The role of mitochondrial oxidative stress and the tumor microenvironment in radiation-related cancer

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ABSTRACT

The health risks associated with low-dose radiation, which are a major concern after the Fukushima Daiichi nuclear power plant accident (the Fukushima accident), have been extensively investigated, and the cancer risks from low-dose radiation exposure (below \( \sim 100 \) mSv) are thought to be negligible. According to World Health Organization and the United Nations Scientific Committee on the Effects of Atomic Radiation reports, the level of radiation exposure from the Fukushima accident is limited, estimating no significant increased risk from the accident. Radiation-induced cell injury is mainly caused by oxidative damage to biomolecules, including DNA, lipids, and proteins. Radiation stimulates metabolic activation within the mitochondria to provide energy for the DNA damage response. Mitochondrial respiratory chain complexes I and III are the most important intracellular source of reactive oxygen species (ROS) during oxidative phosphorylation in eukaryotic cells. Manganese superoxide dismutase and glutathione are key players in redox control within cells. However, perturbation of the antioxidant response leads to chronic oxidative stress in irradiated cells. Excess ROS of mitochondrial origin is reported in cancer-associated fibroblast and promotes carcinogenesis. The aim of this review paper is to discuss critical roles of mitochondria in radiation-related cancer by introducing our recent studies. In particular, elevated mitochondrial ROS in stromal fibroblasts potentiate transforming growth factor-beta (TGF-\( \beta \)) signaling, which triggers smooth muscle actin (\( \alpha \)-SMA) expression to stimulate myofibroblast differentiation. Radiation-induced myofibroblasts promote tumor growth by enhancing angiogenesis. Thus, radiation affects both malignant cancer cells and neighboring stromal cells through secretion of soluble factors.

Keywords: mitochondria; reactive oxygen species; cancer; radiation; tumor microenvironment

INTRODUCTION

Tumor-associated stroma has an important impact on tumor development. Cancer-associated fibroblasts (CAFs) are thought to be a major cellular component of the tumor stroma in many tumors and are distinguishable from their normal counterparts. There is mutual crosstalk between tumor cells and CAFs, which facilitates the growth and invasion of tumor cells by modulating the tumor microenvironment. CAF release a variety of growth factors, chemokines and matrix-degrading proteases to communicate with cancer cells. On the other hand, tumor cells stimulate the ability of CAF to promote angiogenesis, extracellular matrix (ECM) remodeling and metastasis [1–3].

CAFs are generated from various cell types, including fibroblast, epithelial, endothelial and mesenchymal cells [4]. CAFs are thought to arise from activation of quiescent fibroblasts. Basic fibroblast growth factor (bFGF), transforming growth factor-beta (TGF-\( \beta \)) and platelet-derived growth factor (PDGF) activate fibroblasts for connective tissue repair and regeneration following radiation injury [5, 6]. Cellular senescence is associated with clearance of activated fibroblasts to restore a dormant fibroblast state in wound healing [7]. In contrast, chronic tissue wound healing response causes irreversible fibroblast activation and tissue fibrosis, which is likely to be associated with acceleration of tumor progression. However, the roles of tumor...
microenvironments in radiation-induced carcinogenesis remain to be elucidated.

**Fukushima Accident and Epidemiological Studies**

The Fukushima Daiichi nuclear power plant accident occurred on 11 March 2011, following the Great East Japan earthquake. Radioactive materials were emitted from the damaged nuclear plant and spread over the surrounding area. This severe nuclear accident caused radioactive contamination of the environment in Fukushima. In order to mitigate public exposure to radiation, mandatory evacuations from the radiation-affected areas and monitoring and restriction of the distribution of contaminated food products were implemented after the Fukushima accident [8]. The World Health Organization and the United Nations Scientific Committee on the Effects of Atomic Radiation conducted dose assessment after the accident, assessed the health risks and concluded that health risks would not be expected because of the limited radiation exposure among the public [9, 10]. However, mental health problems, such as psychological distress from fear of radiation exposure, were reported among evacuees [11]. Fears of low-dose-radiation health risks, especially the ‘risk of cancer due to radiation exposure’ and ‘genetic effects on offspring’, are a major concern among Japanese citizens.

According to an epidemiological study on the effects of radiation among the children of the Hiroshima and Nagasaki atomic-bomb victims, transgenerational genetic effects did not increase as parental gonadal dose increased [12, 13]. High-dose ionizing radiation can cause leukemia and solid cancer among radiation victims. Radiation-induced cancer is thought to occur because of stochastic effects without a dose-threshold level depending on the absorbed dose: the incidences of leukemia and solid cancer increase in proportion to the radiation dose. Epidemiological studies of the atomic-bomb survivors of Hiroshima and Nagasaki indicated that excess leukemia appeared ∼2 years after radiation exposure, and the incidence of leukemia peaked 6–7 years after the bombing [14, 15]. Solid cancers tended to appear ∼10 years after exposure, and the risk of solid cancer increased for life in survivors [14, 16–18]. Radiation cancer risks depend on the dose received, the age at exposure and sex. The cancer risks among atomic-bomb survivors are slightly higher for females than for males [18].

The radiation effects of internal radiation exposure to 131I were reported after the Chernobyl accident in 1986. An increased risk of pediatric thyroid cancer related to internal radiation exposure to 131I from contaminated milk was evident in epidemiological studies of Chernobyl victims [19–21].

High natural background radiation is present in some areas, including Ramsar in Iran, Kerala in India and Yangjiang in China. Some areas have natural background radiation levels 2–10 times greater than the average level in Japan. Epidemiological studies are underway to identify the health effects associated with the high radiation levels of Kerala, India [22] and Yangjiang, China [23]. In Kerala, the median outdoor radiation levels are >4 mGy/year and the maximum level is as high as 70 mGy/year. However, an increased risk of cancer has not been observed in those areas. In France, the association between environmental factors (including terrestrial and cosmic gamma radiation and indoor radon levels) and acute leukemia incidence was investigated, but no evidence of an association was found [24]. However, the wide confidence intervals in these epidemiological studies indicate the uncertainty of the cancer risks associated with radiation below ∼100 mSv as low-dose radiation effects are thought to be too small to detect as radiation health risks. The health risks associated with long-term, low-dose radiation exposure remain to be elucidated and are currently being investigated intensively to understand the Fukushima case [10, 11].

**Biomarkers of Radiation-Induced Tumorogenesis**

Ionizing radiation biomarkers have been exclusively investigated to elucidate the mechanisms of radiation-related cancers in the field of basic radiation biology. Scientific evidence is required to create a biological model of carcinogenesis to understand the cancer risks of low-dose radiation [25]. Identification of ionizing radiation biomarkers may help in overcoming the limitations of epidemiological studies. However, most potential biomarkers have been demonstrated only in the laboratory, and further development and progress are essential prior to their use for epidemiological studies. The main biological target of radiation is DNA. Double-strand breaks (DSBs) in DNA pose a severe hazard to the genome as erroneous rejoining of DSBs can lead to loss of genomic information. In order to maintain genome integrity in irradiated cells, mammalian cells harbor cell defense systems such as DNA repair, cell cycle checkpoints and apoptosis [26]. Mutational signatures of ionizing radiation associated with radiation-related cancer have been investigated. Hematopoietic tissue is known to be highly vulnerable to radiation, and many cases of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) have been diagnosed among atomic-bomb survivors [27]. Chromosomal translocations of acute myeloid leukemia 1 (AML1), which encode a DNA binding subunit of a component of the AML1–core binding factor β (CBFβ) transcriptional factor complex, are associated with AML [28]. Radiation-induced AML1 mutations are likely to be associated with the transformation of hematopoietic progenitors. Rearrangements in the RET/PTC genes were found to be major events in the development of thyroid cancer in atomic bomb survivors [29]. RET/PTC and BRAF rearrangements have been proposed as radiation signatures for ionizing radiation-associated thyroid cancer [30]. Currently, these have not been thought of as radiation signatures [31]. The radiation signatures among various cancers are not fully understood.

The biologically-based mathematical model of carcinogenesis promises better understanding of the radiation carcinogenesis process [25]. Armitage and Doll proposed a multistage model of carcinogenesis that suggests that multiple successive gene mutations are associated with cancer progression [32]. The model extends to the two-stage clonal expansion model and stochastic multistage clonal expansion models [32, 33]. These models can be applied in understanding the risk of radiation-related carcinogenesis using human epidemiological data [34].
**MITOCHONDRIA AS A BIOLOGICAL RADIATION TARGET**

Radiation-induced cell injury is mainly caused by oxidative damage to biomolecules, including oxidative DNA damage, lipid peroxidation and protein carbonylation [35]. Radiation exposure leads to the generation of reactive oxygen species (ROS) in the radiolysis of water or as a byproduct of oxidative phosphorylation (OXPHOS) within the mitochondria [36]. Mitochondrial respiratory chain complexes I and III are the most important intracellular sources of ROS in eukaryotic cells [37, 38]. Rotenone treatment blocks electron transfer at complex I, and electron leakage leads to the generation of superoxide and the induction of mitochondrial oxidative damage. In contrast, blocking electron transfer in complex III by treatment with antimycin A does not induce mitochondrial damage [39]. These results indicate that ROS release from complex I predominantly cause mitochondrial damage. The mitochondria possess cellular antioxidant defense systems, including manganese superoxide dismutase (MnSOD) and antioxidant glutathione (GSH). Glutathione peroxidases use reduced glutathione to convert hydrogen peroxide into water. Cellular responses to oxidative stress are activated in response to oxidative insults. Nuclear factor E2-related factor 2 (Nrf2) plays a role in the maintenance of cellular redox homeostasis and in the transcriptional control of antioxidant response elements of several cytoprotective genes [40]. Nrf2 also affects mitochondrial respiration and biogenesis [41]. Physiologically controlled ROS levels regulate cell proliferation by activating intracellular signaling pathways [42–44]. However, ionizing radiation-induced excess ROS disrupt mitochondrial functions. Mammalian cells contain mitochondrial DNA (mtDNA), which encodes rRNA, tRNA, and respiratory complexes to control mitochondrial replication and transcription. The mtDNA that is close to intracellular ROS generation sites is more vulnerable to ROS toxicity than the DNA in the nucleus [45, 46]. Mutations in mtDNA alter the expression of proteins required for respiration. Thus, mitochondrial ROS impair OXPHOS, which increases ROS production and damages the mitochondria, establishing a mitochondrial ‘vicious cycle’ [47]. Changes in mitochondrial morphology due to the fusion of healthy mitochondria with damaged mitochondria can be induced by ionizing radiation [48]. Severe mitochondrial damage induces the selective degradation of damaged mitochondria, referred to as ‘mitochondrial autophagy’ or ‘mitophagy’ [49]. During mitophagy, the E3 ubiquitin ligase, parkin, is selectively recruited to impaired mitochondria to promote their degradation [50]. The other aspect of mitochondrial regulation is apoptosis, in which cells die in a programmed manner. Cytochrome c is released from the mitochondria through transition pores, activating an apoptotic cascade, including the activation of caspases [51].

Our recent study sheds light on mitochondrial radiation responses and the role of the mitochondria in radiation-related cancer [39, 52–55]. Radiation stimulates the metabolic processes within the mitochondria to provide energy for DNA damage responses [56]. Studies using normal human fibroblasts have indicated that an acute single-radiation exposure at low or moderate doses transiently increases the mitochondrial ROS level, which is then restored to normal levels 24 h after radiation exposure [39]. In contrast, high doses of acute single-radiation or repeated exposures to radiation increase the mitochondrial ROS level for days. 8-Hydroxy-2′-deoxyguanosine (8-OHdG), a marker of oxidative damage in the DNA, can be used to detect radiation-induced mitochondrial oxidative damage [54]. MnSOD and GSH are key regulators of cellular redox control. Perturbations in antioxidant systems are associated with the induction of chronic oxidative stress in irradiated cells [39]. Further examination is necessary to clarify the mechanisms of radiation-induced mitochondrial ROS and the subsequent induction of oxidative stress. Collectively, radiation induces mtDNA mutation and the loss of mitochondrial function. Mitochondria are thought to be a major target of radiation and oxidative stress via ROS generation in irradiated cells [53, 56].

**ASSOCIATION BETWEEN NUCLEAR DNA DAMAGE RESPONSE AND MITOCHONDRIAL RADIATION RESPONSE**

Figure 1 depicts a schematic diagram of the underlying mechanisms involved in the cross-talk between nuclear DNA damage and mitochondria in irradiated cells. A DNA damage sensor kinase, ataxia telangiectasia mutated (ATM), acts as a master regulator for recognizing DNA DSBs and transmitting damage signals to downstream target molecules, thereby executing DNA damage responses [57]. ATM also controls mitochondrial quality following radiation exposure [39, 52]. ATM phosphorylates AMP-activated protein kinase (AMPK), which senses the AMP/ATP ratio [58]. DNA damage activates the ATM–AMPK–peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α) signaling pathway, which stimulates mitochondrial biogenesis [59]. Previously, we demonstrated that radiation enhances mitochondrial biogenesis via the upregulation of PGC1α expression in an ATM-dependent manner [52]. These results indicate that ATM is a key molecule in the relationship between the nucleus and mitochondria under stress conditions inflicted by ionizing radiation. ATM loss leads to defects in the mitochondrial damage response in human fibroblasts, and severe mitochondrial damage is observed as mitochondrial fragmentation in irradiated cells [52]. Similarly, an abnormality in the mitochondria is reported in vivo in ATM-null mice [60]. The lack of the ATM-mediated mitochondrial damage response is likely to be associated with the highly radiosensitive phenotypes in ATM-deficient cells.

**THE ROLE OF MITOCHONDRIAL OXIDATIVE STRESS IN RADIATION-RELATED CANCER**

Low levels of ROS are required for normal cellular proliferation [61], while excess ROS of mitochondrial origin have been reported in cancer cells and promote carcinogenesis [62, 63]. Antioxidants can prevent the growth of cancer cells and inhibit the metabolic shift required for tumorigenesis [64]. In tumor cells, ROS accumulate because of insufficient antioxidant capacity, and these endogenous ROS damage the DNA, leading to genomic instability, which may contribute to cancer progression [65]. These results indicate a possible role of oxidative stress in carcinogenesis. Metabolic change is a key event in carcinogenesis. Instead of mitochondrial OXPHOS, tumor cells produce a large portion of ATP via glycolysis. Loss of mitochondrial function is caused by mtDNA mutations, which are reported in various types of tumor [66, 67]. Mitochondrial genomic instability is also associated with an increased risk of age-related diseases, including vascular disease...
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**Fig. 1.** Schematic diagram of mutual communication between the nuclei and mitochondria following radiation in mammalian cells.

and neurodegeneration, as well as cancer [68–71]. Therefore, mitochondrial functional defects are thought to be important in radiation-induced carcinogenesis.

**THE ROLE OF THE TUMOR MICROENVIRONMENT IN RADIATION-RELATED CANCER**

In mouse thymic lymphomas, the radiation target is not only the thymocytes but also other tissues. Thymic lymphomas were induced when normal thymus gland freshly isolated from normal donors was transplanted into irradiated hosts [72]. This observation suggested an indirect mechanism for tumor development [73]. Thus, the interaction of the malignant cancer cells with the tumor microenvironment contributes to carcinogenesis.

Tumor-associated macrophages (TAM) are major players in cancer-related inflammation [74]. Macrophages are a common phagocytic cell and are classified into two categories, classical M1-type or the alternative M2-type macrophages. M1 macrophages function as the positive immune response and provide antitumor immunity. In contrast, M2 macrophages have a role in the anti-inflammatory response, immunosuppressive function and pro-tumorigenic properties. TAM have an M2 phenotype and promote tumor cell survival, proliferation and metastasis [75]. Ionizing radiation can alter the tumor microenvironment and leads to immune cell priming via the generation and release of tumor-specific neoantigens and cytokines. The macrophages are actively recruited into tumors and act as pivotal players in the alteration of the tumor microenvironment [76].

Since dormant stem cells will acquire the mutations necessary for tumor development, stem cell populations are believed to be the targets of radiation-induced cancer [77]. The local environment is important to maintain stem cell–niche interactions and asymmetric cell division in the generation of daughter cells intended for differentiation [78]. The ECM allows stem cells to anchor the basement membrane in the local niche environment and to communicate with niche cells. However, disruption of the ECM perturbs stem cell expansion and differentiation, resulting in the generation of cancer stem cells [79, 80]. Matrix metalloproteinase (MMP) is an enzyme that degrades fibrillar collagen for the remodeling of the ECM. MMP is also associated with TGF-β activation in the release of secreted inactive TGF-β-containing latency-associated protein (LAP) [81]. ROS can also activate TGF-β by directly oxidizing LAP [82]. Ionizing radiation has been shown to induce rapid and persistent activation of TGF-β during normal tissue injury and fibrosis [82–84]. This pathway is also implicated in tumor microenvironment formation associated with radiation-induced tumors. Recently, the role of fibroblasts in tumor microenvironment formation associated with radiation-induced cancer was summarized (T. Shimura, submitted for publication). This paper described the role of cancer-associated fibroblasts in radiation-induced carcinogenesis. Nakamura proposed a model of radiation carcinogenesis describing the role of stromal cells in tumor microenvironment formation [85]. The tumor stroma is associated with an increased number of active fibroblasts (Fig. 2) [2, 3]. Stromal fibroblasts release tumor cell growth factors, which communicate with malignant tumor cells. We recently revealed...
the role of stromal fibroblasts in radiation-induced carcinogenesis (Fig. 2) [55]. Radiation alters the morphology of normal human diploid fibroblasts that express myofibroblasts and CAF markers, such as smooth muscle actin (α-SMA) and PDGF receptors [55]. Mitochondrial ROS activate TGF-β signaling, which, in turn, induces α-SMA expression to achieve myofibroblast differentiation [55]. Radiation-induced myofibroblasts promote tumor growth by releasing bFGF and vascular endothelial growth factor (VEGF) to induce angiogenesis in vivo [55]. Furthermore, growth recovery following radiation is associated with myofibroblast clearance, which is essential for restoring stromal cell dormancy and preventing tumor microenvironment formation. However, chronic oxidative stress activates

Fig. 2. Mitochondrial ROS-mediated tumor microenvironment formation associated with radiation-related cancer.
cellular signaling for the maintenance of radiation-induced myofibroblasts, facilitating tumor microenvironment formation. Therefore, targeting ROS using antioxidants is likely to be effective in the mitigation of radiation-related cancer.

CONCLUSION

Induction of mitochondrial oxidative stress in fibroblasts is a key event in radiation-induced cancers via tumor microenvironment formation. Radiation affects not only malignant cancer cells but also the surrounding stromal cells. Further investigation is needed to understand the mechanisms involved in the cross-talk between tumor cells and stromal fibroblasts. Continuing efforts in the study of radiation biology can provide appropriate scientific evidence to aid understanding of the health risks of low-dose radiation.

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CONFLICT OF INTEREST

No potential conflicts of interest were disclosed.

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