Review article

Targeting of cellular redox metabolism for mitigation of radiation injury

Bagher Farhood[a], Milad Ashrafizadeh[b], Ehsan khodamoradi[c], Mojtaba Hoseini-Ghahfarokhi[c], Shima Afrashi[c], Ahmed Eleojo Musa[d], Masoud Najafi[c,*]

[a] Department of Medical Physics and Radiology, Faculty of Paramedical Sciences, Kashan University of Medical Sciences, Kashan, Iran
[b] Department of Basic Science, Veterinary Medicine Faculty, Tabriz University, Tabriz, Iran
[c] Radiology and Nuclear Medicine Department, School of Paramedical Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran
[d] Department of Medical Physics, Tehran University of Medical Sciences (International Campus), Tehran, Iran

ABSTRACT

Accidental exposure to ionizing radiation is a serious concern to human life. Studies on the mitigation of side effects following exposure to accidental radiation events are ongoing. Recent studies have shown that radiation can activate several signaling pathways, leading to changes in the metabolism of free radicals including reactive oxygen species (ROS) and nitric oxide (NO). Cellular and molecular mechanisms show that radiation can cause disruption of normal reduction/oxidation (redox) system. Mitochondria malfunction following exposure to radiation and mutations in mitochondria DNA (mtDNA) have a key role in chronic oxidative stress. Furthermore, exposure to radiation leads to infiltration of inflammatory cells such as macrophages, lymphocytes and mast cells, which are important sources of ROS and NO. These cells generate free radicals via upregulation of some pro-oxidant enzymes such as NADPH oxidases, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Epigenetic changes also have a key role in a similar way. Other mediators such as mammalian target of rapamycin (mTOR) and peroxisome proliferator-activated receptor (PPAR), which are involved in the normal metabolism of cells have also been shown to regulate cell death following exposure to radiation. These mechanisms are tissue specific. Inhibition or activation of each of these targets can be suggested for mitigation of radiation injury in a specific tissue. In the current paper, we review the cellular and molecular changes in the metabolism of cells and ROS/NO following exposure to radiation. Furthermore, the possible strategies for mitigation of radiation injury through modulation of cellular metabolism in irradiated organs will be discussed.

1. Introduction

Nowadays, exposure to ionizing radiation is almost inevitable. Ionizing radiation is applied in some industries such as nuclear industry as well as in the agricultural sector [1]. Radioactive sources are mostly utilized in nuclear power plants. However, ionizing radiation is widely used in medicine for diagnostic or therapeutic aims. Nuclear medicine, radiotherapy and brachytherapy rely heavily on radioactive sources [2]. In spite of the useful aims of ionizing radiation and radioactive sources, there are some concerns for possible threats which are associated with its usage. One of the most devastating disasters from the peaceful usage of ionizing radiations in the nuclear industry is the Chernobyl nuclear power plant accident. This accident caused the release of huge amounts of radioactive elements including iodine and cesium, which led to the death of some exposed people. Furthermore, it had negative effects on the environment, leading to increased incidence of cancer in some exposed people many years after [3].

In addition to the mentioned threats from peaceful applications of radioactive sources, there are serious concerns about the use of radioactive and nuclear sources for war or terror [4]. An example of such incidents was the nuclear bomb explosions in Hiroshima and Nagasaki during World War 2, which also had its own consequences years after. Furthermore, dirty radioactive sources may also be used for terror aims [5]. People who are exposed to ionizing radiation may die or show some serious side effects which affect their quality of life for many years [6]. As a fallout of these threats, it is imperative to implement strategies aimed at preventing these disasters arising from exposure to ionizing radiation. To achieve this aim, it is important to have the knowledge about the mechanisms through which ionizing radiation cause damages to cells, especially in some critical organs such as the bone marrow, lung, heart as well as the gastrointestinal and neurovascular systems [7].

* Corresponding author at: Radiology and Nuclear Medicine Department, School of Paramedical Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran.
E-mail address: najafi_ma@yahoo.com (M. Najafi).

It is well-known that ionizing radiation attack DNA directly or via radiolysis of water molecules. Classical hypothesis in radiobiology suggests that the final consequences of ionizing radiation are as a result of DNA damage at the first moments after exposure. This dogma was challenged following some evidences which showed that damages to non-irradiated cells was due to the release of some unknown clastogenic factors from irradiated cells. Furthermore, it was observed that exposure to ionizing radiation can lead to continuous production of free radicals [8]. The use of antioxidants some days after exposure to radiation has confirmed that a remarkable amount of toxic consequences of ionizing radiation is as a result of some changes within the cells, thereby leading to an increase in the production of free radicals and amplification of radiation toxicity [9].

Results of experimental studies suggest that abnormal changes in the metabolism of reactive oxygen species (ROS) and nitric oxide (NO) have a key role in potentiating genotoxic effects of ionizing radiation [10]. Evidence suggests that exposure to radiation causes an increase in the ROS/NO sources within tissues [11]. In this paper, we aimed to review the cellular and molecular mechanisms of reduction/oxidation (redox) reactions following exposure to radiation and their potential targets for mitigation of radiation injury.

2. Radiation-induced DNA damage triggers systemic redox reactions

Interactions involving ionizing radiation and free radicals with DNA introduce biological consequences in irradiated and also non-irradiated cells/tissues. Although it is well known that damage to other organelles such as membrane and mitochondria by radiation is involved in radiation toxicity, it seems that chronic oxidative stress and inflammatory reactions are as a result of massive DNA damage and cell death following exposure to radiation. This issue has been observed in studies showing that heavy charged particles cause severe release of damage-associated molecular pattern (DAMP) and chronic oxidative stress (a phenomenon that may occur in low severity following low linear energy transfer (LET) radiation) [12,13]. Indeed, inflammatory cells detect DNA damage and cell death through apoptotic bodies after apoptosis or release of DAMPs including high-mobility-group Box 1 (HMGB1), uric acid, oxidized DNA, ATP, heat shock proteins (HSPs) and some other molecules that will be released following necrosis, necroptosis and autophagy [14]. Apoptotic bodies will be absorbed by macrophages, leading to tolerogenic responses as well as the release of TGF-β and IL-10. However, the release of danger alarms triggers inflammatory responses through upregulation of toll like receptors (TLRs), NF-κB, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and finally inflammatory cytokines such as IL-1, IL-6, IL-8, IL-33, TNF-α and IFN-γ. These changes are associated with infiltration of inflammatory cells and also the release of intercellular mediators such as vesicles, exosomes and microRNAs [15,16].

Released cytokines and other intercellular mediators are able to migrate to other adjacent cells or distant organs, leading to activation of inflammatory reactions and redox interactions. This causes amplification of radiation toxicity in irradiated tissues and also induction of oxidative injury and DNA damage in non-irradiated cells [17]. Thus, identification of intercellular communication and mediators that are involved in the bystander effect may help the exploration of new targets for mitigation of radiation injury. Experimental studies have confirmed this hypothesis. For example, the suppression of TGF-β, TNF-α and COX-2 in bystander cells or non-targeted tissues has been shown to ameliorate radiation-induced oxidative injury [18–20]. Targeting of these proteins has also been shown to ameliorate early and late consequences of exposure to radiation in irradiated organs [21–23]. It seems that inflammation and oxidative stress have close relation with each other in irradiated or bystander tissues. Thus, inhibition of some inflammatory targets such as COX-2 and iNOS can lead to reduction of

ROS and NO, which lead to mitigation of radiation injury [24–28].

3. Redox metabolism after radiation

Emerging evidences have shown that cellular metabolism plays a key role in endogenous production of free radicals a long time after exposure to ionizing radiation [29,30]. ROS generating sources within cells play a key role in the regulation of metabolism and cell proliferation. The mitochondria are known as the energy supplier of cells via generation of ATP. It supplies ROS which trigger cell proliferation in most cell types [31]. NADPH oxidase enzymes also produce ROS that is critical for degradation of pathogens. However, ROS derived NADPH oxidase has a role in proliferation of stem/progenitor cells [32]. The role of NADPH oxidase in bone marrow stem cell function is critical. The production of hydrogen peroxide (H_2O_2) by NADPH oxidase enzymes can trigger differentiation of stem/progenitor cells in the bone marrow, while lower levels of H_2O_2 cause stemness of stem/progenitor cells [33]. Ionizing radiation can cause an abnormal increase in mitochondria and NADPH oxidase activity, leading to the generation of an unusual amount of ROS. This causes overwhelming antioxidant cell defense and damage to stem/progenitor cells. For example, upregulation of NOX4 or higher activity of mitochondria in the bone marrow following exposure to radiation is associated with an increase in ROS, apoptosis and senescence in stem cells [34,35]. Metformin, which is known as an inhibitor of mitochondria, is able to mitigate radiation toxicity in bone marrow stem cells [36]. Metabolisms of glucose and lipids are also increased following exposure to radiation, and it seems to be involved in the progression of radiation-induced normal tissue toxicity [37]. Mammalian target of rapamycin (mTOR) and peroxisome proliferator-activated receptor (PPAR) are two important metabolic mediators whose roles have been confirmed in radiation toxicity. Activation of these mediators also plays a key role in tumor growth and angiogenesis [38,39]. Thus, inhibition of these targets may be suggested for protection of normal tissues without negative effects on tumor response to radiotherapy. Thus, targeting oxygen, glucose and lipid metabolism can be suggested for protection and mitigation of radiation injury.

4. mTOR

mTOR is a critical protein kinase that is involved in the regulation of cell growth, cell death, cancer and metabolism [40]. Targeting mTOR by rapamycin is a known strategy for tumor suppression [41,42]. However, some studies have suggested that it may play a role in normal tissue injury. MTOR can be upregulated by PI3K/Akt and MEK1/2–ERK1/2 pathways as well as some growth factors and hormones [41]. mTOR targeting may cause reduction of stem cells' apoptosis and triggers their proliferation, leading to mitigation of radiation toxicity. Treatment of irradiated mice with rapamycin has been shown to stimulate cell proliferation and reduce apoptosis. This caused an increase in CD133+ renal stem cells. Rapamycin has also been shown to attenuate the upregulation of NF-κB and TGF-β in the kidney, two important mediators of fibrosis and inflammation [43,44]. Similar results have been obtained for parotid acinar cells following head and neck irradiation in mice. The parotid gland has highly radiosensitive stem cells. Damages to these cells may be involved in mucositis and xerostomia for patients that undergo radiotherapy for head and neck cancers. Inhibition of mTOR by CCI-779 in mice has shown promising results for preserving cells in parotid glands [45]. It seems that inhibition of mTOR can protect normal tissues via suppression of radiation-induced senescence. A study has shown that rapamycin reduces senescence after irradiation in primary human keratinocytes and epithelial stem cells. Further analyses showed that rapamycin triggers activation of MnSOD, which neutralizes free radicals and prevents senescence. This is associated with reduction of stem cell death and mucositis in mice tongue [46]. Senescence plays a key role in the
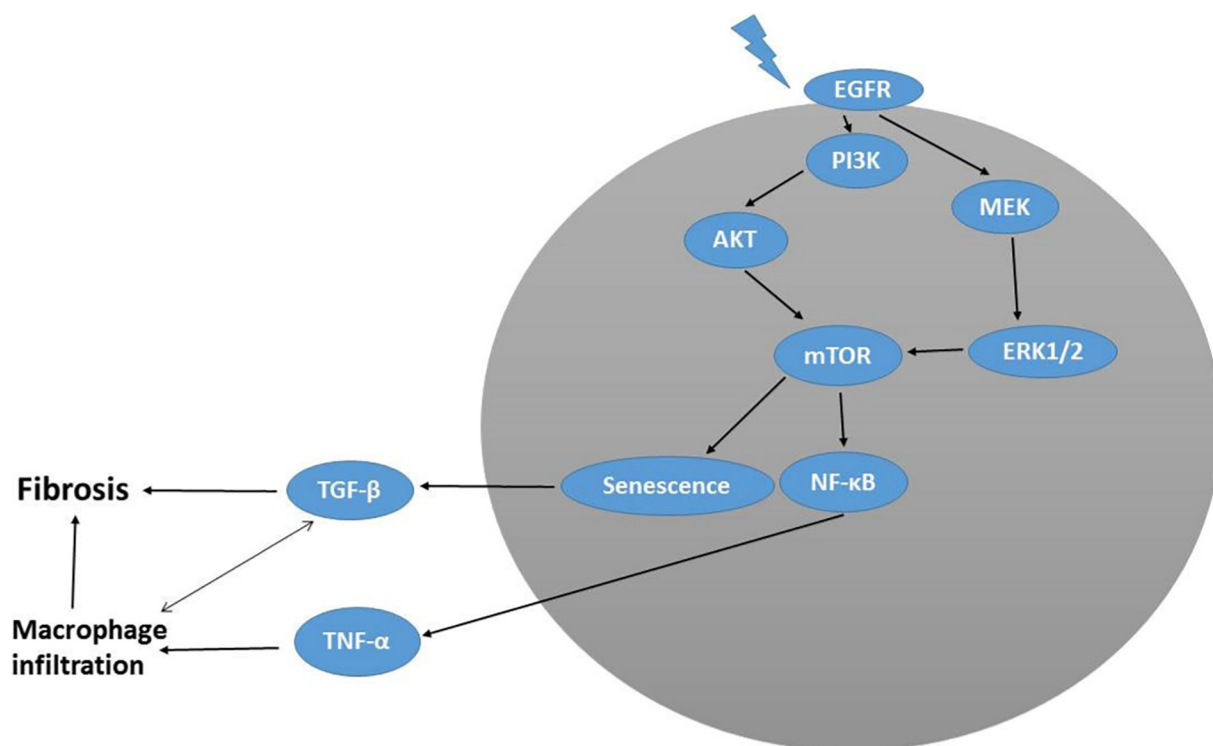
Fig. 1. Mechanisms of mTOR activation after exposure to radiation and its role in the development of fibrosis.

development of fibrosis following pulmonary exposure to radiation [47]. Therefore, it is predictable that suppression of mTOR may be an appropriate candidate for amelioration of fibrosis. This hypothesis has been confirmed in an experimental study by Dai et al. [48]. Chung et al. investigated the effect of mTOR inhibition for mitigation of radiation-induced pulmonary fibrosis. They showed that treatment with rapamycin is associated with reduction of senescence and pro-fibrotic cytokines including IL-1β and TGF-β [49] (Fig. 1).

5. PPARs

PPARs are ligands that regulate several metabolic pathways such as metabolism of fatty acids [50]. They include some subfamilies such as PPARα, PPARβ, PPARδ and PPARγ [51]. The proteins have an important role in the metabolism of fatty acids and glucose, and also in the immune system [52]. Due to their role in the regulation of immune system, it has been suggested that PPARs may have a critical role in inflammatory responses as observed after radiotherapy. One of the important functions of PPARs is suppression of NF-κB, a critical regulator of many inflammatory responses. This effect is mediated through upregulation of IκBα, which can degrade NF-κB [53]. PPARα knock out has been shown to increase the expression of NF-κB. However, this reduces apoptosis and radiosensitivity in the kidney [54]. PPARs also have a negative relationship with the expression of TGF-β in irradiated cells [55,56]. These properties of PPARs may aid protection and mitigation against toxic consequences of ionizing radiation on normal tissues. In vitro studies have confirmed that activation of PPARα and PPARδ can reduce inflammation and oxidative stress in microglia cells [57,58]. An in vivo study also confirmed that PPARα agonist can reduce injury to microglia and preserve hippocampal neurogenesis following whole brain irradiation [59].

Mangoni et al. have shown that PPAR agonists can protect the intestine via suppression of NF-κB. They used rosiglitazone, an agonist of PPARγ before whole-body irradiation of mice. Treatment with PPARγ agonist reduced damages to crypts and villi as well as ameliorated inflammation in the intestine. Rosiglitazone also reduced the induction of

apoptosis. Molecular assays showed that rosiglitazone suppresses the expression of NF-κB and TGF-β, two important inducers of inflammation and apoptosis. This was associated with reduction in inflammatory cytokines' levels [60]. Similar results have been obtained for the lung. Suppression of both NF-κB and TGF-β using rosiglitazone ameliorated pneumonitis and fibrosis [61]. These consequences of PPAR activation by rosiglitazone have also been suggested to reduce the severity of radiation-induced mucositis [62].

6. NADPH oxidases

NADPH oxidases are a group of enzymes that generate H_2O_2 via transferring an electron to oxygen. These enzymes include five subfamilies that are known as NADPH oxidase (NOX)1–5. Furthermore, two other enzymes including dual oxidase (Duox)1 and 2 are involved in ROS production. Under normal conditions, ROS production by these enzymes helps immune cells kill pathogens and pre-cancerous cells. The activity of these enzymes is regulated by some cytokines and growth factors such as IL-1, IL-4, IL-13 and TGF-β. These cytokines are potent inducers of fibrosis. Thus, there is a possibility that NADPH oxidase enzymes play a key role in the development of fibrosis following exposure to ionizing radiation. It has been confirmed that upregulation of NOX1 in the lung can potentiate differentiation of fibroblasts. Furthermore, NOX2 and NOX4 can produce ROS for long periods of time after lung irradiation, thereby mediating oxidative stress [63]. The production of superoxide by NOX1 has also been shown to potentiate apoptosis induction in salivary gland acinar cells. Selective inhibition of NOX1 in the salivary gland acinar cells (NS-SV-AC) showed reduction of apoptosis, which is mediated by ROS derived NOX1 enzyme [64].

Upregulation of NOX2 and NOX4 may be involved in radiation-induced bystander and non-targeted effect, a phenomenon that causes ROS generation in neighboring non-irradiated cells or distant organs [17]. It has been shown that NOX4 and NOX5 have a role in oxidative stress in human fibroblast cells. Inactivation of these enzymes using fulvene-5, a NOX-specific inhibitor showed reduction of ROS level and DNA damage after irradiation [65]. One of the most interesting

properties of NOX system is continuous generation of ROS in highly radiosensitive organs including the bone marrow and intestine. It seems that NOX2 and NOX4 are the most important redox enzymes which amplify radiation toxicity in these organs. In mice bone marrow cells, upregulation of NOX4 has been investigated for 8 weeks after whole-body irradiation. Inactivation of NOX4 has been shown to augment survival and reduce genomic instability. The upregulation of NOX enzymes has also been investigated in mice intestine and was suggested as a source of ROS which mediates chronic oxidative stress for a long time after irradiation. It seems that upregulation of NOX enzymes also plays a key role in chronic inflammation via triggering infiltration of inflammatory cells.

Duox1 and Duox2 are other subfamilies that produce H_2O_2. The most common action of these enzymes is regulating thyrocyte cell function to release thyroxin (T4) and triiodothyronine (T3) [66]. It has been confirmed that high activities of these enzymes are associated with genomic instability and carcinogenesis [67]. In response to ionizing radiation, Duox1 is upregulated and generates higher amount of H_2O_2 for long periods of time. It has been confirmed that an increase in IL-13 is responsible for continuous production of ROS by Duox1 [68]. It seems that upregulation of both Duox1 and Duox2 is involved in the late effect of ionizing radiation which occurs following an increase in IL-4 or IL-13. Infiltration of inflammatory cells and fibrosis have been associated with upregulation of these enzymes [67].

7. Mitochondria

It seems that the mitochondria are the main source of ROS generation for most cells. During oxidative phosphorylation (OXPHOS) the redox reactions in electron transient chain (ETC) lead to superoxide production [69]. Under normal conditions, superoxide is neutralized by superoxide dismutase (SOD) and catalase to prevent toxic effects on vital organelles. Any disruption in the normal OXPHOS can cause overproduction of superoxide by mitochondria, leading to overwhelming antioxidant defense by SOD and catalase. This causes oxidative damage to DNA, organelles, proteins and also lipids within cells and in membrane [70]. Ionizing radiation is able to cause several mutations in mitochondrial DNA (mtDNA) [71]. Studies have shown that mutations in mtDNA after exposure to radiation lead to chronic overproduction of superoxide, which itself triggers production of ROS from other pro-oxidant enzymes in a positive feedback loop [72]. Damages to the DNA nucleus can also trigger mitochondria ATP generation. It seems that cell-cycle kinase CDK1 plays a key role in this pathway. After DNA damage, an increase in CDK1 can stimulate ATP generation and OXPHOS in the mitochondria. This is associated with DNA damage and inhibition of DNA repair [73]. Also, cell death after exposure to ionizing radiation has a key role in stimulating oxygen metabolism and generation of ROS by the mitochondria and other pro-oxidant enzymes. Apoptosis and senescence trigger the release of TGF-β by macrophages, while necrosis may lead to the release of inflammatory cytokines that amplify radiation toxicity via triggering ROS generation [29,74,75]. As earlier mentioned, senescence can stimulate some side effects such as fibrosis following exposure to ionizing radiation. Mitochondrial dysfunction can trigger senescence after exposure to ionizing radiation. It has been shown that radiation-induced mitochondrial respiratory complex II dysfunction causes overproduction of superoxide, which is associated with oxidative stress and cell death through senescence [76]. In mice intestine, ROS production by the mitochondria has been investigated for one year after irradiation [12]. Furthermore, in vitro studies have shown that the mitochondria are responsible for ROS overproduction and genomic instability in the progeny of irradiated cells [77,78]. Damage to mtDNA can also release vesicles that cause oxidative injury in bystander cells [79]. This shows that the mitochondria play a key role in chronic oxidative injury after exposure to ionizing radiation, thus its targeting could help protect and mitigate radiation-induced oxidative injury.

To date, many studies have shown that suppression of mitochondria via selective inhibitors or some other agents mitigates oxidative stress. On the other hand, some agents that neutralize free radicals to prevent mitochondrial injury can mitigate radiation injury, probably via reducing mitochondrial malfunction [80]. Targeting the mitochondria via mitochondrial inhibitors or mitochondrial antioxidants has been shown to reduce ROS generation, DNA damage and cell death [81–84]. One of the interesting mitochondrial inhibitors is metformin [85]. Pre-irradiation treatment with metformin has been shown to reduce genotoxic effects of radiation such as DNA damage, micronuclei formation and apoptosis [86]. Pre and post-irradiation treatments with metformin has shown radioprotective effects for some organs such as intestine, colon, kidney, lung and heart [87–91]. Administration of metformin only after irradiation can mitigate radiation-induced hematopoietic injury, pneumonitis and fibrosis, which are associated with reduction of TGF-β [92–94]. In a clinical study, administration of metformin was shown to improve the number of peripheral white cells in patients treated with radioactive iodine for thyroid cancer [95].

One of the interesting strategies for targeting the mitochondria is the use of mitochondria antioxidants or some agents that neutralize superoxide generated by the mitochondria selectively [96]. The delivery of SOD2 (MnSOD) into the mitochondria has been shown to reduce radiation damage in irradiated cells [97–100]. JP4-039 is a mitochondria-targeted nitroxide that is concentrated in the mitochondria, neutralizes and acts as an SOD mimic. Administration of JP4-039 after whole body irradiation with a lethal dose of radiation can mitigate radiation injury and increase survival [101]. It has been shown to reduce oxidative stress and cell death in the bone marrow, thus mitigates lethal effects of ionizing radiation [102–105]. Rajagopalan et al. evaluated the effect of JP4-039 for mitigation of radiation injury following exposure to a lethal dose of radiation. They showed that JP4-039 helps DNA repair after irradiation [106]. JP4-039 can reduce epithelial cells' necrosis in the small intestine and help stem cells' recovery. These are associated with suppression of inflammation in the intestine [107]. The protective effect of JP4-039 on epithelial cells has also been confirmed for oral and esophageal mucus, while it showed no protective effect for cancer cells [108–110]. To date, some different analogues of JP4-039 have been explored to increase protective and mitigatory effects against radiation [111,112]. Furthermore, some other types of mitochondrial antioxidants such as MnTE-2-PyP5+, tempol and glutathione peroxidase 4 mimic Mito-Ebselen have been developed [113–119].

8. Nitric oxide synthases (NOSs)

NO is a product of macrophages via upregulation of iNOS. NO has a higher half-life compared to ROS and is able to attack DNA. Also, it can combine with superoxide generated by mitochondria to generate peroxynitrite. Peroxynitrite can attack the DNA and cause nucleotide damage directly. Interaction of peroxynitrite with the DNA leads to formation of 8-nitroguanine or oxidation of deoxyribose, which give rise to an abasic site and single strand break (SSB). Peroxynitrite can also cause oxidation and degradation of guanine [120]. Another mechanism of peroxynitrite is induction of DNA damage and cell death through modulation of DNA repair mechanisms. Peroxynitrite causes nitroacetylation of 8-Oxoguanine DNA Glycosylase (Ogg1), a key enzyme in base excision repair (BER) pathway. Nitroacetylation of Ogg1 causes attenuation of its activity and accumulation of unrepaired 8-oxodeoxyguanine (8-oxodG). In this condition, unrepaired SSBs can cause upregulation of poly(ADP-ribose) polymerase 1 (PARP1) and induction of apoptosis [121]. Many years ago, it was suggested that modulation of NO can reduce radiation toxicity in normal tissues [122]. It has been confirmed that production of NO after exposure to radiation is involved in bone marrow dysplasia and enteritis [123,124]. Inhibition of NO generation in the bone marrow has been shown to be associated with radioresistance of hematopoietic and marrow stromal cells [125]. Activation of iNOS in the bone marrow stroma after exposure to radiation

Table 1
Summary results of redox metabolism targeting for mitigation of radiation-induced normal tissue injury.

Route	Cells/tissues	Radiation dose	Targets	Findings	References
In vitro	CHO-K1	3 Gy	Mitochondria	Inhibition of mitochondria by dihydropyridines (DHPs) reduces the level of ROS generation which cause amelioration of oxidative DNA damage.	[81]
Mice	Intestine	9–10 Gy	Mitochondria	JP4-039 can mitigate lethal effect of radiation via preventing epithelial necrosis, helps recovery of stem cells, reduction of inflammatory cells infiltration and release of TNF-α.	[107]
Mice	Bone marrow	8 Gy	Mitochondria	JP4-039 attenuates apoptosis in bone marrow cells.	[104]
Mice	Head and neck	30 Gy	Mitochondria	JP4-039 can reduce severity of mucositis.	[109]
Rats	Lung	20 Gy	Mitochondria	Treatment with metformin after irradiation ameliorates fibrosis.	[93]
Mice	Bone marrow	7 Gy	Mitochondria	Metformin can mitigate radiation injury in hematopoietic cells and increases survival.	[94]
Rats	Lung and heart	15 Gy	Mitochondria	Treatment with metformin pre-and post-irradiation reduced the level of IL-4, pneumonitis and fibrosis.	[90,89]
Mice	Kidney	8 Gy	mTOR	Rapamycin administration after whole-body irradiation can induce repopulation of renal stem cells, thus ameliorates nephrotoxicity.	[43]
Mice	Parotid glands	5 Gy	mTOR	Inhibition of mTOR by rapalogue (CCI-779) can augment the number of cells in parotid glands, while no hyperplasia was observed for mandibular glands.	[45]
Mice	Lung	5 × 6 Gy	mTOR	Treatment with rapamycin starting 2 days before irradiation for 16 weeks led to reduction of senescence and pulmonary fibrosis. Rapamycin also caused reduction of macrophage infiltration into the lung.	[49]
Mice	Tongue	5 × 6 Gy	mTOR	Rapamycin induced the activation of MnSOD, which prevented senescence in stem cells as well as mucositis.	[46]
In vitro	Murine microglia cells	10 Gy	PPARδ	Reduction in the level of inflammatory mediators and cytokines, and also reduced oxidative stress observed following activation of PPARδ.	[57]
In vitro	Murine microglia cells	2–10 Gy	PPARα	Activation of PPARα caused a reduction in inflammatory markers including NF-κB and AP-1.	[58]
Mice	Brain	10 Gy	PPARα	Activation of PPARα using fenofibrate could preserve the hippocampus after whole-brain irradiation.	[59]
Mice	Kidney	10 Gy	PPARα	Knock out of PPARα increased the expression of NF-κB; however, it protected kidney cells via inhibition of apoptosis.	[54]
Mice	Intestine	12 Gy	PPARγ	Activation of PPARγ can reduce radiation toxicity in the intestine via suppression of apoptosis and inflammation.	[60]
Mice	Lung	19 Gy	PPARγ	Rosiglitazone as an agonist of PPARγ attenuated pneumonitis and fibrosis following downregulation of NF-κB and TGF-β.	[61]
Mice	Head and neck	16.5 Gy	PPARγ	Suppression of NF-κB and TGF-β following PPARγ activation showed amelioration of mucositis.	[62]
In vitro	Human pulmonary artery endothelial cells (HPAECs)	5 Gy	NOX1	NOX1 potentiates pulmonary fibrosis.	[63]
In vitro	salivary gland acinar cells (NS-SV-AC)	10 Gy	NOX1	Superoxide production by NOX1 mediates apoptosis in irradiated cells.	[64]
In vitro	Human fibroblasts	2–10 Gy	NOX4&5	Inactivation of NOX4 and NOX5 can reduce oxidative stress and DNA injury after exposure to radiation.	[65]
Rats	Lung	2 Gy	NOX2&4	Localized pelvis irradiation led to upregulation of NOX2 and NOX4. Administration of melatonin could attenuate the upregulation of these genes.	[138]

can cause damage to transplanted hematopoietic or stem cells [123]. Infiltration of macrophages after exposure to ionizing radiation can be a sign of NO production. Pneumonitis after lung exposure to radiation is a known consequence of infiltration of inflammatory cells. Production of NO by macrophages has a role in the progression of pneumonitis. Inhibition of iNOS by N(G)-nitro-L-arginine methyl ester (L-NAME) in a rat model showed remarkable reduction in inflammation and fibrosis [126]. In contrast to these results, some evidences have shown that disruption of NO generation and overproduction of ROS by NADPH oxidase are responsible for vascular injuries after exposure to ionizing radiation [127]. Anti-inflammatory and antioxidant agents which neutralize both ROS and NO could be appropriate for mitigating radiation injury via boosting DNA repair and overcoming oxidative stress. NO has a dual effect on cancer and may cause both cell death and angiogenesis [128]. Although selective targeting of NO may not be applicable for radiotherapy, NO scavengers are promising for mitigation of radiation toxicity [111,129].

9. Epigenetic regulators of redox reactions

It has been confirmed that some epigenetic modulators such as microRNAs are able to change redox state through regulation of antioxidant or pro-oxidant agents [130] (Table 1). MiR-21 is one of the most common players in the epigenetic modulation of oxidative stress following exposure to ionizing radiation. It has been shown that miR-21 is triggered by TGF-β [131,132]. On the other hand, miR-21 causes suppression of SOD2, leading to overcoming superoxide generation on the antioxidant defense of cells [133,134]. MiR-22 can also increase ROS derived mitochondria, thus induces apoptosis [135]. Recently, it has been shown that this pathway plays a role in apoptosis of bone marrow stem cells [136]. Its inhibition and that of some other

microRNAs such as miR-214 that are involved in oxidative injury, have been shown to ameliorate radiation toxicity in irradiated cells [137] (Fig. 2).

10. Conclusion

As explained in this review, cell metabolism is an important target for mitigation of radiation injury. The most part of cell metabolism for mitigation of radiation injury is related to abnormal increased generation of free radicals including both ROS and NO. Mitochondria are the major source of free radicals for a wide range of cells. Targeting mitochondrial ROS by mitochondrial ROS scavengers and SOD mimicking antioxidants confirms its pivotal role in radiation toxicity. NADPH oxidase enzymes are involved in radiation-induced injury in some organs such as bone marrow, intestine and lung. These enzymes can be activated by both immune and non-immune cells. COX-2 and iNOS are regulated mainly by inflammatory cells and have a role in inflammatory reactions and fibrosis in some organs like lung, neurovascular and gastrointestinal system. Treatments with antioxidants and anti-inflammatory agents have been shown to mitigate radiation toxicity via neutralization of free radicals and attenuation of these enzymes. Moreover, some other mechanisms including mTOR and PPARs which are activated by ionizing radiation mediate cell death mainly through senescence. This is very important for late effects of ionizing radiation such as fibrosis.
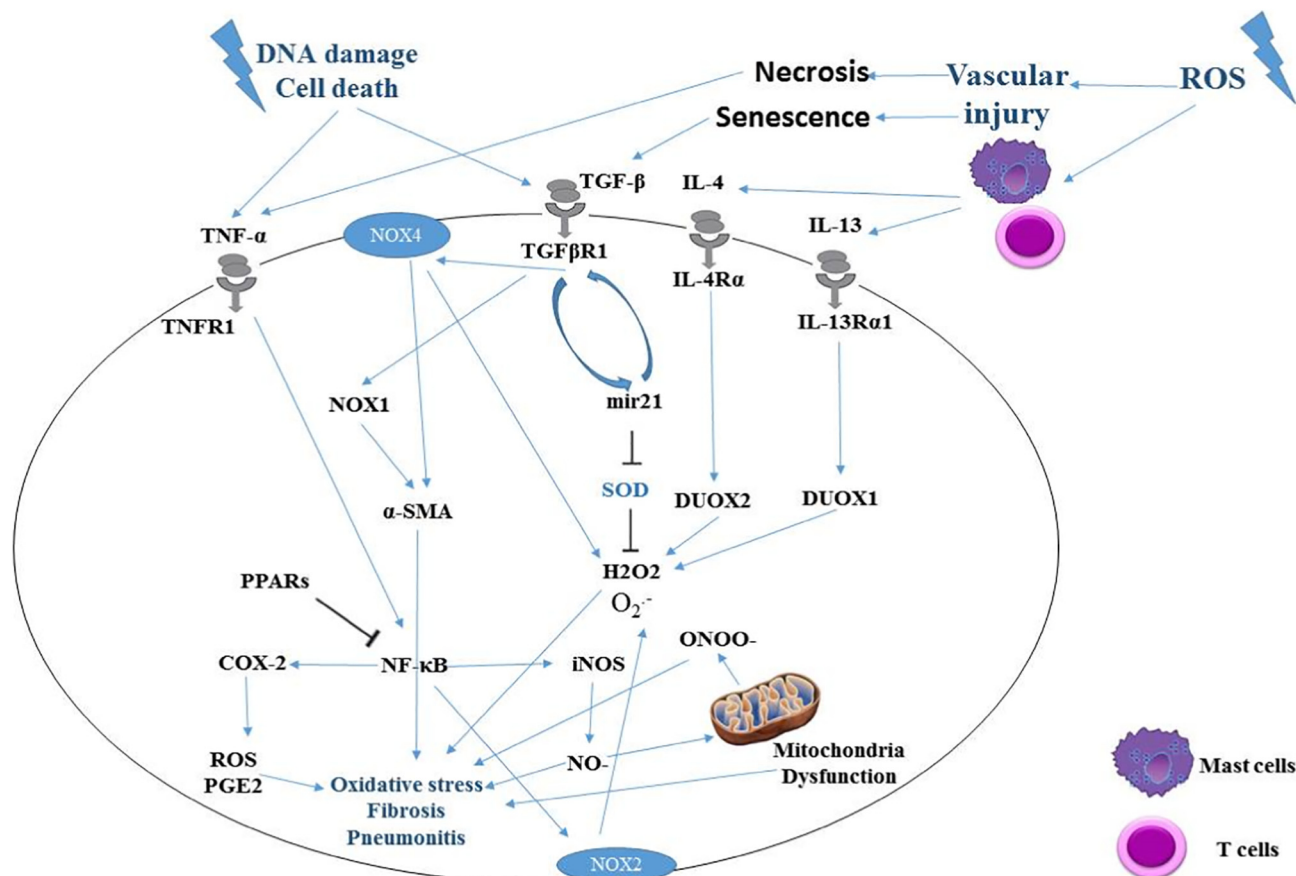
Fig. 2. Redox metabolisms following exposure to ionizing radiation.

Declaration of competing interest

None.

References

[1] I. Piri, M. Babayan, A. Tavassoli, M. Javaheri, The use of gamma irradiation in agriculture, Afr. J. Microbiol. Res. 5 (2011) 5806–5811.

[2] J.W. Jones, I.L. Jackson, Z. Vujaskovic, M.D. Kaytor, M.A. Kane, Targeted metabolomics identifies pharmacodynamic biomarkers for BIO 300 mitigation of radiation-induced lung injury, Pharm. Res. 34 (2017) 2698–2709.

[3] E. Cardis, M. Hatch, The Chernobyl accident—an epidemiological perspective, Clin. Oncol. 23 (2011) 251–260.

[4] M. Williams, D.C. Sizemore, Biologic, chemical, and radiation terrorism review, StatPearls [Internet], StatPearls Publishing, 2019.

[5] S.H. Kim, E.-T. Park, J.H. Kim, Study of police response to radiation accidents and terrorism, Journal of the Korean Society of Radiology 11 (2017) 646–653.

[6] M. Hirosaki, T. Ohira, S. Yasumura, M. Maeda, H. Yabe, M. Harigane, H. Takahashi, M. Murakami, Y. Suzuki, H. Nakano, Lifestyle factors and social ties associated with the frequency of laughter after the Great East Japan Earthquake: Fukushima Health Management Survey, Qual. Life Res. 27 (2018) 639–650.

[7] J.S. Parrish, G. Seda, Disasters resulting from radiologic and nuclear events, Crit. Care Clin. 35 (2019) 619–631.

[8] K.N. Mishra, B.A. Moftah, G.A. Alsbeih, Appraisal of mechanisms of radioprotection and therapeutic approaches of radiation countermeasures, Biomed. Pharmacother. 106 (2018) 610–617.

[9] N. Paraswani, A. Ghosh, M. Thoh, Cellular redox status mediates adaptive response to ionizing radiation in human peripheral blood mononuclear cells, Free Radic. Biol. Med. 108 (2017) S22.

[10] B. Farhood, K. Mortezaee, E. Motevaseli, H. Mirtavoos-Mahyari, D. Shabeeb, A. Eleojo Musa, N.S. Sanikhani, M. Najafi, A. Ahmadi, Selenium as an adjuvant for modification of radiation response, J. Cell. Biochem. 120 (2019) 18559–18571.

[11] K. Mortezaee, M. Najafi, B. Farhood, A. Ahmadi, D. Shabeeb, A. Eleojo Musa, Genomic Instability and Carcinogenesis of Heavy Charged Particles Radiation: Clinical and Environmental Implications, vol. 55, Medicina, Kaunas, Lithuania, 2019, p. 591.

[12] K. Datta, S. Suman, B.V. Kallakury, A.J. Fornace Jr., Exposure to heavy ion radiation induces persistent oxidative stress in mouse intestine, PLoS One 7 (2012) e42224.

[13] I.V. Mavragani, Z. Nikitaki, S.A. Kalospyros, A.G. Georgakilas, Ionizing radiation and complex DNA damage: from prediction to detection challenges and biological significance, Cancers 11 (2019) 1789.

[14] Z. Nikitaki, I.V. Mavragani, D.A. Laskaratou, V. Gika, V.P. Moskvin, K. Theofilatos, K. Vougas, R.D. Stewart, A.G. Georgakilas, Systemic mechanisms and effects of ionizing radiation: a new old paradigm of how the bystanders and distant can become the players, Semin. Cancer Biol. 37-38 (2016) 77–95.

[15] H. de Andrade Carvalho, R.C. Villar, Radiotherapy and immune response: the systemic effects of a local treatment, Clinics 73 (2018).

[16] T. Szatmári, D. Kis, E.N. Bogdándi, A. Benedek, S. Bright, D. Bowler, E. Persa, E. Kis, A. Balogh, L.N. Naszályi, Extracellular vesicles mediate radiation-induced systemic bystander signals in the bone marrow and spleen, Front. Immunol. 8 (2017) 347.

[17] B. Farhood, E. khodamoradi, M. Hoseini-Ghahfarokhi, E. Motevaseli, H. Mirtavoos-Mahyari, A. Eleojo Musa, M. Najafi, TGF-β in radiotherapy: mechanisms of tumor resistance and normal tissues injury, Pharmacol. Res. (2020) 104745.

[18] Y. Chai, R.K. Lam, G.M. Calaf, H. Zhou, S. Amundson, T.K. Hei, Radiation-induced non-targeted response in vivo: role of the TGFbeta-TGFBR1-COX-2 signalling pathway, Br. J. Cancer 108 (2013) 1106–1112.

[19] T.J. Wang, C.C. Wu, Y. Chai, R.K. Lam, N. Hamada, Y. Uchihori, P.K. Yu, T.K. Hei, Induction of non-targeted stress responses in mammary tissues by heavy ions, PLoS One 10 (2015) e0136307.

[20] Z. Fang, A. Xu, L. Wu, T.K. Hei, M. Hong, The role of protein kinase C alpha translocation in radiation-induced bystander effect, Sci. Rep. 6 (2016) 25817.

[21] M. Dadrich, N.H. Nicolay, P. Flechsig, S. Bickelhaupt, L. Hoeltgen, F. Roeder, K. Hauser, A. Tietz, J. Jenne, R. Lopez, M. Roehrich, U. Wirkner, M. Lahn, P.E. Huber, Combined inhibition of TGFβ and PDGF signaling attenuates radiation-induced pulmonary fibrosis, Oncoimmunology 5 (2016) e1123366.

[22] L. Liang, D. Hu, W. Liu, J.P. Williams, P. Okunieff, I. Ding, Celecoxib reduces skin damage after radiation: selective reduction of chemokine and receptor mRNA expression in irradiated skin but not in irradiated mammary tumor, Am. J. Clin. Oncol. 26 (2003) S114–S121.

[23] M.A. El-Ghazaly, A.S. Nada, R.M. El-Hazek, M.T. Khayyal, Effect of selective COX-2 inhibitor, celecoxib on adjuvant-induced arthritis model in irradiated rats, Int. J. Radiat. Biol. 86 (2010) 1079–1087.

[24] J.G. Kiang, J.T. Smith, N.G. Agravante, Geldanamycin analog 17-DMAG inhibits iNOS and caspases in gamma-irradiated human T cells, Radiat. Res. 172 (2009) 321–330.

[25] C.-W. Hong, Y.-M. Kim, H. Pyo, J.-H. Lee, S. Kim, S. Lee, J.M. Noh, Involvement of inducible nitric oxide synthase in radiation-induced vascular endothelial damage, J. Radiat. Res. 54 (2013) 1036–1042.

[26] R. Malaviya, A.J. Gow, M. Francis, E.V. Abramova, J.D. Laskin, D.L. Laskin, Radiation-induced lung injury and inflammation in mice: role of inducible nitric oxide synthase and surfactant protein D, Toxicol. Sci. 144 (2015) 27–38.

[27] S. Ghosh, D.K. Maurya, M. Krishna, Role of iNOS in bystander signaling between macrophages and lymphoma cells, Int. J. Radiat. Oncol. Biol. Phys. 72 (2008) 1567–1574.

[28] S. Ohta, S. Matsuda, M. Gunji, A. Kamogawa, The role of nitric oxide in radiation damage, Biol. Pharm. Bull. 30 (2007) 1102–1107.

[29] E.I. Azzam, J.-P. Jay-Gerin, D. Pain, Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury, Cancer Lett. 327 (2012) 48–60.

[30] B. Kura, A.K. Bagchi, P.K. Singal, M. Barancik, T.W. LeBaron, K. Valachova, L. Šoltés, J. Slezák, Molecular hydrogen: potential in mitigating oxidative-stress-induced radiation injury, Can. J. Physiol. Pharmacol. 97 (2019) 287–292.

[31] L. Diebold, N.S. Chandel, Mitochondrial ROS regulation of proliferating cells, Free Radic. Biol. Med. 100 (2016) 86–93.

[32] J.C. Huetsch, K. Suresh, L.A. Shimoda, Regulation of smooth muscle cell proliferation by NADPH oxidases in pulmonary hypertension, Antioxidants (Basel, Switzerland) 8 (2019) 56.

[33] N. Urao, M. Ushio-Fukai, Redox regulation of stem/progenitor cells and bone marrow niche, Free Radic. Biol. Med. 54 (2013) 26–39.

[34] Y. Wang, L. Liu, S.K. Pazhanisamy, H. Li, A. Meng, D. Zhou, Total body irradiation causes residual bone marrow injury by induction of persistent oxidative stress in murine hematopoietic stem cells, Free Radic. Biol. Med. 48 (2010) 348–356.

[35] L. Shao, Y. Luo, D. Zhou, Hematopoietic stem cell injury induced by ionizing radiation, Antioxid. Redox Signal. 20 (2014) 1447–1462.

[36] G. Xu, H. Wu, J. Zhang, D. Li, Y. Wang, Y. Wang, H. Zhang, L. Lu, C. Li, S. Huang, Metformin ameliorates ionizing irradiation-induced long-term hematopoietic stem cell injury in mice, Free Radic. Biol. Med. 87 (2015) 15–25.

[37] C.B. Zhao, L. Shi, H.H. Pu, Q.Y. Zhang, The promoting effect of radiation on glucose metabolism in breast cancer cells under the treatment of cobalt chloride, Pathol Oncol Res 23 (2017) 47–53.

[38] V. Purohit, D.M. Simeone, C.A. Lyssiotis, Metabolic regulation of redox balance in cancer, Cancers 11 (2019) 955.

[39] E. Chattopadhyay, B. Roy, Altered mitochondrial signalling and metabolism in cancer, Front. Oncol. 7 (2017).

[40] R.A. Saxton, D.M. Sabatini, mTOR signaling in growth, metabolism, and disease, Cell 168 (2017) 960–976.

[41] T. Tian, X. Li, J. Zhang, mTOR signaling in cancer and mTOR inhibitors in solid tumor targeting therapy, Int. J. Mol. Sci. 20 (2019) 755.

[42] S. Tavakol, M. Ashrafizadeh, S. Deng, M. Azarian, A. Abdoli, M. Motavaf, D. Poormoghadam, H. Khanbabaei, E. Ghasemipour Afshar, A. Mandegary, Autophagy modulators: mechanistic aspects and drug delivery systems, Biomolecules 9 (2019) 530.

[43] L. Shao, W. Yang, R. Xu, S. Zhu, Y. Huang, H. Li, X. Wu, M. Yue, X. Xiong, X. Chen, B. Kuang, G. Fan, Q. Zhu, H. Zeng, Inhibition of mTORC1 signaling protects kidney from irradiation-induced toxicity via accelerating recovery of renal stem-like cells, Stem Cell Res Ther 9 (2018) 219.

[44] M. Ashrafizadeh, Z. Ahmadi, N.G. Kotla, E.G. Afshar, S. Samarghandian, A. Mandegary, A. Pardakhty, R. Mohammadinejad, G. Sethi, Nanoparticles targeting STATs in cancer therapy, Cells 8 (2019) 1158.

[45] M. Morgan-Bathke, Z.I. Harris, D.G. Arnett, R.R. Klein, R. Burd, D.K. Ann, K.H. Limesand, The Rapalogue, CCI-779, improves salivary gland function following radiation, PLoS One 9 (2014) e113183.

[46] R. Iglesias-Bartolome, V. Patel, A. Cotrim, K. Leelahavanichkul, A.A. Molinolo, J.B. Mitchell, J.S. Gutkind, mTOR inhibition prevents epithelial stem cell senescence and protects from radiation-induced mucositis, Cell Stem Cell 11 (2012) 401–414.

[47] Y. He, D. Thummuri, G. Zheng, P. Okunieff, D.E. Citrin, Z. Vujaskovic, D. Zhou, Cellular senescence and radiation-induced pulmonary fibrosis, Transl. Res. 209 (2019) 14–21.

[48] Y.-h. Dai, X.-q. Li, D.-p. Dong, H.-b. Gu, C.-y. Kong, Z. Xu, P27 promotes TGF-β-mediated pulmonary fibrosis via interacting with MTORC2, Can. Respir. J. 2019 (2019) 9.

[49] E.J. Chung, A. Sowers, A. Thetford, G. McKay-Corkum, S.I. Chung, J.B. Mitchell, D.E. Citrin, Mammalian target of rapamycin inhibition with rapamycin mitigates radiation-induced pulmonary fibrosis in a murine model, Int. J. Radiat. Oncol. Biol. Phys. 96 (2016) 857–866.

[50] J. Auwerx, K. Schoonjans, J.C. Fruchart, B. Staels, Regulation of triglyceride metabolism by PPARs: fibrates and thiazolidinediones have distinct effects, J. Atheroscler. Thromb. 3 (1996) 81–89.

[51] A unified nomenclature system for the nuclear receptor superfamily, Cell 97 (1999) 161–163.

[52] M.B. Debril, J.P. Renaud, L. Fajas, J. Auwerx, The pleiotropic functions of peroxisome proliferator-activated receptor gamma, J Mol Med (Berl) 79 (2001) 30–47.

[53] P. Delerive, P. Gervois, J.-C. Fruchart, B. Staels, Induction of IκBα expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor-α activators, J. Biol. Chem. 275 (2000) 36703–36707.

[54] W. Zhao, S. Iskandar, M. Kooshki, J.G. Sharpe, V. Payne, M.E. Robbins, Knocking out peroxisome proliferator-activated receptor (PPAR) α inhibits radiation-induced apoptosis in the mouse kidney through activation of NF-κB and increased expression of IAPs, Radiat. Res. 167 (2007) 581–591.

[55] V. Subramanian, I. Seemann, J. Merl-Pham, S.M. Hauck, F.A. Stewart, M.J. Atkinson, S. Tapio, O. Azimzadeh, Role of TGF beta and PPAR alpha signaling pathways in radiation response of locally exposed heart: integrated global transcriptomics and proteomics analysis, J. Proteome Res. 16 (2016) 307–318.

[56] S.A. Nuwormegbe, J.H. Sohn, S.W. Kim, A PPAR-gamma agonist rosiglitazone suppresses fibrotic response in human pterygium fibroblasts by modulating the p38 MAPK pathway, Invest. Ophthalmol. Vis. Sci. 58 (2017) 5217–5226.

[57] C.I. Schnegg, M. Kooshki, F.C. Hsu, G. Sui, M.E. Robbins, PPARdelta prevents radiation-induced proinflammatory responses in microglia via transrepression of NF-kappaB and inhibition of the PKCalpha/MEK1/2/ERK1/2/AP-1 pathway, Free Radic. Biol. Med. 52 (2012) 1734–1743.

[58] S. Ramanan, M. Kooshki, W. Zhao, F.C. Hsu, M.E. Robbins, PPARalpha ligands inhibit radiation-induced microglial inflammatory responses by negatively regulating NF-kappaB and AP-1 pathways, Free Radic. Biol. Med. 45 (2008) 1695–1704.

[59] S. Ramanan, M. Kooshki, W. Zhao, F.C. Hsu, D.R. Riddle, M.E. Robbins, The PPARalpha agonist fenofibrate preserves hippocampal neurogenesis and inhibits microglial activation after whole-brain irradiation, Int. J. Radiat. Oncol. Biol. Phys. 75 (2009) 870–877.

[60] M. Mangoni, M. Sottili, C. Gerini, I. Desideri, C. Bastida, S. Pallotta, F. Castiglione, P. Bonomo, I. Meattini, D. Greto, S. Cappelli, L. Di Brina, M. Loi, G. Biti, L. Livi, A PPAR-gamma agonist protects from radiation-induced intestinal toxicity, United European Gastroenterol J 5 (2017) 218–226.

[61] M. Mangoni, M. Sottili, C. Gerini, P. Bonomo, A. Bottoncetti, F. Castiglione, C. Franzese, S. Cassani, D. Greto, T. Masoni, I. Meattini, S. Pallotta, A. Passeri, A. Pupi, E. Vanzi, G. Biti, L. Livi, A PPAR-gamma agonist attenuates pulmonary injury induced by irradiation in a murine model, Lung Cancer 90 (2015) 405–409.

[62] M. Mangoni, M. Sottili, C. Gerini, I. Desideri, C. Bastida, S. Pallotta, F. Castiglione, P. Bonomo, I. Meattini, D. Greto, E. Olmetto, F. Terziani, C. Becherini, C. Delli Paoli, L. Trombetta, M. Loi, G. Biti, L. Livi, A PPAR gamma agonist protects against oral mucositis induced by irradiation in a murine model, Oral Oncol. 64 (2017) 52–58.

[63] S.H. Choi, M. Kim, H.J. Lee, E.H. Kim, C.H. Kim, Y.J. Lee, Effects of NOX1 on fibroblastic changes of endothelial cells in radiationinduced pulmonary fibrosis, Mol. Med. Rep. 13 (2016) 4135–4142.

[64] Y. Tateishi, E. Sasabe, E. Ueta, T. Yamamoto, Ionizing irradiation induces apoptotic damage of salivary gland acinar cells via NADPH oxidase 1-dependent superoxide generation, Biochem. Biophys. Res. Commun. 366 (2008) 301–307.

[65] U. Weyemi, C.E. Redon, T. Aziz, R. Choudhuri, D. Maeda, P.R. Parekh, M.Y. Bonner, J.L. Arbiser, W.M. Bonner, Inactivation of NADPH oxidases NOX4 and NOX5 protects human primary fibroblasts from ionizing radiation-induced DNA damage, Radiat. Res. 183 (2015) 262–270.

[66] I. Szanto, M. Pusztaszeri, M. Mavromati, H(2)O(2) metabolism in normal thyroid cells and in thyroid tumorigenesis: focus on NADPH oxidases, Antioxidants (Basel, Switzerland) 8 (2019) 126.

[67] D.P. Carvalho, C. Dupuy, Role of the NADPH oxidases DUOX and NOX4 in thyroid oxidative stress, European Thyroid Journal 2 (2013) 160–167.

[68] R. Ameziane-El-Hassani, M. Talbot, M.C. de Souza Dos Santos, A. Al Ghuzlan, D. Hartl, J.M. Bidart, X. De Deken, F. Miot, I. Diallo, F. de Vathaire, M. Schlumberger, C. Dupuy, NADPH oxidase DUOX1 promotes long-term persistence of oxidative stress after an exposure to irradiation, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 5051–5056.

[69] J.A. Smeitink, M. Zeviani, D.M. Turnbull, H.T. Jacobs, Mitochondrial medicine: a metabolic perspective on the pathology of oxidative phosphorylation disorders, Cell Metab. 3 (2006) 9–13.

[70] L.V. Yuzefovych, S.I. Musiyenko, G.L. Wilson, L.I. Rachek, Mitochondrial DNA damage and dysfunction, and oxidative stress are associated with endoplasmic reticulum stress, protein degradation and apoptosis in high fat diet-induced insulin resistance mice, PLoS One 8 (2013) e54059.

[71] S. Prithivirajsingh, M.D. Story, S.A. Bergh, F.B. Geara, K. Kian Ang, S.M. Ismail, C.W. Stevens, T.A. Buchholz, W.A. Brock, Accumulation of the common mitochondrial DNA deletion induced by ionizing radiation, FEBS Lett. 571 (2004) 227–232.

[72] W.W. Kam, R.B. Banati, Effects of ionizing radiation on mitochondria, Free Radic. Biol. Med. 65 (2013) 607–619.

[73] L. Qin, M. Fan, D. Candas, G. Jiang, S. Papadopoulos, L. Tian, G. Woloschak, J. Grdina David, J. Li Jian, CDK1 enhances mitochondrial bioenergetics for radiation-induced DNA repair, Cell Rep. 13 (2015) 2056–2063.

[74] B.P. Tseng, E. Giedzinski, A. Izadi, T. Suarez, M.L. Lan, K.K. Tran, M.M. Acharya, G.A. Nelson, J. Raber, V.K. Parihar, C.L. Limoli, Functional consequences of radiation-induced oxidative stress in cultured neural stem cells and the brain exposed to charged particle irradiation, Antioxid. Redox Signal. 20 (2014) 1410–1422.

[75] C. Mothersill, T.D. Stamato, M.L. Perez, R. Cummins, R. Mooney, C.B. Seymour, Involvement of energy metabolism in the production of 'bystander effects' by radiation, Br. J. Cancer 82 (2000) 1740–1746.

[76] A. Lafargue, C. Degorre, I. Corre, M.-C. Alves-Guerra, M.-H. Gaugler, F. Vallette, C. Pecqueur, F. Paris, Ionizing radiation induces long-term senescence in endothelial cells through mitochondrial respiratory complex II dysfunction and superoxide generation, Free Radic. Biol. Med. 108 (2017) 750–759.

[77] S. Rajendran, S.H. Harrison, R.A. Thomas, J.D. Tucker, The role of mitochondria in the radiation-induced bystander effect in human lymphoblastoid cells, Radiat. Res. 175 (2010) 159–171.

[78] M. Najafi, R. Fardid, G. Hadadi, M. Fardid, The mechanisms of radiation-induced bystander effect, Journal of Biomedical Physics & Engineering 4 (2014) 163–172.

[79] K. Ariyoshi, T. Miura, K. Kasai, Y. Fujishima, A. Nakata, M. Yoshida, Radiation-induced bystander effect is mediated by mitochondrial DNA in exosome-like vesicles, Sci. Rep. 9 (2019) 9103.

[80] T. Shimura, M. Koyama, D. Aono, N. Kunugita, Epicatechin as a promising agent to countermeasure radiation exposure by mitigating mitochondrial damage in human fibroblasts and mouse hematopoietic cells, FASEB J. 33 (2019) 6867–6876.

[81] Y. Zhang, J. Wang, Y. Li, F. Wang, F. Yang, W. Xu, Synthesis and radioprotective activity of mitochondria targeted dihydropyridines in vitro, Int. J. Mol. Sci. 18 (2017) 2233.

[82] J. Atkinson, A.A. Kapralov, N. Yanamala, Y.Y. Tyurina, A.A. Amoscato, L. Pearce, J. Peterson, Z. Huang, J. Jiang, A.K. Samhan-Arias, A. Maeda, W. Feng, K. Wasserloos, N.A. Belikova, V.A. Tyurin, H. Wang, J. Fletcher, Y. Wang, I.I. Vlasova, J. Klein-Seetharaman, D.A. Stoyanovsky, H. Bayîr, B.R. Pitt, M.W. Epperly, J.S. Greenberger, V.E. Kagan, A mitochondria-targeted inhibitor of cytochrome c peroxidase mitigates radiation-induced death, Nat. Commun. 2 (2011) 497.

[83] J.H. Lee, S.Y. Kim, I.S. Kil, J.-W. Park, Regulation of ionizing radiation-induced apoptosis by mitochondrial NADP + -dependent isocitrate dehydrogenase, J. Biol. Chem. 282 (2007) 13385–13394.

[84] M.W. Epperly, J.G. Pierce, T. Dixon, D. Franicola, P. Wipf, J.S. Greenberger, The mitochondrial targeted GS-nitroxide JP4-039 is radioprotective in vitro and in vivo, International Journal of Radiation Oncology, Biology, Physics 72 (2008) S82.

[85] M. Ashrafizadeh, H. Yaribeygi, S.L. Atin, A. Sahebkar, Effects of newly introduced antidiabetic drugs on autophagy, Diabetes & Metabolic Syndrome: Clinical Research & Reviews 13 (2019) 2445–2449.

[86] M. Cheki, A. Shirazi, A. Mahmoudzadeh, J.T. Bazzaz, S.J. Hosseinimehr, The radioprotective effect of metformin against cytotoxicity and genotoxicity induced by ionizing radiation in cultured human blood lymphocytes, Mutation Research/Genetic Toxicology and Environmental Mutagenesis 809 (2016) 24–32.

[87] M. Najafi, M. Cheki, G. Hassanzadeh, P. Amini, D. Shabeeb, A.E. Musa, The radioprotective effect of combination of melatonin and metformin on rat duodenum damage induced by ionizing radiation: a histological study, Adv Biomed Res 8 (2019) 51.

[88] M. Najafi, M. Cheki, G. Hassanzadeh, P. Amini, D. Shabeeb, A. Eleojo Musa, Protection from radiation-induced damage in rat's ileum and colon by combined regimens of melatonin and metformin: a histopathological study, Antiinflamm Antiallergy Agents Med Chem (2019).

[89] R. Azmoonfar, P. Amini, H. Saffar, S. Rezapoor, E. Motevaseli, M. Cheki, R. Yahyapour, B. Farhood, F. Nouruzi, E. Khodamoradi, D. Shabeeb, A. Eleojo Musa, M. Najafi, Metformin protects against radiation-induced pneumonitis and fibrosis and attenuates upregulation of dual oxidase genes expression, Adv Pharm Bull 8 (2018) 697–704.

[90] R. Yahyapour, P. Amini, H. Saffar, S. Rezapoor, E. Motevaseli, M. Cheki, B. Farhood, F. Nouruzi, D. Shabeeb, A. Eleojo Musa, M. Najafi, Metformin protects against radiation-induced heart injury and attenuates the upregulation of dual oxidase genes following rat's chest irradiation, Int J Mol Cell Med 7 (2018) 193–202.

[91] C. Xiong, D. Yin, J. Li, Q. Huang, M.K. Ravoori, V. Kundra, H. Zhu, Z. Yang, Y. Lu, C. Li, Metformin reduces renal uptake of radiotracers and protects kidneys from radiation-induced damage, Mol. Pharm. 16 (2019) 808–815.

[92] B. Farhood, A. Aliasgharzadeh, P. Amini, A. Rezaeyan, A. Tavassoli, E. Motevaseli, D. Shabeeb, A.E. Musa, M. Najafi, Mitigation of radiation-induced lung pneumonitis and fibrosis using metformin and melatonin: a histopathological study, Medicina (Kaunas) 55 (2019).

[93] J. Wang, Y. Wang, J. Han, H. Mei, D. Yu, Q. Ding, T. Zhang, G. Wu, G. Peng, Z. Lin, Metformin attenuates radiation-induced pulmonary fibrosis in a murine model, Radiat. Res. 188 (2017) 105–113.

[94] R.C. Miller, J.S. Murley, D.J. Grdina, Metformin exhibits radiation countermeasures efficacy when used alone or in combination with sulfhydryl containing drugs, Radiat. Res. 181 (2014) 464–470.

[95] A. Bikas, D. Van Nostrand, K. Jensen, S. Desale, M. Mete, A. Patel, L. Wartofsky, V. Vasko, K.D. Burman, Metformin attenuates 131I-induced decrease in peripheral blood cells in patients with differentiated thyroid cancer, Thyroid: Official Journal of the American Thyroid Association 26 (2016) 280–286.

[96] M. Ashrafizadeh, R. Mohammadinejad, S. Tavakol, Z. Ahmadi, S. Roomiani, M. Katebi, Autophagy, anoikis, ferroptosis, necroptosis, and endoplasmic reticulum stress: potential applications in melanoma therapy, J. Cell. Physiol. 234 (2019) 19471–19479.

[97] M.W. Epperly, J.E. Gretton, C.A. Sikora, M. Jefferson, M. Bernarding, S. Nie, J.S. Greenberger, Mitochondrial localization of superoxide dismutase is required for decreasing radiation-induced cellular damage, Radiat. Res. 160 (2003) 568–578.

[98] X. Zhang, M.W. Epperly, M.A. Kay, Z.Y. Chen, T. Dixon, D. Franicola, B.A. Greenberger, P. Komanduri, J.S. Greenberger, Radioprotection in vitro and in vivo by minicircle plasmid carrying the human manganese superoxide dismutase transgene, Hum. Gene Ther. 19 (2008) 820–826.

[99] M.W. Epperly, M. Carpenter, A. Agarwal, P. Mitra, S. Nie, J.S. Greenberger, Intraoral manganese superoxide dismutase-plasmid/liposome (MnSOD-PL) radioprotective gene therapy decreases ionizing irradiation-induced murine mucosal cell cycling and apoptosis, In Vivo 18 (2004) 401–410.

[100] M.W. Epperly, J.A. Bray, P. Esocobar, W.L. Bigbee, S. Watkins, J.S. Greenberger, Overexpression of the human manganese superoxide dismutase (MnSOD) transgene in subclones of murine hematopoietic progenitor cell line 32D cl 3 decreases irradiation-induced apoptosis but does not alter G2/M or G1/S phase cell cycle arrest, Radiat. Oncol. Investig. 7 (1999) 331–342.

[101] M.W. Epperly, J.R. Sacher, T. Krainz, X. Zhang, P. Wipf, M. Liang, R. Fisher, S. Li, H. Wang, J.S. Greenberger, Effectiveness of analogs of the GS-nitroxide, JP4-039, as total body irradiation mitigators, In Vivo 31 (2017) 39–43.

[102] M.W. Epperly, T.M. Dixon, S. He, D. Shields, P. Wipf, S. Li, J.S. Greenberger, Combination mitigators, GS-nitroxide JP4-039 and water soluble oxetanyl sulfoxide MMS350 improve survival of lethally irradiated mice, Blood 124 (2014) 2751.

[103] M.W. Epperly, J.G. Pierce, T. Dixon, D. Franicola, S. Nie, X. Zhang, I.I. Vlasova, P. Wipf, V. Kagan, J. Greenberger, A mitochondrially targeted nitroxide JP4-039

protects and mitigates against total body irradiation induced hematopoietic syndrome, Blood 112 (2008) 4721.

[104] K. Gupta, M.W. Epperly, D. Franicola, X. Zhang, J.G. Pierce, P. Wipf, J. Greenberger, Mitigation of irradiation induced potentially lethal damage (PLD) in hematopoietic cells by mitochondrial localized GS-nitroxide, JP4-039, Blood 112 (2008) 4725.

[105] J.P. Goff, M.W. Epperly, T. Dixon, H. Wang, D. Franicola, D. Shields, P. Wipf, S. Li, X. Gao, J.S. Greenberger, Radiobiologic effects of GS-nitroxide (JP4-039) on the hematopoietic syndrome, In Vivo 25 (2011) 315–323.

[106] M.S. Rajagopalan, K. Gupta, M.W. Epperly, D. Franicola, X. Zhang, H. Wang, H. Zhao, V.A. Tyurin, J.G. Pierce, V.E. Kagan, P. Wipf, A.J. Kanai, J.S. Greenberger, The mitochondria-targeted nitroxide JP4-039 augments potentially lethal irradiation damage repair, In Vivo 23 (2009) 717–726.

[107] L. Wei, B.J. Leibowitz, M. Epperly, C. Bi, A. Li, J. Steinman, P. Wipf, S. Li, L. Zhang, J. Greenberger, J. Yu, The GS-nitroxide JP4-039 improves intestinal barrier and stem cell recovery in irradiated mice, Sci. Rep. 8 (2018) 2072.

[108] M.W. Epperly, A. Shinde, H. Berhane, B.H. Rhieu, R. Kalash, K. Xu, D. Franicola, X. Zhang, T. Dixon, D. Shields, H. Wang, P. Wipf, K. Parmar, E. Guinan, V. Kagan, Y. Tyurina, R.L. Ferris, S. Li, J.S. Greenberger, Abstract 3340: intraoral administration of mitochondrial targeted GS-nitroxide (JP4-039) radioprotects the oral mucosa but not orthotopic tumors in Fancd2−/− mice, Cancer Res. 75 (2015) 3340.

[109] J. Willis, M.W. Epperly, R. Fisher, X. Zhang, D. Shields, W. Hou, H. Wang, S. Li, P. Wipf, K. Parmar, E. Guinan, J. Steinman, J.S. Greenberger, Amelioration of head and neck radiation-induced mucositis and distant marrow suppression in Fanca (−/−) and Fancg(−/−) mice by intraoral administration of GS-nitroxide (JP4-039), Radiat. Res. 189 (2018) 560–578.

[110] H. Kim, M.E. Bernard, M.W. Epperly, H. Shen, A. Amoscato, T.M. Dixon, A.S. Doemling, S. Li, X. Gao, P. Wipf, H. Wang, X. Zhang, V.E. Kagan, J.S. Greenberger, Amelioration of radiation esophagitis by orally administered p53/Mdm2/Mdm4 inhibitor (BEB55) or GS-nitroxide, In Vivo 25 (2011) 841–848.

[111] J.C. Rwigema, B. Beck, W. Wang, A. Doemling, M.W. Epperly, D. Shields, J.P. Goff, D. Franicola, T. Dixon, M.C. Frantz, P. Wipf, Y. Tyurina, V.E. Kagan, H. Wang, J.S. Greenberger, Two strategies for the development of mitochondrion-targeted small molecule radiation damage mitigators, Int. J. Radiat. Oncol. Biol. Phys. 80 (2011) 860–868.

[112] M.W. Epperly, P. Wipf, R. Fisher, D. Franicola, J. Beumer, S. Li, R.M. Brand, L.D. Falo Jr., G. Erdos, J.S. Greenberger, Evaluation of different formulations and routes for the delivery of the ionizing radiation mitigator GS-nitroxide (JP4-039), In Vivo 32 (2018) 1009–1023.

[113] D. Stoyanovsky, J. Jiang, M. Murphy, M. Epperly, S. Li, J.S. Greenberger, V. Kagan, H. Bayir, Mitigation of irradiation damage in vitro and in vivo by mitochondrial targeted glutathione peroxidase 4 mimic mito-ebselen, International Journal of Radiation Oncology, Biology, Physics 93 (2015) E541.

[114] J.B. Mitchell, M.R. Anver, A.L. Sowers, P.S. Rosenberg, M. Figueroa, A. Thetford, M.C. Krishna, P.S. Albert, J.A. Cook, The antioxidant tempol reduces carcinogenesis and enhances survival in mice when administered after nonlethal total body radiation, Cancer Res. 72 (2012) 4846–4855.

[115] J. Liebmann, A.M. DeLuca, A. Epstein, S.M. Steinberg, G. Morstyn, J.B. Mitchell, Protection from lethal irradiation by the combination of stem cell factor and tempol, Radiat. Res. 137 (1994) 400–404.

[116] S.M. Hahn, M.C. Krishna, A.M. DeLuca, D. Coffin, J.B. Mitchell, Evaluation of the hydroxylamine Tempol-H as an in vivo radioprotector, Free Radic. Biol. Med. 28 (2000) 953–958.

[117] B. Gauter-Fleckenstein, K. Fleckenstein, K. Owzar, C. Jiang, I. Batinic-Haberle, Z. Vujaskovic, Comparison of two Mn porphyrin-based mimics of superoxide dismutase in pulmonary radioprotection, Free Radic. Biol. Med. 44 (2008) 982–989.

[118] B. Gauter-Fleckenstein, J.S. Reboucas, K. Fleckenstein, A. Tovmasyan, K. Owzar, C. Jiang, I. Batinic-Haberle, Z. Vujaskovic, Robust rat pulmonary radioprotection by a lipophilic Mn N-alkylpyridylporphyrin, MnTnHex-2-PyP(5+), Redox Biol. 2 (2014) 400–410.

[119] B. Gauter-Fleckenstein, K. Fleckenstein, K. Owzar, C. Jiang, J.S. Reboucas, I. Batinic-Haberle, Z. Vujaskovic, Early and late administration of MnTE-2-PyP5+ in mitigation and treatment of radiation-induced lung damage, Free Radic. Biol.

[120] B.U. Islam, S. Habib, P. Ahmad, S. Allarakha, Ali A. Moinuddin, Pathophysiological role of peroxynitrite induced DNA damage in human diseases: a special focus on poly(ADP-ribose) polymerase (PARP), Indian Journal of Clinical Biochemistry: IJCB 30 (2015) 368–385.

[121] R. Wang, C. Li, P. Qiao, Y. Xue, X. Zheng, H. Chen, X. Zeng, W. Liu, I. Boldogh, X. Ba, OGG1-initiated base excision repair exacerbates oxidative stress-induced parthanatos, Cell Death Dis. 9 (2018) 628.

[122] J. Liebmann, A.M. DeLuca, D. Coffin, L.K. Keefer, D. Venzon, D.A. Wink, J.B. Mitchell, In vivo radiation protection by nitric oxide modulation, Cancer Res. 54 (1994) 3365–3368.

[123] N.V. Gorbunov, K.L. Pogue-Geile, M.W. Epperly, W.L. Bigbee, R. Draviam, B.W. Day, N. Wald, S.C. Watkins, J.S. Greenberger, Activation of the nitric oxide synthase 2 pathway in the response of bone marrow stromal cells to high doses of ionizing radiation, Radiat. Res. 154 (2000) 73–86.

[124] Y. Erbil, C. Dibekoglu, U. Turkoglu, E. Ademoglu, E. Berber, A. Kizir, S. Mercan, G. Toker, Nitric oxide and radiation enteritis, The European journal of surgery 164 (1998) 863–868.

[125] M.W. Epperly, S. Cao, X. Zhang, D. Franicola, H. Shen, E.E. Greenberger, L.D. Epperly, J.S. Greenberger, Increased longevity of hematopoiesis in continuous bone marrow cultures derived from NOS1 (nNOS, mtNOS) homozygous recombinant negative mice correlates with radioresistance of hematopoietic and marrow stromal cells, Exp. Hematol. 35 (2007) 137–145.

[126] Y. Nozaki, Y. Hasegawa, A. Takeuchi, Z. Fan, K. Isobe, I. Nakashima, K. Shimokata, Nitric oxide as an inflammatory mediator of radiation pneumonitis in rats, Am. J. Phys. Lung Cell. Mol. Phys. 272 (1997) L651–L658.

[127] A.I. Soloviev, S.M. Tishkin, A.V. Parshikov, I.V. Ivanova, E.V. Goncharov, A.M. Gurney, Mechanisms of endothelial dysfunction after ionized radiation: selective impairment of the nitric oxide component of endothelium-dependent vasodilation, Br. J. Pharmacol. 138 (2003) 837–844.

[128] S.K. Choudhari, M. Chaudhary, S. Bagde, A.R. Gadbail, V. Joshi, Nitric oxide and cancer: a review, World Journal of Surgical Oncology 11 (2013) 118.

[129] J.S. Greenberger, D. Clump, V. Kagan, H. Bayir, J.S. Lazo, P. Wipf, S. Li, X. Gao, M.W Epperly, Strategies for discovery of small molecule radiation protectors and radiation mitigators, Front. Oncol. 1 (2012) 59.

[130] M. Ashrafizadeh, Z. Ahmadi, R. Mohammadinejad, T. Farkhondeh, S. Samarghandian, MicroRNAs mediate the anti-tumor and protective effects of ginsenosides, Nutr. Cancer (2019) 1–12.

[131] Y. Jiang, X. Chen, W. Tian, X. Yin, J. Wang, H. Yang, The role of TGF-β1−miR-21−ROS pathway in bystander responses induced by irradiated non-small-cell lung cancer cells, Br. J. Cancer 111 (2014) 772.

[132] X. Yin, W. Tian, L. Wang, J. Wang, S. Zhang, J. Cao, H. Yang, Radiation quality-dependence of bystander effect in unirradiated fibroblasts is associated with TGF-β1-Smad2 pathway and miR-21 in irradiated keratinocytes, Sci. Rep. 5 (2015) 11373.

[133] W. Tian, X. Yin, L. Wang, J. Wang, W. Zhu, J. Cao, H. Yang, The key role of miR-21-regulated SOD2 in the medium-mediated bystander responses in human fibroblasts induced by α-irradiated keratinocytes, Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 780 (2015) 77–85.

[134] S. Xu, N. Ding, H. Pei, W. Hu, W. Wei, X. Zhang, G. Zhou, J. Wang, MiR-21 is involved in radiation-induced bystander effects, RNA Biol. 11 (2014) 1161–1170.

[135] Z. Liu, T. Li, Fu S. Deng Sn, X. Zhou, Y. He, Radiation induces apoptosis and osteogenic impairment through miR-22-mediated intracellular oxidative stress in bone marrow mesenchymal stem cells, Stem Cells Int. 2018 (2018).

[136] Z. Liu, T. Li, F. Zhu, Sn Deng, X. Li, Y. He, Regulatory roles of miR-22/Redd1-mediated mitochondrial ROS and cellular autophagy in ionizing radiation-induced BMSC injury, Cell Death Dis. 10 (2019) 227.

[137] S. Zhang, W. Wang, Q. Gu, J. Xue, H. Cao, Y. Tang, X. Xu, J. Cao, J. Zhou, J. Wu, W.-Q. Ding, Protein and miRNA profiling of radiation-induced skin injury in rats: the protective role of peroxiredoxin-6 against ionizing radiation, Free Radic. Biol. Med. 69 (2014) 96–107.

[138] M. Najafi, A. Shirazi, E. Motevaseli, G. Geraily, P. Amini, L.F. Tooli, D. Shabeeb, Melatonin modulates regulation of NOX2 and NOX4 following irradiation in the lung, Curr. Clin. Pharmacol. 14 (2019) 224–231.