cells from tumor vessels [176]. Increasing oxygenation of tumors following met-

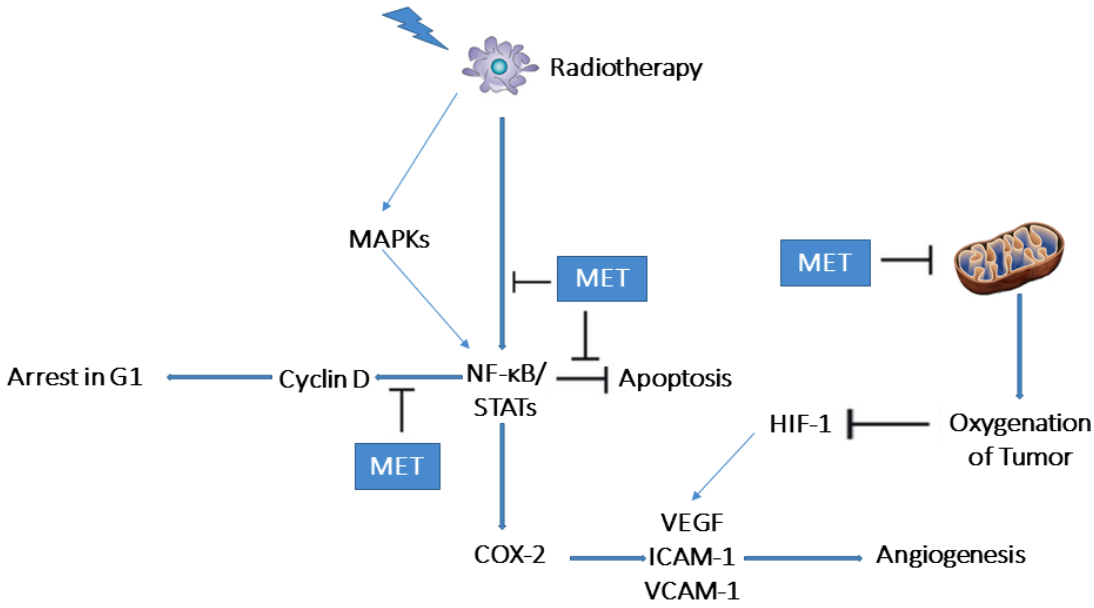

Fig. (4). Molecular mechanisms of radiation sensitization of tumor cells by metformin.

formin treatment has been confirmed using 2-nitroimidazole hypoxia markers, positron emission tomography (PET) and immunohistochemistry in prostate and colorectal cancer xenografts [177].

CLINICAL EVIDENCES OF METFORMIN IN RADIOTHERAPY

Metformin is an FDA approved an anti-diabetic drug that is used by millions of people worldwide. Also, there are several patients with cancer and diabetes that use metformin or other anti-diabetic drugs during radiotherapy. Emerging evidences have shown an anti-cancer effect of metformin in patients with diabetes [178-186]. Some studies have been conducted to evaluate the possible effect of metformin usage in preventing side effects of radiotherapy to normal tissues or preventing tumor regression and patient survival [104, 187]. Although the numbers of these studies are limited, their results are promising. A study by Ferro *et al*., evaluated the incidence of dermatitis in patients with breast cancer who had undergone radiotherapy. Patients included people with diabetes that used metformin or other alternative drugs. Results showed a 56% incidence of desquamation in patients who received metformin compared to 49% desquamation in non-diabetic patients. Interestingly, diabetic patients who received other alternative anti- diabetic drugs showed only 32% incidence of desquamation [188].

Metformin has shown inhibitory effect on tumor progression of patients with hepatocellular carcinoma. Patients with diabetes type 2 that received 500 to 2000 mg metformin per day were compared to other diabetic patients with same cancer that did not receive metformin. All patients were treated with hypofractionated radiotherapy or stereotactic body radiotherapy (SBRT). A three-year follow-up showed that patients who used metformin had a 39% higher survival compared to patients who did not receive metformin [189]. Metformin also has shown preventive effects on rectal cancers [183, 190-194]. A clinical study by Young *et al*., showed that metformin has a synergic effect on rectal tumor control by radiotherapy. Their study involved non-diabetic patients as well as diabetic patients with rectal cancer who had used metformin or other anti-diabetic drugs. Patients received radiotherapy and capecitabine or 5-fluorouracil (FU) for cancer treatment. Their results showed a significant increase in tumor response for patients who used metformin compared to other anti-diabetic drugs. Also, tumor response was more obvious for patients who used metformin compared to non-diabetic patients who did not use metformin or other anti-diabetic drugs [195]. Wink investigated the possible synergic effect of metformin on the therapeutic ratio of chemo/radiation therapy for patients with NSCLC. Patients were treated with cisplatin, carboplatin or etoposide and radiotherapy with at least 50 Gy. A five-year follow-up showed a significant increase in survival of patients with metastasis, locoregional recurrence and progression-free survival, while there was no improvement in the overall survival [196].

CONCLUSION

As mentioned in this review, metformin has interesting properties that could make it a potential adjuvant for radiotherapy. This is due to its radioprotective and radiosensitive effects as revealed in several *in vitro* and *in vivo* experiments. Some studies have revealed the antioxidant effects of metformin against ionizing radiation, however, its main effect seems to be related to stimulating DNA repair pathways such as BER and HR pathways through upregulation of AMPK. In addition, metformin is a potent inhibitor of mitochondria, which plays a central role in chronic oxidative stress following exposure to ionizing radiation. Suppression of endogenous ROS production by mitochondria as well as other pro-oxidant enzymes such as NADPH oxidase can reduce oxidized DNA and cell death after exposure to radiation. Metformin has shown that through modulation of these mediators, it ameliorates chronic oxidative injury, inflammation and fibrosis in different organs and cells. Redox modu-

lation by metformin is a promising property for mitigation of radiation injury after a radiological terror, nuclear explosion or an accidental radiation event that is possible in industrial or medical applications. Experimental studies have proposed that metformin inhibits increased production of ROS by hematopoietic stem cells, which is very crucial for the mitigation of hematopoietic failure and death because of lymphopenia and increased risk of infection.

The most limiting factors for using a radioprotector in radiotherapy are high toxicity and possible protection of tumor cells. Although amifostine has received FDA approval for reducing xerostemia and mucositis in patients with head and neck cancers, its high toxicity could lead to discontinuation of radiotherapy, thereby increasing the possibility of tumor regression. On the other hand, the use of classical antioxidants for normal tissue preservation during radiotherapy has its risks in terms of protection of tumor cells and failure of radiotherapy outcomes. Metformin has shown that in addition to the protection of normal cells, it is able to sensitize cancer cells to radiation, thus augments therapeutic ratio of radiotherapy. Inhibition of mitochondria and some related genes like mTOR can disrupt the metabolism of tumor cells. Tumor cells need a high concentration of oxygen because of its high proliferation rate compared to normal cells, leading to hypoxia in distant cells to vascular. As hypoxia causes the resistance of cancer cells to radiotherapy, as well as leading to upregulation of some angiogenesis factors such as HIF-1 and VEGF, targeting of angiogenesis is one of the most interesting methods for tumor sensitization to radiotherapy. Metformin *via* attenuation of cell metabolism and oxygen consumption, causes oxygenation of hypoxic cells, leading to radiosensitization of cancer and attenuation of tumor growth. Metformin is also able to induce tumor growth inhibition through increasing DNA damage and accumulation of cells in G_1 or G_2 phase of cell cycle, which are more sensitive to ionizing radiation.

Results of *in vitro* and murine models' studies have shown that metformin may be an effective adjuvant for radiotherapy. Although clinical evidences for the radioprotective effect of metformin are limited, emerging evidences have emphasized its potential anti-tumor activity on different cancers such as breast, rectal and lung cancers. As metformin has potent anti-apoptosis effect on normal cells, evaluating metformin on blood parameters and its side effects on the gastrointestinal system such as mucositis and enteritis for patients with abdomen/pelvis cancers could prove useful. Results of clinical studies can aid this hypothesis and may be used in the future for clinical radiotherapy.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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