Original Article

Modulation of Radiation-induced Base Excision Repair Pathway Gene Expression by Melatonin

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Abstract

Objective: Approximately 70% of all cancer patients receive radiotherapy. Although radiotherapy is effective in killing cancer cells, it has adverse effects on normal cells as well. Melatonin (MLT) as a potent antioxidant and anti-inflammatory agent has been proposed to stimulate DNA repair capacity. We investigated the capability of MLT in the modification of radiation-induced DNA damage in rat peripheral blood cells. **Materials and Methods:** In this experimental study, male rats (n = 162) were divided into 27 groups (n = 6 in each group) including: irradiation only, vehicle only, vehicle with irradiation, 100 mg/kg MLT alone, 100 mg/kg MLT plus irradiation in 3 different time points, and control. Subsequently, they were irradiated with a single whole-body X-ray radiation dose of 2 and 8 Gy at a dose rate of 200 MU/min. Rats were given an intraperitoneal injection of MLT or the same volume of vehicle alone 1 h prior to irradiation. Blood samples were also taken 8, 24, and 48 h postirradiation, in order to measure the 8-oxoguanine glycosylase1 (*Ogg1)*, *Apex1,* and *Xrcc1* expression using quantitative real-time-polymerase chain reaction. **Results:** Exposing to the ionizing radiation resulted in downregulation of *Ogg1*, *Apex1,* and *Xrcc1* gene expression. The most obvious suppression was observed in 8 h after exposure. Pretreatments with MLT were able to upregulate these genes when compared to the irradiation-only and vehicle plus irradiation groups ($P < 0.05$) in all time points. **Conclusion:** Our results suggested that MLT in mentioned dose may result in modulation of *Ogg1*, *Apex1,* and *Xrcc1* gene expression in peripheral blood cells to reduce X-ray irradiation-induced DNA damage. Therefore, administration of MLT may increase the normal tissue tolerance to radiation through enhancing the cell DNA repair capacity. We believed that MLT could play a radiation toxicity reduction role in patients who have undergone radiation treatment as a part of cancer radiotherapy.

Keywords: Base excision repair, gene expression, melatonin, radiation, real-time-polymerase chain reaction

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Introduction

Radiotherapy has been used for the treatment of patients with various types of cancer.[1] It is estimated that about 70% of clinical oncology treatments are performed with radiotherapy or a combination of radiotherapy and chemotherapy.[2] Reactive oxygen species produced in radiotherapy induces a variety of DNA lesions, including oxidized base damage, single-strand breaks (SSBs), and double-strand breaks in normal cells. If these lesions remain unrepaired, it may result in cell death through mitotic catastrophe and apoptosis.[3]

DNA repair plays a critical role in protecting normal cells from malignancy.[4] DNA damage and massive cell death following radiotherapy may lead to severe toxicity in normal tissues and limit radiation dose delivered to patients. Massive cell death after irradiation can also result in long-term side effects such as pneumonia, fibrosis, heart damage, myelopathy, and change in kidney function. Administration of different radiation modifiers including melatonin (MLT) has been proposed for the management of these side effects.[5-8]

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The DNA repair mechanism is controlled by a specific set of pathways and encoding genes of the enzymes that catalyze cellular responses to DNA damage. The lack of repair function or impaired control of repair processes may cause very serious consequences in cells and individuals.[9] However, a high percentage of oxidative DNA damage induced by radiation is repaired by the base excision repair (BER) pathway. Defects in BER have shown to result in hypersensitivity to ionizing radiation (IR).[10,11]

Radiation may upregulate or downregulate the expression of several repair genes. Changes in the expression of these genes can affect the ability of cells to repair DNA damage.[12] The 8-oxoguanine glycosylase1 (*Ogg1)*, *Apex1,* and *Xrcc1* genes are most important for the repair of free radical-induced DNA damage in BER pathway.[13,14] *Ogg1* is a DNA repair gene which removes 7,8-dihydro-8-oxodeoxyguanine.[10,12] Apurinic/apyrimidinic endonuclease *(Apex1)* plays a role in several biological contexts. *Apex1* gene has a key role in BER pathway of DNA damage and transcriptional regulation of several eukaryotic genes.[15] X-ray repair cross-complementing group 1 (*Xrcc1*) is a DNA repair gene involved in rejoining DNA strand breaks.[16] MLT, a hormone secreted by the pineal gland, not only plays an important role in the regulation of circadian rhythm, but also has an anticancer effect by the stimulation of anti-inflammatory and antioxidative pathways.[17] Due to its amphiphilicity, MLT can be found in any cellular compartment.[17,18] In addition, several studies suggest that the MLT can be localized inside the nucleus to protect DNA from oxidative damage and promote DNA stability.[19]

The main purpose of our study was to assess the effect of radiotherapy on BER pathway gene expression in rat lymphocyte cells and investigate protective properties of MLT through improvement of DNA repair capacity in white blood cells (WBCs).

Materials and Methods

Animal preparation

The experimental procedure was in accordance with the guidelines for care and use of laboratory animals as adopted by the Ethics Committee of the School of Allied Medicine, Tehran University of Medical Sciences, Tehran, Iran. Animals were housed in animal facility, with room temperature maintained at 20°C–22°C, relative humidity of 50%–70%, and an airflow rate of 15 exchange/h. Also, a time-controlled system provided 08:00–20:00 h light and 20:00–08:00 h dark cycles. All rats were given standard rodent chow diet and water from sanitized bottle fitted with stopper and sipper tubes.

A total of 162 male Wister rats with body weights of 180–200 g were divided into 27 groups using the "simple random sampling method." The present study was performed in the Research Center of School of Allied Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Melatonin administration

MLT (Sigma Aldrich, USA) at a dose of 100 mg/kg was administered 1 h prior to irradiation to animals by intraperitoneal injection during the designated night period. MLT was first dissolved in a small amount of absolute ethanol and then diluted with phosphate-buffered saline in final ethanol concentration of 5%.

Experimental design and irradiation

In this experimental study, 162 male rats were divided into 27 groups ($n = 6$ in each group) in 3 different time points:

- Groups 1–3: Control (8, 24, and 48 h)
- Groups 4–6: Vehicle (5% absolute ethanol in phosphate-buffered saline) (8, 24, and 48 h)
- Groups 7–9: 2 Gy whole-body gamma radiation (8, 24, and 48 h)
- Groups 10–12: Vehicle + 2 Gy whole-body gamma radiation (8, 24, and 48 h)
- Groups 13–15: MLT (100 mg/kg body weight) +2 Gy whole-body X-ray radiation (8, 24, and 48 h)
- Groups 16–18: MLT (100 mg/kg body weight) (8, 24, and 48 h)
- Groups 19–21: 8 Gy whole-body gamma radiation (8, 24, and 48 h)
- Groups 22–24: Vehicle + 8 Gy whole-body gamma radiation (8, 24, and 48 h)
- Groups 25–27: MLT (100 mg/kg body weight) +8 Gy whole-body X-ray radiation (8, 24, and 48 h).

The rats were irradiated with a 6 MV X-ray linear accelerator machine (Elekta Compact 6 MV, China) with a fixed field size of 35 cm × 35 cm at room temperature (22°C ± 2°C). Before irradiation, to ensure the output of the accelerator, dosimetry and calibration were performed using an ionizing chamber based on International Atomic Energy Agency TRS-398 standard. The selection of 1 h interval between MLT injection and exposure to X-ray radiation was largely based on previous studies.[20,21] Additionally, the MLT concentrations and the dose of X-ray radiation selected were based on the experience from the studies performed by other researchers.[20,22]

RNA extraction and quantitative polymerase chain reaction

Analysis of the *Ogg1*, *Apex1,* and *Xrcc1* mRNA expression in WBCs was carried out by quantitative polymerase chain reaction (PCR) after incubation of control and pretreated cells for 8, 24, and 48 h postirradiation. The total cellular RNA was isolated from WBCs. RNA purification was performed by Trizol and the purified samples was isolated using high-pure RNA extraction kit (Gene all, Seoul, South Korea) based on the manufacturer's instructions. Integrity of RNA was evaluated by electrophoresis in ethidium bromide stained in 1% agarose-Tris-borate ethylenediaminetetraacetic acid gels. The result of absorbance ratio of A260 nm/A280 nm was >1.9. For cDNA synthesis, 1 µg of total RNA was denatured at 65°C for 10 min and reverse transcribed for 30 min at 45°C using a cDNA synthesis kit (Gene all, Seoul, South Korea) in a final volume of 20 µl, in the presence of 500 ng of 12–18 primers oligo.[23]

Gene Runner software (Version 3.05; Hastings software Inc., Hastings, USA) was used for primer design. All rat primers were

designed by Gene Runner software. The sequences of forward and reverse primers were as follows. *Ogg1 (NM_030870),* forward primer: *5'-CAA CAT TGC TCG CAT CAC TGG-3'*, reverse primer, *5'-GGC TTT AGC ACT GGC ACA TAC A-3', Apex1(NM_024148),* forward primer: *5'-GAT GAA TGC CCG CTC TAA G-3',* reverse primer: *5'-GTG TCA CAG TGC TAG GTA A-3', Xrcc1(NM_053435)*, forward primer: *5'-CCC ATC TGA GAG CCG AAG T-3',* reverse primer: *5'-CGT AGG GTG AGT CCT TGC TG-3',* and glyceraldehyde-3-phosphate dehydrogenase (Gapdh) *(NM_017008)* forward primer: *5'-TTC ACC ACC ATG GAG AAG GC-3'*, reverse primer: *5'-GGC ATG GAC TGT GGT CAT GA-3'*. The level of expression of Gapdh gene served as an internal control.

The amplification conditions and cycle protocol were applied as follows: 95°C for 2 min for initial denaturation and then 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 15 s, and elongation at 72°C for 15 s. We determined the real-time PCR efficiency for target genes and internal control genes with the slope of a linear regression model.[24] Each cDNA sample was bulked and used as the PCR template ranging from 2 to 50 ng.[5] The PCR efficiency of both target and internal control genes was calculated by measuring the threshold cycle (CT) in a specific threshold for a serial dilution of bulked cDNA. Three blood samples were assessed for each group.

For each sample, assays were run in triplicate. The comparative $2^{-\Delta\Delta CT}$ was used to show the relative fold changes in the expression of target genes (*Ogg1*, *Apex1*, and *Xrcc1*) to normalize an endogenous reference (GAPDHgene), a relevant untreated and unirradiated control.[25] ΔΔCT is the difference between the mean ΔCT (treatment group) and mean ΔCT (control group), whereas ΔCT is the difference between the mean CT gene of interest and the mean CT internal control gene in each sample.

Statistical analysis

Each data point represents mean ± standard error of the mean of at least three independent experiments per group. A one-way analysis of variance was performed to compare different groups, followed by Tukey's multiple comparison tests. $P < 0.05$ was considered to represent a statistically significant difference.

Results

mRNA expression of BER pathway

As shown in Figure 1a, in the irradiation-only group, the expression of *Ogg1* was significantly decreased at the initial 8, 24, and 48 h relative to control groups (0.62 ± 0.04 folds, 0.64 ± 0.05 folds, and 0.69 ± 0.17 folds, respectively, $P < 0.05$). In the vehicle plus irradiation group, the expression of *Ogg1* compared to irradiation-only group did not present any significant difference at all time points. However, in the MLT pretreatment group, *Ogg1* expression was significantly upregulated in comparison with irradiation-only and vehicle plus irradiation groups at 8, 24, and 48 h postirradiation (3.07 ± 0.09 folds, 3.42 ± 0.13 folds, and

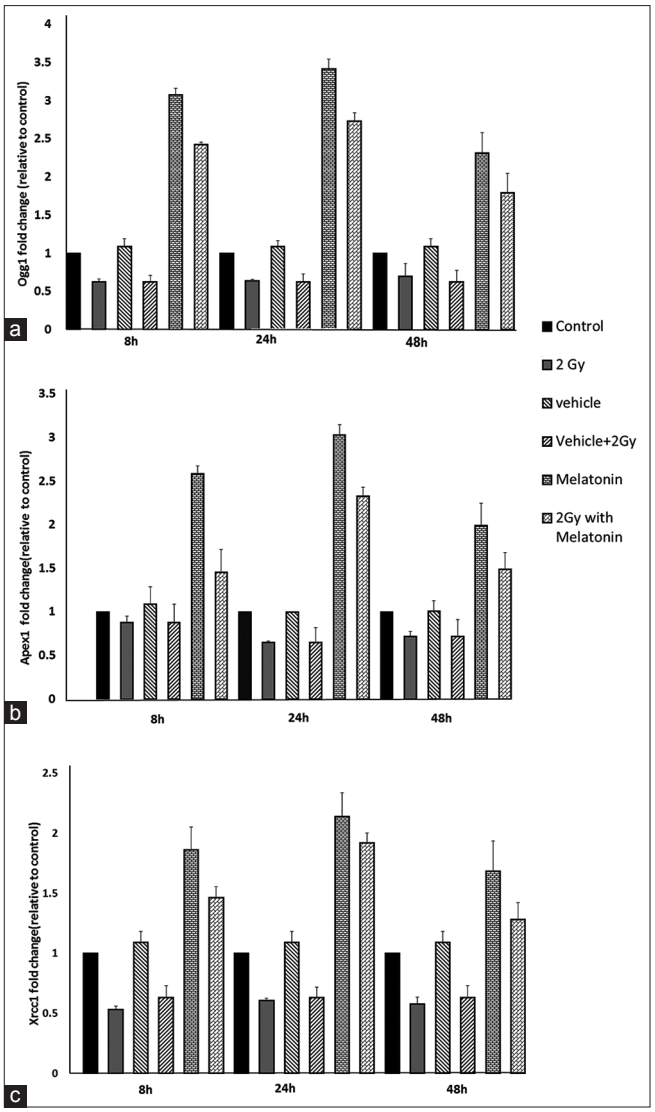

Figure 1: (a-c) Real-time polymerase chain reaction analysis of the fold changes of *Apex1*, *Ogg1,* and *Xrcc1* at various time points after irradiation (relative to control). Values are expressed as mean ± standard error of the mean of three independent samples, each performed in triplicate. (a) ($P < 0.01$) compared to the control group, (b) ($P < 0.01$) melatonin with 2 Gy compared to the 2 Gy groups

2.3 ± 0.27 folds, respectively, $P < 0.05$). Additionally, MLT pretreatment with 2 Gy irradiation significantly increased *Ogg1* gene expression than 2 Gy irradiation alone in all time points (2.14 ± 0.03 folds, 2.72 ± 0.11 folds, 1.79 ± 0.25 folds, respectively, $P < 0.05$). *Ogg1* expression in MLT pretreatment of 2 Gy irradiation was lower compared to MLT alone group. As shown in Figure 2a, also *Ogg1* expression in 8 Gy irradiation significantly decreased at the initial 8, 24, and 48 h relative to control groups (0.57 ± 0.05 fold, 0.47 ± 0.2 fold, and 0.38 ± 0.02 fold, respectively). In the vehicle plus irradiation group, the expression of *Ogg1* compared to irradiation-only group did not present any significant difference at all time points. Data showed that MLT significantly increased *Ogg1* expression after exposed to 8 Gy radiation (1.44 ± 0.23 folds, 1.53 ± 0.06 folds, and 1.38 ± 0.26 folds).

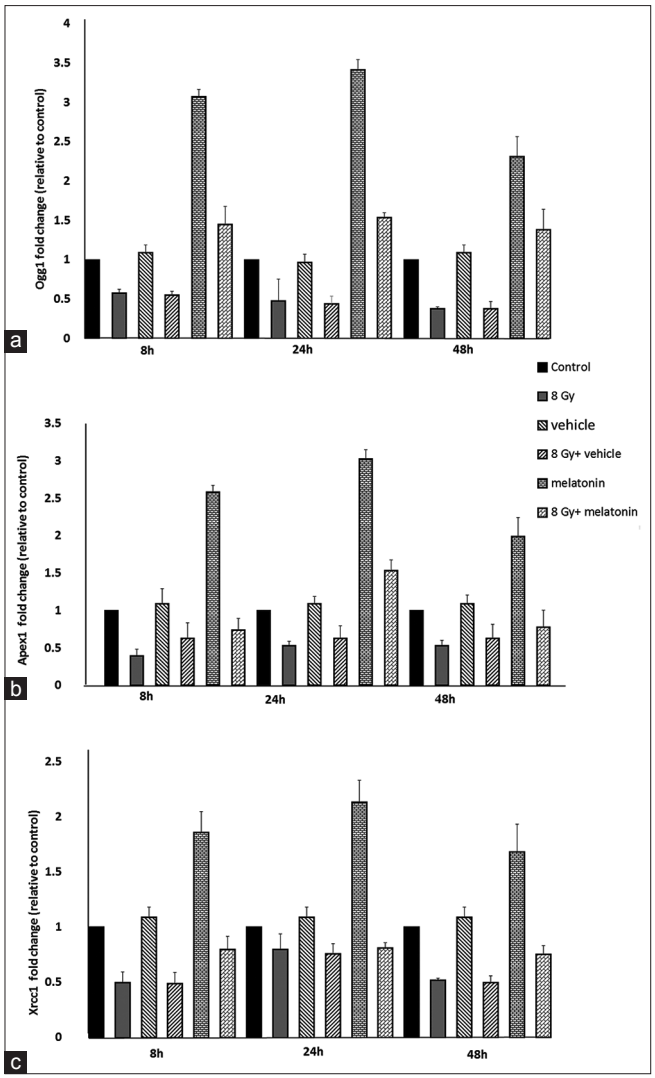

Figure 2: (a-c) Real-time quantitative real-time-polymerase chain reaction analysis of the fold changes of *Apex1*, *Ogg1,* and *Xrcc1* at various time points after irradiation (relative to control). Values are expressed as mean ± standard error of the mean of three independent samples each performed in triplicate. (a) ($P < 0.01$) compared to the control group, (b) ($P < 0.01$) melatonin with 8 Gy compared to the 8 Gy groups

As shown in Figure 1b, *Apex1* gene expression in the irradiation-only group was significantly decreased at the initial 8, 24, and 48 h after radiation in comparison with control group (0.88 ± 0.07 fold, 0.64 ± 0.06 fold, 0.71 ± 0.06 fold, respectively, $P < 0.05$). In the vehicle plus irradiation group, the expression of *Apex1* compared to irradiation-only group did not show significant difference at all time points. In the MLT pretreatment group, *Apex1* expression was significantly upregulated in comparison with irradiation-only and vehicle plus irradiation groups at 8, 24, and 48 h postirradiation (2.58 ± 0.09 fold, 3.03 ± 0.12 fold, 1.98 ± 0.26 fold, respectively, $P < 0.05$). Also, pretreatment with 2 Gy irradiation increases the *Apex1* gene expression compared to the 2 Gy irradiation alone in all time points (1.45 ± 0.26 fold, 2.33 ± 0.1 fold, and 1.49 ± 0.19 fold, respectively, $P < 0.05$). *Apex1* expression in MLT pretreatment of MLT with 2 Gy irradiation was lower when compared to the MLT alone group. However, as shown in Figure 2b, *Apex1* expression in 8 Gy irradiation decreased at the initial 8, 24, and 48 h relative to control groups (0.39 ± 0.09 fold, 0.53 ± 0.08 fold, and 0.32 ± 0.02 fold, respectively). In the vehicle plus irradiation group, the expression of *Apex1* compared to irradiation-only group did not present any significant difference at all time points. Data showed that MLT enhances *Apex1* expression after exposed to 8 Gy radiation in 8, 24, and 48 h after radiation (0.73 ± 0.17 fold, 0.77 ± 0.05 fold, and 0.6 ± 0.1 fold, respectively).

As shown in Figure 1c, in 2 Gy irradiation-only group, the expression of *Xrcc1* was significantly decreased at the initial 8, 24, and 48 h compared to control group (0.53 ± 0.05 fold, 0.6 ± 0.1 fold, and 0.57 ± 0.15 fold, respectively, $P < 0.05$). In the vehicle plus irradiation group, the *Xrcc1* expression did not show any significant difference at all time points when compared to the irradiation-only group. However, in the MLT pretreatment group, *Xrcc1* expression was significantly upregulated in comparison with irradiation-only and vehicle plus irradiation groups at 8, 24, and 48 h postirradiation (1.85 ± 0.19 folds, 2.13 ± 0.2 folds, and 1.68 ± 0.2 folds, respectively, $P < 0.05$). Moreover, MLT pretreatment with 2 Gy irradiation resulted in more significant increase in *Xrcc1* expression than 2 Gy irradiation alone (1.46 ± 0.07 folds, 1.91 ± 0.08 fold, and 1.28 ± 0.14 folds, respectively, $P < 0.05$). Although the*Xrcc1* expression in MLT pretreatment with 2 Gy irradiation was lower compared to MLT alone group, it has been shown in Figure 2c that *Xrcc1* expression in 8 Gy irradiation decreased at the initial 8, 24, and 48 h relative to control groups (0.49 ± 0.1 fold, 0.79 ± 0.25 fold, and 0.51 ± 0.08 fold, respectively). In the vehicle plus irradiation group, the expression of *Xrcc1* compared to irradiation-only group did not present any significant difference at all time points. Data showed that MLT enhances *Xrcc1* expression after exposed to 8 Gy radiation (0.79 ± 0.12 fold, 0.81 ± 0.04 fold, and 0.75 ± 0.06 fold).

Discussion

BER pathway plays a key role in DNA damage responses and cell survival after exposure to IR.[26] Suppression of different genes involved in this pathway, such as *Ogg1*, *Apex1,* and *Xrcc1,* has been shown to be associated with different cancers.[27] *Ogg1,* an important repair enzyme involved in the BER pathway, plays an essential role in cell survival and repair of 8-hydroxyguanine in damaged DNA.[26] In addition, *Ogg1* and *Apex1* have a pivotal role in the simulation of BER pathway.[28]

Mutation in these genes results in hypersensitivity to a wide range of mutagenic agents including IR. A reduction in *Ogg1*, *Apex1,* and *Xrcc1* gene expression can lead to decreased SSB repair capacity that may warrant genomic stability. Also, *Apex1* has a crucial role in the stimulation of *Ogg1* gene expression.[29,30]

In this study, we investigated whether the exposure to radiation can inhibit the expression of genes involved in BER

pathway. Our data suggested that the irradiation suppresses the expression of *Ogg1*, *Apex1,* and *XRCC1* genes during 8, 24, and 48 h after exposure. As previous studies have shown, the expression of BER pathway genes is dependent on *Apex1* and *Ogg1*.[31,32] Hence, inhibition of *Ogg1* and *Apex1* postirradiation can result in suppression of other genes involved in BER pathway. Nitric oxide (NO) produced by IR can inhibit 8-oxodG BER mechanism, resulting in accumulation of DNA damages.[29] Inhibition of BER pathway by NO has been investigated for a homolog of *Ogg1* protein. This may be related to a nitrosylation of cysteine residues in the zinc-finger motif of the *Ogg1* protein after exposing to NO.[33] The repair failure of 8-oxodG increases the mutagenesis and is expected to promote cancer initiation and progression. Studies shown that mutations of the *Ogg1* gene could be associated with several human cancers such as lung, kidney, and gastric cancer.[34] Therefore, stimulation of these genes may facilitate DNA repair capacity and increases cell survival after exposure to IR.

MLT, a potent antioxidant and anti-inflammatory agent, has been proposed to stimulate DNA repair pathways.[32,35] Since MLT was initially identified as a free radical scavenger in 1993, many studies have shown that MLT may protect DNA against free radical damages by stimulating DNA repair capacity and antioxidative enzymes, as well as scavenging free radicals.[6,23,36,37] In the current study, we examined the effects of preadministration of MLT on SSB response pathway including *Ogg1*, *Apex1,* and *Xrcc1* after exposure to IR. Our result showed that pretreatment with MLT upregulates the expression of *Ogg1*, *Apex1,* and *Xrcc1* genes for 8, 24, and 48 h postirradiation. These results indicate a positive effect of MLT on DNA repair capacity after exposure to IR. The stimulatory effects of MLT in BER pathway observed are aligned with previous findings.[38,39]

In previous studies, we showed that pretreatment with MLT can enhance the expression of genes involved in homologous recombination (HR) and nonhomologous end joining. MLT showed increase in the expression of Ku70 at 8 h and 24 h after IR but not at 48 h. Moreover, melatonin could increase the expression of Xrcc4 in 8, 24, and 48 h after IR.[40,41]

In our previous study, we showed that administration of MLT before IR enhances the expression of two HR genes including Cdkn1a and Rad50.[42]

However, the exact mechanisms of stimulatory effects of MLT on the expression of DNA damage response genes remain unknown. Some mechanisms such as the scavenging effect of NO and inducible NO synthase gene silencing through inhibiting p52 acetylation may be involved in the upregulation of these genes during the administration of MLT.[43] Upregulation of p53 activity after administration of MLT is another mechanism that may be involved in inducing BER pathway following irradiation. MLT can enhance p53 acetylation through downregulation of murine double minute-2 gene expression.[44] On the other hand, p53 enhances the removal of 8-oxoG during oxidative stress conditions.[45] Furthermore, p53 stimulates BER pathway by interacting with DNA polymerase β and *APEX1*/*REF1*.[38]

Conclusion

Although the molecular mechanisms of MLT in cancer-related biological pathways are still largely unclear, our data suggest that MLT may contribute to the amelioration of DNA damage and genomic instability in cancer patients who are undergoing radiotherapy. Our data suggest that MLT may participate in the regulation of several key genes involved in DNA damage repair pathways. However, further *in vivo* and clinical trial studies are needed to clarify DNA repairing mechanism and other properties of this agent. Future investigations might therefore utilize alternative strand break visualization technologies, including phospho-H2AX labeling, to provide conformational evidence, to assess the kinetics of MLT's effect on DNA repair, and to measure the response in specific biological pathways that lead to MLT induction at the protein level.

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Conflicts of interest

There are no conflicts of interest.

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