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RESEARCH ARTICLE

NADPH Oxidase as a Target for Modulation of Radiation Response; Implications to Carcinogenesis and Radiotherapy

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Abstract: ***Background:*** Radiotherapy is a treatment modality for cancer. For better therapeutic efficiency, it could be used in combination with surgery, chemotherapy or immunotherapy. In addition to its beneficial therapeutic effects, exposure to radiation leads to several toxic effects on normal tissues. Also, it may induce some changes in genomic expression of tumor cells, thereby increasing the resistance of tumor cells. These changes lead to the appearance of some acute reactions in irradiated organs, increased risk of carcinogenesis, and reduction in the therapeutic effect of radiotherapy.

Discussion: So far, several studies have proposed different targets such as cyclooxygenase-2 (COX-2), some toll-like receptors (TLRs), mitogen-activated protein kinases (MAPKs) etc., for the amelioration of radiation toxicity and enhancing tumor response. NADPH oxidase includes five NOX and two dual oxidases (DUOX1 and DUOX2) subfamilies that through the production of superoxide and hydrogen peroxide, play key roles in oxidative stress and several signaling pathways involved in early and late effects of ionizing radiation. Chronic ROS production by NOX enzymes can induce genomic instability, thereby increasing the risk of carcinogenesis. Also, these enzymes are able to induce cell death, especially through apoptosis and senescence that may affect tissue function. ROS-derived NADPH oxidase causes apoptosis in some organs such as intestine and tongue, which mediate inflammation. Furthermore, continuous ROS production stimulates fibrosis via stimulation of fibroblast differentiation and collagen deposition. Evidence has shown that in contrast to normal tissues, the NOX system induces tumor resistance to radiotherapy through some mechanisms such as induction of hypoxia, stimulation of proliferation, and activation of macrophages. However, there are some contradictory results. Inhibition of NADPH oxidase in experimental studies has shown promising results for both normal tissue protection and tumor sensitization to ionizing radiation.

Conclusion: In this article, we aimed to review the role of different subfamilies of NADPH oxidase in radiation-induced early and late normal tissue toxicities in different organs.

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INTRODUCTION

Radiotherapy is a treatment modality for cancer and can be used in combination with surgery, chemotherapy or immunotherapy [1, 2]. In comparison to surgery, radiotherapy is less invasive and also has lesser systematic side effects compared to chemotherapy [3, 4]. Although, immunotherapy has the lowest side effects and is a growing modality for cancer treatment, it needs further studies. In recent years, some studies have proposed that the combination of radiotherapy with immunotherapy may be more effective compared to other modalities for tumor eradication [5-8]. Besides the beneficial therapeutic effects of radiotherapy on tumor suppression, normal tissue toxicity is the most limiting factor for the delivery of a sufficient radiation dose to tumor cells

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[9, 10]. So far, several studies have been conducted to ameliorate normal tissue damage following exposure to ionizing radiation [11]. Amifostine is the most common radioprotector. However, its high toxicity may cause the discontinuation of radiotherapy [12-14]. So far, several experiments have shown promising results for low toxic natural and herbal agents [15].

A knowledge of the mechanisms by which ionizing radiation causes toxicity in normal cells is essential for the development of effective radiation modifiers. However, sensitization of tumor cells to ionizing radiation can increase therapeutic ratio as well as a reduction in the dose required for tumor eradication [16, 17]. Studies have proposed that inhibition of several targets following exposure to ionizing radiation can attenuate radiation-induced normal tissues complications [18]. Studies have shown that the side effects of exposure to radiation may originate from the accumulation of DNA damage and cell death, which occurs immediately some hours after irradiation [19, 20]. Oxidized DNA damage and cell death trigger several signaling pathways that are involved in early and late effects of radiotherapy. Oxidized DNA and necrotic cells through some toll-like receptors (TLRs) such as TLR2, TLR4, TLR5 and TLR9 stimulate the regulation of transcription factors like NF-kB, leading to the release of inflammatory cytokines by macrophages and lymphocytes [21, 22]. On the other hand, apoptosis induction, which is a common cell death type following exposure to radiation leads to the release of tolerogenic cytokines such as IL-10 and TGF-β [23, 24].

Both pro-inflammatory and tolerogenic cytokines are able to stimulate the upregulation of pro-oxidant enzymes. These enzymes, through the continuous production of different types of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) affect cell function by amplifying radiation toxicity [25]. The simplest effects of free radical production by pro-oxidant enzymes are more DNA damage and cell death, in addition to triggering reduction/oxidation reactions that cause further production of ROS and nitric oxide (NO) [26, 27]. The most common pro-oxidant enzymes include NADPH oxidase, cyclooxygenase-2 (COX-2), iNOS, lipoxygenases (LOX) [28]. The mitochondria, a source of energy supply within cells, have a close relation withthese enzymes and may produce superoxide during oxidative stress conditions [29]. NADPH oxidase enzymes include some subfamilies that produce H_2O_2 for a long time following exposure to a foreign stimulus such as ionizing radiation [30, 31]. In recent years, some studies have proposed a key role for NADPH oxidase enzymes in the development of various side effects such as oxidative injury, genomic instability, inflammation, fibrosis, and the bystander effect.

NADPH OXIDASE SYSTEM FUNCTION

Several studies have shown that NADPH oxidase system is the main source of endogenous ROS, which is activated following cell exposure to internal or external stimulus [32, 33]. NADPH oxidase has seven isoforms which include NOX1-5 as well as DUOX1 and DUOX2. NOX subfamilies have a core structure comprising six transmembrane protein domains. In addition, each NOX subfamily uses some combination subunits which are required for the activation of this core [34, 35]. Among NOX enzymes, NOX1, NOX2, and NOX4 are membrane-dependent. These enzymes require a subunit for their function which is named p22phox. Production of superoxide anion (O_2^-) by NOX1 and NOX2 enzymes needs other subunits in addition to RAC GTPase. NOX4 does not require these proteins for its activity while NOX5 is activated by calcium signaling. In contrast to other subfamilies of NADPH oxidase, NOX4 and DUOX1&2 produce H_2O_2 [36, 37]. In response to internal danger alarms or foreign stimulus, NOX system activates and augments the level of O_2^- and H_2O_2 within cells [38]. One of the most important functions of NADPH oxidase enzymes is its phagocytic activity that leads to the killing of foreign bodies such as microbes [39].

DISTRIBUTION OF NADPH OXIDASE

NOX family (including DUOX1 and DUOX2) can be expressed in various cells. Moreover, in a specific cell type, the expression of more than one of these genes is probable [40]. NOX1 can be expressed in the epithelium of some organs such as colon, lung and vascular [41]. NOX2 plays a key role in phagocytosis in different organs, and is also expressed in the thyroid, bone marrow, gastrointestinal system and kidney cells [42, 43]. Similar to NOX2, NOX3 has phagocyte activity and can be expressed by phagocyte cells. NOX4 can be expressed by several cells in the lung, kidney, brain, vascular, bone marrow, etc. The expression of NOX5 has been found in fibroblast cells, spleen, testis, and lymph nodes. DUOX1 and DUOX2 isoforms are involved in the production of thyroid hormones and highly expressed in thyrocyte cells [44-46].

NADPH OXIDASE AND CARCINOGENESIS

Several evidences have shown that chronic inflammation plays a key role in cancer initiation [47]. Some studies proposed that inflammation is responsible for the incidence of up to half of cancers [48-50]. It has been revealed that upregulation of inflammatory cytokines such as IL-1 and TNF-α, TGF-β, etc., can increase ROS production following upregulation of NADPH oxidase [51]. In addition, some growth factors such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) are able to trigger upregulation of these enzymes [52-55]. It is well known that chronic oxidative stress is a hallmark for genomic instability and cancer induction [56, 57]. Potential carcinogenesis role of NADPH oxidase was first identified by Chiera *et al*. They used a model of HeLa cells with high expression of NOX1. Overexpression of NOX1 in Hela cells was associated with the chronic production of ROS and RNS, DNA damage and 3-fold increase in HPRT mutation. Authors suggest that chronic oxidative injury in this cell type causes saturation of DNA repair responses (DRR), leading to genomic instability [58]. NOX1 is also able to prevent apoptosis of precancerous cells through inhibition of p53 Lys382 acetylation, which is necessary for apoptosis of malignant cells [59].

Increased expression of NADPH oxidase has been observed to be associated with pre-malignant lesions. Upregulation of NOX1, NOX2, and DUOX2 in adenomas of the

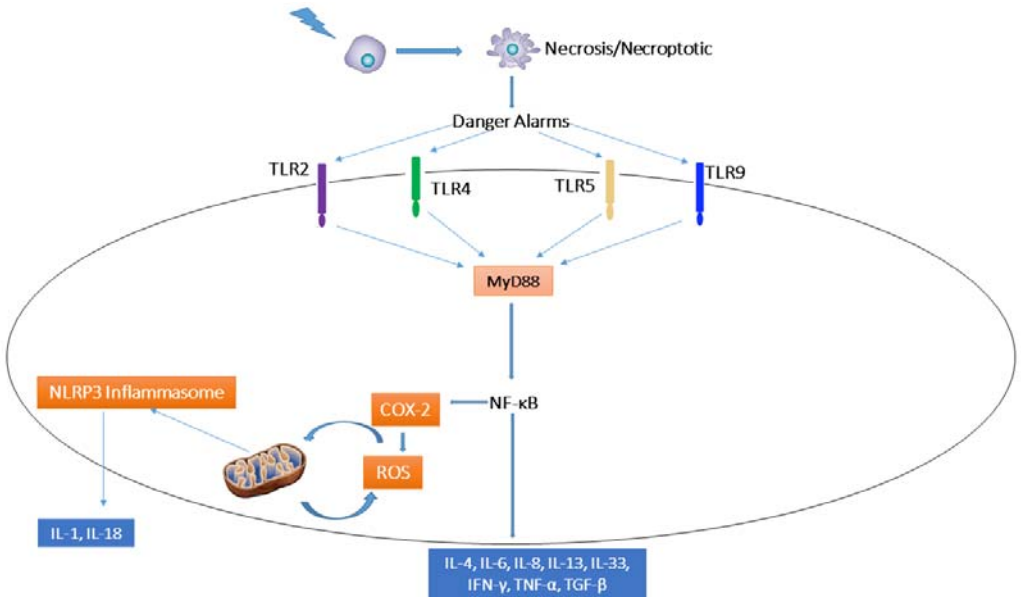

Fig. (1). Mechanisms of radiation-induced release of danger alarms and cytokine release by macrophages and Lymphocytes-T.

colon and inflammatory bowel diseases has been detected [25, 60, 61]. It is supposed that dysregulation of pathogen recognition in the intestine or other organs may induce chronic upregulation of NADPH oxidase system and chronic oxidative injury [62]. Upregulation of NADPH oxidase is associated with increased risk of some other tumor types. Also, inhibition of these enzymes has been proposed for reducing the probability of cancer incidence. Han *et al*., in a systematic review and meta-analyses, proposed that there is a close link between overexpression of NOX system genes and the incidence of lung cancer [63]. Experimental studies propose a role for NOX1-5 in the promotion of mutagenesis. NOX1 can cause mutation of the K-RAS oncogene that plays a key role in the development of some malignancies such as lung cancer [64]. Increased expression of NOX4 has been observed in chronic myeloid leukemia [65]. Also, it has been proposed that increased expression of NOX4 has a role in the initiation of breast and ovarian cancers [66].

In addition, to direct ROS production by NADPH oxidase enzymes, the interaction of these enzymes with other redox mediators has a key role in oxidative injury, genomic instability, and carcinogenesis [67]. The mitochondria have a close relation with NADPH oxidase. Under normal conditions, oxidative phosphorylation with mitochondria causes the production of oxygen metabolites including 5% superoxide. These superoxide molecules are naturally neutralized by antioxidant defense enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx) and glutathione reductase (GR). However, during oxidative stress conditions, mutations in the mitochondrial DNA (mtDNA) increases the production of superoxide by the mitochondria and amplify oxidative injury [68]. In this situation, antioxidant enzymes are not able to neutralize abnormally increased superoxide production, leading to the activation of redox interactions which cause chronic oxidative stress and genomic instability [69]. Some studies proposed that NADPH oxidase activation leads to mutations in mtDNA and increased superoxide production [70-72]. Furthermore, NOX4 is able to transport a GFP into the mitochondria, leading to ROS production within the mitochondria [66].

NADPH OXIDASE IN RADIATION-INDUCED NORMAL TISSUE TOXICITY; POTENTIAL MODIFIERS

NADPH Oxidase and Radiation-Induced ROS Production and Genomic Instability

As earlier mentioned, chronic upregulation of NADPH oxidase enzymes is associated with continuous ROS production which may induce genomic instability and carcinogenesis. An *in vitro* study showed that inhibition of NOX4 or NOX5 can mitigate DNA damage caused by ionizing radiation. This study showed that inhibition of these genes with fulvene-5 is capable of reducing 35% of double-strand breaks (DSBs) in human peripheral blood mononuclear cells. Other NOX and DUOXs subfamilies genes did not show remarkable expression in these cells [73]. Wang *et al*. in an *in vivo* study, evaluated chronic oxidative injury in bone marrow stem cells following whole body irradiation of mice with 6.5 Gy. They observed, up to 2-fold increase in the production of ROS in hematopoietic stem cells (HSCs) 2 weeks after irradiation. Increased production of ROS continued up to the 8^{th} week. Further analyses showed that when mice were treated with diphenyleneiodonium chloride (DPI) (an inhibitor for all isoforms of NOX system), the production of ROS and oxidative DNA damage were significantly attenuated. However, treatment with other inhibitors such as COX-2 inhibitor, lipoxygenase inhibitor, and mitochondrial inhibitor could not attenuate oxidative injury. Also, they showed that when mice were treated with apocynin, NOX enzymes except NOX4 were inhibited, while the oxidative injury was not mitigated [74]. Another study by Pazhanisamy *et al*. evaluated the role of NOX4 in mice bone marrow in genomic instability. They showed that chronic upregulation of NOX4 is responsible for genomic instability in mice HSCs. Inhibition of NOX4 showed that the number of unstable

chromosomal aberrations reduces significantly. The protective effect of NOX4 inhibition had a similar effect on mice when treated with a potent antioxidant [75]. Similar results were obtained after irradiating mice with high energy protons. Results showed a significant reduction in the number of HSCs 22 weeks after irradiation. Results showed a 1.8-fold increase in NOX1, a 3-fold increase in NOX2, and a 36-fold increase in the expression of NOX4 [76]. Another study by Kyung *et al.* confirmed the role of NOX1 in the radiation-induced ROS production and DNA damage in irradiated cells. Irradiation of human Jurkat T cells leads to a remarkable increase in the production of ROS and formation of micronuclei. Similar to some other findings, activation of MAPK enzymes plays a key role in ROS production in these cells. Inhibition of NOX1 showed reduced ROS production and micronuclei formation [77].

It seems that NOX system activation in bone marrow cells is induced by upregulating the release of TGF-β by immune cells, resulting from the high incidence of apoptosis. A study by Zhang *et al*. showed that inhibition of TGF-β with SB431542 can mitigate radiation toxicity in mice bone marrow mononuclear cells (BMMNCs), hematopoietic progenitor cells (HPCs) and HSCs. Moreover, their results indicated that TGF-β activation following exposure to radiation induces toxicity through upregulation of NOX1, NOX2, and NOX4 [78]. Some studies showed that attenuation of these genes is involved in radioprotection against ionizing radiation. Li *et al*. evaluated the potential modulatory effect of melatonin and 5-methoxytryptamine-α-lipoic acid (a combination of melatonin and α-lipoic acid) on the expression of NOX4 and ROS production in mice hematopoietic cells following whole body irradiation. They showed that both melatonin and 5-methoxytryptamine-α-lipoic acid are able to attenuate ROS production and DNA damage through modulation of NOX4 upregulation [79]. Similar results have been observed for dark tea extract [80]. Also, treatment with metformin or resveratrol can reduce long-term production of ROS in hematopoietic cells through suppression of TGF-β – NOX4 pathway [81, 82].

In addition to the NOX system, upregulation of DUOX1 and DUOX2 may be involved in radiation-induced toxicity in some tissues with high expression of these genes. A study by El-Hassani *et al*. revealed high expression of these genes following exposure to radiation in thyrocyte cells. They showed that exposure of thyrocyte cells to radiation caused chronic upregulation of DUOX1 through activation of p38 by IL-13. They showed that targeting of IL-13 and p38 led to a significant reduction in the expression of DUOX1 and DSBs. Authors suggested that since DUOX1 is upregulated in thyroid cancers, it is possible that radiation through chronic upregulation of DUOX1 and continuous production of free radicals triggers genomic instability and thyroid cancer [83].

NADPH Oxidase and Radiation-Induced Cell Death

Cell death after exposure to ionizing radiation is the most critical response of radiosensitive normal tissues that cause the appearance of side effects in these organs. Apoptosis induction in the bone marrow, intestine, and tongue isone of the most important reasons for organ failure following exposure to an acute high dose of radiation. Chronic ROS production by pro-oxidant enzymes such as NADPH oxidase enzymes is a reason for cell death, especially through apoptosis induction. Tateishi *et al*. evaluated the role of ROS production by NOX1 in the apoptosis induction following irradiation of salivary gland cells. Their study showed that inhibition of NOX1 in normal salivary gland cell lines such as NS-SV-AC and NSSV-DC leads to significant reduction in ROS production and apoptosis. This study showed no significant increase in the expression of other NOX system genes including NOX2, NOX3, NOX4 or NOX5 [84]. In contrast, a study by Wang *et al*. showed a significant increase in the expression of all NOX subfamilies following irradiation of HepG2 cells with carbon ions. Their results confirmed that upregulation of NOX family enzymes induces cell death through the production of free radicals [85].

In addition to the direct production of ROS, NADPH oxidase enzymes are able to stimulate superoxide production by mitochondria, leading to cell death [86]. It has been proposed that NADPH oxidase stimulates mitochondrial-ROS production in hematopoietic stem and progenitor cells, leading to death and inhibition of proliferation following exposure to radiation [87].

NADPH Oxidase and Radiation-Induced Bystander Effect

The bystander effect is a radiobiological phenomenon which is involved in radiation toxicity through the initiation of redox reactions in adjacent non-irradiated cells. Several experimental studies have shown that bystander effect causes free radical production and genomic instability. Thus, it may be involved in second primary cancers after radiotherapy [88-91]. For the first time, Narayanan *et al*. showed that irradiation of human lung fibroblasts with alpha particles leads to the generation of intracellular superoxide and hydrogen peroxide. Their analyses showed that membrane-dependent NOX enzymes are responsible for the production of free radicals. Moreover, they showed that the activation of these enzymes and ROS production do not require direct exposure of cells to ionizing radiation and may be induced in adjacent cells [92]. Azzam *et al.* evaluated the possible role of NADPH oxidase signaling in micronuclei formation in non-irradiated human lung fibroblasts. They showed that irradiation of these cells leads to the upregulation of MAPK genes such as p38 and ERK, as well as transcription factors such as activator protein 1 (AP-1) and NF-kB. This was associated with the production of hydrogen peroxide and superoxide, while incubation of cells with DPI or ROS scavengers suppressed these changes [93].

It has been proposed that the release of oxidized cell-free DNA following cell death is a possible mechanism for the induction of oxidative stress and genomic instability in bystander cells [69]. A study by Sergeeva *et al*. has shown that irradiation of mesenchymal stem cells (MSCs) with a low dose of radiation caused upregulation of NOX4 and increased ROS production in bystander cells. They showed that increased NOX4 expression is detectable 30 minutes after irradiation [94]. Although experimental studies illustrating the direct role of NOX system in bystander effect are limited, there are some evidences for the upregulation of

upstream genes of NOX family in bystander cells and tissues. Increased expression of TGF-β, p38, ERK1/2, NF-kB and other inflammatory mediators such as mitochondrial ROS has been confirmed by several studies [95-101].

NADPH Oxidase and Radiation-Induced Inflammation

Some evidences have given an indication about the role of NADPH oxidase in inflammatory responses following exposure to radiation. Inhibition of NOX system has shown promising results for mitigation of some organs. Inhibition of NOX enzymes by apocynin ameliorates the infiltration of inflammatory cells, epithelial damage and apoptosis induction in rat's intestine [102]. Su *et al.* showed that upregulation of NADPH oxidase and chronic ROS production by these enzymes are involved in radiation-induced inflammatory response in mice skin. This study showed that ROS production by NADPH oxidase induces p38 and NF-kB, leading to the release of prostaglandins (PGs) and inflammatory cytokines such as TNFα, IL-1β, and IL-6. Targeting NADPH oxidase by 18β-Glycyrrhetinic acid could mitigate these changes and subsequent skin damage [103].

Senescence is a type of cell death that has a close relation to the NOX system and triggers inflammation. It has been shown that irradiation of primary mouse embryonic fibroblasts leads to a significant increase in the incidence of senescence. Their results showed a 5-fold increase in the level of β-galactosidase (a marker of senescence). Also, this study showed that among different types of NOX and DUOX isoforms, the expression of NOX4 increased significantly. Interestingly, this study showed that NOX4 is not responsible for senescence, while senescence triggers the expression of NOX4 and free radical production. Although, NOX4 gene expression is independent of NF-kB, its ROS production stimulates the infiltration of inflammatory cells [104].

NOX2 plays a key role in chronic oxidative stress in the brain cells. Irradiating mice brain (40 Gy/8 fractions/4 weeks) showed association with increased level of TNF-α and MCP-1, as well as ROS. These changes were more obvious on the 8th week after irradiation. Selective inhibition of NOX-2 with NOX-2 agonist antibody showed a reduction in free radicals (less than 20%) compared with irradiated mice without NOX-2 agonist antibody [105]. Irradiation of rat's brain microvascular endothelial cells also showed increased ROS production through upregulation of NOX2 and NOX4. Also, it has been shown that NOX2 is involved in the upregulation of NF-kB and ICAM-1 [106].

NADPH Oxidase and Radiation-Induced Fibrosis

In addition to inflammation, there are some evidences suggesting that NADPH oxidase plays a role in the differentiation of myofibroblasts and in the development of fibrosis. An *in vitro* study showed that irradiating primary mice prostate fibroblast cells leads to the activation of NOX4, cell senescence and activation of fibroblasts. While inhibition of NOX4 leads to inactivation of fibroblasts, reduced cell death and inhibition of TGF-β – Smad2/3 signaling pathway, which plays a central role in the development of fibrosis [107]. The pivotal role of NOX system and ROS production in the development of radiation-induced fibrosis has been confirmed by another study by Park *et al.* This study showed that irradiation of lung fibroblast cells leads to immediate ROS production by NOX4. They showed that upregulation of p38 and Akt, but not Erk has a role in chronic upregulation of NOX4 and ROS production. Inhibition of NOX4, p38 or Akt caused the inhibition of ROS production and attenuated the increased expression of α-smooth muscle actin (α-SMA), fibronectin (FN) and extracellular matrix (ECM) accumulation. Results of this study indicated that NOX4 plays a central role in redox activation in fibroblast cells, which stimulate fibrosis through continuous ROS production [108]. Inhibition of p38 following exposure to ionizing radiation has been shown to attenuate redox activation and continuous production of ROS. This was associated with reduced cell senescence, induced by the NOX system [109].

Choi *et al*. evaluated the possible role of NOX1, NOX2, and NOX4 in the myofibroblasts following irradiation of human pulmonary artery endothelial cells (HPAECs). They showed that inhibition of NOX2 and NOX4 had no remarkable effect on fibrotic changes. However, NOX1 inhibition caused significant attenuation of fibrotic changes. In addition, targeting each of these genes led to a reduction in ROS production. Also, in an *in vivo* study, they showed that suppression of NOX1 is associated with a reduction in the expression of pro-fibrotic genes such as α-smooth muscle actin (αSMA) as well as amelioration of collagen deposition [110]. Results of this study were confirmed by another study conducted by Citrin *et al*. They evaluated the expression of different genes using microarray analysis and also histological changes following irradiation of mice lungs. Results showed that radiation-induced cell death through senescence in airway cells plays a key role in the initiation of fibrosis.

Since the NOX system is responsible for senescence, they investigated the role of NOX genes in this process. Their results showed that when NOX enzymes were inhibited by DPI, the induction of senescence in airway cells and fibrosis was attenuated remarkably [111]. Chen *et al*. detected the ROS production and collagen deposition at 1 and 5 months after irradiating murine lungs with 15 Gy. They showed significantly elevated production of ROS at 1 month and collagen deposition at both 1 and 5 months after irradiation. Moreover, their results showed that ROS production by NOX2 and NOX4 in activated macrophages is the main source of free radical production. Also, ROS-derived NOX enzymes could induce myofibroblast activity and collagen deposition. In contrast to another study, this study showed that NOX1 had no significant role in the production of ROS by activated macrophages. Upregulation of NOX system was attenuated through the inhibition of NF-kB [112].

NADPH OXIDASE IN TUMOR RESPONSES TO RADIOTHERAPY

The activation of immune cells and redox reactions have complicated effects on killing and resistance of tumor cells to therapeutic strategies, including radiotherapy. Activated macrophages play a key role in redox activity via regulation of some pro-oxidant enzymes such as NADPH oxidase and iNOS [113]. The role of ROS and NO produced by these enzymes is a double-edged sword [114]. ROS and NO have detrimental effects on cancer cells because of the induction of DNA damage and suppression of DNA repair mechanisms

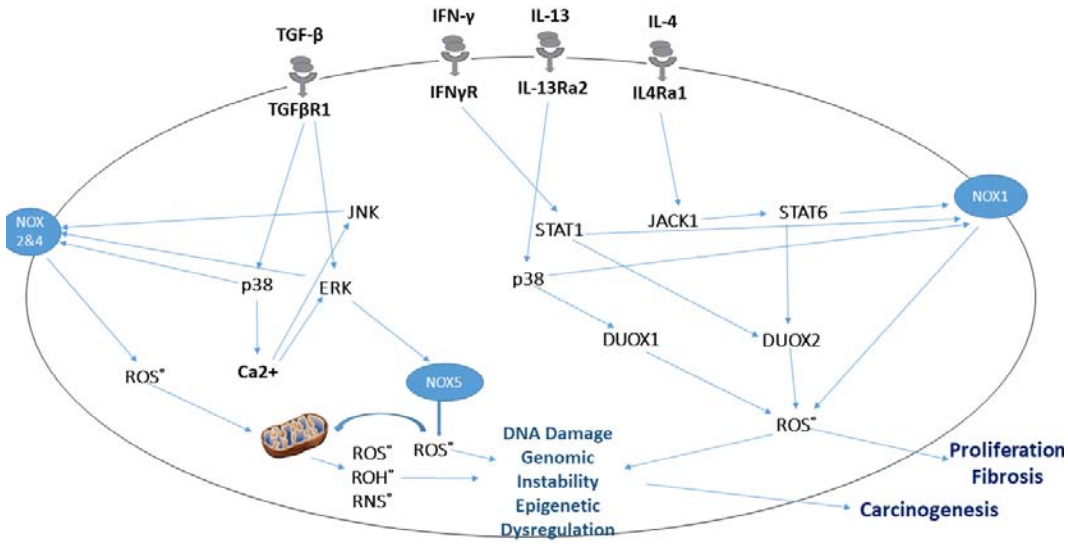

Fig. (2). The mechanisms of radiation-induced NADPH oxidase activation and normal tissue toxicity.

[114-116]. NO derived activated macrophage is able to induce apoptosis through the accumulation of p53 which is necessary for the initiation of apoptosis. Moreover, it can stimulate the downregulation of Bcl-2 and causes damage to mitochondria [117]. Moreover, NO through S-nitrosylation of p50 inhibits the regulation of NF-kB and downstream signaling including the release of prostaglandins by COX-2 [118]. NF-kB – COX-2 signaling plays a key role in tumor resistance via inhibition of apoptosis [119]. Thus, NO can facilitate apoptosis through targeting of this pathway [120]. By contrast, there are several studies that proposed an adaptation of cancer cells to radiation following exposure to free radicals [121, 122]. It has been suggested that the adaptive response of cancer cells results from high rate mutation in cancer genome, induction of hypoxic factors, angiogenesis, and stimulation of cancer stem cells proliferation [123, 124].

In contrast to the toxic effects of NOX and DUOX enzymes on normal cells, some experimental studies have shown a radioresistance role for these enzymes in tumor cells. It has been revealed that upregulation of NOX4 through stimulation of the PI3k/Akt pathway increases both the resistance of cancer cells to apoptosis and survival [65]. Similar results have been observed for non-small cell lung cancer (NSCLC). Zhang *et al.* showed that the expression of NOX4 is increased in samples obtained from patients with NSCLC. They showed that upregulation of NOX4 has a close correlation with tumor resistance and patient survival. Moreover, their results indicated that NOX4 has a direct relationship with the PI3k/Akt pathway and tumor invasion [125]. You *et al.* evaluated the expression of different subfamilies of NADPH oxidase in gastric cancer cells. They showed a high expression of NOX1, NOX2, and NOX4, but no significant increase in the expression of NOX5 as well as DUOX1 and DUOX2 is observed. Moreover, their results indicated that high expression of NOX4 has an indirect correlation with patient survival [126].

It has been confirmed that NADPH oxidase activity can induce resistance to radiotherapy and chemotherapy agents, thus reducing the therapeutic effects of these modalities. Lu *et al.* evaluated the role of NOX family in prostate cancer cell response to ionizing radiation. This is crucial for androgen therapy, which is a common modality for increasing therapeutic ratio. Hormonal therapy was associated with a significant increase in the expression of NOX2 and NOX4, but not NOX5. Inhibition of these enzymes by apocynin or DPI could sensitize prostate cancer cells to ionizing radiation [127]. The role of NADPH oxidase in radioresistance of tumor cells through induction of hypoxia has been detected. Hypoxia is a well-known phenomenon in different types of solid tumors, which play a key role in tumor growth and resistance to radiotherapy. In the hypoxic area of the tumor microenvironment, NOX4 stimulates the regulation of HIF-1, which is a key modulator of tumor angiogenesis through the upregulation of VEGF. Inhibition of NOX4 in glioblastoma-bearing mice tumor attenuates resistance of this tumor to radiation [128].

ROS production by NOX enzymes or other pro-oxidant enzymes is responsible for HIF-1 upregulation and tumor radioresistance [129]. Moreover, ROS production by NOX system can upregulate p38, which promotes radioresistance of G_0 cells [130]. Also, it causes the activation of PTEN and its downstream genes such as protein kinase B (Akt), which is similar to p38 in stimulating the proliferation of tumor cells. This may be involved in the radioresistance of glioblastoma cells [131]. A study by Wu *et al.* proposed the role of ROS-derived NOX2 in the development of inflammation and radioresistance of advanced rectal cancer cells. They showed that NOX2, through stimulation of ataxia telangiectasia mutated (ATM) kinase stimulates the activation of macrophages and their infiltration in the tumor. They proposed that targeting NOX2 may lead to poorer resistance of tumor cells through the reprogramming of macrophage infiltration in tumor cells [132].

In contrast to the aforementioned evidences for the role of NADPH oxidase in radioresistance of tumor cells, it has been reported that DUOX2 upregulation sensitizes gastric cancer cells [133]. Also, it has been shown that the upregula-

tion of NADPH oxidase in prostate cancer cells attenuates antioxidant defense through suppression of reduced thioredoxin and also the inhibition of FOXO3a, which causes attenuation of SOD and CAT. Hence, stimulation of NADPH oxidase by parthenolide induced-ROS elevation and attenuation of antioxidant defense in prostate cancer cells, leads to sensitization of PC3 cells to radiation [134].

CONCLUSION

As mentioned in this review, there are some evidences suggesting that all subfamilies of NADPH oxidase (including NOX1-5 and DUOX1&2) are involved in toxic effects of ionizing radiation on normal tissues through amplification of ROS production. Evidences from *in vitro* and *in vivo* studies proposed that it is possible that NADPH oxidase activity is initiated some minutes after exposure to radiation and may continue for a long time, depending on the irradiated cells/organs. Some *in vivo* studies showed upregulation of NOX enzymes in bone marrow cells some weeks after irradiation, while ROS production is obvious even after one year in intestinal cells. In addition to the direct role of NOX1-5 and DUOX1&2 in ROS production, these enzymes are able to trigger other redox mediators such as the mitochondria that amplify oxidative stress in a positive feedback loop. This leads to more DNA damage and cell death by stimulating more release of pro-inflammatory and pro-fibrotic cytokines.

Several studies showed that NOX1-5 and DUOX1&2 play a key role in acute reactions like apoptosis of bone marrow and gastrointestinal system as well as late effects of radiotherapy such as inflammation and fibrosis. Acute cell death in radiosensitive organs such as bone marrow, tongue, and small intestine is a major cause of mucositis and lymphopenia for patients with head and neck as well as abdomen and pelvic cancers. Modulation of appropriate targets is one of the most important aims in alleviating radiation toxicity in these organs. In experimental studies, inhibition of NOX system and DUOX1&2 showed promising results for suppression of apoptosis, as well as amelioration of genomic instability, which is a hallmark for increased risk of second primary cancers following radiotherapy. Moreover, suppression of NOX1, NOX2, NOX4, and NOX5 has shown interesting results which can help attenuate fibrosis following radiotherapy. Experimental studies indicate potential modulatory effects of some radioprotectors such as metformin, melatonin, and resveratrol on the expression of these genes.

In contrast to toxic effects on normal tissues, some evidences have shown that different subfamilies of the NOX system increase the resistance of tumor cells to ionizing radiation. Evidences from some limited studies proposed that ROS production by NOX system plays a central role in the proliferation of tumor cell, via induction of hypoxia and stimulation of angiogenesis. Hence, suggesting that the activation of p38 by ROS-derived NOX enzymes is a key mediator for the proliferation of tumor cells.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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

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Declared none.

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