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REVIEW ARTICLE

Reduction–oxidation (redox) system in radiation-induced normal tissue injury: molecular mechanisms and implications in radiation therapeutics

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Abstract

Every year, millions of cancer patients undergo radiation therapy for treating and destroying abnormal cell growths within normal cell environmental conditions. Thus, ionizing radiation can have positive therapeutic effects on cancer cells as well as post-detrimental effects on surrounding normal tissues. Previous studies in the past years have proposed that the reduction and oxidation metabolism in cells changes in response to ionizing radiation and has a key role in radiation toxicity to normal tissue. Free radicals generated from ionizing radiation result in upregulation of cyclooxygenases (COXs), nitric oxide synthase (NOSs), lipoxygenases (LOXs) as well as nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), and their effected changes in mitochondrial functions are markedly noticeable. Each of these enzymes is diversely expressed in multiple cells, tissues and organs in a specific manner. Overproduction of reactive oxygen radicals (ROS), reactive hydroxyl radical (ROH) and reactive nitrogen radicals (RNS) in multiple cellular environments in the affected nucleus, cell membranes, cytosol and mitochondria, and other organelles, can specifically affect the sensitive and modifying enzymes of the redox system and repair proteins that play a pivotal role in both early and late effects of radiation. In recent years, ionizing radiation has been known to affect the redox functions and metabolism of NADPH oxidases (NOXs) as well as having destabilizing and detrimental effects on directly and indirectly affected cells, tissues and organs. More noteworthy, chronic free radical production may continue for years, increasing the risk of carcinogenesis and other oxidative stress-driven degenerative diseases as well as pathologies, in addition to late effect complications of organ fibrosis. Hence, knowledge about the mechanisms of chronic oxidative damage and injury in affected cells, tissues and organs following exposure to

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ionizing radiation may help in the development of treatment and management strategies of complications associated with radiotherapy (RT) or radiation accident victims. Thus, this medically relevant phenomenon may lead to the discovery of potential antioxidants and inhibitors with promising results in targeting and modulating the ROS/NO-sensitive enzymes in irradiated tissues and organ injury systems.

Keywords Radiation · Redox · Normal tissue injury · Inflammation · NADPH oxidase

Introduction

Annually, more than 12 million new cases of cancer are reported worldwide [1]. Approximately, more than half of these patients require radiation therapy (RT) either alone or in combination with other modalities such as surgery, immunotherapy, hyperthermia, chemotherapy and hormone therapy. In addition to clinical applications, several people are exposed to lethal or sub-lethal doses of ionizing radiation resulting from radiation accident or terrorist activities [2, 3]. So far, several studies have been conducted to understand the mechanisms of minimizing the detrimental effects of exposure to ionizing radiation (IR) on normal tissues.

Exposing cells to IR causes immediate free radical formation with a nanoseconds half-life. For many years, it has been believed that these free radicals, as well as direct radiation interaction with DNA, are responsible for the side effects of exposure to IR. However, the discovery of new phenomena in radiobiology such as radiation-induced bystander effect, non-targeted effect and genomic instability, have challenged this central dogma [4]. In recent years, studies have demonstrated that changes in the normal functions of the reduction/oxidation (redox) systems are involved in several damages following exposure to IR [5]. Free radical production by the redox system begins some few hours after exposure and may continue for several years [6, 7]. The redox system plays a key role in acute radiation syndrome. It is also responsible for several early and late effects of exposure to IR such as bystander effect, out-of-field effect, inflammation, fibrosis and others [8].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the main sources of damage to normal tissues after exposure to IR. During normal cell function, ROS and RNS are essential mediators for several cellular processes such as immune responses, cell signaling, microbial defense, differentiation, cell adhesion, apoptosis and others [9]. Antioxidant systems include enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) as well as peptides such as glutathione (GSH). They neutralize additional free radicals and protect cells against the detrimental effects. Exposure to IR causes excessive production of free radicals over the antioxidant system potency, resulting in oxidative damage to DNA, proteins and lipids. These effects cause damages to normal cell function and may lead to genomic instability that increases the risk of malignancies [10].

Redox system biology and enzymology, redox chemistry and ionizing radiation interactions

Free radicals including ROS, ROH and RNS are recognized as dual role players which can have both deleterious and beneficial consequences. Free radicals formed via redox system biology can act as messengers in cell signaling and changes in gene expression patterns. For example, several types of protein kinases and transcription factors are stimulated by oxidation reactions, while protein phosphatases are inactivated. These changes result in the activation of several ROS/RNS-producing enzymes which may continue for hours, days, months or years [5]. So far, several types of oxidoreductases with the ability to produce superoxide in cells have been identified. They include cyclooxygenase (COX), lipoxygenase, nitric oxide synthase (NOS), cytochrome P450 enzymes, xanthine oxidase, NADPH oxidase and mitochondrial electron transfer chain. It is generally accepted that mitochondrial ROS production and expression of other genes involved in redox system such as NF-kB, COX-2, iNOS and NADPH oxidase amplify each other [11].

A large number of studies have been conducted to reveal the roles of interacting redox biology and chemistry systems in oxidative-mediated damages induced by IR. Recent studies have demonstrated that redox activity is involved in both early and late effects of exposure to IR. Moreover, the results of different studies have indicated that the expression of genes involved in the redox system is tissue dependent. Hence, an understanding of the specific functions of such redox biological and chemical systems' interactions with IR is specifically described further.

Radiation-induced inflammation triggers redox activation

Inflammation plays a key role in redox activation. Exposure of normal cells directly to IR or ROS will result in both nucleus and mitochondria DNA damages, which may cause cell death through apoptosis, mitotic catastrophe or necrosis.

Mitotic catastrophe is not immunogenic, while apoptosis and necrosis trigger immune activation through the release of danger signals [12]. Although apoptotic bodies are omitted by macrophages and do not activate inflammatory response, necrotic cells release various signals to immune cells including mast cells and lymphocytes which will result in the secretion of inflammatory cytokines [13]. Apoptosis can trigger the release of anti-inflammatory cytokines such as IL-10 and TGF-β, while necrosis may lead to the release of inflammatory cytokines such as IL-1, IL-6, IL-8, IL-13, IL-33 and TNF-α, as well as other inflammatory mediators [14]. In the absence of suppression of these responses by the immune system, chronic inflammation may continue for a long time after exposure. This is associated with chronic oxidative damage, which lead to genomic instability and damage to the normal function of organs [15] (Fig. 1).

NADPH oxidases (NOXs) functions and interactions

NADPH oxidase enzymes are a group of oxidoreductases that transfer electron from NADPH to oxygen molecules. So far, several subtypes of these enzymes have been detected within cells. NADPH oxidase genes have been discovered in both phagocytic and non-phagocytic cells and are involved in immune system responses and inflammation. It was suggested that the NADPH oxidase system is involved in signaling pathways that mediate cell growth, cell survival and death. In response to inflammatory stimuli, macrophages and neutrophils produce superoxide via the phagocytic NADPH oxidase (NOX). On the other hand, ROS produced by non-phagocytic isoforms of NADPH oxidase (such as NOX2 in the membrane) are involved in regulating intracellular signaling cascades in various types of non-phagocytic cells [16]. NOX1-5, DUOX1 and DUOX2 are the most important subtypes of NADPH oxidase enzymes involved in respiratory burst following exposure to radiation. In contrast to other sources of ROS, NADPH oxidase (NOX) is a professional and distinct ROS generator through conversion of O_2 to superoxide anion O_2^- [17, 18].

These enzymes have high stability, resulting in continuous ROS production following exposure. Each of these enzymes is activated in specific tissue type of cells. In addition, enzymes can be stimulated by a large group of stressors and stimuli, as well as different types of cytokines and growth factors. A large group of inflammatory cytokines, chemokines and hormones such as IL-1, TNF-α, TGF-β, IFN-γ and angiotensin II are implicated in NOX system activation [19].

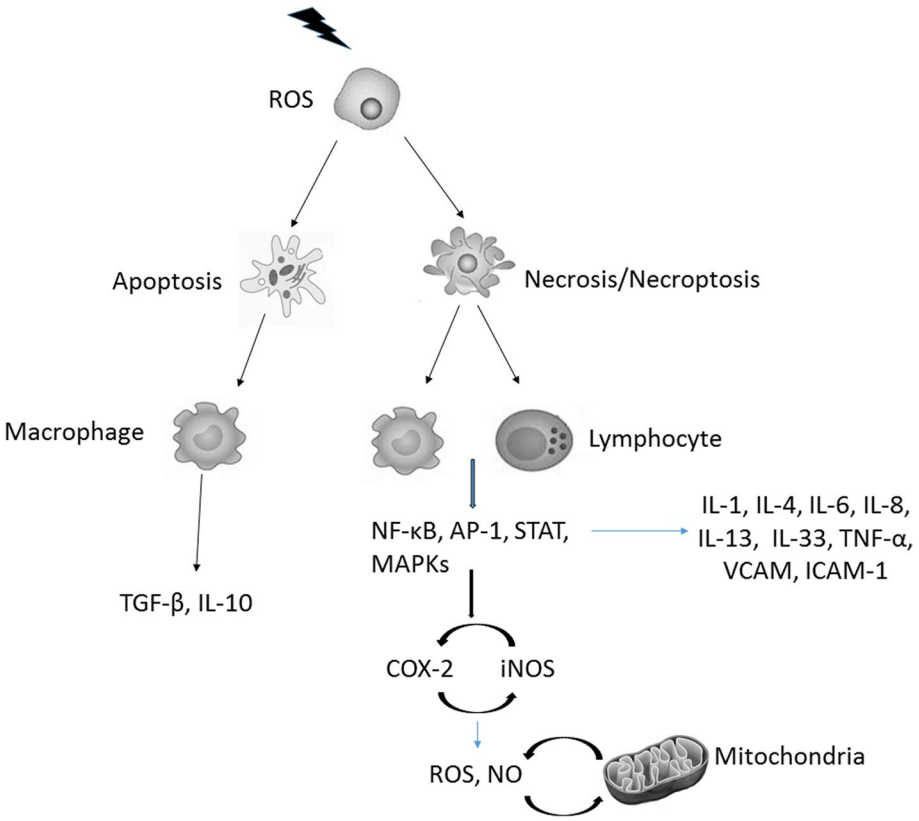

Fig. 1 Mechanisms of cell death trigger inflammation and free radical production after exposure to ionizing radiation

NOX1

NOX1 is expressed in several types of cells such as endothelial cells, in the placenta, prostate and uterus, and osteoclasts, as well as in some malignancies such as melanoma and colon cancer [20–22]. Hence, overexpression of NOX1 may be involved in some malignancies. In addition to risk of carcinogenesis, the role of NOX1 in radiation fibrosis has been proposed. Choi et al. showed that inhibiting NOX1 but not NOX2 or NOX4 ameliorates collagen deposition and pulmonary fibrosis markers such as α-SMA and fibroblast-specific protein 1 (FSP1) in C57BL/6J mice following lung irradiation. Furthermore, ROS production following NOX1 inhibition decreased significantly [23].

NOX2

There are some evidences that NOX2 expressions in both phagocytic and non-phagocytic cells are regulated by some cytokines and growth factors such as IFN-γ, TGF-β and IL-12 [16, 24]. Studies have demonstrated that some mediators such as TLR-2 have an intermediate role in NOX2 activation. In response to IR, NOX2 has an important role in continuous ROS production. Narayanan et al. showed that irradiating human lung fibroblasts with alpha particle produces $O_2^{\cdot-}$ and H_2O_2. Analysis showed that the plasma membrane-bound NOX2 is primarily responsible for $O_2^{\cdot-}$ and H_2O_2 production [25]. The upregulation of upstream genes such as NF-KB, Raf-1, ERK 1/2, c-Jun, p38, activator protein 1 (AP-1) and calcium signaling is involved in this process. Scavenging of free radicals by SOD or catalase inhibited these signaling pathways and subsequent NOX2 upregulation [26–28]. The involvement of NOX2 in radiation-induced salivary gland injury was demonstrated by Kim et al. They showed that exposing rats to 18 Gy of X-ray (2 Gy/min) increases NOX2 gene expression in salivary glands at least 7 days after irradiation. Their results indicated that apoptotic genes such as caspase-9 as well as MAPKs including p-38 and JNK are involved in NOX2 signaling cascades [29]. NOX2 is involved in persistent ROS production in the intestine as well. Datta et al. have shown that irradiating mice with gamma rays and high LET ^{56}Fe radiation causes stimulation of ROS production in the intestinal epithelial cells for 1 year after exposure. The result of this study indicated that NOX1, NOX2 and mitochondria malfunction are responsible for persistent oxidative damage [30].

NOX3

Studies conducted to depict the role of NOX3 in radiation damage are very limited. A study by Shin et al. showed that exposure to radiation upregulates the expression of NOX3 in the oral mucosa of rat. Increased NOX3 expression was associated with necrotic inflammatory exudates and ulceration in the oral mucosa [31].

NOX4

NOX4 is one of the most important subtypes of the NOX system in response to IR. Some studies have proposed a role for NOX4 in radiation-induced bone marrow toxicity. Evaluating different ROS/NO producing enzymes in mice bone marrow showed that increase in NOX4 activity following exposure has a central role for ROS production and bone marrow stem cells damage after exposure. Analysis showed persistent ROS production 8 weeks after exposure. Inhibiting the NOX system resulted in better survival and decreased bone marrow damage [32–34]. As TGF-β has a pivotal role in bone marrow toxicity following exposure to radiation, it seems that TGF-β–NOX4 pathway is responsible for the continuous ROS/NO production and subsequent genomic instability in bone marrow following exposure [35]. Moreover, studies have shown that other mediators such as TLR-4 and MyD88 have an intermediate role in NOX4 activation. The C-terminal region of NOX4 has an interaction with the tail of TLR-4. However, it was proposed that other mediators such as MyD88 and IRAK were involved in this pathway. These interactions are essential for ROS production from NOX4 [36].

NOX5

NOX5-induced ROS production is due to Ca^{2+} flux through Ca^{2+}-binding sites [37]. This gene is absent in rodents. Evidences for its role in radiation oxidative damages are limited. A study by Weyemi et al. on human primary fibroblasts have revealed that inhibition of both NOX4 and NOX5 leads to reduced levels of DNA damage associated with increased cell survival. Results showed that the levels of protection by inhibition of these genes are similar to administering two potent radioprotectors: *N*-acetylcysteine (NAC) and fulvene-5 [38].

DUOX1/DUOX2

Some evidences support the role of DUOX1/DUOX2 in chronic oxidative stress and subsequent consequences of IR such as fibrosis. However, data for responses of these genes to IR are very limited. IFN-y, IL-4 and IL-13 have pivotal roles in the upregulation of DUOX1 and DUOX2. Hassani et al. showed that DUOX1 gene expression is upregulated for several days after exposing human thyrocytes to radiation. Analysis showed that IL-13-p38 MAPK is responsible for persistent DUOX1-induced H_2O_2 production following irradiation [39]. IL-4 and IL-13 induce DUOX2 production as

well as increased ROS production. IL-4–STAT6 pathway is responsible for upregulating DUOX2. The increased expression of these genes may be involved in the development of pancreatic and gastrointestinal malignancies [40]. Further studies are needed to depict possible roles of these genes in radiation-induced carcinogenesis and other side effects.

COX-2

COX-2 has a central role in inflammatory responses which convert arachidonic acid liberated from membrane phospholipids to prostaglandins (PGs). During the synthesis of PGE_2, production of ROS is a common secondary effect of arachnoid acid metabolism [41]. Several studies have indicated that upregulation of COX-2 is involved in different toxicities following exposure to IR [42]. Increased COX-2 expression was reported for its association with radiation toxicity in the gastrointestinal system such as the intestine and colon [43]. Furthermore, upregulated COX-2 gene expression is involved in radiation toxicities in the lung, heart, brain, kidney and others [44]. COX-2 upregulation causes accumulation of immune system cells and appearance of inflammation signs. Inflammatory cells including macrophages and lymphocytes further enhance oxidative damage through secretion of NO and ROS. These changes cause activation of matrix metalloproteinases (MMPs) which change the normal function of tissues through deposition of collagen and fibronectin. Thus, COX-2 mediates pathological damages induced by ionizing radiation such as fibrosis, atherosclerosis and vascular damage [45].

It was suggested that COX-2 can stimulate carcinogenesis through signal modulation involved in cell proliferation and apoptosis [46]. In addition, ROS produced by COX-2 play a key role in mutagenesis and genomic instability. Increased COX-2 expression has been reported for its association with several malignancies such as breast, esophageal, gastric, colorectal and lung cancer [47]. Some studies have also shown the role of COX-2 in oxidative damage in non-irradiated cells. Overexpression of COX-2 in the distant lung and bronchial after pelvis or abdominal irradiation in rats and mice have been reported. These effects lead to increased ROS production and DNA oxidative damage through bystander and non-targeted responses [48, 49].

Lipoxygenases

Lipoxygenases (LOXs) are iron-containing enzymes which catalyze the deoxygenation of unsaturated fatty acids. This process is associated with ROS production and initiating lipoperoxidation of membranes as well as some changes in the cell metabolism [50]. Thus, LOXs can stimulate DNA damage, genomic instability and also cell death, especially apoptosis [51]. In mammals, increased LOXs have been found to be associated with some inflammatory diseases and cancer. A study by Matyshevskaia et al. showed an increase in LOX activity of lymphocytes within 6 h post-irradiation of Wistar rats with 1 Gy X-ray. They also observed that inhibiting LOX activity leads to a remarkable reduction in DNA damage. The results of this study proposed that LOX activity was involved in ROS production during early hours after exposure [52]. In another study, Grichenko et al. have shown that LOX activity was obvious 1 h after exposure but not at later times [53].

Nitric oxide in DNA damage and inflammation

Nitric oxide (NO) is an important mediator which affects a number of targets within cells. iNOS is the main source of NO during stress conditions such as inflammation and plays a key role in oxidative stress and carcinogenesis. NO is generated by macrophages via iNOS enzyme in response to inflammatory stimulus. NO is highly reactive, interacting with mitochondria-derived superoxide to form higher reactive peroxynitrite. On the other hand, high level of NO competes with O_2 in the ETC and may suppress respiration in the mitochondria. This effect may lead to increased superoxide generation which amplifies oxidative stress [54].

Several studies have indicated that reactive nitrogen species (RNS) are involved in radiation-induced normal tissue injury [55–57]. In addition, NO has a pivotal role in radiation-induced bystander and non-targeted effects [48, 58]. Increased NO level following radiation exposure has shown association with DNA damage and genomic instability [59]. A study by Ohta et al. proposed that increase in NO level has a direct relation to radiation dose. Moreover, they observed that increased serum level of NO occurs in the early hours after exposure [55]. However, it seems that increased NO production by iNOS in exposed tissues continues for several days or months after exposure. This is associated with long-term pathological changes in irradiated tissues [60].

One important mechanism for NO-induced normal tissue damage is nitro-acetylation and subsequent epigenetic changes in some enzymes such as DNA repair enzymes. NO produced by macrophages and neutrophils has a role in inhibiting DNA repair enzymes involved in the base excision repair (BER) and mismatch repair (MMR) pathways [61]. Elevated NO production which can be seen following exposure to radiation may suppress the activity of some DNA repair enzymes in these pathways [62]. Studies have proposed that chronic upregulation of nitric oxide synthases (NOS) especially iNOS, causing nitro-acetylation and decreased half-life of ogg1 and AGT [63, 64]. These effects on DNA repair responses result in incomplete repair of DNA damage which may provide grounds for mutation, chromosomal instability, genomic instability and finally carcinogenesis [65, 66].

Mitochondrial functions as an energy and free radical reservoir

Mitochondria sources energy for the cells through reducing oxygen to water and synthesizing adenosine triphosphate (ATP). This process is known as oxidative phosphorylation. It accounts for the consumption of 90% of all oxygen taken up by mammals [67]. Oxidative phosphorylation occurs within the electron transport chains, a series of enzymes embedded within the inner mitochondrial membrane. During electron transportation, a percentage of the oxygen molecules undergo a one-electron reduction to superoxide [68]. In normal conditions, antioxidant defense systems such as catalase, superoxide dismutase and glutathione peroxides neutralize superoxide and form free radicals [69]. Thus, this system protects cells from oxidative damage resulting from mitochondria activity. O_2 radicals may be released to the cytosol to generate reactive oxygen species. Furthermore, these radicals may react with NO, producing peroxynitrite [70].

Although several studies have shown that the main source for radiation-induced ROS production is the mitochondria, its activation mechanisms have not been recognized completely. A study by Yamamori et al. indicated that irradiating human lung carcinoma A549 cells increases the mitochondrial contents of the cells. Furthermore, they showed that exposure of cells to radiation increases the mitochondrial membrane potential and also stimulates the mitochondrial electron transport chain (ETC) function [71]. In addition, some studies reported that irradiation leads to an increase in the mitochondrial mass in different cell lines [72, 73].

An investigation by Tulard et al. revealed a persistent and dose-dependent increase in mitochondrial ROS in human colon cells after exposure to gamma radiation [74]. Evaluation of intracellular and mitochondrial ROS after irradiation has shown that a dose-dependent increase in both sources of ROS is due to radiation. However, analysis showed a difference in the intracellular and mitochondrial ROS profile. Intracellular ROS increased soon after exposure, subsided after 24 h, and thereafter increased for another 3 days. In contrast to intracellular ROS, mitochondrial ROS level rises gently and attains its peak 3 days after exposure. This may remain high for a week after irradiation [75]. These results have been confirmed by other studies [76–78]. It was suggested that mitochondrial ROS are involved in DNA damage and tumor development [79]. In different tumor cells, the increased level of mitochondrial ROS was confirmed. For example, it was observed that mtDNA mutations are associated with malignancies such as lung, stomach and breast cancer as well as leukemia and lymphoma [80, 81].

However, the exact mechanisms for increased oxidative phosphorylation in mitochondria remain to be elucidated. Mitochondrial dysfunction, inflammation and intracellular ROS play a key role. Increased the level of ROS production stimulates mitochondria response through a phenomenon known as ROS-induced ROS. Secretion of calcium ions into cytosol plays a key role in this signaling. Following secretion of Ca^{2+} in the endoplasmic reticulum to cytosol during stress conditions, these ions are accumulated in the mitochondria, leading to the disruption of the normal ETC activity which causes increase in ROS production [82, 83].

Mitochondria malfunction is another reason for ROS production. It was proposed that mutation in mtDNA and ETC are involved in this process. Yoshida et al. have shown that exposure of rats' A7r5 cells to 5 Gy gamma rays causes decreased activity of complex I and increased ROS production. Complex I (NADH dehydrogenase) is the most important complex involved in the release of ROS from the ETC [78]. In another study, Dayal et al. showed that dysfunction of complex II after exposure to 10 Gy X-rays played a key role in ROS production and oxidative stress in GM10115 cells. Irradiation of the heart of mice with 2 Gy has shown that succinate-stimulated respiration decreased significantly compared to the control mice. This study has shown that cytochrome C as well as ETC1 and three activities were reduced [84]. This could be because unstable mitochondria produce more hydrogen peroxide compared to normal cells, resulting from decreased respiratory rate [85]. Evidences show that the NADPH oxidase system and mitochondria have a synergic effect on each other in response to stress signaling [86]. Also, studies proposed that the ROS-derived NOX system is involved in mitochondrial dysfunction and subsequent ROS production in this organelle [87, 88].

Epigenetics of redox activation

Evidences indicate that the redox system is related to epigenetic regulation. On one hand, free radicals including both ROS and NO regulate epigenetic processes such as DNA methylation, histone methylation and acetylation. On the other hand, changes in some miRNAs can increase or decrease oxidative damage [89]. Exposure to IR increases the expression of miRNAs involved in ROS production such as let-7 family, mir-15b, mir-21, mir-128 and mir-636 [90–92]. The best example for redox activation of epigenetic modulators is upregulation of mir-21 following exposure to radiation and bystander cells. mir-21 is activated in oxidative stress conditions, as well as following upregulation of some cytokines such as TGF-β [93]. mir-21 induces oxidative stress via targeting of SOD and TNF [94]. Following exposure to radiation, it can suppress detoxification of superoxide by targeting SOD3. In addition, it subdues the regulation of TNF-a, resulting in reducing SOD2 levels [94].

The role of mir-21 in ROS production in bystander cells has also been investigated. Xu et al. showed that the upregulation of mir-21 in bystander MRC-5 cells is associated with increased oxidative and DNA damage. Their results showed that expression of SOD3 was reduced significantly [95]. Tian et al. [96] achieved similar results for MnSOD or SOD2 in bystander WS1 cells following alpha particle irradiation of HaCaT keratinocytes. TGF-β is responsible for the oxidative damage in bystander cells through mir-21. An investigation by Jiang et al. [97] showed that inhibiting TGF-βR1 via a selective inhibitor resulted in abolishing mir-21 and oxidative stress in bystander cells. Since free radicals can upregulate mir-21 and TGF-β, it is possible that mir-21 via a positive loop feedback plays a role in continuous oxidative damage following exposure to ionizing radiation (Fig. 2).

Hypoxia

Hypoxia results from vascular damage and tissue injury. Some changes associated with hypoxia have a regulatory role in tissue remodeling and wound healing. Following exposure to IR, increased inflammatory cytokines and chemokines stimulate accumulation of macrophages in the injured area. This phenomenon is associated with increased oxygen consumption by activated macrophages leading to a low oxygen state and hypoxia in the injured tissues. This hypoxic state stimulates free radical production of some redox system agents such as Ca^{2+} flux, mitochondria and NADPH oxidase enzymes [98, 99]. Evidences indicate that hypoxia is involved in chronic oxidative stress and radiation-induced late normal tissue injury such as fibrosis [100, 101]. These and many other factors are responsible for this development.

Bystander/non-targeted effect-induced redox activation

Radiation-induced bystander effect refers to a phenomenon in which irradiated cells secrete signals to adjacent non-irradiated cells that cause damage to them. Studies have shown that ROS and NO play a pivotal role in DNA damage and genomic instability in bystander cells. Results from several studies proposed that upregulating some genes involved in redox system such as COX-2, iNOS, NADPH oxidase and also mitochondria plays a central role in this phenomenon [11]. COX-2 and iNOS have a synergic effect on each other [102]. Furthermore, ROS and NO produced by these enzymes increase the activity of the electron transfer chain (ETC) in the mitochondria. Several experiments have been conducted to reveal the signaling pathways that cause oxidative damage in bystander cells. Exposing cells to radiation cause damages to DNA and other structures such as the membrane and mitochondria. These damages result in the release of various products such as exosomes, miRNAs, oxidized DNA and other danger alarms from irradiated cells. In response to these products, macrophages and lymphocytes release several cytokines such as IL-1, IL-6, IL-8, IL-33,

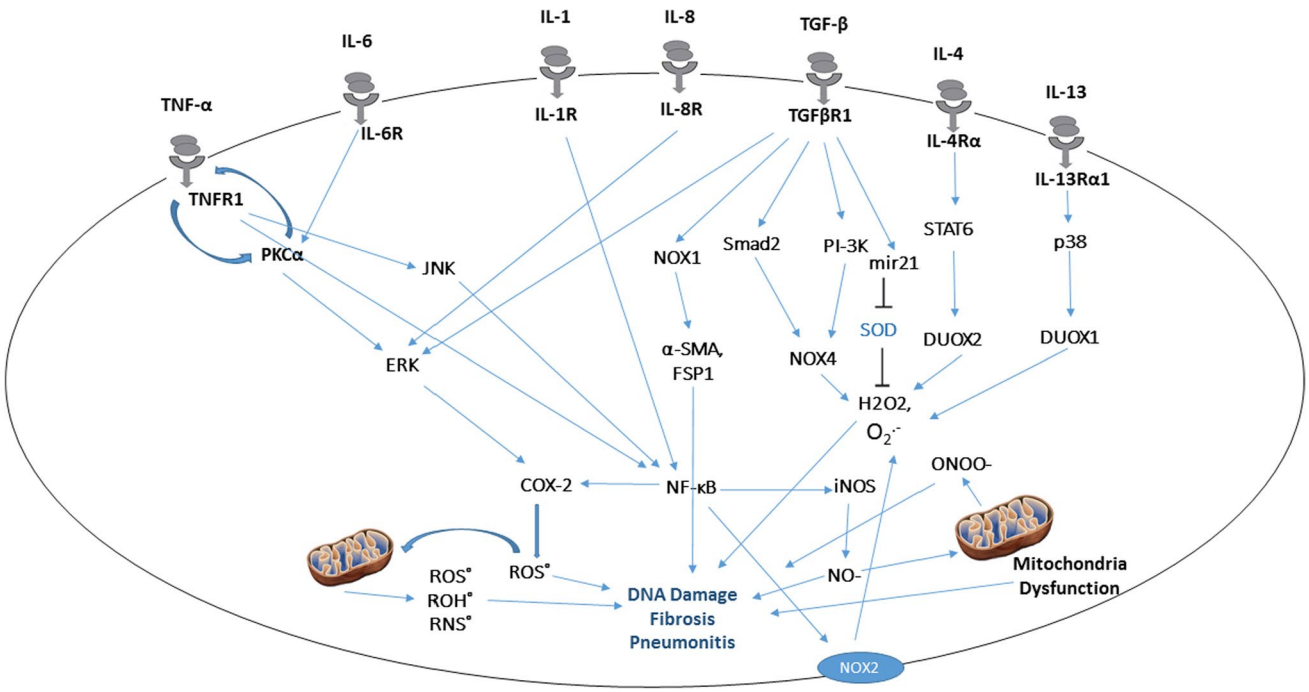

Fig. 2 Mechanisms of redox system activation following exposure to radiation

TNF-α, TGFβ and others [103]. Migration of these signals to other cells leads to long-term detrimental changes that may give rise to secondary effects of ionizing radiation on normal tissues. In vitro studies have revealed that gap junction intercellular communication (GJIC) plays a key role in the transfer of bystander signals to non-irradiated cells [104–106]. Inhibiting GJIC by specific inhibitors resulted in the suppression of DNA damage in bystander cells [107, 108].

Several studies have shown that inflammatory responses to DNA damage, DNA repair and cell death play a key role in the secretion of factors involved in activating redox systems [8, 109]. As regards the ability of several of these factors to migrate to distant organs, it is acceptable that redox system activity increases in non-targeted organs. The released clastogenic signals can migrate to distant tissue and stimulate ROS/NO production [110]. Several in vivo studies have shown that local irradiation of a limited area causes ROS production and oxidative damage in out-of-field tissues [48, 111]. A study by Chai et al. showed that the TGF-β–TGFβR1–COX-2 pathway plays an important role in ROS production and oxidative DNA damage in distant lung tissues. However, non-targeted induced oxidative damage affects non-irradiated tissues in a tissue specific manner. The expression of TGβR1 and its cascades such as COX-2 and ROS in the lung was obvious, but not for the liver [112]. As oxidative DNA damage causes hypomethylation and other epigenetic changes, this effect may result in a tissue-specific epigenetic change in non-irradiated tissues. For example, cranial irradiation resulted in long-term hypomethylation and changes in miRNAs profile in rat's spleen. These changes were not observed in the skin. A better understanding of the basic mechanisms of this phenomenon may help improvements in the therapeutic ratio of RT [113].

Genomic instability is a phenomenon seen in several types of malignancies. It is associated with chromosomal aberrations such as increased mutation frequency within the genome, damage to DNA repair genes, alteration in mitochondrial function, mutation in mitochondrial DNA, change in energy balance within cells, attenuation of antioxidant enzymes and so on [114–120]. These abnormal changes in cells lead to persistent ROS production, oxidative stress and mutation in chromosome, making cells susceptible to carcinogenesis [121].

Evidences indicated that most cancers have a type of genetic instability. This phenomenon in many tumors causes a large number of genetic alterations. Although, so far, the mechanisms involved in genomic instability remain unknown, the results of several studies have proposed a role for ROS. A study by Limoli et al. has shown that clones derived from cells exposed to ionizing radiation have abnormal increase in ROS levels. In addition, the number of dysfunctional mitochondria was higher compared to normal clones [122]. A further study observed that using some scavengers such as DMSO and cationic thiol cysteamine reduces genomic instability after irradiation. This implies the role of continuous ROS production following exposure to radiation in the induction of genomic instability. Furthermore, results indicated that the abnormal functions of mitochondria have a key role in chronic oxidative stress [85]. Genomic instability induced by different types of radiations such as low and high LET radiations has been revealed in both in vitro and in vivo studies [123] (Fig. 3).

Targeting of the redox system for mitigation of radiation-induced normal tissue injury

Normal tissue protection during RT is an active area of research in radiation oncology. It is widely known that radiation induces a wide range of clinical disorders which reduce the outcomes of RT. For many years, radiation protectors have attracted a great deal of attention and various agents have been tested for different tissues [124]. It has been observed that the responses of different organs to IR are distinct [49]. On the other hand, results of a large number of studies have indicated that no agent can protect all organs against IR. Moreover, in clinical applications, protection of normal tissues against RT is a complex biological process and finding selective drugs requires advanced biological experiments. In recent years, researchers have investigated a variety of approaches to obtain the best biological mechanism of radiation protection. It was revealed that these mechanisms are tissue dependent and differ based on the structure of organs as well as functions, in addition to the immune system response [125]. Hence, selection of an appropriate radioprotector is based on tissue responses.

In this present radiation biology era, to find the best clinical radioprotector, new approaches such as redox mechanisms of irradiated organs are of particular interest [109]. As a good example, targeting NOX2 and NOX4 genes has a critical role in bone marrow sparing following exposure to radiation. Several studies have indicated that some radiation modifiers cause reduction of NOX4 gene expression and amelioration of bone marrow toxicity following exposure. Guoshun et al. showed that total body irradiation (TBI) of mice results in long-term upregulation of NOX4 and increase in ROS production in bone marrow hematopoietic stem cells (HSCs). These changes were involved in radiation-induced chronic oxidative damage and long-term injury in the bone marrow. Moreover, they showed that treatment with metformin significantly attenuated ROS production and ameliorated micronuclei formation via NOX4 downregulation in HSCs [126]. Similar results were obtained after administering 5-methoxytryptamine-α-lipoic acid and resveratrol [127, 128]. Moreover, inhibition of these genes may

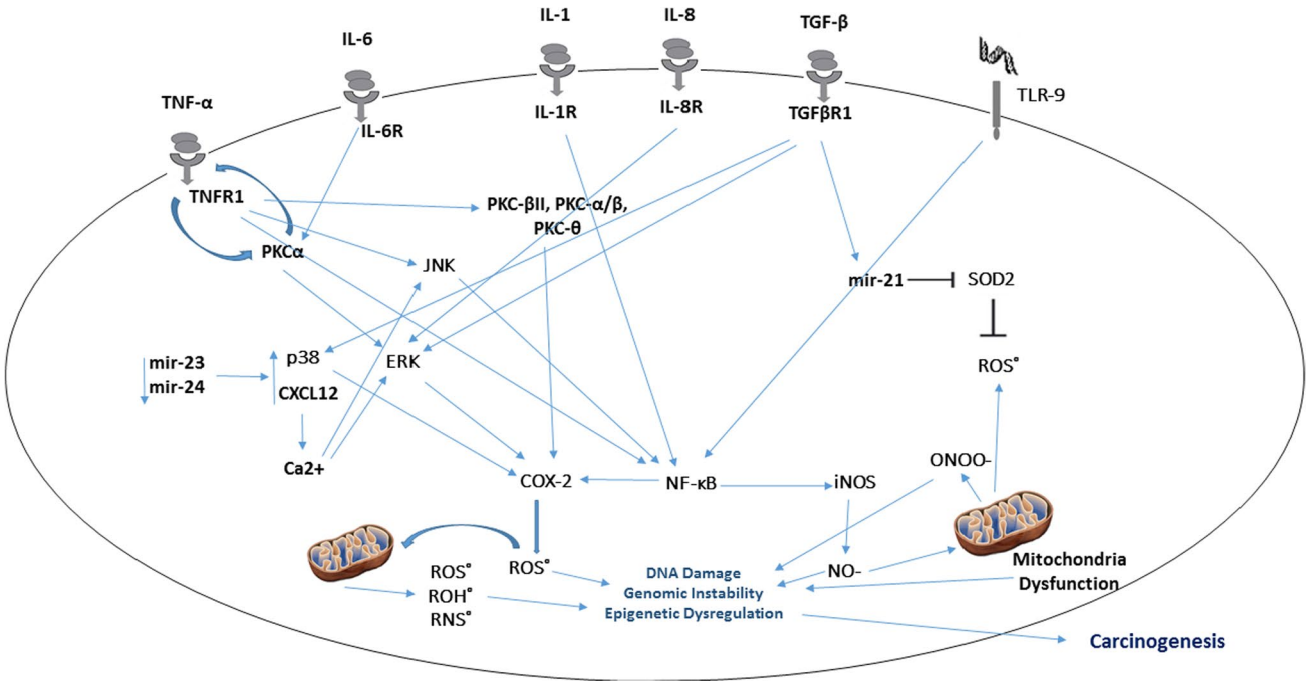

Fig. 3 Mechanisms of radiation-induced bystander/non-targeted effect

protect the lungs and cardiovascular system from fibrosis [129].

NO plays a key role in radiation-induced chronic injury in irradiated cells, as well as bystander cells [130, 131]. iNOS as the main source of NO is expressed in several tissues such as lungs and gastrointestinal organs. Hence, inhibiting this enzyme is an approach for normal tissue protection against inflammatory responses to ionizing radiation. Some inhibitors like *N*-nitro-L-arginine methyl ester and amino guanidine have shown ability to ameliorate radiation injury in the lung [132, 133]. In another study by Erbil et al., it was observed that treatment with N(omega)-nitroarginine methyl ester (L-NAME), which is another type of iNOS inhibitor, can ameliorate radiation-induced enteritis [134]. However, in the gastrointestinal system, NOX1, NOX2, cyclooxygenase-2 (COX-2) and mitochondria are other sources of ROS [109, 135].

Targeting mitochondria-induced ROS has been proposed by some studies for mitigating radiation injury. Rwigema et al. evaluated three types of mitochondrial targeting drugs including JP4-039, MCF201-89 and BEB55. The study was conducted as both in vitro for mice hematopoietic progenitor cell line and in vivo for mice. Their results showed that administering these drugs after exposure to a lethal dose of radiation (9.5 Gy) can reduce death of progenitor cells as well as increase survival of irradiated mice [136]. It has been shown that targeting mitochondria is associated with arresting cell cycle in G1, leading to increased DNA damage repair [137]. Reducing apoptosis, especially in apoptosis-prone cells like hematopoietic system cells is another property of mitochondria-targeting agents, which can prolong survival [138]. Similar results have been confirmed for other mitochondria-targeting agents [139, 140] (Table 1).

Summary and conclusion

This review has presented the mechanisms of ROS production and oxidative damage derived from some enzymes and mitochondria. It is clear that these systems contribute to acute and late deleterious effects of ionizing radiation. In addition, results from several studies propose that ROS as well as NO production by mitochondria and inflammatory cells is involved in oxidative damage to bystander cells and non-targeted tissues. Although the roles of some inflammatory mediators such as lipoxygenases, COX-2 and iNOS have been confirmed in radiation toxicity, recent studies indicate that mitochondria malfunction and upregulation of NADPH oxidase enzymes have pivotal roles. NADPH oxidase enzymes including NOXs and DUOX1-2 are H_2O_2-producing enzymes with abilities to stimulate continuous oxidative damage and genomic instability for a long time after exposure. However, recent studies have highlighted the roles of these enzymes in other consequences of exposure to radiation such as pneumonitis, fibrosis and vascular injury. With regard to the role of NADPH oxidase in radiation-induced oxidative stress, it is

Table 1 Redox enzyme responses in radiation-induced normal tissue injury

Target	Organs	Effects	Inhibitors	Refs.
NOX1	Lung, intestine	Fibrosis, enteritis, oxidative damage	Apocynin, diphenyleneiodonium (DPI)	[23]
NOX2	Salivary gland, intestine	Mucositis, enteritis, oxidative damage	Apocynin, DPI	[29]
NOX3	Oral mucosa	Mucositis, ulceration	Apocynin, DPI	[31]
NOX4	Bone marrow, fibroblast cells	Genomic instability, senescence of stem cells, hematopoietic system syndrome	Metformin, resveratrol, melatonin, *N*-acetylcysteine, fulvene-5, DPI	[126]
NOX5	Fibroblast cells	Genomic instability	*N*-acetylcysteine, fulvene-5	[38]
DUOX1&2	Thyroid	Chronic oxidative stress, fibrosis	IC_{50}	[141]
COX-2	Joints, heart, lung	Arthritis, pneumonitis, DNA damage	Celecoxib, resveratrol	[142]
iNOS	Lung, intestine	Enteritis, pneumonitis	*N*-nitro-L-arginine methyl ester, amino guanidine, L-NAME	[109, 135]
Mitochondria	Bone marrow, gastrointestinal system, pulmonary epithelial cells	Enteritis, oxidative DNA damage, fibrosis	Metformin, JP4-039, MCF201-89, BEB55, cationic rhodamine 19	[143, 144]

possible to detect involvement of some other sub-families of NADPH oxidase in radiation injury after some years. This is very vital for normal tissue regeneration in the abdominal or cardiovascular systems.

In recent years, numerous studies have indicated that epigenetic modulators play a key role in redox induced normal tissue injury following exposure to ionizing radiation. Upregulation of some immune mediators such as TGF-β and free radicals stimulates the expression of various miRNAs. For example, TGF-β, which has a direct relationship with radiation dose and oxidative damage, upregulates the expression of miRNA21. On the other hand, miRNA21 can suppress SOD activity, resulting in the amplification of oxidative damage induced by inflammatory mediators. The interaction of ionizing radiation, inflammation and epigenetic modulators is very complicated and requires further studies to illustrate this complicated interrelationship between them.

Despite the complicated interrelationships between these factors, it has been confirmed that all mentioned inflammatory mediators, free radicals and some epigenetic modulators amplify each other, resulting in a positive feedback loop. During these interactions, free radicals continually attack genome and cell structure. In addition, oxidative damage changes the expression of various genes involved in the long-term detrimental effects of radiation. Modulation of ROS production from these interactions has been shown to ameliorate lethality, pathological damages as well as genomic instability. Hence, the management of each of these enzymes depends on the irradiated organs. Moreover, knowledge of the mechanisms of redox activation in each organ can help in the production of novel radioprotectors and mitigators with higher efficacy.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Disclaimer All opinions are the personal and professional opinions of the authors and are not the opinions of their respective academic affiliations and agencies. Masoud Najafi and Vilmar Villa share both senior authorship.

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