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Review Article

A Holistic standpoint of Melatonin supplementation on cancer treatment outcome

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Abstract

Background for the study: Melatonin (N Acetyl 5 methoxy tryptamine), a biological synchronizer of human circadian clock, is a pluripotent pineal hormone. Melatonin, upon binding with its membrane receptors MT1 and MT2 regulate circadian and seasonal rhythms of biological clock, while it exerts its immunomodulatory and antitumor effects upon binding with its nuclear RZR/ROR class receptor. The present study was aimed to evaluate Melatonin's ability to address the major hallmarks of cancer, its shortcomings, its potential synergism with conventional chemotherapeutics and discussion on available preclinical and clinical data.

Results: Melatonin is found to attenuate almost all hallmarks of cancer progression. Melatonin reduces NAD+, downregulates HIF1α induced activation of Pyruvate dehydrogenase, thus attenuating Warburg effect. Melatonin also impedes SOX9 and HIF1α mediated epithelial to mesenchymal transition; attenuates sustained proliferation through downregulation of NFκβ, PI3K, HIF1α, ERα, cMyc-Nestin pathways in cancer. Melatonin also attenuates tumor angiogenesis while promoting would healing in normal tissues. Melatonin has strong immunomodulatory role through upregulation of T helper and NK cells and was found to impede cancer progression through Indolamine 2,3 Dioxygenase induced rapid tryptophan depletion from tumor microenvironment. However, dual reports of Melatonin's role in DNA damage repair process in cancer, necessitates in depth molecular analysis. Different clinical trial settings on adjunct Melatonin treatment along with different class of chemotherapeutics showed that adjunct Melatonin is best effective along-with antimetabolite chemotherapies.

Conclusions: Despite of earlier studies on Melatonin's efficacy as an oncostatic agent, a detailed analysis of Melatonin's effect in complex living system would unravel its true oncostatic potential.

Keywords: Melatonin, Melatonin Metabolism, Melatonin and cancer hallmarks, Preclinical and clinical studies

Introduction

Melatonin (N- Acetyl-5-methoxy tryptamine), a biological synchronizer of human circadian clock, is considered a wonder biomolecule, endowed with a myriad of clinical potential. Although various earlier studies have endorsed for its enormous oncostatic potential, no single study has ever elucidated the complete spectra of its functionality as well as its limitations in cancer treatment.

Melatonin is synthesized from the pineal gland upon sympathetic nervous system mediated activation from the suprachiasmatic nuclei (SCN). The synthesis and secretion of Melatonin occurs optimally during night, regulating a set of genes in a rhythmic pattern,[1] thereby regulating a diverse physiological function in the body, including regulation of sleep-wake cycle (circadian rhythm) and an enhancement of body's defence mechanisms.[2] Upon being synthesized from the pineal gland melatonin controls various essential physiological processes like regulation of circadian rhythm of biological system and neuroendocrine processes through activation of

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two G protein–coupled receptors, termed MT1 and MT2.[3] While both MT1 and MT2 regulate circadian and seasonal rhythms of biological clock, there are a second class of nuclear orphan receptors, the nuclear receptor superfamily RZR/ROR that mediate immunomodulatory and antitumor effects of Melatonin.[4] Association of Melatonin on tumor progression is intrinsically complex. The aim of the present study is to compile the available data and to understand the caveat in our existing knowledge on role of Melatonin in oncogenic progression.

Results from reported studies

I. Physiological role of Melatonin: Melatonin's primary physiological function relies on conveying the information about circadian light and dark cycle to the living system. The circadian clock is an orchestra, regulated by suprachiasmatic nuclei (SCN) and Melatonin, regulating a diverse group of physiological rhythmic functions of the body, viz. glucose homeostasis, circadian thermoregulation regulation of neuroendocrine control on diverse physiological functions.[5]

II. Melatonin metabolism in normal tissue and the context of tumor progression: In biological system, Tryptophan is metabolized through Kynurenine pathway and Serotonin pathway. Melatonin is synthesized from Tryptophan through Serotonin pathway in the biological system. Briefly, tryptophan is broken down by Tryptophan Hydroxylase (TPH1/2) and 5-Hydroxy Tryptophan decarboxylase into Serotonin, which through a series of reaction, produce Melatonin (N Acetyl 5 methoxy tryptamine) (Figure 1). The enzyme Indolamine 2, 3 Dioxygenase (IDO1/2) that regulate Tryptophan metabolism to form N-Formyl Kynurenine is the rate limiting factor for onset of Kynuranine pathway. In normal tissue, IDO1 is important in Tryptophan homeostasis and in induction of T cell's tolerance to foreign antigens viz. allergens through promoting differentiation of naïve CD4+ T cells into Foxp3+ Tregs.[6] IDO1 is reported to be upregulated in cancers.[7] The end result of Kynurenine pathway rapidly replenishes cellular pool of NAD+[8] and thus, Kynurenine pathway is a favoured pathway in many chemoresistant tumors.[9] It may be concluded that Melatonin which is also metabolized by Indolamine 2.3 Dioxygenase (IDO1) (Figure 1),[10] might deplete the pool of IDO1 from tumor cells, to form AFMK (N1 Acetyl N2 Formyl 5 Methoxy Kynuramine).[8, 11] Both Melatonin and AFMK could restore cellular NADH+ pool[12] (Figure 1), thus restoring mitochondrial function.[13]

III. Melatonin on cancer hallmarks: From various earlier reports, Melatonin has been associated with development of cancer and an early review suggested that Melatonin can impede or modulate almost all hallmarks of cancer progression[14] (Figure 2). The brief mechanism of oncostatic mechanism of Melatonin can be summarized as follows:

A. Induction of cell death and apoptosis

In synergism with Doxorubicin, Melatonin was reported to induce apoptosis in breast cancer cell line MCF-7.[15] Other reports suggested that while Melatonin exerts its proapoptotic effect on tumors, it differentially protects immunocytes and neurocytes from reactive oxygen triggered apoptosis.[16]

B. Melatonin on sustained cellular proliferation process

Melatonin induced downregulation of NFκβ, PI3K signalling, Cyclins and CDKs, estrogenetc impact sustained proliferative signalling in cancer cells.[14]

i) Role on HIF1 α signalling: Hypoxia is an important contributor of sustained proliferation signalling. In normal tissues normal Hypoxia induces nuclear translocation and SUMOylation of HIF1α, which binds to VHL, leading to ubiquitination and proteasomal degradation. A previous report stated that Melatonin nuclear receptor of RZR/ROR family subtype γ to be overexpressed in gastric cancer cell line and Melatonin downregulated RZR/RORγ and prevented SENP1 (SUMO-specific protease 1) induced nuclear stabilization of HIF1α.[4] Melatonin induced HIF1α destabilization also occurs through downregulation of sphingosine kinase 1 (SPHK1) a HIF-1α modulator, as observed in PC-3 prostate cancer cells under hypoxia.[14] Melatonin also inhibits the Akt/ glycogen synthase kinase-3β (GSK-3β) signaling mediated stabilization of HIF-1α .[14]

ii) Role on c-Myc-Nestin Pathway: c-Myc-Nestin signalling play an important role in maintaining stemness of tumors. c-Myc is upregulated in invasive tumors like glioblastoma and a positive correlation of Nestin upregulation with c-Myc was observed, resulting in poor prognosis of the tumor. Melatonin induced attenuation of small ubiquitin-related modifier-1 (SUMO-1) disturbed nuclear translocation of nestin for its direct binding to c-Myc, thereby preventing stemness of cultured glioblastoma cells.[17, 18]

iii) Role on Nuclear factor-kappa Beta (NF-κB) signalling pathway: NF-κB activation has implication in sustaining proliferative signalling through inflammatory response. Earlier study revealed Melatonin's efficacy in preventing nuclear translocation of NF-κB, thereby enhancing the anticancer effect of berberine against lung cancer.[14] Also, invasiveness of hepatocellular carcinoma cells were attenuated

through Melatonin mediated NF-κB inhibition.[14]

iv) **Role on Phosphoinositide 3-kinase (PI3Ks) signalling pathways:** In normal tissue, Melatonin, through its interaction with circadian clock component Bmal1, increases cellular survival through increased phosphorylation of AkT, ERK-1/2, PDK1, mTOR, PTEN, GSK-3αβ, and p70S6K.[19] While PI3k/AkT activation is associated with Melatonin's neuroprotective effect on normal tissue,[20] Melatonin mostly showed inhibitory effect on PI3K signaling pathway in tumors. Melatonin alone or along with vitamin D3 was reported to inhibit breast cancer cell proliferation.[14] In melanoma cells, melatonin alongwith endoplasmic reticulum stress-inducers (thapsigargin or tunicamycin) resulted in cell death through inhibition of PI3K/Akt/mTOR pathway.[14]

v) **Role on estrogen signalling pathways:** Melatonin inserts its anti-estrogenic anti tumor promoting function through the following pathways, viz. Melatonin can alter the effect of estrogen in three different ways:
   (a) direct inhibition of steroid synthesis by gonads;
   (b) inhibition of aromatase leading to andro gen synthesis;
   (c) binding with estrogen receptor to prevent its transactivation[14] of Calcium/Calmodulin complex and activation of proteins like Rac, Cdc42, IQGAP etc that are associated with tumor cell metastasis.[21,22] An earlier report showed Melatonin not only directly ERα mRNA expression but MT1 induced activation of G (αi2 and subsequent reduction of cAMP level, prevented estrogen induced activation of and estrogen-induced transcriptional activity of the ERα through MEL receptors 1 (MT 1)-induced activation of ERα.[23]

vi) **Role on Hippo signalling:** Hippo signalling pathway is a negative regulator of cell proliferation and is reported be dysregulated in human cancers. A recent study showed that Melatonin downregulated YAP, an inhibitor of Hippo signalling and inhibition of YAP resulted in downregulation of Bcl-2 and GLUT-3 in hepatocellular carcinoma cells leading to apoptosis.[24]

C. Melatonin on tumor metabolic hallmarks

Melatonin, through downregulation of HIF1α, downregulates HIF1α induced activation of Pyruvate dehydrogenase by phosphorylation, thereby preventing Warburg effect through increased lactate production.[14] Melatonin was also reported to downregulate MYC activation and associated genes associated with glucose metabolism viz. glycolytic enzymes, PDK1, lactate dehydrogenase, glucose transporters like GLUT4 etc.[14,25] G protein coupled receptor mediated modulation of insulin signalling pathways,[25] indicating its potent regulatory role in metabolite uptake. In normal tissue, Melatonin in duces rapid glucose uptake in muscle tissues through insulin receptor substrate.[26]

Melatonin binding to its receptors, viz. MT1 and MT2, inhibits adenylate cyclise activation, thus lowering the intracellular level of cyclic adenosine monophosphate (cAMP). This in turn reduces cellular pool of protein kinase C (PKC), protein kinase A (PKA), mitogen-activated protein kinases (MAPKs) which in turn downregulates a set of genes viz. c-fos, c-myc etc, that are associated with cellular proliferation c-fos, c-myc and downregulation of genes associated with the process of proliferation.[27]

D. Melatonin on epithelial to mesenchymal transition (EMT)

EMT, the process by which polarized epithelial cells that progressively lose their functionality to form undifferentiated mesenchymal phenotype, is an indispensable process involved in cancer cell metastasis, apart from its implication in various physiological processes like embryogenesis, wound healing etc.[28] While type 2 EMT is connected with wound healing, the type 3 EMT is responsible for cancer metastasis.[28] Earlier studies suggested that Melatonin attenuated hypoxia induced upregulation of CCL20 and induction of EMT in glioma cells.[29] Also in osteosarcoma stem cells, Melatonin suppressed EMT through SOX mediated signalling.[30]

E. Interference with DNA damage repair process in tumors

Melatonin, through upregulation of p53 and reduction of RAD51 and DNA-PKcs, reduces the chances of DNA damage repair especially through the NHEJ (Non-Homologous End Joining) pathway in tumors.[31] A second class of DNA damage repair system include SIRT1, a class III histone deacetylase and previous report in prostate cancer cells corroborated the antagonizing effect on Melatonin on SIRT1 expression and attenuation of DNA damage repair.[32] Melatonin has also been shown to increase DNA repair capacity in breast and colon cancer cell lines as observed through comet assay.[33] While an earlier study affirmed that both Melatonin and AFMK could enhance chemosensitivity of Gemcitabine in pancreatic cancer cells (PANC1),[34] their ability to induce DNA damage repair in tumors might compromise their efficacy when given in combination with chemotherapeutics that exert their function

through generation of free radicals. For normal tissue, upregulated phosphorylation of p53 at Ser-15 residue prevents DNA damage accumulation, through recruitment of ATM and ATR protein kinases.[35, 36]

F. Melatonin on immune surveillance and immune escape

Escape of immune surveillance is one important hallmark of cancer progression. One fundamental mechanism of escape of immune surveillance by tumor cells, is through upregulation of IDO1 (Indolamine 2, 3 Dioxygenase), an important regulator of Kynurenine pathway that depends on Tryptophan bioavailability. Upregulated IDO1 in resident tumor cells sequesters Tryptophan from the tumor microenvironment, rendering the lymphocytes early senescence and rapid depletion of tumor infiltrating T helper lymphocytes with upregulation of T regulator cells[37,38] (Figure 3). Moreover, tumors overexpressing IDO1, promote N- formyl-kynurenine, which promotes tolerogenic immune response in tumor microenvironment. Melatonin, like all indolamines is also a substrate for Indolamine 2, 3 dioxygenase (IDO1). Based on earlier report of Melatonin to downregulate IDO1 and Kynurenine in Melanoma cells,[39] it may be stated that Melatonin, through quenching overexpressed IDO1, possibly prevents the rapid depletion of Tryptophan from tumor microenvironment. As evident from earlier literature, CD4+ T cells express membrane as well as nuclear Melatonin receptor and exogeneous Melatonin is reported to augment their proliferation.[40]

As evident from pinealectomized animals, a total depletion of circulating lymphocytes and absence of lymphoblasts along with significant reduction of structural components of major immune organs viz. lymph nodes, thymus and spleen showed that Melatonin to be an indispensable factor for immunocyte maturation. Prolonged administration of Melatonin was found to increase the amount and the activity of circulating NK cells.[41] Although several earlier studies indicated that Melatonin significantly decreases Treg cell population and upregulates T_H cell population,[41] impact of Melatonin supplementation of patient undergoing immunotherapy is still elusive.

An earlier report suggested that Melatonin supplementation during immunotherapy with Nivolumab, significantly increased the objective response rate in patients.[42]

G. Effect on angiogenesis

Factors inducing tumor angiogenesis viz. VEGF (vascular endothelial growth factor), PDGF (platelet-derived growth factor), EGF (epidermal growth factor), and HGF (hepatocyte growth factor) are reportedly downregulated upon Melatonin treatment.[14] In contrast, Melatonin supplementation augmented angiogenesis and wound healing in normal tissues as evident from previous reports[43] (Figure 2).

H. Effect on tumor promoting inflammation

Inflammation has a direct association with cancer progression through induction of several mediators like cyclooxygenase-2 (COX-2), NF-κB, tumor necrosis factor alpha (TNF-α) etc. In normal rat model, Melatonin at a dosage of 10mg/ml was effective to treat colitis.[14] In human breast carcinoma MDA-MB-231 cells, Melatonin induced up-regulation in the expression of the proapoptotic protein Bim and down-regulation of COX-2 expression were reported as a mechanism of action of melatonin to inhibit tumor progression.[14]

I. Epigenetic regulation of tumor

Melatonin, through its MT1 receptor, induced chromatin hyperacetylation leading to suppression of tumor cell proliferation and apoptosis.[27] Melatonin was reported to counteract different histone deacetylases, leading to suppression of tumor cell proliferation.[27] Earlier reports showed that Melatonin attenuated oral cancer progression through suppression of histone lysine specific demethylases.[27] Melatonin was reported to downregulate miR-24, a micro RNA that promotes the genes related to cell division.[27]

Preclinical and clinical data on Melatonin supplementation on tumor progression

Earlier reports showed that in animal models, Melatonin prevented obesity and reduced the activity of aromatase, suggesting its probable association in reducing the risk of breast cancer development in animal models.[44] In rat mammary carcinogenesis model, Melatonin in combination with pravastatin, significantly affected mammary tumor formation.[45] Intraperitoneal and oral administration of Melatonin prevented hepatic and pancreatic tumor progression respectively.[27] Melatonin also prevented DMBA induced ovarian tumor formation in rats.[27] In animal model of melanoma, Melatonin halted the progression of tumor through G2/M dependent cell cycle arrest and through redistribution of cell surace F- actin.[46]

In clinical settings, a Meta-analysis by Wang et al (2018) showed Melatonin supplementation to improve tumor remission rate, overall survival rate as well as incidences of chemotherapy side effects in cancer patients.[47] In a recent study, Melatonin attenuated the adverse effects of adjuvant chemotherapy through improved neuroprotection, cognitive function etc.[48]

Melatonin on efficacy of chemotherapeutics at the molecular level

A recent report suggested synergistic effect of Melatonin on anticancer effect of shikonin (SHK) through inhibition of SIRT3/SOD2-AKT pathway.[49] A synergism of Melatonin with 5-fluorouracil in colon cancer was also observed suppressing PI3K/AKT and NF-κB/iNOS signaling pathways.[50] An earlier report suggested that, Melatonin, through its interference with Akt and MAPK signaling pathways, affects drug resistance in tumors.[51]

Analysis of Metatonin's efficacy on different clinical settings

In order to study the efficacy of Melatonin on different clinical trials as adjunct therapy, different clinical trials were set up earlier. A study by Lissoni et al (1997) showed limited therapeutic efficacy of Melatonin when given in combination with DNA damaging drugs viz. Cisplatin and Etoposide (Melatonin with chemotherapy vs. chemotherapy alone (11/34 vs. 6/35; OR:2.391; p value 0.132), although 1 year survival benefit was higher in the Melatonin treatment arm than control group.[52] Melatonin also showed limited efficacy in combination with Irinotecan alone (stable disease in 5 of 16 patients treated with Irinotecan alone and in 7 out of 14 patients treated with Irinotecan and Melatonin).[53] However reports from other clinical trials demonstrated Melatonin's efficacy in combination with Gemcitabine and Cisplatin or along with Taxane, Cisplatin and 5-FU (Fluorouracil)[53,54] indicating that Melatonin functions better in combination with antimetabolite chemotherapies. A recent report affirmed Melatonin's efficacy in combination with anti PD-1 monoclonal antibody, Nivolumab[42] (Table 1).

Table 1: Different class of chemotherapeutics under present review

Class of drug	Name
DNA damage inducer	Cisplatin, Carboplatin
Topoisomerase inhibitor	Etoposide, Irinotecan
Microtubule stabilizer	Taxanes eg: Paclitaxel
Antimetabolite	Gemcitabine, 5FU
Immune checkpoint blocker	Nivolumab

Figure 1: Melatonin metabolism in biological system. Briefly, the Serotnin pathway and the Kynurenine pathway maintain the balance in Melatonin metabolism. In cancers, the rate limiting enzyme of Kynurenine pathway, viz. IDO1/2 is upregulated, which, coupled with dysregulated Melatonin synthesis, leads to accumulation of cellular NAD+ pool which could contribute to Warburg effect of increased aerobic glycolysis. The antioxidant effect of Melatonin and its metabolite AFMK is expected to reduce the cellular pool of NAD+ in cancer.

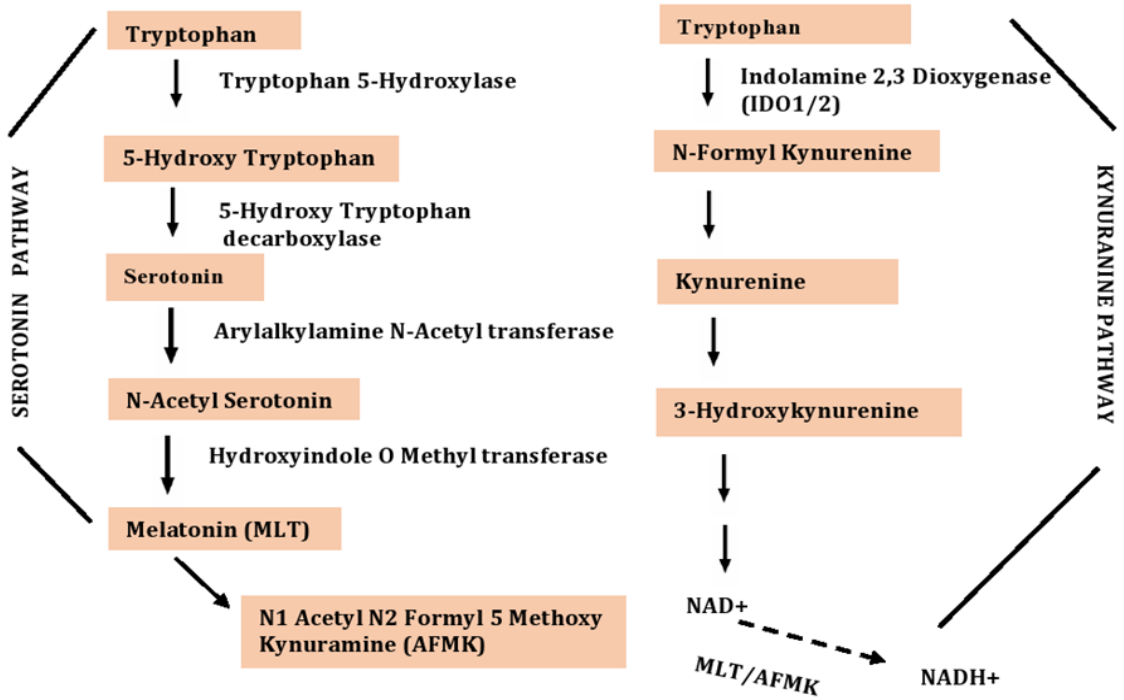


Figure 2: Pluripotent efficacies of Melatonin in normal and cancer tissues. While Melatonin showed a plethora of effects like anti-inflammatory effect, anti-Warburg effect, antiangiogenic effect, immunomodulatory and immune evasion antagonistic effect that support its oncostatic role, some studies showed that it could also upregulate DNA damage repair pathways in tumors (indicated in red) which might counterpoise the effects of DNA intercalating chemotherapeutics.

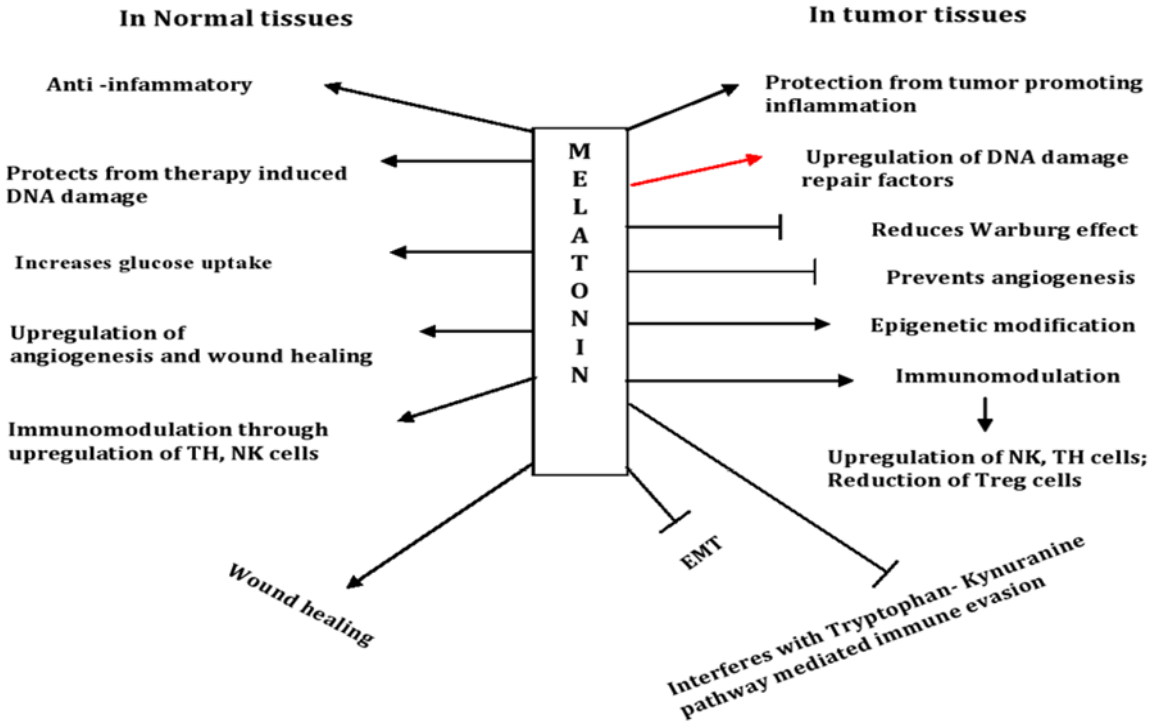
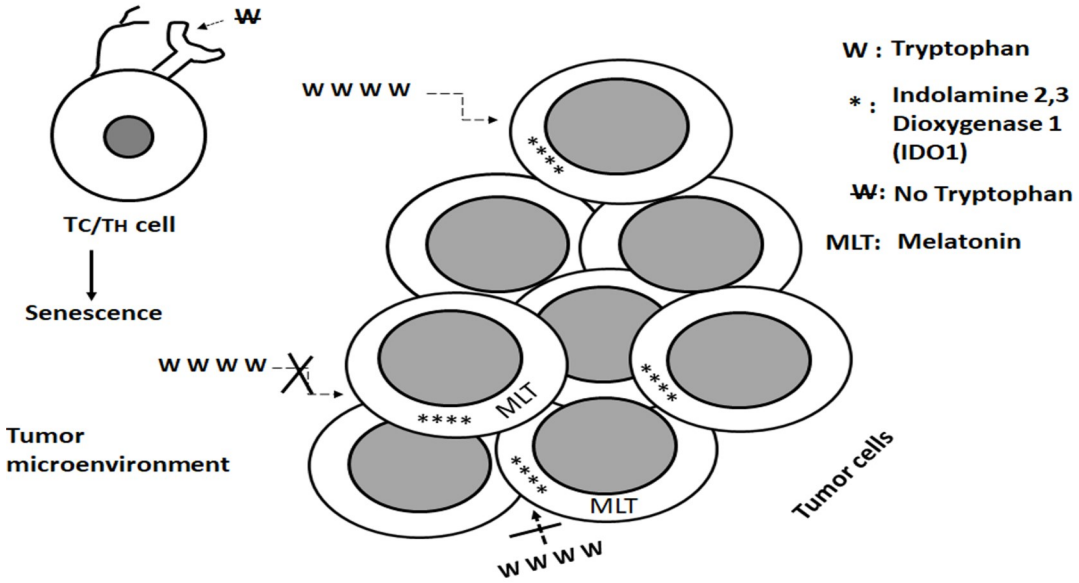


Figure 3: Melatonin on immune-evasion of tumor cells. Indolamine 2,3 Dioxygenase (IDO1) which is overexressed in tumors, renders the tumor microenvironment Tryptophan deficient, resulting in early senescence of infiltrating T effector (TH, TC) cells. Melatonin, like Tryptophan is also a substrate of IDO1, thus reduce the cellular pool of the enzyme, keeping the free Tryptophan in tumor microenvironment available for T effector cells to mature.



Controversies on Melatonin efficacy as anticancer agent

Although Melatonin is a standalone naturally occurring oncostatic biomolecule, there are reports on its adverse effect. Earlier studies showed that Melatonin administered during morning can stimulate tumor growth.[56] Another report in Glioma showed that Melatonin pretreatment upregulated cell proliferation associated genes and a high therapeutic concentration of Melatonin (>5 mM) only resulted in cell death.[57] Based on these reports, it may be concluded that the antitumor effect of Melatonin lies on its dosage as well as the time of administration.

Discussion

Melatonin was reported to affect almost all hallmarks of cancer. We observed that the first and foremost anticancer effect of Melatonin lies on its biosynthesis mechanism, where both Serotonin and Kynurenine pathway maintain the balance in Melatonin metabolism and in cancers, owing to dysregulated Melatonin synthesis, cellular pool of NAD+ contribute to Warburg effect of anaerobic glycolysis to promote cancer (Figure 1). The anti-Warburg effect of Melatonin was also conspicuous through prevention of Pyruvate dehydrogenase activation, c-Myc activation, PDK1, lactate dehydrogenase, glucose transporters like GLUT4 etc.[14,25] Contrarily in normal tissues, Melatonin induces rapid glucose uptake, as was observed from earlier studies.[26] Earlier studies confirmed that Melatonin attenuates different tumor survival pathways viz. HIF1α signaling, c-Myc-Nestin pathway, NF-κB signalling pathway, Phosphoinositide 3-kinase signalling pathways, Estrogen signaling pathways.[4, 14, 17, 18, 19, 21,&22] Melatonin was also found to upregulate Hippo signaling pathway, one important negative regulator of tumor proliferation.[24] Melatonin induced apoptosis in tumors, attenuated hypoxia induced upregulation of CCL20 and EMT in glioma cells,[29] SOX mediated EMT in osteosarcoma stem cells.[30] However, controversial reports are observed in terms of Melatonin's role on DNA damage repair in tumors, as discussed before. Previous studies showed Melatonin to be efficient in combination with antimetabolites and less efficient in chemotherapy system having only DNA damaging or replication inhibitor drugs.

An important hallmark of Melatonin's role in oncostasis is through inhibition of Indolamine 2,3 dioxygenase mediated upregulation of Kynurenine pathway that otherwise confers a tolerogenic microenvironment in tumors. Melatonin promotes upregulation of T helper cells and NK cells and thus augment tumor immune response.[37-39]

Melatonin also exerted its antiproliferative effect in preclinical as well as clinical system and it was found to be well tolerated even at high dosage.[58] It is worthy to be noted that low Melatonin level night shift workers makes them susceptible to several metabolic diseases including cancer,[59] implicating immense therapeutic potential of Melatonin in cancer.

Although a lot of studies have been conducted on Melatonin's efficacy as an oncostatic agent, a detailed analysis of Melatonin's effect in complex living system would be beneficial for renovating anticancer defence in human system.

Conclusion

The present study highlighted the role of Melatonin, the circadian clock regulating pineal hormone, on different molecular pathways of cancer development and its differential impact on tumor and normal tissues, preclinical and clinical aspects of adjunct Melatonin supplement on tumor progression, different molecular mechanisms of Melatonin's synergistic effect on various anticancer therapies and endorse its enormous oncostatic potential which need more extensive research.

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Conflict of interest

Authors declare that there is no potential conflict of interest.

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