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Review article

Boosting immune system against cancer by melatonin: A mechanistic viewpoint

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A R T I C L E I N F O

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A B S T R A C T

Cancer is a disease of high complexity. Resistance to therapy is a major challenge in cancer targeted therapies. Overcoming this resistance requires a deep knowledge of the cellular interactions within tumor. Natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) are the main anti-cancer immune cells, while T regulatory cells (Tregs) and cancer associated fibroblasts (CAFs) facilitate immune escape of cancer cells. Melatonin is a natural agent with anti-cancer functions that has also been suggested as an adjuvant in combination with cancer therapy modalities such as chemotherapy, radiotherapy, immunotherapy and tumor vaccination. One of the main effects of melatonin is regulation of immune responses against cancer cells. Melatonin has been shown to potentiate the activities of anti-cancer immune cells, as well as attenuating the activities of Tregs and CAFs. It also has a potent effect on the mitochondria, which may change immune responses against cancer. In this review, we explain the mechanisms of immune regulation by melatonin involved in its anti-cancer effects.

1. Introduction

Cancer treatment is one of the most challenging issues in medicine. Each year, millions of people are diagnosed with neoplastic diseases which need immediate therapeutic strategies for increasing their probability of survival [1]. Solid tumors are very complex. They contain a pack of multi-tasking cells including immune system cells, cancer clonogenic cells, fibroblasts, vascular, etc. [2]. Clonogenic cells are responsible for the division and generation of new cells which lead to tumor growth. However, besides cancer clonogenic cells, other cells within the tumor such as fibroblasts, immune cells including dendritic cells, macrophages and different subfamilies of lymphocyte-T cells play key roles in tumor growth and response to different therapeutic modalities [3].

Immune system within tumor has a pivotal role in both inhibition and invasion of cancer cells. In response to apoptosis or necrosis of cancer cells following radiotherapy or chemotherapy, several signaling pathways are activated after the interaction of apoptosis or necrosis products with immune system cells. Responses of immune cells are different depending on the type of cell death and interactions [4]. Usually, macrophages digest apoptotic bodies and release tolerogenic cytokines which lead to the attenuation of immune system's activity against tumor cells. Moreover, some secreted cytokines from macrophages or lymphocytes trigger the development of new vessels through stimulation of angiogenesis factors [5,6]. Modulation of immune system responses is an interesting strategy for suppressing tumor growth as well as eradication of clonogenic cells within tumor, which can lead to complete treatment of cancer [4]. In recent times, numerous experimental studies have shown promising results for tumor inhibition by targeting some immune mediators either alone or in combination with radiotherapy or chemotherapy [7,8]. Natural agents are interesting for targeting tumor invasion because of their high bioavailabilities and low toxicities [9]. Melatonin is among the natural agents that has shown interesting effects on immune system responses. Furthermore, the

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interesting properties of melatonin make it a promising agent for use as an adjuvant for cancer immune modulation and improving tumor control [10].

To date, numerous studies have been conducted to explain the immunoregulatory effects of melatonin in normal cells, and also in different type of cancers [11–13]. In the current review, we provide a mechanistic viewpoint for immunomodulation of tumor by melatonin, which may facilitate the response of cancer cells to radiotherapy, chemotherapy and immunotherapy. For this aim, PubMed database was searched to obtain the most suitable and recent related articles. The criteria for selection of articles were based on the quality of studies and journals.

1.1. Tumor resistance to therapy

Tumor microenvironment (TME) includes several types of cells such as immune system cells. Cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells are the most common tumor suppressor cells [3]. Increased release of danger alarms such as heat shock protein 70 (HSP70), adenosine triphosphate (ATP) and high mobility group box 1 (HMGB1) stimulates proliferation and activation of CTLs. Activation of CTLs lead to the release of anti-cancer cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α, to induce apoptosis in cancer cells [3]. In contrast to CTLs, NK cells do not require danger alarms to act against cancer cells. NK cells are able to kill cancer cells via detection of cell membrane antigens (major histocompatibility complex (MHC) class 1). Cancer cells usually lose MHC class 1. NK cells, in response to the absence of MHC class 1, release lytic enzymes including perforin and granzymes. On the other hand, T regulatory cells (Tregs), M2 type of macrophages, neutrophils and immature myeloid cells can promote growth of tumor via regulating the proliferation of cancer stem cells (CSCs) and angiogenesis [14]. Cancer cells are differentiated form of CSCs; however, they have positive cross-talks with each other to stimulate survival and proliferation of each other. Tregs play a central role in the resistance of cancer cells to apoptosis. Tregs via release of immune suppressor cytokines such as transforming growth factor-β (TGF-β), interleukin (IL)-4 and IL-13 limit the anti-cancer activities of CTLs and NK cells [15].

Increasing the activities of Tregs, CSCs, cancer cells and M2 macrophages is critical for resistance of tumors to different cancer therapy modalities such as radiotherapy, chemotherapy, targeted therapy and immunotherapy [16]. It has been shown that in response to anti-cancer agents such as radiation, Tregs release immunosuppressive cytokines such as TGF-β to suppress proliferation of CTLs, and to promote proliferation of CSCs and CD4+FOXP3+ Tregs [17–19]. TGF-β can also stimulate the polarization of macrophages towards M2 type. These changes show that tumor promoting cells within TME amplify the activities of each other to enhance proliferation of cancer cells and CSCs. These responses lead to resistance of cancer cells to subsequent therapeutic doses. In response to some chemotherapy drugs, immune responses within TME may shift in favour of Treg activation [20]. For radiotherapy, the response of TME is highly dependent on the dose of radiation in each fraction. Usually, using the conventional radiotherapy dose (2 Gy per fraction) causes conquering of Tregs to CTLs and NK cells, leading to further release of immunosuppressive cytokines. In contrast, following irradiation with 10 Gy (which is a common dose for stereotactic and grid radiotherapy), CTLs to Tregs ratio is high and enhances killing of cancer cells [3]. In this situation, release of IFN-γ and TNF-α from CTLs induce apoptosis in CSCs and cancer cells more potently, and also suppress activities of Tregs and M2 cells. Studies have suggested that although this dose of radiation modulates the immune system against cancer, after some days, Tregs release some factors to suppress CTLs and also attenuate apoptotic induction in cancer cells [3]. The most important factor released in this situation is programmed cell death-1 (PD-1). PD-1 ligand (PDL-1) is expressed by some cells such as cancer cells and CTLs within TME. The engagement of PD-1 with PDL-1 can abrogate the release of cytotoxic cytokines by CTLs [2]. Furthermore, this engagement is able to downregulate anti-apoptotic phosphoinositide-3-kinase (PI3K)/protein kinase B (AKT) pathway, leading to CTL apoptosis. On the other hand, cytotoxic T-lymphocyte associated protein 4 (CTLA-4), a glycoprotein that is expressed on the CTLs exerts long time activity of CTLs following exposure to danger alarms and the expression of MHC1. For these reasons, boosting CTLs and NK cells is a crucial issue in cancer therapy. Also, inhibition of Tregs as well as polarization of macrophages into M1 type macrophages are important strategies for suppressing tumor growth [3]. In recent years, immunotherapy and immune checkpoint inhibitors provide a new window for this aim. However, it seems that this strategy alone is not sufficient to overcome tumor resistance (Fig. 1).

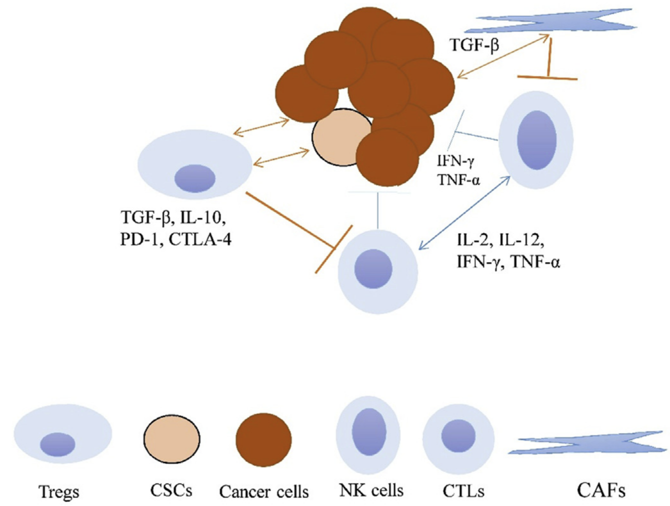

Fig. 1. Interactions of cancer cells with immune system within TME.

1.2. Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a natural hormone that has been recognized in nearly all existing organisms such as plants, fungi and animals [21]. Melatonin is a chronobiotic hormone that is mainly synthesized and secreted by the pineal gland from the amino acid tryptophan. Its synthesis involves four essential enzymes: tryptophan-5-hydroxylase, 5-hydroxytryptophan decarboxylase, serotonin N-acetyltransferase and hydroxyindole-O-methyltransferase [22]. Melatonin is secreted in the evening with a robust circadian rhythm reaching a maximum plasma peak in the middle of the night. Therefore, this neurohormone is broadly involved in the regulation of circadian rhythms, sleep, food intake, etc. [23]. Indeed, during the last decades, different melatonin-rich foods such as rice, olive, fish and eggs which aid sleep have been identified [24]. Moreover, melatonin is also produced in extra-pineal sites, tissues and organs where it acts as an autocrine and paracrine signal [25]. Numerous studies have revealed other effects of melatonin on cells [22]. Melatonin is a well-known endogenous free radical scavenger, as well as an indirect antioxidant involved in the regulation of cellular redox status [26]. Melatonin also exhibits other activities, such as tumor growth inhibitor, boosting immune defense, thermoregulation amongst others [27]. The effectiveness of melatonin relies on its high lipophilicity and low hydrophobicity, that allow it to pass through cellular membranes easily. Therefore, this hormone is distributed throughout all the subcellular compartments, mostly enriched in the nucleus and mitochondria [28]. The main effects of melatonin are induced through its interaction with MT1 and MT2 G protein-coupled receptors [22].

1.3. Immune system and melatonin

Melatonin has a close relation with immune cells which is mediated through melatonin receptors. It has been suggested that ablation of the pineal gland is associated with loss of immune cells [29]. Some cytokines and growth factors such as IFN-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) are able to stimulate melatonin secretion, while IL-1 may inhibit it. It has been reported that immune cells such as $CD4^+$ T helper cells and CTLs express melatonin receptors including MT1 and MT2. Melatonin is able to stimulate the release of IL-2 by upregulation of MT1, which ultimately leads to an increase in the number of NK cells [29]. Melatonin also enhances antigen presentation by macrophages to T-lymphocytes, leading to activation and proliferation of CTLs. Melatonin via triggering the release of anti-tumor cytokines such as IFN-γ, TNF-α and IL-6 as well as suppression of IL-4 may help the proliferation of CTLs and anti-cancer activity of immune system within TME [21]. The anti-cancer activity of melatonin in some cancers may be associated with a reduction in the proliferation of $CD4^+$ T helper cells to CD4(+) CD25(+) Tregs [19]. The different effects of melatonin on the immune system and immune responses within TME will be discussed in the following sections.

1.4. Modulation of immune responses by melatonin against tumor

1.4.1. Melatonin modulates immune system through mitochondrial function remodeling

Mitochondria have long been studied as essential organelles in eukaryotic cells for meeting the majority of cellular energy requirements. The generation of metabolic energy is mainly produced by the mitochondria through oxidative phosphorylation (OXPHOS) machinery that sustains most physiological functions of the cell. Therefore, the mitochondria play a crucial role in the regulation of wide range of cell signaling events such as calcium metabolism, reactive oxygen species (ROS) generation and apoptotic processes [30,31]. In addition, it has been recently uncovered that a new connection exists between mitochondrial and inflammatory responses. Mitochondria are organelles with high dynamic plasticity that experience fusion and fission events to rapidly adapt mitochondrial function and morphology in response to different stimuli and cellular needs [32]. Studies have shown that mitochondrial dynamics and its redistribution are dependent on antigen-specific activation of immune responses [33]. Mitochondria are localized in sites with high ATP demand. Thus, movement of mitochondria through microtubules and actin filaments is mediated by kinesin and dynein protein families in response to physiological signals [34]. Activation and regulation of T-cell responses require two important hits, involving the regulation of calcium balance and the antigen-specific presentation. Mitochondria have a critical role for the maintenance of both calcium homeostasis and immune synapse stability. Mitochondria move towards the immune synapse mediated by integrin adhesion and cell polarization [33,35,36]. Baixauli and colleagues demonstrated that the fission factor dynamin-related protein 1 (Drp1) is involved in the regulation of mitochondrial movement towards immune synapse. Actually, the lack of Drp1 abolishes mitochondrial redistribution in response to T-cell receptor assembly [35]. Consequently, altered plasticity of mitochondria is not only associated with disrupted cellular bioenergetics, but also favours tumor progression.

Cancer is associated with a metabolic reprogramming based on the preferential use of glucose via aerobic glycolysis rather than mitochondrial respiration [37]. The metabolic reprogramming exerted by cancer cells impacts mitochondrial function. Altered mitochondrial function contributes to tumor anabolism due to its direct involvement in the regulation of intracellular redox status, calcium homeostasis and cell death [38]. Although tumor-infiltrating T cells are identified in several tumors, there are large number of tumors where T cells are undetectable. Zhang et al. demonstrated that the presence of tumor-infiltrating T cells is correlated with improved clinical outcomes [39]. Given that the mitochondria exert control in the concomitant activation and homeostasis of T cells, several cancer therapeutic strategies are focused on modulating mitochondrial function and immune system [40].

Recent findings have considered melatonin as a mitochondrial protector due to its role as a metabolic regulator. Indeed, mitochondria were found to be the main sites for melatonin synthesis and metabolism [41,42]. These studies are supported by the fact that melatonin is accumulated in the mitochondria which drives the regulation of diverse mechanisms [28]. Melatonin regulates several cellular functions with pleiotropic effects on the immune system. Melatonin is involved in the protection of electron transport chain (ETC) and the prevention of mitochondrial oxidative damage, thereby regulating redox balance. Hence, increased ATP generation by mitochondria also prevents the decay of the membrane potential and regulates apoptotic responses [43–45]. Furthermore, melatonin maintains mitochondrial bioenergetics and redox homeostasis via controlling mitochondrial dynamics [46]. Thus, melatonin is a master regulator of both mitochondrial function and fusion-fission dynamics, and can be critical for boosting the immune system and apoptotic processes in cancer.

Abundant evidence indicates that melatonin displays anticancer properties via its effects on mitochondrial function regulation. It has been demonstrated that melatonin repressed aerobic glycolysis, survival signaling and metastasis [47]. Regulation of pyruvate kinase complex and the pyruvate dehydrogenase kinase are considered potential therapeutic targets in cancer [48]. New research has proposed that melatonin may improve TME through the inhibition of mitochondrial enzyme pyruvate dehydrogenase kinase (PDK). Melatonin was found to enhance mitochondrial oxidative phosphorylation; a response that may reduce cancer proliferation and improve chemotherapy outcomes [49]. Moreover, melatonin treatment prevented pathophysiological alterations by modulating mitochondrial dynamics. The restoration of mitochondrial network formation resulted in an increased apoptosis that led to the reduction of cell growth in several cancers such as lung, breast and colon [50–52]. However, the regulatory actions of melatonin should be selectively cytotoxic towards cancer cells whereas they should protect healthy cells [53]. The phosphoinositide 3-kinase (PI3K)-AKT pathway is essential for regulation of cell proliferation, mitochondrial dynamics and apoptosis and therefore is critical for tumor development and maintenance [54]. AKT signaling is commonly upregulated in cancer. It has been shown that inhibition of AKT signaling reduces proliferation of cancer cells and promotes apoptosis [55]. Especially, melatonin activates proliferative signaling via PI3K-AKT pathway in healthy cells, whereas this hormone is able to reduce cell viability and proliferation in cancer cells by blocking the negative feedback from the downstream effector mammalian target of rapamycin complex 1 (mTOR) [56–58]. Shen and colleagues suggested that melatonin administration can be used as an adjuvant with rapamycin because melatonin improves the effectiveness of treatment through regulation of mitochondrial function and apoptotic responses while preventing harmful effects [56]. It has been proposed that the selective action of melatonin is due to the switching of G-protein between Gi and Gs influenced by calcium signaling [59,60]. Melatonin receptors modulate PI3K/AKT pathway, which is essential for regulating mitochondrial dynamics and apoptotic responses [61]. Moreover, PI3K-AKT-mTOR axis is considered as a crossroad between cancer and cellular immune system. PI3K signaling is involved in the modulation of immune cells' effector function, in which the mitochondria are vital [62]. However, future studies on the use of melatonin in different cancers should be carried out to further investigate the effects of melatonin on mitochondrial dynamics, immune system and apoptotic processes.

1.4.2. Melatonin facilitates apoptosis via suppression of nuclear factor kappa B (NF-κB) signaling

NF-κB is a key transcription gene that mediates regulation of several signaling pathways such as proliferation, inflammation, DNA repair, apoptosis and others [63,64]. It activates DNA repair responses including homologous recombination (HR) following exposure to clastogenic agents [65]. Moreover, NF-κB through upregulation of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) promotes proliferation and resistance to apoptosis, thus increases cell survival [66,67]. COX-2 is an important inflammatory mediator which produces prostaglandins (PGs) and ROS. Evidences from studies have shown that most cancers have high expression of COX-2. This shows that COX-2 may be involved in the development and progression of cancers. In addition, upregulation of COX-2 plays a key role in the resistance of tumor cells to therapeutic modalities such as chemotherapy and radiotherapy. Some studies have revealed that COX-2 upregulation is associated with tumor proliferation and growth. PGs derived from COX-2 triggers proliferation of CSCs, leading to tumor repopulation [24,68]. Moreover, COX-2 inhibits the activity of immune system against cancer via attenuation of NK cells, dendritic cells and lymphocyte-T cells [69]. It has been proposed that targeting COX-2 can sensitize tumors to radiotherapy and chemotherapy [70].

Melatonin as a potent anti-inflammatory agent is able to target NF-κB-COX-2, thus ameliorates the production of PGs and facilitates apoptosis. Apoptotic induction is one of the most promising strategies for sensitizing tumor cells to anti-cancer agents. It has been shown that suppression of NF-κB by melatonin has a direct relation with decreased tumor volume [71]. Melatonin can abrogate binding of NF-κB to COX-2, thus prevents upregulation of COX-2. Also, melatonin attenuates the activity of p300 histone acetyltransferase (HAT), thus prevents acetylation of p52, which is an activator of COX-2 [72]. Melatonin has shown that through suppression of NF-κB-COX-2 pathway, potentiates anti-tumor activity of curcumin on bladder cancer cells [73]. Similar results have shown that melatonin via inhibiting binding of NF-κB to COX-2, potentiates anti-tumor activity of fisetin and berberine through activation of mitochondrial apoptotic pathway [74,75].

Melatonin is able to induce apoptosis in MDA-MB-231 cells when combined with tunicamycin. Tunicamycin activates NF-κB and upregulates COX-2 via stimulation of p38 mitogen activated protein kinase (MAPK). However, its combination with melatonin leads to inhibition of NF-κB and COX-2, leading to apoptotic induction [76]. The synergic effect of melatonin in combination with chemotherapy and radiotherapy has been confirmed to play a key role for targeting NF-κB and its downstream genes. Gao et al. showed a synergic therapeutic effect of melatonin on human colon cancer cell line when combined with 5-Fluorouracil (5-FU), one of the most common chemotherapeutic drugs. They found that melatonin potentiates apoptotic induction mediated through activation of caspase and poly ADP ribose polymerase (PARP). Further analyses showed that suppression of NF-κB and PI3K/AKT pathway is responsible for the synergic role of melatonin. Melatonin could prevent translocation and binding of NF-κB to iNOS promotor [77].

In addition to chemotherapy, targeting NF-κB by melatonin has been shown to be involved in tumor sensitization to ionizing radiation. A study showed that melatonin suppresses cell viability and induces apoptosis of thyroid cancer cell lines in a dose-dependent manner. This study showed that while irradiation of thyroid cancer cells leads to upregulation of NF-κB, treatment of cells with melatonin before irradiation causes potent suppression of NF-κB, leading to increasing apoptosis. Interestingly, results showed that melatonin further amplifies ROS production. *In vivo* evaluations have confirmed these results [78] (Fig. 2).

1.5. Regulation of immune cells within TME by melatonin

1.5.1. Melatonin enhances anti-tumor immunity via stimulation of NK cells

NK cells are at the first layer of immune system defense against tumor development. It has been established that NK cells play a key role in the lysis of cancer cells, especially hematopoietic origin malignancies [79]. It has also been well-known for a long time that melatonin can amplify lytic activity of NK cells [80]. IL-2 plays a central role in the regulation of proliferation of NK cells. Melatonin also potentiates the proliferation and activities of NK cells via triggering the release of IL-2. Melatonin stimulates T helper type 1 lymphocytes and monocytes to secrete IL-2 [81]. In an animal study, administration of melatonin for 1 or 2 weeks caused a significant increase in the number of NK cells in the spleen and bone marrow. This study proposed that increased proliferation of monocytes in the bone marrow triggers proliferation of NK cells following treatment with melatonin [82]. In addition to IL-2, it has been suggested that the release of other cytokines by monocytes and T helper-1 lymphocytes such as IL-6, IL-12, IL-27 and IFN-γ plays a key role in the proliferation of NK cells [83,84]. Melatonin can augment the release of these cytokines following enhancement of T helper-1 lymphocytes [21]. In a mice bearing leukemia model, it has been shown that administration of melatonin can augment NK cell numbers and improve the duration of survival [85]. A clinical study showed that administration of 20 mg melatonin per day for patients who underwent chemotherapy could not improve the recovery of NK cells [86]. There is a need to explore possible effects of higher doses of melatonin for patients who undergo radiotherapy and/or chemotherapy.

1.5.2. Melatonin enhances anti-tumor immunity via stimulation of CTLs

Lymphocytes are able to secrete melatonin, which has a positive effect on the activities of lymphocytes. It has been shown that melatonin released by lymphocytes can abrogate the suppressive effect of PGE2 on the release of IL-2 by lymphocytes [87]. Melatonin is able to trigger the secretion of IL-2, which causes proliferation of $CD8^+$ cytotoxic T cells [88]. IL-2 prevents apoptosis of matured T cells in thymus and provides a prolonged augment in the number of CTLs [88]. Melatonin is able to trigger $CD4^+$ T helper lymphocytes to release IFN-γ and TNF-α so as to potentiate the activity of CTLs [29]. It has been suggested that melatonin can also increase the number and activity of $CD8^+$ cytotoxic T cells following stimulation of hematopoiesis in bone marrow, while some clinical studies failed to confirm this effect of melatonin [89,90]. In a rat model, it has been shown that a reduction in the level of melatonin following pinealectomy leads to attenuation of T cell maturation in thymus, which causes reduction of circulating $CD8^+$ cytotoxic T cells [91]. RNA-Seq study showed that melatonin can upregulate the expression of TNF alpha-induced protein 8 like 2 (TNFAIP8L2) in TME. Upregulation of this gene can augment the activities of $CD8^+$ and NK cells, while reducing the activity of myeloid-derived suppressor cells (MDSCs) [92]. Melatonin can reduce epithelial mesenchymal transition (EMT) in cancer cells via stimulation of this gene [93]. This gene has shown high expression in the immune cells and is involved in the suppression of tumor growth and metastasis via phosphorylation of AKT and p38 [94].

1.5.3. Melatonin suppresses Tregs activity

Studies evaluating melatonin's effect on Tregs are very few. IFN-γ is a potent inhibitor of Tregs induced following administration of melatonin, while important inducers of Tregs such as TGF-β and IL-4 are suppressed by melatonin [95]. A study by Liu et al. attested the role of melatonin on the function of Tregs. In this study, mice bearing gastric cancer cells (murine foregastric carcinoma) were treated with different doses of melatonin (25, 50 and 100 mg/kg) for 7 days. Results showed a significant reduction in the tumor weight for 50 and 100 mg/kg melatonin. Furthermore, the melatonin dose of 100 mg/kg showed a significant effect on the reduction of Tregs ratio and Foxp3 expression in murine tumors [96]. Administration of melatonin to patients with

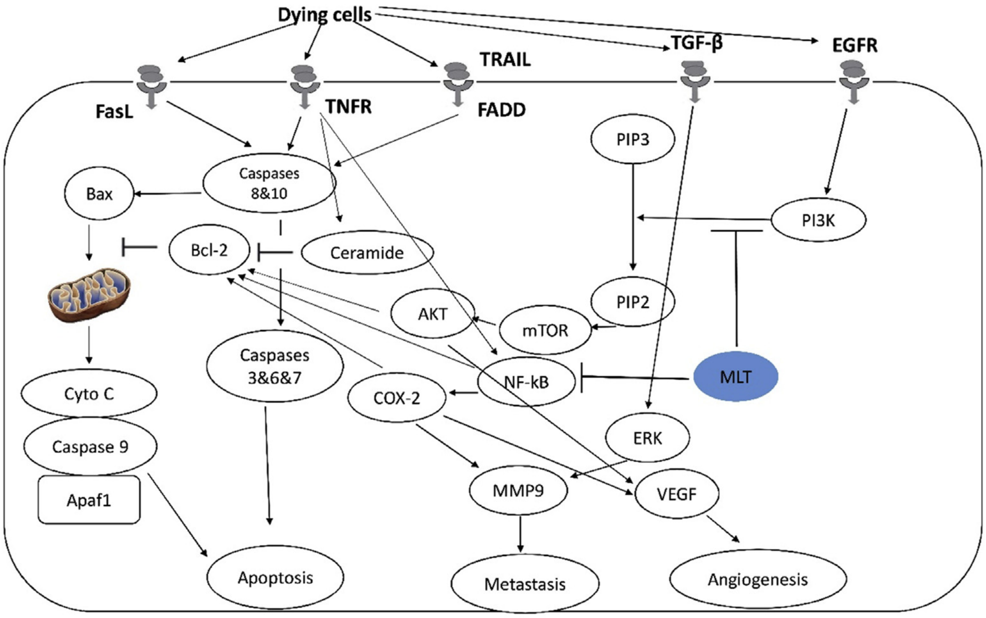

Fig. 2. Mechanisms of apoptotic resistance of cancer cells and sensitizing effect of melatonin. NF-κB plays a central role in the resistance of cancer cells to apoptosis. Melatonin via preventing nuclear translocation of NF-κB augments apoptosis rate. Inhibition of PI3K/mTOR pathway by melatonin is also involved in the progression of apoptosis in cancer cells.

untreatable metastatic solid tumor has shown a reduction in Tregs [97]. However, there is the need for further studies to investigate the possible role of melatonin on Tregs when combined with other cancer therapy modalities such as chemotherapy and radiotherapy.

1.5.4. Melatonin and CAFs

TGF-β is one of the most potent immune suppressive cytokines which aids tumor progression. Cancer associated fibroblasts (CAFs) are one of the main sources for the release of TGF-β and PGE2 to TME [98]. These secretions attenuate the activity of anti-tumor cells like NK cells and $CD8^+$ cytotoxic T cells [99]. Therefore, attenuation of CAFs and suppression of TGF-β can help eradicate cancer cells. Treatment of CAFs isolated from breast tumors with melatonin has been shown to suppress PGE2 and aromatase (an enzyme responsible for biosynthesis of estrogens, which also plays a key role in the progression of breast cancer) [100]. An *in vitro* study also showed that treatment of breast cancer cells co-cultured with CAFs led to reduction of angiogenesis factors [101]. Studies exploring the direct effect of melatonin on CAFs are very few. However, numerous studies have shown that melatonin is able to reduce the level of PGE2 and TGF-β in cancer cells following exposure to anti-cancer agents including radio- and/or chemotherapy [27,102].

1.5.5. Melatonin may reduce stemness in cancer

Stemness is associated with cancer resistance, EMT, invasion and metastasis. Some evidences have shown that melatonin reduces stemness in cancers [103]. Treatment of ovarian cancer cells with melatonin has been shown to reduce EMT and MMP-9, which are associated with attenuation of CSCs invasion and migration. Melatonin has also shown to reduce resistance to apoptosis in CSCs via downregulation of MAPKs and PI3K [104]. Similar results were observed for colon and breast CSCs [105,106]. A study showed that the suppressive effect of melatonin on melanoma CSCs is mediated via suppression of NF-κB. This study showed that inhibition of NF-κB p50/65 reduced iNOS and hTERT activities, leading to reduction of stemness in melanoma cancer cells [107].

1.6. Melatonin enhances therapeutic effect of immunotherapy

Immunotherapy is one of the most promising strategies for eradication of cancer cells. The combination of melatonin with other agents and therapeutic modalities may increase the chances of tumor control. Melatonin has been used in combination with IL-2 for many years [108]. Results suggested a better response of tumor and lower side effects compared to chemotherapy with cisplatin [109,110]. In recent years, some studies have been conducted to investigate possible positive effects of melatonin combination with immunotherapy and tumor vaccination. A study by Moreno et al. showed positive combination of melatonin and gDE7-based vaccine in mice bearing tumors. Melatonin was shown to amplify the release of IFN-γ, thus increases the therapeutic efficiency of tumor vaccination [111]. Also, melatonin in combination with human papillomavirus (HPV)-16 E7 DNA vaccine can amplify the anti-tumor activity of the immune system via triggering the release of IFN-γ and TNF-α, as well as increasing the number of $CD8^+$ cytotoxic T cells [112]. Melatonin has a role in increasing the number of $CD8^+$ cytotoxic T cells via suppressing PD-1 expression. Treatment of hepatocellularcarcinoma cells (HCC) with melatonin causes the release of some exosomes that suppress the regulation of PD-1 on macrophages [113].

2. Conclusion

The immune system plays a central role in the elimination or eradication of cancer cells. Modulation of immune responses within TME is one of the major mechanisms in cancer therapy. Radiotherapy, immunotherapy and tumor vaccination work via enhancing immune system responses against cancer cells. Melatonin as a natural hormone can modulate the immune system to aid eradication of cancer cells. It can induce apoptosis in cancer cells via suppression of anti-apoptotic mediators, especially via NF-κB and COX-2 signaling pathways. These genes are able to stimulate DNA repair and upregulate survival of cancer cells via suppression of pro-apoptotic genes. Melatonin can also reduce survival of cancer cells via actions on mitochondrial function. This is associated with downregulation of PI3K-AKT and mTOR pathways. In addition to direct effects of melatonin on cancer cells, it can change immune response within TME. Melatonin is able to increase NK and $CD8^+$ cytotoxic T cells via triggering the release of TNF-α and IFN-γ, while it reduces Tregs because of suppression of TGF-β. Melatonin has also been shown to suppress PD-1 expression, thus further amplifies the activity of $CD8^+$ cytotoxic T cells. These properties of melatonin may enhance therapeutic efficiency of anti-cancer therapy modalities such as immunotherapy. However, there is the need for further studies to explain the mechanisms of immunoregulatory effects of melatonin in combination with radiotherapy and chemotherapy.

Declaration of competing InterestCOI

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

Acknowledgment

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