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REVIEW ARTICLE

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Melatonin: A pleiotropic molecule that modulates DNA damage response and repair pathways

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Abstract

DNA repair is responsible for maintaining the integrity of the genome. Perturbations in the DNA repair pathways have been identified in several human cancers. Thus, compounds targeting DNA damage response (DDR) hold great promise in cancer therapy. A great deal of effort, in pursuit of new anticancer drugs, has been devoted to understanding the basic mechanisms and functions of the cellular DNA repair machinery. Melatonin, a widely produced indoleamine in all organisms, is associated with a reduced risk of cancer and has multiple regulatory roles on the different aspects of the DDR and DNA repair. Herein, we have mainly discussed how defective components in different DNA repair machineries, including homologous recombination (HR), nonhomologous end-joining (NHEJ), base excision repair (BER), nucleotide excision repair (NER), and finally DNA mismatch repair (MMR), can contribute to the risk of cancer. Melatonin biosynthesis, mode of action, and antioxidant effects are reviewed along with the means by which the indoleamine regulates DDR at the transduction, mediation, and functional levels. Finally, we summarize recent studies that illustrate how melatonin can be combined with DNA-damaging agents to improve their efficacy in cancer therapy.

KEYWORDS

cancer, chemotherapy, circadian rhythm, DNA, genome stability, pineal gland

1 | INTRODUCTION

DNA repair machinery is essential for maintaining genomic integrity, and it is often altered in human cancers.[1] To preserve the integrity of the genome, cells trigger a specific cellular response, referred to as DNA damage response (DDR), which consists of a complex series of interlocking mechanisms, intra- and intercellular signaling events, and different enzymatic activities.[2] DDR is initiated with the detection of DNA damage and involves the regulation of DNA replication, cell cycle arrest, and the repair or bypass of DNA damage; in the case of unrepairable damage, DDR takes part in cell fate decisions including induction of cell death.[3] Several forms of DNA damage trigger responses by various repair machineries

and signaling pathways, and finally, different DDR pathways may compensate in the absence of the optimal repair.[2]

In human cells, DNA repair is carried out through five major mechanisms, namely base excision repair (BER), homologous recombination repair (HRR), and nonhomologous end-joining (NHEJ), nucleotide excision repair (NER), and the mismatch repair (MMR). Modified bases, oxidative lesions, alkylation products, and the DNA single-strand breaks (SSBs) are the most common forms of DNA damage; they are repaired by the BER pathway.[4] DNA double-strand breaks (DSBs) are the most deleterious form of DNA damage, because they are associated with chromosome breaks and translocations during cell division. DSBs are repaired by two major pathways, namely HRR and NHEJ. HRR is a conservative process, which acts primarily in the S and G phases of the cell cycle and depends on the presence of undamaged homologous chromatid DNA.[5] In contrast to HRR, which is accurate and very efficient, NHEJ pathways (C-NHEJ and alt-NHEJ) are less accurate and occur throughout the cell cycle.[5] NHEJ pathway works on replicated DNA with potential DNA deletions and mutations. The NER pathway is activated by modified nucleotides or bulkier single-strand lesions that distort the DNA helical structure. Such damage can be induced by UV and platinum salts.[6] Finally, MMR pathway is capable of repairing replication errors, including single-base mismatches as well as a wide variety of small-nucleotide insertions and deletions in the genome.[6] As DNA repair plays a substantial role in the development, progression, and response to therapy in a wide range of cancers, it is not surprising that there is substantial effort to validate DDR and repair proteins as therapeutic targets for cancer drug development.[2,7]

Melatonin (N-acetyl-5-methoxytryptamine) is a small lipophilic molecule secreted by the pineal gland and produced in many other organs[8,9]; in the pineal gland, its synthesis varies in a circadian and seasonal pattern. The biological effects of melatonin are mediated by several major mechanisms, some of which employ G protein-coupled membrane receptor signaling[10-13] and some of which are receptor independent.[14] A cytosolic melatonin receptor, which has been identified quinone reductase 2 (QR2), is a known detoxifying enzyme. It exhibits rapid ligand association/dissociation kinetics and an exclusive pharmacological profile in different tissue types.[15] Melatonin has also been demonstrated to bind to nuclear orphan receptors, RZR/RORα to change the transcription of several genes that have important functions in cellular proliferation, such as p21 and 5-LOX.[16-18] Accumulating studies have indicated that high levels of melatonin (N-acetyl-5-methoxytryptamine), secreted from pineal gland, decrease the risk of developing cancer.[19,20] It is suggested that melatonin is involved with the activation of tumor suppressor pathways that participate in the prevention of malignant transformation.[21] In addition, melatonin is a powerful antioxidant acting directly by scavenging free radical species[22-24] and by regulating the gene expression profile of antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase.[25,26] Herein, we focus on the cellular response to DNA damage and major roles of DDR in cells. After a general overview of how such lesions are generated, signaled, and repaired, we outline recent work characterizing the regulatory effects of melatonin on different aspects of the DDR and DNA repair processes.

2 | THE DNA DAMAGE RESPONSE AND DNA REPAIR

The DNA damage response (DDR) is a functionally complex network encompassing signal transduction, cell cycle regulation, DNA repair, replication bypass mechanisms, and apoptosis; it has been well conserved in many organisms from yeast to humans.[27] To preserve genomic stability and integrity, cells have developed the DDR to overcome DNA lesions that are caused by various genotoxic agents arising from different sources.[28] DDR is initiated by early phosphorylation-driven signaling cascades that sense DNA damage, and is regulated by various mediator signaling proteins, which finally activate downstream effectors to determine cell fate by arresting the cell cycle either to repair damaged DNA or to initiate cell death, when the damage is irreparable.[29] DDR pathways contain three major components (some with overlapping functions): sensors, mediators, signal transducers, and effectors.[30]

2.1 | DDR sensors/transducers

To properly preserve the genome, all forms of DNA structural damage must be recognized, including gaps, nicks, SSBs, DSBs, and a myriad of alterations that prevent DNA replication. There are five independent molecular complexes that act as sensors and signal transducers of different types of DNA damage.[31] Three families of the phosphoinositide-3-kinase-related kinases, ATM, ATR and DNA-PKcs, are the most extensively studied transducers in the DDR pathway.[32] DSBs are recognized by Mre11–Rad50–Nbs1 (MRN) mediator complex which recruits ATM to broken DNA molecules.[33] Therefore, in response to DSBs, the inactive dimer ATM is stimulated by dissociation and eventual intermolecular phosphorylation of multiple residues. This frees the monomers for phosphorylation of other downstream substrates.[34] ATM kinases also phosphorylate Chk2, p53, and BRCA1, which are involved in regulating the induction of DNA repair and cell cycle checkpoints. In addition, MRN might recruit substrates to ATM.[35] Moreover, a variety of other proteins such as PP5 phosphatase and the histone acetyltransferase, Tip60, have been shown to be involved in ATM activation.[34]

When DNA polymerases are triggered, DNA unwinding continues ahead of the replication fork by the MCM replicative helicases, leading to the production of ssDNA, which is then

bound by the single-strand binding protein complex RPA,[35] The ssDNA–RPA complex plays two pivotal functions: first, recruitment of ATR through its regulatory subunit ATRIP and second, the recruitment and activation of Rad17 clamp loader which then attaches the PCNA-related 911 (Rad9–Rad1–Hus1) complex onto DNA.[36] Thereafter, ATR phosphorylates Rad17 and 911, which are important for downstream signaling. ATR is activated during this process.[37] Overall, ATR kinases are induced by ultraviolet light-mediated damage and detection of SSD molecules, such as stalled replication forks and DSBs.[37] ATR kinases principally phosphorylate Chk1 kinases and control genomic stability and cell cycle checkpoints. Another key molecule in this context is TopBP1.[38] TopBP1 is required for DNA replication and checkpoint signaling. In addition to its role in DNA replication, TopBP1 binds to the 911 complex and contains a domain that binds ATRIP and activates ATR kinase. TopBP1 is a substrate for ATR and ATM, and its phosphorylation appears to be required for its ability to function in checkpoint signaling.[33] DNA-PKcs are induced upon the detection of DSBs and subsequently autophosphorylate as well as phosphorylate and other substrates. PKcs play an important role in DSB repair through NHEJ.[34]

2.2 | DDR mediators

Mediators promote the interactions between ATM and ATR kinases and their downstream effector kinases. They also contribute to the recruitment of other substrates and act as scaffolds onto which complexes are assembled.[31] Upon DNA damage, ATM, ATR, and DNA PK phosphorylate the histone variant H2AX on Ser-139 and recruit Mdc1 to amplify H2AX phosphorylation, possibly by tethering ATM or preventing H2AX dephosphorylation.[39] Mdc1 and H2AX also facilitate the recruitment of several other proteins to sites of damage leading to the production of IR-induced foci (IRIF). 53BP1 is then recruited to IRIFs, which is a H2AX- and Mdc1-dependent process. An E3 ubiquitin ligase, Ubc13-Rnf8, is recruited by phosphorylated Mdc1 and subsequently ubiquitinates H2AX and likely additional proteins. This leads to the recruitment of 53BP1 and the BRCA1 "A complex".[40-42] Ubiquitin foci at IRIFs rely on Ubc13, Rnf8, and BRCA1, the last one being a ubiquitin ligase itself.[43] There are various causes of DNA damage including oncogenic mutilation, telomere shortening, and chemotherapy. Each of these effects is similar, leading to the activation of p53 resulting in senescence, cell cycle arrest, or apoptosis, thereby suppressing tumorigenesis.[44]

2.3 | DDR effectors

Over the past decade, biased screens for ATM and ATR substrates and single protein analyses discovered more than 700 proteins phosphorylated in response to UV or IR.[32] These studies showed a large number of proteins that potentially contribute to DNA replication; these included ORCs, MCMs, GINS, RFC, TopBP1, and DNA polymerase complexes, findings that explain how fork stability and slowing of DNA replication are regulated in response to DNA damage.[45,46] Enrichment was also revealed for factors with distinct functions in direct DNA repair such as nucleotide metabolism, transcription-coupled repair, bypass polymerases, global excision repair, cross-link repair, MMR, and HR. In addition to the critical role of DDR in controlling cell cycle, these connections emphasize the essential function of the DDR pathways in directly controlling genomic stability and DNA repair.[31] For example, the Chk1-mediated phosphorylation of Rad51 is crucial for HR, and ATR-mediated phosphorylation of FANCD2 regulates cross-link repair. Most interestingly, there are a huge number of novel connections to pathways and protein groups not previously known to be directly involved in the DDR such as RNA splicing, insulin signaling, nonsense-mediated decay, mitotic spindle and kinetochore proteins, the spindle checkpoint, chromatin remodeling, tumor suppressors, and a multitude of transcription factors.[31] Moreover, a not unexpected series of connections to the master circadian clock has appeared.[47] Furthermore, the substrates of kinases, including Chk1, Chk2, casein kinases, and MK2 kinase, are under investigation for their potential implication in the DDR response.

Cell cycle status governs the cellular response to DNA damage. Because cells in G1 phase do not have sister chromatid DNA available as a template, they depend on NHEJ repair system for correction of DSBs. In addition, there are significant variations in the key roles of checkpoints at various stages of the cell cycle and in the DDR factors that are involved. Hence, DDR factors, including ATM, p53, and CHK2, regulate the G1/S checkpoint, which check the repair of DNA damage prior to the start of DNA replication in order to remove obstacles against DNA synthesis.[48] The intra-S phase checkpoint proteins ATR, DNAPK, WEE1, and CHK1 can delay replication origin firing to provide time to deal with any unrepaired DNA damage, therefore preventing under-replicated DNA regions being taken beyond S phase.[49] G2/M checkpoint proteins including MYT1, CHK1, and WEE1 cause a rise in phosphorylated CDK1, thereby keeping it in its inactive state and delaying mitotic entry. The pivotal and complicated role of the DDR in maintaining the genomic integrity is a good reason why DDR represents an important source of anticancer drug targets.[49]

3 | FUNCTION OF MELATONIN IN DDR

3.1 | Melatonin: an antioxidant that prevents of DNA damage

An essential feature of the high efficacy of melatonin as an antioxidant is its amphiphilic nature, and its ready passage

through all morphophysiological barriers.[50,51] Its cytoprotective effects are unequivocally linked to its direct and indirect antioxidant properties.[22,24,52-56] The experimental evidence has repeatedly documented melatonin's role as a direct free radical and ROS scavenger. Research has also proven that melatonin is as an indirect antioxidant which it executes this action via several mechanisms: (i) activating antioxidant enzymes, (ii) protection of antioxidative enzymes from oxidative damage, (iii) stimulation of glutathione synthesis, (iv) enhancing the activities of other antioxidants (or vice versa), (v) binding of transition metals, (vi) inhibition of prooxidative enzymes through epigenetic mechanisms, and (vii) increasing the efficiency of mitochondrial electron transport chain (ETC), thereby lowering electron leakage and reducing free radical generation,[24,57] etc. As a result of these multiple actions, melatonin readily controls DNA damage and more generally molecular damage, in response to oxidative stress.

The antioxidant potential of melatonin has been exhaustively investigated using numerous animal and human models, under conditions such as age-related neurodegeneration, left ventricular hypertrophy, and drug-induced nephrotoxicity and hepatotoxicity.[58-60] Furthermore, this versatile agent shields DNA from the deleterious effects of physical agents, ionizing radiation, toxins, and lipopolysaccharides.[61-63] The DNA damage as a result of these exposures is a function of toxic free radical generation. Because the major cause of DNA damage are the elevated ROS levels, the protective effect of melatonin is likely attributed to its antioxidant potential. The ability of the low molecular weight melatonin to pass through cell membranes[51] enables it to provide on-site protection of DNA against locally generated free radicals.[64,65] Table 1 provides a comprehensive list of studies about the protective roles of melatonin against the DNA damage induced by various factors.

3.2 | Regulation of DDR sensors/transducers

In response to DNA damage, ATM is activated by autophosphorylation on serine residues, and then phosphorylates the downstream effectors of DDR. Recently, melatonin was shown to protect the male reproductive system against γ-radiation-induced injury in mice.[95] In this report, melatonin protected against radiation-induced sperm abnormalities, motility and viability and lipid peroxidation as well as DNA strands breaks; this was associated with an increase in the total antioxidant capacity of the tissue. To assess the potential role of melatonin in regulating ATM-dependent DDR signaling in testes of irradiated mice, the authors evaluated the expression of ATM after γ-irradiation by western blotting. ATM was upregulated postirradiation and showed roughly 10-fold rise in comparison with the control. Pretreatment with melatonin suppressed the expression of ATM fivefold in irradiated mice, compared to the untreated irradiated controls. Thus, the protective actions of melatonin against radiation-induced DNA damage were found to be ATM dependent. In contrast, in another study by Santoro et al.,[96] melatonin inhibited DNA damage induced by caffeine in an ATM-independent manner. Melatonin-induced reduction in DNA damage did not require ATM activity as it occurred in both ATM−/− and ATM+/+ human fibroblasts and upon caffeine-mediated inhibition of ATM kinase activity. As melatonin was found to reduce DNA fragmentation even in the absence of ATM, it was concluded that the protective role of melatonin may have been independent of ATM. In addition, the authors performed immunofluorescent staining of phosphorylated ATM in MCF-7 and HCT116 cell lines treated with either melatonin or cisplatin alone or in combination. While cisplatin alone induced ATM phosphorylation, melatonin by itself failed to do so and combining the two compounds did not result in a further rise in phosphorylated ATM, indicating that other kinases might be potentially involved in melatonin-mediated reduction in DNA damage.[96]

In addition to ATM, DNA-PKcs is a key player in the NHEJ. Cells lacking components of the NHEJ are defective in DSB repair and highly sensitive to ionizing radiation. Moreover, a rise in the expression of DNA-PKcs enhances the repair of ionizing radiation-induced, etoposide-induced, and doxorubicin-induced DNA DSBs. Alonso-Gonzales et al.[97] found that melatonin sensitizes human MCF-7 breast cancer cells to ionizing radiation by downregulating DNA-PKcs. They showed that radiation alone limited DNA-PKcs mRNA expression in MCF-7 cells. Pretreatment with melatonin before radiation led to a significantly higher drop in the expression of DNA-PKcs mRNA compared with radiation alone. Decreased levels of DNA-PKcs upon melatonin administration strengthen the hypothesis that the radiosensitizing effect of melatonin on human breast cancer cells may involve the impairment of DSB repair.

3.3 | Regulation of DDR mediators

With certain types of DNA damage including DSBs, the phosphorylation of the histone variant H2AX on serine 139-a form called γH2AX, by ATM and ATR kinases, has been identified as an early event. This post-translational modification is critical for the subsequent recruitment of multiple DDR effectors including checkpoint and DNA repair proteins to the damaged site.[98] Multiple studies have noted that melatonin modulates DDR by regulating H2AX phosphorylation. Santoro et al.[96] documented that melatonin reduced DNA damage-induced cisplatin. They reported that this effect is in part mediated by H2AX, a mediator of DDR. They measured the levels of H2AX phosphorylated on Ser-139 (γH2AX) in MCF-7 and HCT116 cells and, interestingly, observed an increase in γH2AX protein levels in both MCF-7 and HCT116 cell lines. It was also confirmed that the rise

TABLE 1 Protective roles of melatonin and, in a few cases, its metabolites against DNA damage

Targets	DDR-induced toxins	Melatonin conc.	Effects	Ref.
Calf thymus DNA	Fenton reagents, chromium(III), hydrogen peroxide (H_2O_2)	3.6 μmol/L	Reduced 8-OH-dG formation	66
Calf thymus DNA	Paraquat or ultraviolet light	1.5 mmol/L	DNA damage prevented, as assessed by agarose gel electrophoresis	67
Calf thymus DNA	Cyanide, kainate, glutathione/ Fe^{3+}/O2, or H_2O_2/Fe^{2+}	1.0 or 1.5 mmol/L	DNA damage suppressed by melatonin, as assessed by agarose gel electrophoresis	68
Calf thymus DNA	Chromium(III) and H_2O_2	0.5 μmol/L	Limited 8-OH-dG formation	63
Fetal rat brain	Ischemia and reperfusion	10 mg/kg	Decreased 8-OH-dG formation	69
Female senescence-accelerated mice	Age-related damage	2 mmol/L	Decreased 8-OH-dG formation, reduced melatonin levels after DNA damage	70
Rat brain cells	60-Hz magnetic field	1 mg/kg	Blocked DNA single- and double-strand breaks in brain cells, as assayed by a microgel electrophoresis method	71
Human semen from infertile males	Oxidative stress	6 mg/d	Reduced DNA damage, assessed by TUNEL assay	72
Calf thymus DNA	Fenton reagents, chromium(III), H_2O_2	3.6 μmol/L	Limited 8-OH-dG formation	73
Plasmid pUC19 DNA	5-Aminolevulinic acid, Fe^{2+}	1 mmol/L	Decreased 8-OH-dG formation	74
U-937 cells	H_2O_2	1 μmol/L	Decreased DNA damage as assessed by cytokinesis-block micronucleus technique	75
Human full-thickness skin	UVR	1 mmol/L	Caused an enhancement of antioxidative enzyme gene expression (CAT, GPx, SOD), prevented UVR-induced depletion, decreased 8-OH-dG formation	76
C57BL mice	X-ray	0.3 μmol/L	Ameliorated 8-OH-dG formation,	77
Hamsters	Opisthorchis Viverrini	5, 10, 20 mg/kg	Decreased 8-oxo-7,8-dihydro-2′-deoxyguanosine and 8-nitroguanine, reduced expression of heme oxygenase-1 and cytokeratin 19, nitrate/nitrite levels, and bile duct proliferation in the liver, reduced expression levels of NF-kB, and cyclooxygenase-2, TNF-a and IL-1b, increased expression levels of Nrf2 and manganese superoxide dismutase	78
Rat brain	Phosphine	0.1 mmol/L	Limited 8-OH-dG formation	79
Murine bone marrow cells	Cyclophosphamide	20 mg/kg	Increased MnPCEs (micronucleated polychromatic erythrocytes) as an indication of DNA damage	80
Rat model	Phenytoin sodium	4 mg/kg	Decreased DNA damage as assessed by comet assay	81
Human peripheral blood lymphocytes	Diazinon	400 μmol/L	Reduced DNA damage as assessed by micronucleus formation	82
Rat germ cells	Bisphenol-A	5 mmol/L	Quelled DNA damage as assessed by comet assay and the meiotic spread method	83
Kidney of rats	$KBrO_3$	10 mg/kg	Decreased 8-OH-dG formation	84
The hippocampus of adult male Wistar rats.	Kainate	4 × 2.5 mg/kg	Reduced DNA damage as assessed by TUNEL staining	85
Rat liver	Safrole	0.2-0.4 mg/kg	Decreased DNA damage as assessed by a nuclease Pl-enhanced 32P-postlabeling assay	86
Mouse spermatocyte-derived cell line	Mobile phone radiation	200 nmol/L	Limited DNA damage as assessed by alkaline comet assay	87

(Continues)

TABLE 1 (Continued)

Targets	DDR-induced toxins	Melatonin conc.	Effects	Ref.
Bovine cumulus cells	During in vitro maturation (IVM)	10^{-9} mol/L	Decreased DNA damage as assessed by comet assay	88
CHO cells	H_2O_2- and cyclophosphamide	0.1-3 mmol/L	Reduced DNA damage as assessed by cytogenetic and cytofluorimetric analyses	89
Rat kidney	Ferric nitrilotriacetate	25 or 50 mg/kg	Lowered 8-OH-dG formation	90
Hamster kidney	Estradiol	64.5 μmol/kg	Limited 8-OH-dG formation	91
Rat lymphocytes	50 Hz magnetic field	0.5 or 1.0 mmol/L	Inhibited DNA damage as assessed by comet assay	92
Bovine cerebral endothelial cells	Hyperoxia	0.04-5 mmol/L	Lowered DNA damage as assessed by DNA ladder	93
Calf thymus DNA	Chromium(III) and H_2O_2	0.1 μmol/L	Lowered 8-OH-dG formation	94

in melatonin-induced H2AX phosphorylation was not due to an increase in the total level of H2AX protein. To determine whether DNA repair machinery was triggered after treatment with melatonin and cisplatin alone or in combination, immunofluorescent staining of γH2AX was also carried out. Melatonin and cisplatin alone, as well as in combination, induced γH2AX foci formation in both MCF-7 and HCT116 cells.

In a related study by Tanabe et al.,[99] it was demonstrated that melatonin protects the integrity of granulosa cells by lowering oxidative stress in nuclei, mitochondria, and plasma membrane and by inducing a reduction in DNA damage, mitochondrial dysfunction, lipid peroxidation, and apoptosis. Fluorescence-based immunocytochemistry using specific antibodies for γH2AX was used to evaluate phosphorylated H2AX levels in the target cells. They showed that H_2O_2 treatment of cells promoted a rise in the fluorescence intensity of γH2AX, with this increase completely suppressed by melatonin treatment. Similar results were reported by Chua et al.,[100] in which the protective effects of melatonin on doxorubicin-induced DNA damage were investigated in rat model of cardiorenal syndrome. Melatonin caused a significant reduction in the expression of γH2AX after doxorubicin treatment (H2AX4). Additionally, Chen et al.[101] observed that melatonin pretreatment contributes to the efficacy of menadione against hepatic ischemia–reperfusion injury in rats and that this effect is mediated by suppression of DNA damage associated with a reduction in H2AX expression as well as mitochondrial permeability transition. The effects of melatonin in ameliorating DNA damage in germ cells were also evaluated by Wu et al.[83] They found that bisphenol-A administration caused a significant rise in the levels of thiobarbituric acid-reactive substances and a reduction in SOD activity that were in parallel with the enhanced DNA migration within male germ cells and γH2AX foci formation on the autosomes of spermatocytes. Melatonin pretreatment significantly protected these cells from bisphenol-A effects. They speculated that bisphenol-A promotes DNA damage in germ cells through the induction of oxidative stress, which is suppressed by melatonin. Taken together, melatonin could potentially serve as a promising molecule for preventing the potential genotoxicity of DNA-damaging agents (Figure 1).

3.4 | Regulation of DDR effectors

3.4.1 | Effectors of cell cycle checkpoints

Cell cycle checkpoints are cellular supervision pathways that delay or arrest cell cycle progression in response to DNA damage. The ATR-Chk1 and ATM-Chk2 pathways are two key checkpoint signaling pathways that have been described in mammalian cells. Both pathways include damage sensors, mediators, signal transducers, and effectors. The downstream events related to these signaling pathways involve the

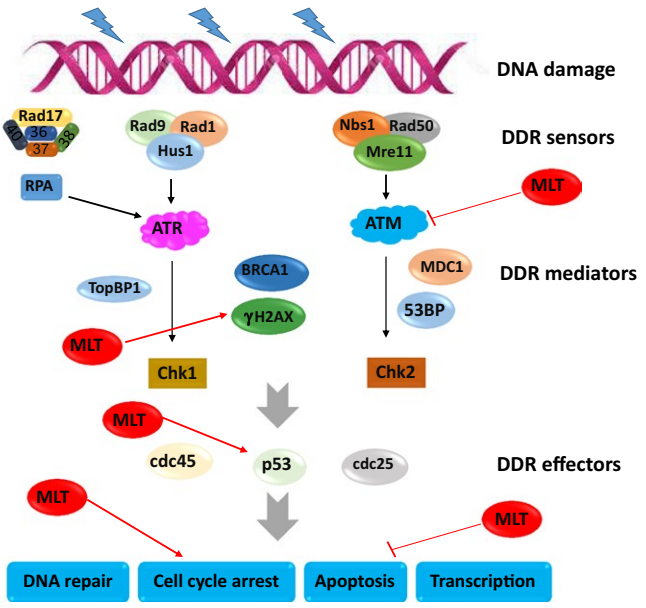

FIGURE 1 The role of melatonin in DNA damage response pathways. The pathways encompass damage sensors, mediators, and effectors. MLT, melatonin

phosphorylation of effector proteins including Cdc25, Cdc45, and p53 and finally suppression of two key kinases, Cdk2 and Cdc2; these cause cell cycle arrest at G1/S and G2/M, respectively.[102] Multiple studies have focused on the effects of melatonin in the regulation of cell cycle checkpoints. In MCF-7 breast cancer cell lines, melatonin pretreatment prior to radiation decreased G2-M phase arrest and subsequently sensitized the cells to the ionizing effects of radiation by limiting cell proliferation.[97] In a human lung adenocarcinoma cisplatin-sensitive cell line (SKLU-1), melatonin induced cell cycle arrest in the S phase, compared to treatment with cisplatin alone. Thus, melatonin potentiated the cisplatin-induced cytotoxicity arresting the cell cycle in the S phase.[103]

The p53 tumor suppressor can interrupt the cell cycle at the G1/S in response to DNA damage. The transcription of p21 is also induced by DNA damage, and the elevated p21 protein level has a pivotal role in the maintenance of G1/S checkpoint by binding to Cdk2 and suppressing its activity. Moreover, p21 binds to PCNA, inhibits DNA replication, and is thus involved in regulating the intra-S checkpoint. Previous studies have shown that melatonin is a key regulator of p21 and p53 proteins under DNA damage-free conditions in various cancer cell lines.[104-108] Santoro et al.[109] showed that melatonin reduces DNA fragmentation following treatment with DNA-damaging agents, such as chemotherapeutic drugs and ionizing radiation. Moreover, they found that this event is mediated by p38 MAPK activation of p53. Because melatonin stimulates signaling pathways by binding to its G protein-coupled membrane receptors MT1 and MT2, the authors also investigated whether the p53- and p38-dependent reduction in DNA fragmentation by melatonin was prompted by MT signaling; they reported that melatonin-induced p53-dependent DDR was mediated by MT1 and MT2. As a consequence, melatonin's function in reducing cell proliferation and p53-dependent DDR is disturbed in the absence of either of these receptors. In a related study, it was noted that melatonin mediates phosphorylation of p53 at Ser-15 in MCF-7 and HCT116 cells, thereby suppressing cell proliferation and protecting against caffeine-induced DNA damage in both normal and transformed cells. The expression of p53 and promyelocytic leukemia (PML) and phosphorylation of p53 at the Ser-15 residue are required for melatonin to function in this capacity. Additionally, melatonin-induced p53 phosphorylation relies on an intact p38 phosphorylation cascade and is severely impaired upon chemical inhibition of p38 MAPK activity. P53-mediated reduction in DNA damage by melatonin was also investigated by Janjetovic et al.[110] in human epidermal keratinocytes. Melatonin treatment significantly reduced the generation of ROS in keratinocyte during UVB exposure, by solely enhancing the expression of p53 phosphorylated at Ser-15. No change in the expression of p53 phosphorylated at Ser-46 or nonphosphorylated p53 was noted. Similar results were also reported by Alonso-González et al.,[97] who demonstrated that melatonin sensitizes human breast cancer cells to ionizing radiation by inducing the expression of p53.

3.4.2 | Effectors of DNA repair

The DNA repair machinery is an important component of DDR, which eliminates DNA damage, restores the continuity of the DNA duplex, and contributes to genome integrity. The role of melatonin in DNA repair pathways was recently illustrated in various studies focusing on the protective role of melatonin against DNA damage (Figure 2). In a genome-wide gene expression analysis of MCF-7 breast cancer cell line treated with melatonin and a mutagen, methyl methanesulfonate, an increase in DNA repair capacity was demonstrated after melatonin treatment. They further detected altered expression of many genes involved in multiple DNA damage-responsive pathways.[111] Bennukul et al.[112] reported that melatonin mitigates cisplatin-induced HepG2 cell death

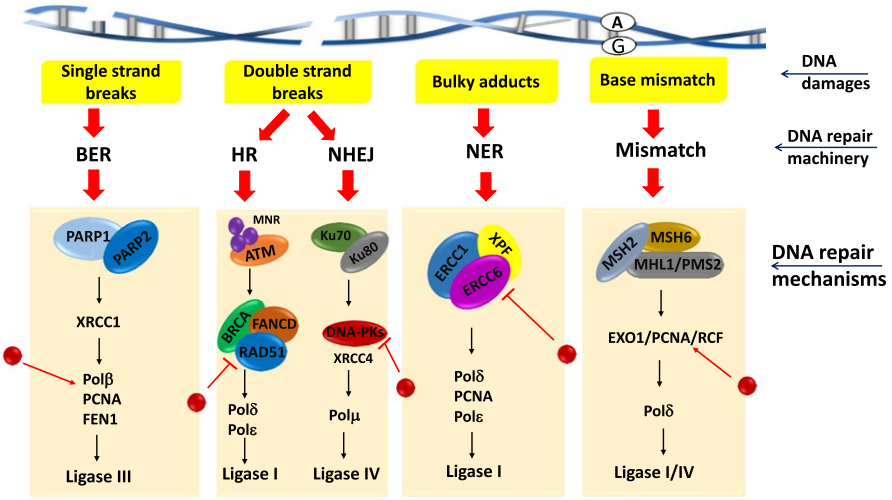

FIGURE 2 The role of melatonin in DNA repair pathways. BER, base excision repair; HR, homologous recombination; DNA, NER, nucleotide excision repair; NHEJ, nonhomologous end-joining; ●, melatonin

by regulating the expression of an endonuclease enzyme identified as excision repair cross-complementary 1 (ERCC1). ERCC1 acts at the incision step of the NER pathway. ERCC1 cleaves damaged DNA at upstream sites, leading to DNA re-synthesis and ligation to restore DNA duplex.[113] When combined with cisplatin, melatonin suppressed ERCC1 overexpression. Melatonin also decreased cisplatin cytotoxicity by lowering the levels of phosphorylated p53 apoptotic protein, cleaved caspase 3, and Bax, as well as augmenting the expression of antiapoptotic Bcl-2 gene and protein. In a combination treatment, melatonin arrested the cell cycle at the S phase. Furthermore, the effects of melatonin on another member of NER, ERCC6, was evaluated in a study by Sun et al.[114] They showed that melatonin protects neurons against ischemic neuronal injury by modulating DNA damage and repair. Transient ischemia was shown to induce the expression of ERCC6 at the mRNA level in the injured neurons. ERCC6 colocalized with PCNA, as manifested in the double-labeled images. Moreover, melatonin significantly enhanced the expression of ERCC6 mRNA in the peri-ischemic region of rat brains. Similarly, a melatonin-mediated rise in PCNA levels also was reported following DNA damage induced by γ-radiation exposure in spermatogenic cells of mice.[95] In addition to NER, melatonin stimulates DNA repair by modulating critical proteins involved in HRR and NHEJ. Alonso-González et al.[97] showed that treatment with melatonin led to a significant drop in mRNA expression of RAD51, a key protein in HRR, and of DNA-PKcs, core proteins in NHEJ, after radiation exposure of a MCF-7 cell line. In this study, the sensitizing effect of melatonin pretreatment on radiation therapy of breast cancer cells included suppressing cell proliferation, induction of cell cycle arrest, and downregulation of proteins involved in double-strand DNA break repair. In a study by Sliwinski et al.,[115] it was reported that melatonin can have a protective effect against oxidative DNA damage by chemical inactivation of a DNA-damaging agent, as well as by activating DNA repair, but key factors in BER, including glycosylases and AP-endonucleases, do not seem to be affected by melatonin. In recent years, the effects of melatonin on DNA repair capacity in dealing with DNA strand breaks has been examined using the comet assay, which is a reliable method for measuring the extent of DNA damage. For example, an increase in the DNA repair efficacy, mediated by melatonin, has been already reported in the case of DNA damage induced by idarubicin, irinotecan, lead, and UVR.[110,116-118]

3.4.3 | Effectors of apoptosis

Induction of apoptosis by melatonin has been described in multiple studies, and the number of papers in this area is increasing rapidly. Apoptosis is a key cell death modality in different pathologic conditions including tissue damage in cerebrovascular and cardiovascular ischemia, which induce extensive cell death in response to ROS produced by transient ischemia.[119] One of the most important advances in cancer biology has been the understanding of the significance of apoptosis in barricading transformed cells from proliferation and, therefore, preventing potential cancer development and/or progression. The mechanism of action of melatonin in apoptosis has been investigated in breast and pancreatic cancer cells as well as in lymphoma and myeloma. The common feature of these studies is that melatonin inhibits cell proliferation and induces apoptosis by simultaneously regulating important signaling pathways involved in the apoptotic cascade. Below, we discuss the involvement of melatonin in modulating DNA damage-induced apoptosis in three certain categories.

In the first category, melatonin acts as a preventive therapeutic molecule against apoptosis induced by ischemia–reperfusion, diabetes, malarial infection, β-amyloid, H_2O_2, UV, cyclophosphamide, indomethacin, and oxaliplatin (Table 2). Melatonin normalizes the expression of proteins and target genes in apoptotic pathways. In addition, melatonin interferes with multiple important processes involved in the mitochondrial and death receptor apoptotic pathways, a property that is most likely associated with the redox capabilities of melatonin. Considering the outcome of these studies, melatonin may be a promising pharmacological agent for ameliorating the potential catastrophic effects of the agents identified.

In the second category, melatonin induces apoptosis and sensitizes cancer cells to chemotherapeutic agent-induced cytotoxicity and apoptosis. Pariente et al.[120] found that melatonin effectively enhanced the sensitivity of cervical cancer cells to cisplatin, 5-FU, and doxorubicin in HeLa cells. In the case of cisplatin, the sensitizing effect was greatest, probably due to the fact that the indoleamine activates (ROS-mediated) initiator caspases, thus rapidly promoting apoptotic cell death. Hence, the concomitant treatment with melatonin and cisplatin significantly increased the ratio of cells entering mitochondrial apoptosis due to ROS overproduction, and as a result augmented the population of apoptotic cells; this resulted in markedly higher DNA fragmentation compared to cisplatin alone.[121] Kosar et al.[122] used a combination of melatonin and doxorubicin to drive MCF-7 breast cancer cells to apoptosis, an effect that may be related to a reduction in Ca2+ entry and activation of caspase-9. The findings suggest that melatonin has an inhibitory effect on the activity of the TRPV1 channel, in a way that the downregulation of TRPV1 channel upon doxorubicin treatment leads to apoptosis and oxidative stress in MCF-7 breast cancer cells. Thus, melatonin could possibly be useful as an adjuvant to increase the therapeutic effects of doxorubicin. A similar synergism was also reported for cisplatin-mediated apoptosis via the inactivation of ERK/p90RSK/HSP27 cascade in ovarian cancer SK-OV-3 cell lines.[121] Moreover, Casado-Zapico et al.[123] identified a synergy in the antitumor effect when melatonin was combined with vincristine or with ifosfamide. This synergism involves

TABLE 2 Melatonin's impact on apoptosis induced by different agents

Target	DDR-induced toxin	Target genes	Effects	Other techniques	Ref.
Melatonin decreases apoptosis					
Leukemic K562 cells and HeLa cancer cells	Idarubicin	Caspase 3	Melatonin diminished the extent of apoptosis Melatonin decreased activation of caspase 3 Melatonin had no effect on DNA fragmentation	Comet assay was used to evaluate DNA fragmentation and DAPI Staining for the detection of morphological changes in chromatin	118
Transient middle cerebral artery (MCA) occlusion model	Ischemia	Bcl-2	Melatonin reduced the number of DNA SSB- and DNA DSB-positive cells Melatonin decreased the number of bcl-2-positive cells	TUNEL technique was performed to detect DNA DSB. PUNT labeling was used to detect DNA SSB.	114
Rat, urinary bladder	Cyclophosphamide	Nrf2 NF-kB	Melatonin reduced DNA damage Melatonin lowered the percentage of TUNEL-positive cells Melatonin reduced oxidative stress	TUNEL assay was performed to detect apoptosis	124
Renal carcinoma Caki cells	Oxaliplatin	PARP, cFLIP, cIAP-2, Bax, Bcl-xL, XIAP, Mcl-1	Melatonin blocked oxaliplatin-induced DNA fragmentation Melatonin markedly reduced oxaliplatin-induced apoptosis	Flow cytometry was performed to detect apoptosis	125
Gastric mucosal cell	Indomethacin	Bax, Bak, Bcl-2, BclxL, Cyt c, Caspases-3, -9	Melatonin prevented the development of mitochondrial oxidative stress Melatonin activated mitochondrial pathway of apoptosis Melatonin downregulated the expression of Bax and Bak and upregulated the Bcl-2 and BclxL Melatonin activated caspase-9 and caspase-3 by blocking the release of cytochrome c	TUNEL assay was performed to detect apoptosis	126
Type 2 diabetic rats	Myocardial ischemia–reperfusion	SIRT1, bcl-2, Bax, foxo-1, Cas-3	Melatonin downregulated the expression of Bax, foxo-1, Cas-3 and upregulated the Bcl-2 Melatonin upregulated SIRT1 expression Melatonin reduced oxidative damage Melatonin suppressed PERK/eIF2a/ATF4 signaling	TUNEL assay was performed to detect apoptosis	127
Cultured thymocytes in mice	Hydroxyl radical	Cas-3	Melatonin completely suppressed the OH-induced apoptosis Melatonin decreased Cas-3 levels	TUNEL assay, DAPI staining, flow cytometry, and DNA laddering were performed to detect apoptosis	128
PC 12 cells	6-Hydroxydopamine	N/A	Prevents melatonin apoptosis as estimated by (i) cell viability assays, (ii) counting of the number of apoptotic cells, and (iii) analysis and quantification of DNA fragmentation	DNA gel electrophoresis and TUNEL assay were performed to detect apoptosis	129
Mesenchymal stem cells	H_2O_2	SIRT1	Melatonin reversed the senescent phenotypes Melatonin improved cell proliferation Melatonin increased SIRT1	FDA staining was used for cell viability assay	130
Mouse cerebellum	High-LET 56Fe particle irradiation	N/A	Melatonin inhibited oxidative damage Melatonin decreased the number of TUNEL-positive cell and apoptosis	TUNEL assay was performed to detect apoptosis	131

(Continues)

TABLE 2 (Continued)

Target	DDR-induced toxin	Target genes	Effects	Other techniques	Ref.
Mice liver	Malarial infection	Bcl-2, Bax, Cas-3	Melatonin downregulated the expression of Bax and upregulated the Bcl-2 Melatonin inhibited oxidative damage Melatonin decreased number of TUNEL-positive cell and apoptosis	TUNEL assay was performed to detect apoptosis	132
HaCaT keratinocytes	UVR	PARP, Cas-3, -9, -7	Melatonin downregulated Cas-3/Cas-7, Cas-9 and reduced PARP activation Melatonin maintained the mitochondrial membrane potential Melatonin inhibited consecutive activation of the intrinsic apoptotic pathway	JC-1 was used to measure mitochondrial membrane potential	133
PC12 cells	Amyloid β	N/A	Melatonin inhibited apoptosis Melatonin suppressed nitric oxide formation Melatonin prevented intracellular calcium overload and alleviated membrane rigidity	Hoechst 33342 nuclei staining, DNA fragmentation analysis, and flow cytometric analysis were performed to detect apoptosis	134
Rat heart	Diabetes	Bcl-2, Cas-3, -8, -9	Melatonin enhanced Bcl-2 expression and blocked the activation of CD95 as well as caspases-9, -8, and -3 Melatonin ameliorated diabetic myocardium injury, apoptosis, reduced the metabolic risk factors, and modulated important steps in both extrinsic and intrinsic pathways of apoptosis	Flow cytometric analysis with annexin V-FITC/PI staining was performed to detect apoptosis	135
Rat	Ischemia–reperfusion	Cas-3	Melatonin prevented lipid peroxidation, cell apoptosis, and necrosis and blocked caspase-3 activity	TUNEL assay was performed to detect apoptosis	136
Urothelial cells	Cyclophosphamide	Cas-3	Melatonin reduced apoptosis and increased proliferation	N/A	137
Human retinal pigment epithelial cells	Ischemia	Bad, Bax, Bcl-x, CPP32, TIAR, ICH	Melatonin decreased the number of apoptotic cells Melatonin decreased ICH and bcl-2 expression levels	DNA gel electrophoresis and TUNEL assay were performed to detect apoptosis	138
Bone marrow Mesenchymal stem cells	Hydrogen peroxide	Bax/Bcl-2, Cas-3	Melatonin attenuated apoptosis Melatonin suppressed the generation of intracellular ROS, expression ratio of Bax to Bcl-2, activation of caspase-3	Flow cytometric analysis with annexin V-FITC/PI staining and TUNEL assay was performed to detect apoptosis	139
Rat brain	Ischemia	Bcl-2	Melatonin prevented the neuronal death and enhanced the expression of bcl-2	N/A	140
Rat heart	Myocardial ischemia–reperfusion	SIRT1, bcl-2, Bax, Cas-3,	Melatonin reduced apoptotic index, diminished serum creatine kinase and lactate dehydrogenase release, upregulated SIRT1, Bcl-2 expression, and downregulated Bax, caspase-3, and cleaved caspase-3 expression.	N/A	141
Melatonin increases apoptosis					
Human cervical cancer HeLa cells	Cisplatin	Cas-3, -9	Melatonin increased the cytotoxic effect of cisplatin Melatonin increased caspase-3 activation Melatonin augmented the population of apoptotic cells and markedly enlarged DNA fragmentation	Flow cytometric analysis with annexin V-FITC/PI staining and DNA fragmentation was performed to detect apoptosis	120

(Continues)

TABLE 2 (Continued)

Target	DDR-induced toxin	Target genes	Effects	Other techniques	Ref.
SK-OV-3 cells	Cisplatin	Cas-3, PARP	Melatonin increased apoptosis Melatonin increased the cleavage of Cas-3 and PARP. Melatonin inhibited the phosphorylation of ERK and dephosphorylation of p90RSK and HSP27	TUNEL assay was performed to detect apoptosis	121
Ewing sarcoma cancer cells	Vincristine and ifosfamide	Cas-3, -8, -9 and Bid	Melatonin increased the activation of Cas-3, -8, -9 and Bid Melatonin potentiated apoptosis, mainly the extrinsic apoptotic pathway	N/A	122
Rat pancreatic AR42J cell line	5-FU, cisplatin, and doxorubicin	N/A	Melatonin increased the population of apoptotic cells Melatonin elevated mitochondrial membrane depolarization Melatonin augmented intracellular ROS production	Flow cytometric analysis with annexin V-FITC/PI staining was performed to detect apoptosis	142
MCF-7 breast cancer cells	Doxorubicin and melatonin	PARP, Cas-3, -9	The intracellular production of reactive oxygen species, mitochondrial membrane depolarization, apoptosis level, procaspase 9 and PARP activities, and Cas-3 and Cas-9 activities were higher in combination treatment	N/A	143

especially cell death, due to the potentiation of the apoptotic rate, and mainly the extrinsic apoptotic pathway. There was a significant increase in the activation of caspases-3, -8, -9 and Bid when melatonin was combined with vincristine or ifosfamide compared to the individual treatments.[123] Collectively, these studies reveal the favorable function of melatonin in exerting a synergistic effect with chemotherapeutic agents in the induction of apoptosis in cancer cells.

In the third category, Majsterek et al.[118] compared the effects of amifostine and melatonin on idarubicin-induced DNA damage and apoptosis in normal and cancer cells. While amifostine reduced apoptosis and DNA damage in normal cells, it increased these phenomena in Hela and K562 cancer cell lines. In this case, melatonin protected both normal and cancer cells against genotoxicity and apoptosis induced by idarubicin. While melatonin is a potent antioxidant, there may be need for caution when melatonin is used in combination with chemotherapeutics, especially in cases of leukemia.

4 | MELATONIN MAY SYNERGIZE WITH DNA-DAMAGING AGENTS IN CANCER

Research has revealed several oncostatic therapeutic actions of melatonin involving its actions as an antioxidant and as an immunomodulatory agent. Melatonin is involved in cancer prevention and has been used for this purpose in clinical trial.[144,145] Recent evidence documents that melatonin suppresses the proliferation of cancer cells by exerting oncostatic and proapoptotic properties.[146] These actions have been recorded in several cancer cell types including breast, colon, ovarian, and cervical cancers. In addition, reduced levels of melatonin have been associated with multiple cancers, aging, neurodegenerative disorders, and immunosenescence.[147-150] In most studies, the combination of a chemotherapeutic with melatonin increases cytotoxicity by enhancing apoptotic cell death. For example, treatment with melatonin and cisplatin in HeLa cells induced mitochondrial apoptosis due to ROS overproduction, emphasizing the link between ROS, mitochondria, and apoptosis in cancer. Melatonin was also shown to sensitize hepatocellular carcinoma cell lines to sorafenib by modulating the mitochondrial stability and turnover through mitophagy.[151] Similar sensitizing effects have been shown for gefitinib TKI-resistant H1975 cells that harbor an EGFR T790M mutation[152] and also for two newly established clofarabine-resistant leukemia cell lines.[153] The sensitizing effects of melatonin with various chemotherapeutic agents including doxorubicin, cytarabine, daunorubicin, and etoposide have also been noted previously.[154,155] The collective results are consistent with the likely beneficial actions of melatonin when given as an adjuvant treatment with conventional therapeutics.

5 | CONCLUSIONS AND PERSPECTIVES

The interactions of melatonin with numerous DNA repair and DDR processes identify its importance in the regulation of cell survival and proliferation. Not only does melatonin

protect against DNA damage by exerting antioxidant functions, but it also interacts with different components of DDR at numerous transduction, mediation, and functional levels. In light of the fact that melatonin influences effectors of apoptosis and has shown synergism with various chemotherapeutics, it holds great promise as an adjuvant to DNA-damaging agents. The efficacy of such combinations must be assessed in clinical trials with the intent of improving anticancer therapies as well as reducing the side effects of these frequently toxic treatments.

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