Biological Effects of Melatonin on Telomere Length in Breast Cancer: A Review Article

Elmira Barari[1] [ID], Golnoosh Azarsina[1], Gordon A. Ferns[2], Saeed Pirouzpanah[1*] [ID]

[1]Molecular Medicine Research Center, Biomedical Institute, Tabriz University of Medical Sciences, Tabriz, Iran.
[2]Department of Medical Education, Brighton and Sussex Medical School, Brighton, BN1 9PH, UK.

Abstract

There has been increasing interest in studying the effects of dietary factors on telomere length. The telomere is a noncoding DNA sequence including "TTAGGG" at the ends of chromosomes of vertebrates. The stability of telomere length is an important factor as a survival signal for cells and cancer prevention. Telomerase is a multi-subunit DNA polymerase that plays a crucial role in maintaining the telomere length, which is critical for the age-related pathogenesis of breast neoplasm. Some regulatory factors interfere with telomerase activity and therefore promote breast tumorigenesis. High telomerase activity and restoring telomere lengths are determined as key factors in progressing tumors to advanced stages of malignancies, which are highly estrogen-dependent in breast carcinogenesis. Melatonin is a hormone-like substance secreted by the pineal gland and has been reported to downregulate telomerase. It may therefore control telomere length in cancer cells. Certain malignancy-related biological pathways have recently been linked to telomere length, and this review provides new insights regarding the effects of melatonin on telomere length by reviewing the anticarcinogenic mechanisms underlying melatonin in relation to telomerase activity in breast carcinogenesis. Experimental insights presenting the effects of melatonin alone or in combination with drugs on enhancing therapeutic protocols were also reviewed, which could assist our understanding of this hormone-like substance and telomeres as prognostic and therapeutic biomarkers in breast cancer.

Introduction

The telomere is a tandem repeat of a noncoding DNA sequence (TTAGGG) located at the end of eukaryotic chromosomes.[1,2] The telomere and its shelterin complex prevent the destruction of the chromosomal endings, by which numerous mitoses and DNA replications at chromosomal endings is possible and may impair telomere maintenance that leading to a reduction in its length.[3,4] Importantly, the length of telomere has been suggested to contribute to cell aging, and many carcinogenic alterations are age-dependent hence telomere shortening may contribute to several biological pathways, including the inactivation of tumor suppressors and promotion of proto-oncogenic activation.[5,6] Experimental studies have supported the notion that telomere shortening is a process actively involved in the initiation of tumorigenesis of breast cancer cells.[7] Observational studies have shown that a reduction in the length of the telomere can increase the risk of breast cancer,[8-10] whereas some epidemiological studies have not confirmed this.[11-14] Telomere length varies at different stages of breast cancer, suggesting that the shorter telomere lengths are associated with advanced stages of breast cancer.[15-18] Sanft *et al.*[19] showed the effects of a six-month weight loss program in overweight and obese women with breast cancer that resulted in telomere lengthening in participants, indicating that telomere length can be affected by environmental factors, such as diet and physical activity.[20] A clinical study has shown that a Mediterranean diet that is associated with a lower risk of cardiovascular disease in women was also associated with telomere lengthening.[21]

Telomerase is an enzyme that contributes to the stability of the telomere length.[22] Telomerase is made up of different proteins and non-protein compartments; each has different functions in regulating and catalyzing the functions of telomerase.[23] It has two important components that contribute to the reverse transcription of telomeres; telomerase reverse transcriptase (TERT) and telomerase RNA (TR).[24] The specific sequence of TR is used as a pattern for the arrangement of nucleotides at the chromosomal termination sites, therefore leading to telomere elongation.[25] Abnormal telomerase activity causes cell damage and could ultimately result in cell death.[26] Mutations in telomerase-encoding genes can lead to the development of several age-associated diseases.[27] Interestingly, the reactivation of telomerase may reverse a

wide range of conditions associated with tissue damage,[26] under certain conditions, such as specific carcinogenic conditions, the activity of telomerase increases.[18,28] Therefore, the process of telomere shortening does not occur during repeated divisions, and thus the aging process is curtailed; but the problem is that telomerase reactivation occurs in an advanced condition of breast cancer and may lead to cell proliferation unrelated to telomere length.[18] Therefore, telomerase inhibitors are the subject of research interest in the treatment of some cancers.[29]

Radiation exposure is an environmental factor that can increase telomerase activity and interestingly makes neoplastic cells resistant to subsequent radiation.[30] Moreover, resistance to treatment is caused by the over-expression of telomerase in patients undergoing chemotherapy; thus, the choice of the type of treatment, as well as the response to treatment, could be partly determined by the level of telomerase activity.[31,32] For these reasons, regulating telomerase activity may be necessary. However, several drugs used to control telomerase activity may have off-target effects, predisposing cells to damage, generating unnecessary cellular responses, genetic alterations, and resistance to treatment.[33-36]

Melatonin (5-methoxy-N-acetyltryptamine), is a hormone secreted by the mammalian pineal gland.[37] It is available as a supplement to treat insomnia and has low reported toxicity which has been demonstrated in both animal and human studies. Melatonin is a natural substance that has several biological effects involved in health.[38] Telomere integrity is another critical factor in breast carcinogenesis that could be affected by melatonin.[39-41] Melatonin is also involved in mitochondrial homeostasis.[42] Hence, it may have potential endogenous antioxidant effects to attenuate cellular oxidative stress.[42,43]

It has been reported that melatonin levels are low in patients with breast tumors.[44,45] Melatonin-related nuclear receptors mediate anti-tumor effects, including: stabilizing telomeres,[46] anti-estrogenic effects, and suppressing estrogen receptor mRNA expression in hormone-dependent cancer,[47,48] anti-angiogenesis effect,[49] inhibition of metastasis,[50] proapoptotic effects on cancer cells,[51] cell cycle modulation[52] and immune system regulation.[53] The telomeric repeat amplification protocol (TRAP) assay has shown that melatonin reduces telomerase activity.[39] Accordingly, cancer susceptibility to metastases and tumor sizes was decreased *in vivo*.[54-56] An *in vitro* study showed that melatonin could downregulate the mRNA expression levels of TERT and TR in MCF-7 cells.[39] Despite the potential effects of melatonin on telomerase activity and telomeres length in cancer, there is limited data on the mechanistic details specifically in breast cancer.

There is a lack of a comprehensive review detailing the interactions between melatonin and telomerase activity, as well as their association with breast cancer. Therefore, this review aimed to provide mechanistic insight, describing the effects of melatonin in association with telomerase reactivity and telomere lengthening in breast cancer.

Telomere
Structure
The telomere is a similar sequence on single or different chromosomes that is rich in guanine repeats constituents "TTAGGG".[57] Telomeres are located at both ends of eukaryotic chromosomes.[58] At the end of this region, there is a T-loop structure that contains attachment points for proteins that are called the Shelterin complex, which plays an important role in telomere function. This protects the T-loop and has six subunits (Figure 1), including telomeric repeat factors (TRF1 and TRF2), TRF-interacting nuclear protein 2 (TIN2), TPP1 (TINT1/PTOP/ PIP1), repressor/activator protein1 (RAP1), and protection of telomeres-1 (POT1).[59] These proteins have specific roles in restoring
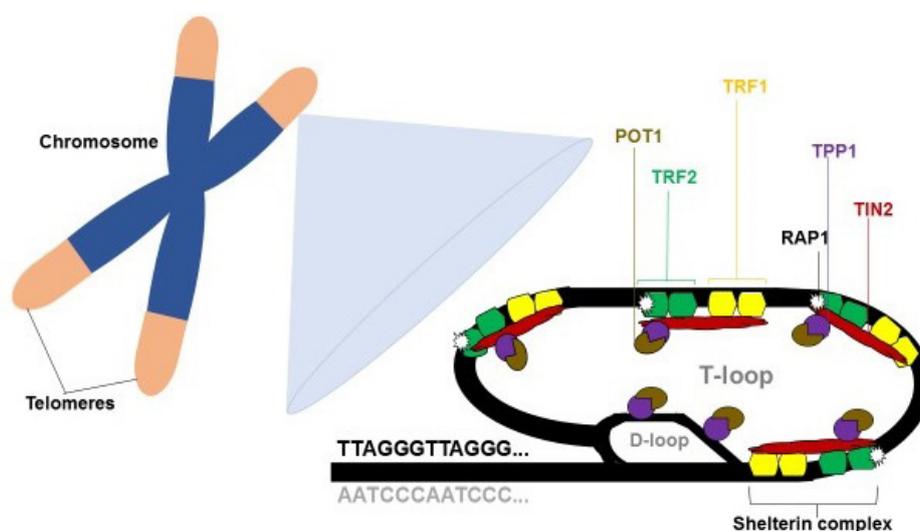

Figure 1. Telomere structure. The telomere comprises the TTAGGG sequence in the terminal region of the chromosome, with T and D loops at the end section. T-Loop contains a complex called shelterin, which is made up of TRF1, TRF2, RAP1, POT1, TPP1, and TIN2 proteins and acts as a telomere protector.[52] TRF1: Telomeric repeat factor-1; TRF2: Telomeric repeat factor-2; RAP1: Repressor/activator protein1; POT1: Protection of telomeres-1; TPP1:TINT1/PTOP/ PIP1; TIN2: TRF2 interacting nuclear protein2.

the telomere length. The DNA molecule, in addition to the shelterin protein complex, is termed a telosome,[25,60] which contains another region called telomeric repeat-containing RNA (TERRA). TERRA is generated by RNA polymerase II and is involved in regulating the factors related to telomeres and telomerase.[25]

Functions

Telomeres, along with the six attached telomere-specific proteins form the telomere complex (telosome), which plays a number of important roles in the processes associated with chromosome longevity and function.[60] They protect the DNA molecule and chromosomes in various ways, such as preventing improper chromosome linkage and also protecting the ends of the chromosomes from being damaged.[61,62] The structure of the telomere protects the end of the chromosome from being destroyed by exonuclease.[63] Similarly, telomeres play key roles in that the chromosomes how located and placed in the nucleus and contribute to the selective silencing of genes adjacent to telomeres.[25,64] Moreover, telomeres prevent the instability of the genome by detecting the double-strand breaks of the DNA.[65-67] Finally, a lack of telomere activity or its reduced length may lead to chromosomal abnormalities.[61,62,68]

Among six protein subunits present in the structure of the telosome, TRF1 and TRF2 are of the most importance. TRFs involve the Myb domain which is a binding site to double strands of DNA and interacts with some other proteins that participate in cell growth and proliferation.[60] Consequently, the action of the telomere may be maintained by being protected on its ends against shortening and deformation.[60,69]

Melatonin
Biosynthesis
The mammalian pineal gland synthesizes and secrets several indoles and peptides.[70] Melatonin is one of the most important substances of the pineal hormones.[71] Tryptophan is an amino acid which actively transported into the pinealocytes and is converted into 5-hydroxytryptophan (5HTP) catalyzing by the tryptophan hydroxylase (TH). The activity of TH is increased in the absence of light. Serotonin (5- hydroxytryptamine, 5-HT) is produced from 5HTP by the action of 5-hydroxytryptophan decarboxylase (5HTPD).[72] Indoleamine N-acetyltransferase (NAT) converts 5HT into the N-acetylserotonin (NAS), which is then, the O-methylation of NAS is a reaction governed by the hydroxyindole-O-methyltransferase (HIOMT) enzyme, and eventually, the end product is melatonin (N-acetyl-5-methoxy tryptamine; Figure 2).[73,74] The rate of melatonin formation is controlled by the activities of the TH and NAT enzymes.[72,75] Two NAT isoforms have been identified. In the rat, sheep, and probably all mammalian pineal glands, both forms of this enzyme are acting at the same time and each is performing their tasks separately.[72,76]

Regulation of melatonin synthesis
Cellular melatonin levels are affected by various factors.[77] The most important environmental stimulus of melatonin production is light.[72,78] Light exposure leads to signals by neurons to the suprachiasmatic nuclei located in the retinohypothalamic fibers of the hypothalamus (the major circadian pacemaker in human CNS) that end in the terminal axons in the pineal gland, and controls melatonin production.[79] Thus, according to the time of day, melatonin concentrations vary. In fact, at night, melatonin is secreted at higher levels than during the day.[72,79] Therefore, melatonin secretion is suppressed in the presence of light.[72] However, if the daytime duration is very long, the body adapts to secret melatonin even if the light is present.[79]

Some cytokines, such as the interferon-gamma)IFN-γ(can stimulate melatonin secretion, while other cytokines, such as the interleukin-1)IL-1(inhibit melatonin secretion.[80] A major factor that may affect the sleep-promoting function of the pineal gland is norepinephrine, which is released by signals from the suprachiasmatic
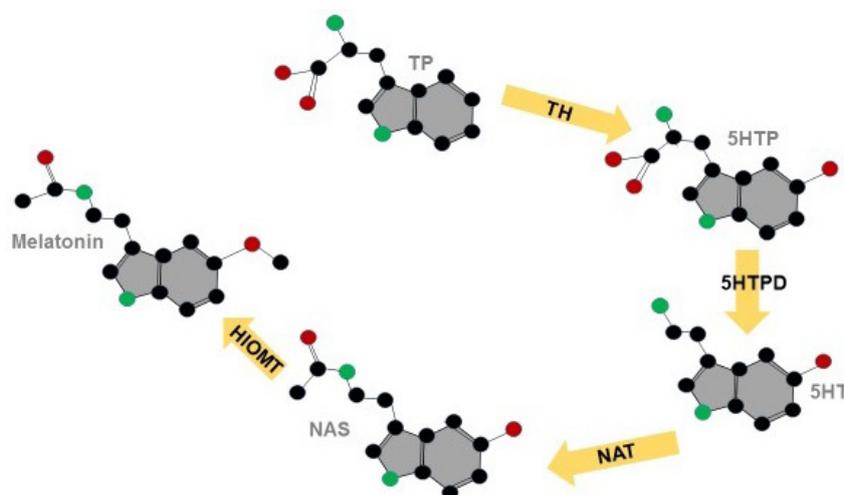

Figure 2. Melatonin production. Tryptophan is converted to melatonin by the TH, 5HTPD, NAT and HIOMT enzymes. TP: tryptophan; TH: tryptophan hydroxylase; 5HTP: 5-hydroxytryptophan; 5HTPD: 5-hydroxytryptophan decarboxylase; 5HT: 5- hydroxytryptamine(Serotonin); NAT: N-acetyltransferase; NAS: N-acetylserotonin; HIOMT: hydroxyindole-O-methyltransferase.

nuclei.[72,79] Therefore, factors that affect norepinephrine levels can influence melatonin levels indirectly.[81] Unlike monoamine oxidase inhibitors and tricyclic antidepressants, β1-adrenergic repressors can reduce the synaptic availability of catecholamine.[79] In addition, dihydropyridine (calcium antagonists) and prostaglandin inhibitors are factors pronounced more likely to influence melatonin secretion.[79,82]

Seasonal variations and long-term exposure to magnetic fields affect melatonin levels.[83,84] Also, levels of melatonin in people suffering from blindness, depression, and insomnia are generally low.[72,78] Nutrition plays an important role in providing melatonin and related substrate (tryptophan). Folate and vitamin B6 are essential cofactors required to catalyze reactions in melatonin production. Therefore, an inadequate intake of certain nutrients that have key roles in the synthesis and secretion of melatonin can significantly disturb the melatonin synthesis,[85-88] thus affecting the telomerase, telomere length, and potentially related risks of cancer.[89-91]

Melatonin receptors

Melatonin is soluble in both water and lipids, thus allowing it to cross the plasma membrane and exerting a wide range of functions.[79] The presence of melatonin receptors in either the cell or nuclear membrane is a determinant defining the type of functions related to melatonin.[92,93] Various melatonin receptors have been identified in vertebrates, including Mel1a, Mel1b, and Mel1c, two of which are found in mammals and have been termed Mel1a (MT1) and Mel1b (MT2).[94]

Generally, MT1 and MT2 are receptors that function through adenylyl cyclase. However, only the MT2 receptor is engaged in inhibition of the guanylyl cyclase-related pathway.[95] They have different distributions in the brain.[94,96] The pars tuberalis (anterior lobe of the pituitary gland) is the neurologic part specifically identified by significant over-expression of melatonin receptors.[97] The MT1 receptor is responsible for melatonin-related signaling that is greater than functions observed by the MT2 receptor.[98] On the other hand, the MT2 receptor is a principal molecule in regulating circadian rhythm and is highly over-expressed in the retina[96]. In addition to membrane receptors (MT1 and MT2), melatonin can bind nuclear receptors, which include retinoic acid-related orphan receptors (RORs)/RZR.[99] Unlike the MT2 membrane receptor, the expression of MT1 and RORα receptors varies in different tissues, including the thymus, spleen, brain, liver, kidney, heart, and lungs.[92,99] The MT2 receptor and nuclear binding receptors particularly have important roles in cancer-related immune response.[100,101]

The functions of melatonin

The functions of melatonin are numerous and have been identified in several organ systems.[77] Melatonin is well-known for controlling circadian and seasonal rhythms.[102] Melatonin has extensive effects on the gastrointestinal tract,[103,104] reproductive system,[105,106] bones,[107] immune system,[108] and other tissues.[78,109] It also plays an important role in cardiovascular disorders, diabetes,[110,111] Alzheimer's diseases,[112] and mood disorders.[113]

Many studies have demonstrated that low concentrations of serum melatonin may increase the risk of cancer.[114-117] Melatonin showed some effects on the onset and progression of cancer, and also different cancer-related factors are still a subject of debate.[118,119] Antioxidant effects of melatonin in response to oxidative stress could exert antitumor effects.[120,121] Melatonin reduces restraining survival signals for cancer cells, and limits proliferation may be across influencing aerobic glycolysis.[122] Also, it may be effective in reducing the proliferation of healthy and cancer cells by activating or blocking specific processes responsible for PI3K/AKT-related metabolism.[123-127] Melatonin supplementation in animal model during the metastatic stages of cancers may contribute to anti-angiogenic effects, including reducing hypoxia-inducible factor-1 (HIF-1α) protein expression[128,129] and levels of vascular endothelial growth factor (VEGF).[130,131] The effects of melatonin on the cell cycle is another antitumorigenic function of melatonin. Melatonin can impact cellular proliferation by inducing cell cycle arrest and therefore putting off the duration of a cell cycle.[132,133]

Melatonin has been shown to have important intracrine, autocrine, and paracrine functions in experimental models to regulate immune system functions. It increases anti-tumor cytokine (such as IFN-γ, tumor necrosis factor (TNF) −α, and IL -6 production in lymphocytes and monocytes. Thus, melatonin can influence the immune responses, in addition to the maturation of lymphocytes.[100,101] Melatonin increases IL-2 by inhibiting the effect of prostaglandin E2 (PGE2), stimulating T helper type1 lymphocytes and monocytes, resulting in CD8+ cytotoxic T cell and NK cell proliferation.[127] It also stimulates the secretion of other cytokines such as IL-6, IL-12, IL-27, and IFN-γ, which helps in the proliferation of the NK cells.[101,134] Melatonin is effective in treating cancer by inhibiting the pyruvate dehydrogenase kinase (PDK) and increasing mitochondrial oxidative phosphorylation.[135] It enhances the production of macrophages despite reducing macrophage nitric oxide (NO) levels by suppressing the nuclear factor kappa B (NF-κB). Therefore, NO- and inflammation-related cell proliferation reduced.[136] Melatonin also plays an important anti-inflammatory role owing to chemotactic activity and the leukocytes' action regulation.[100] An important part of the anti-cancer effects of melatonin is related to the anti-inflammatory contribution of melatonin to tumorigenic molecular events, consequently repressing inflammation-related tumor developments.[137,138]

Association of melatonin with telomerase activity and telomere length

Telomerase activity and telomere length may vary at different stages of cancer.[17,139,140] Due to the tumorigenic effects of increased telomerase activity in advanced breast cancer,

administration of telomerase inhibitors is a topic that has been explored by many experimental studies that would benefit cancer treatment, but it would also prevent telomere length stabilization which shows dual effects of telomerase inhibitors.[140] Melatonin may resolve this problem because it can stabilize telomere length independently while reducing telomerase activity.[55] However, studies support the contribution of melatonin to healthy telomere function and modifying the telomere alterations in cancers are from *in vitro* or animal experimental assays[39,141] and need to be investigated in epidemiological studies or clinical trials. Here are some prominent mechanisms that could underly the anticarcinogenic effects of melatonin in experimental models.

The effects of melatonin on estrogen concentrations

Menopause is a physiological state associated with low levels of estrogen mainly produced in non-ovarian tissues such as adipose tissue.[142] It has been proposed that adipose tissue-derived estrogen may be a significant risk factor for postmenopausal breast cancer.[143,144] Studies in hormone-related malignancies show that telomerase activity could be affected by blood estrogen concentrations.[145,146] For breast cancer cell lines with positive estrogen receptor-*a*, telomerase activity and hTERT expression are both modulated by estradiol-related signaling.[146,147] Research by Jinbo *et al.*[148] on human MCF-7 cells showed that the estradiol-induced increase in telomerase activity is a dose-dependent mode (10^{-10}-10^{-8} mol/L E2).

Melatonin can alter the levels of estrogen in circulation through a variety of pathways.[149] It can indirectly lower blood estrogen concentrations by influencing the activity of the hypothalamic-pituitary axis[150] (Figure 3). Another potential mechanism is reducing the expression levels of estrogen receptor (ER) by inhibiting the binding of estradiol to estrogen receptor complex (E2 – ER) located in estrogen

response element (ERE).[151-152] Melatonin can affect the enzymes involved in estrogen synthesis and might change the estrogenic kinetic, thus affecting intracellular-ER-related signaling to nuclear alterations, most importantly telomerase activity.[52,153]

The ERα is a nuclear receptor that binds estrogen as a specific ligand and plays a significant contribution to cancer cell outgrowth in breast cancer.[142,154,155] The intracellular *ER* protein contains mitogen-activated protein kinase (MAPK) that can track ERα-related signaling, dependent or independent on estradiol, to promote the transcription of specific genes (proto-oncogenes) actively involved in the development of malignant neoplastic diseases.[156,157] In the presence of estradiol, melatonin can exert its anti-cancer effects on MAPK pathway. In the absence of estrogen as a ligand of ER, the mutation-related mitogenic kinase overactivity in ER/MAPK-related signaling pathway was also suppressed using melatonin.[142,158] Cyclic AMP (cAMP) and MAPK pathways interact with each other and possess stimulatory effects on the epidermal growth factor (EGF) and the development of tumors.[159] Increases E2 concentrations elevated cytosolic cAMP levels, which subsequently activates the ERα.[160] Melatonin prevents cancer cell proliferation by regulating the cAMP pathway and reducing its accumulation in cells through the involvement of the melatonin MT1 receptor, expressing somewhat the estrogen interaction in melatonin-related genomic alteration to improve the anti-neoplastic growth.[150,158,161] Another factor that links the effects of melatonin to ERα is calmodulin.[162] The ERα binds to calmodulin and thereby exerts multiple modes of action and pleiotropic effects on cancer cells.[163,164] Melatonin acts as an antagonist of calmodulin, preventing growing effects on MCF-7 cells, more importantly depending on the ERα/ERβ ratio.[165,166] The rising ERα/ERβ ratio, the greater the antiproliferative effects of melatonin, as
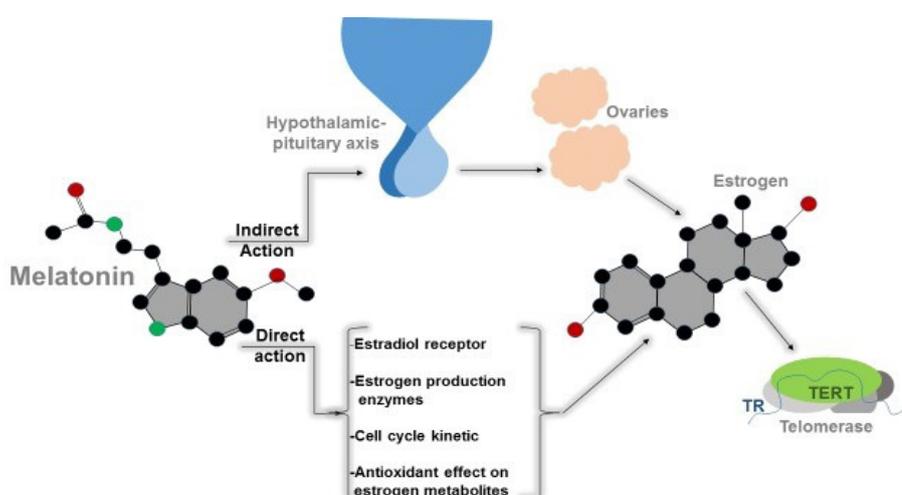

Figure 3. Mechanism of the functions of melatonin on telomerase activity mediated by estrogen-related signaling. There are two general pathways in which melatonin affects estrogen levels including direct actions via estradiol receptors, enzymes, cell cycle arrest, and antioxidant effects which influence telomerase activity. Melatonin indirectly can reduce circulation levels of estrogen implicated by interfering in the pituitary-hypothalamic axis and subsequently ovary endocrines.[144,150] TERT: Telomerase reverse transcriptase; TR: Telomerase RNA.

under such conditions the MCF-7 cells reacted far more to cellular melatonin treatments.[166,167] In fact, melatonin inhibits the calmodulin by binding to it, which reduces the ER's affinity for its ligand, and melatonin by binding to the melatonin receptors in the nucleus without the need for phosphorylation of ER destabilizes the complex E2–ER–ERE.[152] This could be highly dependent on the ratio of levels of binding melatonin to MT-1 receptor to affect the ER-related signaling pathways.[150]

Molis *et al.*[48] have reported that melatonin can reduce *ER* mRNA expression levels at physiological concentrations (10⁻⁹M), independent of estrogen levels and without directly attaching to the ER in the MCF-7 cell line. In addition, it was found that melatonin effects are mostly related to the transcription compartment of related molecular events.[168]

A study in human MCF-7 cells showed that melatonin alone cannot affect *TERT* mRNA expression. The activating effects of E2 or metalloestrogen (cadmium) are necessary for this process.[169,170] Cadmium binds to estrogen receptors that can act like estrogen.[171] The TERT transcription could be inhibited by melatonin while interacting with either E2 or cadmium. Another factor on which the anti-neoplastic effects of melatonin are dependent in breast cancer cells is the status of *ERα* expression. Because the melatonin exhibited an important contribution to inhibiting E2 and cadmium-induced transcription only in the case of ERα-related transcripts and not those of the ERβ.[172] Because the specific amino acid residue that is present in the ERα is the factor that binds cadmium to it. These amino acids include histidine524, cysteine381, cysteine447, aspartic acid538 and glutamic acid523.[173]

Different enzymes are involved in estrogen synthesis.[142,174] Aromatase, estrone sulfatase, 17β- hydroxysteroid dehydrogenase type 1, and estrone sulfotransferase are enzymes regulated, inhibited, or stimulated by physiological concentrations of melatonin, thus affecting the estrogen production in human MCF-7 cells.[175,176] Thereby the melatonin anticancer effects become primarily noticeable in an estrogen-dependent manner.[153,177]

The effects of melatonin on the cell cycle and cellular proliferation

Telomerase activity is profoundly involved in the different cell cycle phases.[178] The ratio between hTERT and hTR is variable dependent on the stage of a cell cycle. During the G1 and G2 phases, there is distinct intranuclear anatomy of hTR and hTERT. In the case of the S phase, however, these two subunits have different distributions such that there exist common intranuclear sites.[179] Consequently, telomerase is activating and involved in inducing the S phase and synthetizes the telomere, particularly occurring in the mid-S.[180] This is apparent that the G1 phase is an important point of the cell cycle in which the cell can choose two pathways. Extracellular signals dictate which path to take. Passing from the G1 phase to the S phase for DNA replication or entering to G0 phase to stop cell division.[179,181,182] Delay in G1-S duration and inhibition

of cell proliferation might be done by melatonin.[132,150,183] Therefore, it can be concluded that telomere synthesis is delayed by the presence of melatonin. An in vitro study has shown that melatonin has no effect whenever estradiol is added to the clonogenic soft agar culture.[184] This indicates a very strong effect of estradiol, and the interesting part of which is that estradiol itself is inhibited by melatonin.[138] In the other *in vitro* study, it was found that melatonin treatment at physiological concentrations increased 15% cell cycle duration in MCF-7 cells prolonging the interphase of cell cycle *vs.* untreated cells.[52] Melatonin affects the G phase and raises the ratio of cells in G1. Tamoxifen (estrogen antagonist) inhibits the functions of estradiol and changes the cell kinetics in a dose-dependent manner, in which melatonin can help tamoxifen and potentiate its effect. Therefore, the entry from the G phase to the S phase of the cell cycle is delayed (Figure 4).

As a result, melatonin hinders breast cancer growth both by counteracting the effects of estradiol on the cell cycle and by directly affecting the G phase (delayed in telomere synthesis).[46,132,185-187] Moreover, during the S phase, the physiological doses of melatonin (1nM) are capable to inhibit the synthesis of the DNA by regulating thymidine kinase activity (the main enzyme that produces thymidine) in human MCF-7 cells (Figure 4); this is, therefore, another reason to put off proliferation.[46]

Other mechanisms regarding the impact of melatonin on the cell cycle include the induction of the regulatory proteins of the cell cycle, such as the P53 tumor suppressor protein.[188] The P53, one of the most important regulators of the cell cycle, delays entry into the S phase of the cell cycle and reduces the cyclin A expression.[188,189] Physiological doses of melatonin increase P53 through nuclear receptors in human MCF-7 cells.[188]
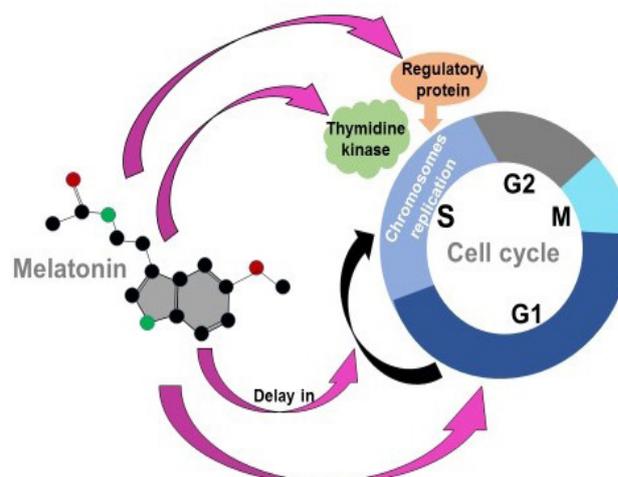

Figure 4. Melatonin and telomere length during the cell cycle. Melatonin can delay G1-S duration, increases telomerase activity and the duration of telomere lengthening. Melatonin directly might increase the proportion of cells in G1. Melatonin delays the entry into S phase by affecting the activity of the enzyme involved in the production of DNA (thymidine kinase) as well as by inducing specific proteins.[170,178] M: Mitosis; G1: Gap1; G2: Gap2; S: Synthesis.

Melatonin, growth factors, and telomeres

The relationship between telomere length, telomerase, and growth factors (Figure 5) has been investigated in a cross-sectional study on elderly men conducted by Move´rare-Skrtla et al.[190] There was a direct relationship between leukocyte telomere length (which may represent the telomere length in other tissues) and serum concentrations of insulin-like growth factor-I (IGF-I), which may be due to the anti-inflammatory effects of IGF-I binding proteins or repressing IGF-1-related telomerase activity. Tu et al.[191] using cord blood mononuclear cells found that IGF-I was able to help PHA increase the h-TERT and affect the telomerase activity. The IGF-I is required for S phase entry and affects the telomeres.[192] All of this demonstrates the profound effect of IGF-1 on telomerase and telomere length.[191] Some studies have suggested that melatonin can reduce IGF-I concentrations, but these studies are inconclusive. Vriend et al.[193] in a study in hamsters showed that the injection of melatonin for 10 weeks could increase IGF-I, which may be related to the enhancement of serotonin in the hypothalamus and brainstem. An in vitro study on human granulosa cells conducted by Schaeffer et al.[194] showed that melatonin (0.01-10 μg/ml) was able to increase the secretion of IGF-1. Based on these results, melatonin might increase the IGF-1-related rates of angiogenesis and tumor growth. On the other hand, Kos-Kudła et al.[195] who studied breast cancer (stage II) patients found that the IGF-I and melatonin in plasma have an inverse relationships. Also, a study of human breast cancer MCF-7 cells by Ishido et al.[196] showed that 10^{-10}M of melatonin temporarily hinders the collective effects of IGF-I and bisphenol A on cell proliferation. It seems that more experiments are needed in this area.

Vascular endothelial growth factor (VEGF) and telomerase have a very close and complex relationship, that may lead to the advanced processing of angiogenesis.[197] Telomerase activity and hTERT gene expression are increased by VEGF.[198] Also, hTERT enhances the expression of the VEGF gene.[199] Melatonin inhibits the function of VEGF by affecting the cell cycle, inducing apoptosis, and arranging the formation of VEGF. Pharmacological doses (1mM) of melatonin can reduce VEGF gene expression in human MCF-7 cells.[200] As melatonin decreases the angiogenesis process, and as hypoxia develops, tumor cells become accustomed to a lack of oxygen such that the hypoxia-inducible factor-1a (HIF-1a) protein helps promote tumors by regulating genes involved in the angiogenesis and cell cycle, such as VEGF and cdc25a.[201-203] Melatonin has also been demonstrated to downregulate this process by reducing the HIF-1a protein levels.[200,204]

Epidermal growth factor receptors (EGFR) include four types of human epidermal growth factor receptor family (HER1, HER2, HER3, and HER4) and mediate cell growth by their intracellular tyrosine kinase activity.[205] Excessive expression of EGFR receptors is associated with increased tumor size, invasion, and angiogenesis in breast cancer.[206] A regulatory pathway of EGFR affects the telomerase. The EGF enhances the expression of the h-TERT gene directly.[207] In a population with endometrial carcinoma, blood concentrations of EGF showed a negative relationship with melatonin levels. Melatonin might exhibit an inhibitory effect on EGF-induced growth,[208] which is not related to the antiestrogenic properties of melatonin. The exact antiproliferative mechanism of melatonin with growth factor involvement is not precisely known yet, but it is distinct that melatonin may reduce or prevent an increase in EGFR.[209]

In human epidermal growth factor receptor 2 (HER2)-positive cells, h-TERT gene expression is increased.[210,211] It was shown that patients with positive HER2 breast cancer have a longer telomere length than those carrying HER2 negative tumors.[15] An in vivo study in rats with ovarian cancer found that the administration of melatonin (200 μg/100 g body weight/day) for 60 days led to HER2 reductions.[212] Decreased HER2 gene expression by melatonin was also found in another study that investigated HER2 in transgenic mice suffering mammary cancer (by 20 mg/l melatonin).[213] Therefore, while melatonin can inhibit telomerase and even affect telomere-related HER2 expressions, telomere could be supposed to influence HER-2 levels of synthesis. Given the available information one can conclude that there is a tide association between melatonin and HER2 expressing breast tumors that would be mediated by telomere length.

Antioxidant effects of melatonin on telomeres

The telomeric structure is rich in guanine, and guanine is highly sensitive to oxidation and redox, thus facilitating the process of telomere shortening.[214] In vitro studies of human WI-38 fibroblasts and in vivo studies in Wistar rats have reported that telomere length is reduced by the effects of oxidative stress.[215-217] Therefore, oxidative stress is a potential risk factor for telomere-related shortened human lifespan.[218] Furthermore, it has been suggested that oxidative stress is a risk factor for metabolic syndrome that might be mediated by telomere shortening processes.[219] A case-control study found a positive relationship between the telomere length and antioxidant agents in the serum of US adults.[220] Another study showed that women who took few vitamins C, E, or beta-carotene had shorter telomere lengths and were at a higher risk of breast cancer.[221] The use of antioxidants can indirectly prevent telomere shortening.[222]

Melatonin and its metabolites not only play important roles in protecting against free radicals (direct effect) but also possess anti-oxidative action (indirect effect).[223] The indole ring (electron conferment), and methoxy and acyl groups in melatonin structure are prominent in detoxifying radicals or radical-affected byproducts.[224-226] DNA damage is caused by various radicals, and the accumulation of mutations and DNA breakages leads to carcinogenesis.[227] The presence of melatonin is important for preventing the damage caused by hydroxyl radical.[228] DNA damage can be reduced by the administration of pharmacological and
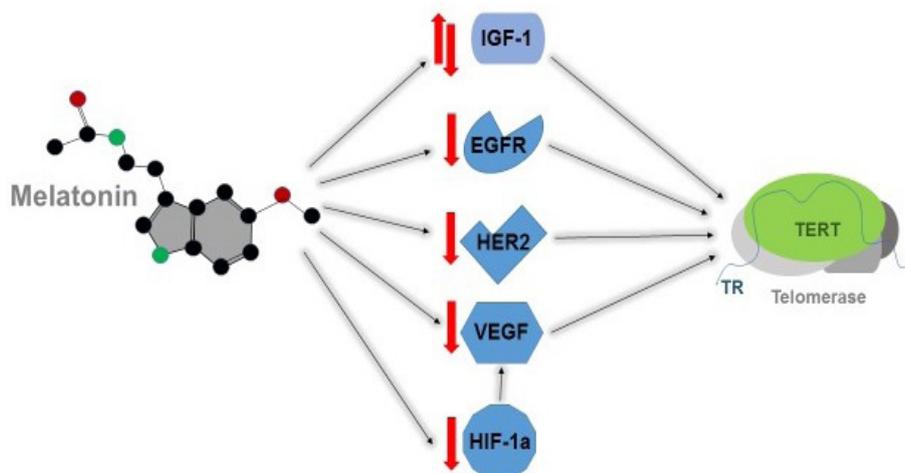
Figure 5. Function of melatonin on telomerase through effects on growth factors and their receptors. Melatonin has different effects on IGF-1-related signaling.[190] The reducing effects of melatonin on EGFR,[208] HER2,[212] and VEGF[200] can dependently contribute to telomerase activity and telomere length.[198,207] HIF-1a can indirectly affect telomerase activity mediated by VEGF.[194] IGF-1: insulin-like growth factor-1; EGFR: Epidermal growth factor receptors; HER2: human epidermal growth factor receptor 2; VEGF: Vascular endothelial growth factor; HIF-1a: hypoxia-inducible factor-1a; TERT: Telomerase reverse transcriptase; TR: Telomerase RNA.

physiological levels of melatonin in rats.[228,229] Melatonin is an antioxidant and also has amphiphilic characteristics.[230] Many antioxidants show their radical scavenging effects by their actions on glutathione. Melatonin can strengthen this antioxidant network, the antioxidant effects of melatonin are individually effective to detoxify radicals as well.[231,232]

Melatonin can increase the activity of antioxidant enzymes via different pathways (Figure 6). Melatonin synergizes with sirtuin-3 to improve the transcription of enzymes that dismute or catalyze the detoxification of radicals (free radicals) from the mitochondria and cytosol, respectively. Since mitochondrial sirtuin-3 levels are elevated by melatonin, it could raise manganese superoxide dismutase (MnSOD; antioxidant enzyme) levels.[233] *In vivo*, high dose or physiological levels of melatonin enhance glutathione peroxidase (GSH-Px) and superoxide dismutases (SOD) activity in several

tissues. Likewise, in rats with pinealectomy, the amount of GSH-Px activity decreases.[234-237] A study by Sewerynek *et al.*[238] observed that in lipopolysaccharide- (LPS) treated rats, the administration of 4 mg/kg melatonin could increase total glutathione (tGSH) concentrations and reduce oxidized glutathione (GSSG) by stimulating the activities of glutamylcysteine synthase and glutathione reductase. Therefore, melatonin can enhance antioxidant enzymes (SODs and GSH-Px) by regulating their gene expression[239] and subsequently ponder the antioxidant pool in favor of the antioxidant defense system.[235] For this purpose, according to neuronal cell line studies, melatonin even at nanomolar concentrations ($\approx$ 1 nM), leads to new protein synthesis.[239] Melatonin is likely to affect transcription factors through MT1/MT2 receptors as well as second messengers (cAMP, phospholipase C, or calcium concentration) and provoke the MAPK pathway.
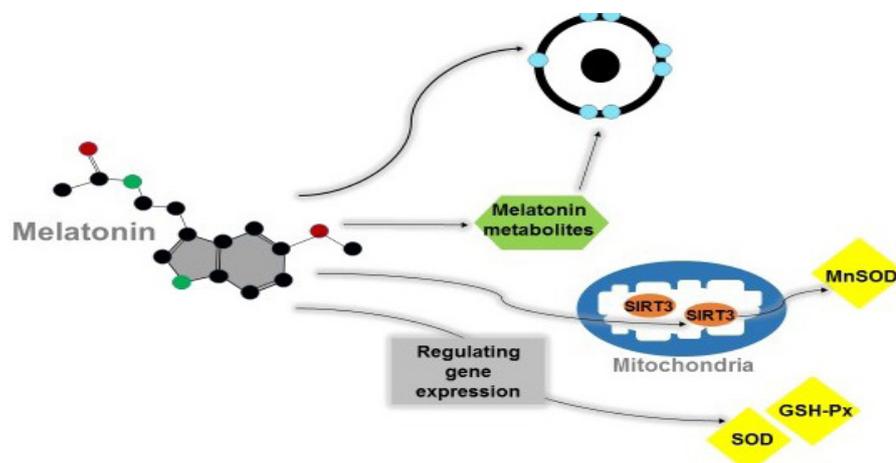

Figure 6. The antioxidant activity of melatonin. Melatonin and its metabolites can reduce free radicals. Melatonin induces the gene expression of antioxidant enzymes mediated by sirtuin-3.[233,240,241] MnSOD: Manganese superoxide dismutase; GSH-Px: glutathione peroxidase; SOD: superoxide dismutases; SIRT3: sirtuin-3.

Accordingly, it could be suggested that it is involved in regulating gene expression of the antioxidant enzymes.[235] N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), N1-acetyl-5-methoxykynuramine (AMK), and cyclic 3-hydroxymelatonin (3OHM) are melatonin metabolites that can reduce free radicals.[242] The performance of these metabolites depends on the polarity or non-polarity of the environment and also the type of free radical.[243] In many conditions, AFMK has a poor capability of eliminating free radicals. The 3OHM and AMK are more efficient, and they also work faster to remove the free radicals.[244] Overall, the antioxidant effects of melatonin could potentially diminish oxidative stress as stimuli of telomere shortening.

Conclusion

Melatonin is a metabolite that may be derived from dietary sources, and restore telomere length in cancer cells. The transactivity of telomerase is a tumorigenic feature that could be linked to tumor metabolism, estrogen signaling, growth factors in the microenvironment, and early or late cell cycle arrest in breast cancer. This review represented melatonin as a potent antioxidant attenuating oxidative stress-related telomere shortening which is a key contributor to cancer prevention. Melatonin shows significant impacts on repressing biological signaling pathways promoting tumor growth and becoming resistant to cancer treatment in breast cancer cells, utmost mediated by regulating the telomerase transactivity. Melatonin administration, according to experimental evidence, demonstrated pharmacogenomic effects on breast carcinogenesis, most notable was interacting with telomerase activity, which is promising for breast cancer treatment.

Author Contributions

Elmira Barari: Investigation, Methodology, Visualization, Writing - Original Draft. Golnoosh Azarsina: Writing - Review & Editing. Gordon R. Ferns: Writing - Review & Editing. Saeed Pirouzpanah: Conceptualization, Methodology, Project administration, Visualization, Review & Editing.

Conflict of Interest

The authors report no conflicts of interest.

References

1. Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, et al. A highly conserved repetitive DNA sequence,(TTAGGG) n, present at the telomeres of human chromosomes. Proc Natl Acad Sci. 1988;85(18):6622-6. doi:10.1073/pnas.85.18.6622

2. Blackburn EH. Telomeres and telomerase: The means to the end (nobel lecture). Angew Chem Int Ed 2010;49(41):7405-21. doi:10.1002/anie.201002387.

3. Blackburn EH. Switching and signaling at the telomere. Cell. 2001;106(6):661-73. doi:10.1016/s0092-8674(01)00492-5

4. Collins K, Mitchell JR. Telomerase in the human organism. Oncogene 2002;21(4):564-79. doi:10.1038/sj.onc.1205083

5. Schmutz I, Mensenkamp AR, Takai KK, Haadsma M, Spruijt L, de Voer RM, et al. Tinf2 is a haploinsufficient tumor suppressor that limits telomere length. Elife. 2020;9:e61235. doi:10.7554/eLife.61235

6. Rahmanto YS, Jung J-G, Wu R-C, Kobayashi Y, Heaphy CM, Meeker AK, et al. Inactivating arid1a tumor suppressor enhances tert transcription and maintains telomere length in cancer cells. J Biol Chem. 2016;291(18):9690-9. doi:10.1074/jbc.M115.707612

7. Kammori M, Sugishita Y, Okamoto T, Kobayashi M, Yamazaki K, Yamada E, et al. Telomere shortening in breast cancer correlates with the pathological features of tumor progression. Oncol Rep. 2015;34(2):627-32. doi:10.3892/or.2015.4063

8. Wang Z, Zhang Z, Guo Y, Shui H, Liu G, Jin T, et al. Shorter telomere length is associated with increased breast cancer risk in a chinese han population: A case-control analysis. J Breast Cancer. 2018;21(4):391-8. doi:10.4048/jbc.2018.21.e52

9. Qu S, Wen W, Shu XO, Chow WH, Xiang YB, Wu J, et al. Association of leukocyte telomere length with breast cancer risk: Nested case-control findings from the shanghai women's health study. Am J Epidemiol. 2013;177(7):617-24. doi:10.1093/aje/kws291

10. Duggan C, Risques R, Alfano C, Prunkard D, Imayama I, Holte S, et al. Change in peripheral blood leukocyte telomere length and mortality in breast cancer survivors. J Natl Cancer Inst. 2014;106(4):dju035. doi:10.1093/jnci/dju035

11. Samavat H, Xun X, Jin A, Wang R, Koh WP, Yuan JM. Association between prediagnostic leukocyte telomere length and breast cancer risk: The singapore chinese health study. Breast Cancer Res. 2019;21(1):50. doi:10.1186/s13058-019-1133-0

12. Pellatt AJ, Wolff RK, Torres-Mejia G, John EM, Herrick JS, Lundgreen A, et al. Telomere length, telomere-related genes, and breast cancer risk: The breast cancer health disparities study. Genes Chromosomes Cancer. 2013;52(7):595-609. doi:10.1002/gcc.22056

13. Gramatges MM, Telli ML, Balise R, Ford JM. Longer relative telomere length in blood from women with sporadic and familial breast cancer compared with healthy controls. Cancer Epidemiol Biomarkers Prev. 2010;19(2):605-13. doi:10.1158/1055-9965.epi-09-0896

14. Herbert B-S, Wright WE, Shay JW. Telomerase and breast cancer. Breast Cancer Res. 2001;3(3):146-9. doi:10.1186/bcr288

15. Barczak W, Rozwadowska N, Romaniuk A, Lipińska N, Lisiak N, Grodecka-Gazdecka S, et al. Telomere length assessment in leukocytes presents potential diagnostic value in patients with breast cancer. Oncol Lett. 2016;11(3):2305-9. doi:10.3892/ol.2016.4188

16. Ennour-Idrissi K, Maunsell E, Diorio C. Telomere length and breast cancer prognosis: A systematic

review. Cancer Epidemiol Prev Biomark. 2017;26(1):3-10. doi:10.1158/1055-9965.EPI-16-0343.

17. Kammori M, Sugishita Y, Okamoto T, Kobayashi M, Yamazaki K, Yamada E, et al. Telomere shortening in breast cancer correlates with the pathological features of tumor progression. Oncol Rep. 2015;34(2):627-32. doi:10.3892/or.2015.4063

18. Artandi SE. Complex roles for telomeres and telomerase in breast carcinogenesis. Breast Cancer Res. 2002;5(1):37. doi:10.1186/bcr553

19. Sanft T, Usiskin I, Harrigan M, Cartmel B, Lu L, Li F-Y, et al. Randomized controlled trial of weight loss versus usual care on telomere length in women with breast cancer: The lifestyle, exercise, and nutrition (lean) study. Breast Cancer Res Treat. 2018;172(1):105-12. doi:10.1007/s10549-018-4895-7

20. Navarro-Ibarra MJ, Hernández J, Caire-Juvera G. Diet, physical activity and telomere length in adults. Nutr Hosp. 2019;36(6):1403-17. doi:10.20960/nh.02673

21. García-Calzón S, Martínez-González MA, Razquin C, Arós F, Lapetra J, Martínez JA, et al. Mediterranean diet and telomere length in high cardiovascular risk subjects from the predimed-navarra study. Clin Nutr. 2016;35(6):1399-405. doi:10.1016/j.clnu.2016.03.013

22. Giardini MA, Segatto M, da Silva MS, Nunes VS, Cano MI. Telomere and telomerase biology. Prog Mol Biol Transl Sci. 2014;125:1-40. doi:10.1016/b978-0-12-397898-1.00001-3

23. Sandin S, Rhodes D. Telomerase structure. Curr Opin Struct Biol. 2014;25(100):104-10. doi:10.1016/j.sbi.2014.02.003

24. Masutomi K, Kaneko S, Hayashi N, Yamashita T, Shirota Y, Kobayashi K, et al. Telomerase activity reconstituted in vitro with purified human telomerase reverse transcriptase and human telomerase rna component. J Biol Chem. 2000;275(29):22568-73. doi:10.1074/jbc.M000622200

25. Jafri MA, Ansari SA, Alqahtani MH, Shay JW. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Med. 2016;8(1):69. doi:10.1186/s13073-016-0324-x

26. Jaskelioff M, Muller FL, Paik J-H, Thomas E, Jiang S, Adams AC, et al. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature. 2011;469(7328):102-6. doi:10.1038/nature09603

27. Bernardes de Jesus B, Blasco MA. Telomerase at the intersection of cancer and aging. Trends Genet. 2013;29(9):513-20. doi:10.1016/j.tig.2013.06.007

28. Akincilar SC, Unal B, Tergaonkar V. Reactivation of telomerase in cancer. Cell Mol Life Sci. 2016;73(8):1659-70. doi:10.1007/s00018-016-2146-9

29. Healy KC. Telomere dynamics and telomerase activation in tumor progression: Prospects for prognosis and therapy. Oncol Res. 1995;7(3-4):121-30.

30. Ameri Z, Ghiasi S, Farsinejad A, Ehsan M, Aghajani S, Pur Yazdan Panah N, et al. Investigation of telomerase inhibition effect on apoptosis of myeloma cell line u266. Sci J Iran Blood Transfus Org. 2017;14(3):204-16.

31. Cerone MA, Londoño-Vallejo JA, Autexier C. Telomerase inhibition enhances the response to anticancer drug treatment in human breast cancer cells. Mol Cancer Ther. 2006;5(7):1669-75. doi:10.1158/1535-7163.MCT-06-0033

32. Lu L, Zhang C, Zhu G, Irwin M, Risch H, Menato G, et al. Telomerase expression and telomere length in breast cancer and their associations with adjuvant treatment and disease outcome. Breast Cancer Res. 2011;13(3):R56. doi:10.1186/bcr2893

33. Yeh YM, Pan YT, Wang TC. Cdc42/rac1 participates in the control of telomerase activity in human nasopharyngeal cancer cells. Cancer Lett. 2005;218(2):207-13. doi:10.1016/j.canlet.2004.06.047

34. Noori-Daloii MR, Hesami SS. Telomerase and it's inhibition in caner: A review article. Tehran Univ Med J. 2009;67(9):607-599.

35. Cunningham A, Love W, Zhang R, Andrews L, Tollefsbol T. Telomerase inhibition in cancer therapeutics: Molecular-based approaches. Curr Med Chem. 2006;13(24):2875-88. doi:10.2174/092986706778521887

36. White LK, Wright WE, Shay JW. Telomerase inhibitors. Trends Biotechnol. 2001;19(3):114-20. doi:10.1016/s0167-7799(00)01541-9

37. Hardeland R, Pandi-Perumal S, Cardinali DP. Melatonin. Int J Biochem Cell Biol 2006;38(3):313-6. doi:10.1016/j.biocel.2005.08.020

38. Pandi-Perumal SR, Srinivasan V, Maestroni G, Cardinali D, Poeggeler B, Hardeland R. Melatonin: Nature's most versatile biological signal? FEBS J. 2006;273(13):2813-38. doi:10.1111/j.1742-4658.2006.05322.x

39. Leon-Blanco MM, Guerrero JM, Reiter RJ, Calvo JR, Pozo D. Melatonin inhibits telomerase activity in the mcf-7 tumor cell line both in vivo and in vitro. J Pineal Res. 2003;35(3):204-11. doi:10.1034/j.1600-079x.2003.00077.x

40. Martínez-Campa CM, Alonso-González C, Mediavilla MD, Cos S, González A, Sanchez-Barcelo EJ. Melatonin down-regulates htert expression induced by either natural estrogens (17β-estradiol) or metalloestrogens (cadmium) in mcf-7 human breast cancer cells. Cancer Lett. 2008;268(2):272-7.

41. Xie Y, Lou D, Zhang D. Melatonin alleviates age-associated endothelial injury of atherosclerosis via regulating telomere function. J Inflamm Res. 2021;14:6799. doi:10.2147/JIR.S329020

42. López A, García JA, Escames G, Venegas C, Ortiz F, López LC, et al. Melatonin protects the mitochondria from oxidative damage reducing oxygen consumption, membrane potential, and superoxide anion production. J Pineal Res. 2009;46(2):188-98. doi:10.1111/j.1600-079X.2008.00647.x.

43. Beyer CE, Steketee JD, Saphier D. Antioxidant properties of melatonin—an emerging mystery. Biochem Pharmacol. 1998;56(10):1265-72. doi:10.1016/s0006-2952(98)00180-4

44. Ram P, Dai J, Yuan L, Dong C, Kiefer T, Lai L, et al. Involvement of the mt1 melatonin receptor in human breast cancer. Cancer lett. 2002;179(2):141-50. doi:10.1016/s0304-3835(01)00873-4

45. Hill SM, Belancio VP, Dauchy RT, Xiang S, Brimer S, Mao L, et al. Melatonin: An inhibitor of breast cancer. Endocr-Relat Cancer. 2015;22(3):R183-R204. doi:10.1530/ERC-15-0030

46. Cos S, Fernandez F, Sanchez-Barcelo E. Melatonin inhibits DNA synthesis in mcf-7 human breast cancer cells in vitro. Life Sci. 1996;58(26):2447-53. doi: 10.1016/0024-3205(96)00249-4

47. Sánchez-Barceló EJ, Cos S, Mediavilla D, Martínez-Campa C, González A, Alonso-González C. Melatonin–estrogen interactions in breast cancer. J Pineal Res 2005;38(4):217-22. doi:10.1111/j.1600-079X.2004.00207.x

48. Molis TM, Spriggs LL, Hill SM. Modulation of estrogen receptor mrna expression by melatonin in mcf-7 human breast cancer cells. J Mol Endocrinol. 1994;8(12):1681-90. doi:10.1210/mend.8.12.7708056

49. Goradel NH, Asghari MH, Moloudizargari M, Negahdari B, Haghi-Aminjan H, Abdollahi M. Melatonin as an angiogenesis inhibitor to combat cancer: Mechanistic evidence. Toxicol Appl Pharmacol. 2017;335:56-63. doi:10.1016/j.taap.2017.09.022

50. Su SC, Hsieh MJ, Yang WE, Chung WH, Reiter RJ, Yang SF. Cancer metastasis: Mechanisms of inhibition by melatonin. J Pineal Res. 2017;62(1):e12370. doi:10.1111/jpi.12370

51. Sainz R, Mayo J, Rodriguez C, Tan D, Lopez-Burillo S, Reiter R. Melatonin and cell death: Differential actions on apoptosis in normal and cancer cells. Cell Mol Life Sci. 2003;60(7):1407-26. doi:10.1007/s00018-003-2319-1

52. Cos S, Recio J, Sánchez-Barceló E. Modulation of the length of the cell cycle time of mcf-7 human breast cancer cells by melatonin. Life Sci. 1996;58(9):811-6. doi:10.1016/0024-3205(95)02359-3

53. Moradkhani F, Moloudizargari M, Fallah M, Asghari N, Heidari Khoei H, Asghari MH. Immunoregulatory role of melatonin in cancer. J Cell Physiol. 2020;235(2):745-57. doi:10.1002/jcp.29036

54. Reiter RJ, Rosales-Corral SA, Tan DX, Acuna-Castroviejo D, Qin L, Yang SF, et al. Melatonin, a full service anti-cancer agent: Inhibition of initiation, progression and metastasis. Int J Mol Sci. 2017;18(4):843. doi:10.3390/ijms18040843

55. Leon-Blanco MM, Guerrero JM, Reiter RJ, Calvo JR, Pozo D. Melatonin inhibits telomerase activity in the mcf-7 tumor cell line both in vivo and in vitro. J Pineal Res. 2003;35(3):204-11. doi:10.1034/j.1600-079x.2003.00077.x

56. Otálora BB, Madrid JA, Alvarez N, Vicente V, Rol MA. Effects of exogenous melatonin and circadian synchronization on tumor progression in melanoma-bearing c57bl6 mice. J Pineal Res. 2008;44(3):307-15. doi:10.1111/j.1600-079X.2007.00531.x

57. Zhong Z, Shiue L, Kaplan S, de Lange T. A mammalian factor that binds telomeric TTAGGG repeats in vitro. Mol Cell Biol. 1992;12(11):4834-43. doi:10.1128/mcb.12.11.4834-4843.1992

58. Zakian VA. Telomeres: The beginnings and ends of eukaryotic chromosomes. Exp Cell Res. 2012;318(12):1456-60. doi:10.1016/j.yexcr.2012.02.015

59. Schmutz I, de Lange T. Shelterin. Current Biol. 2016;26(10):R397-R9. doi:10.1016/j.cub.2016.01.056

60. Xin H, Liu D, Songyang Z. The telosome/shelterin complex and its functions. Genome Biol. 2008;9(9):232. doi:10.1186/gb-2008-9-9-232

61. Bolzán AD. Chromosomal aberrations involving telomeres and interstitial telomeric sequences. Mutagenesis 2012;27(1):1-15. doi:10.1093/mutage/ger052

62. Al-Wahiby S, Slijepcevic P. Chromosomal aberrations involving telomeres in brca1 deficient human and mouse cell lines. Cytogenet Genome Res. 2005;109(4):491-6. doi:10.1159/000084208

63. Greetham M, Skordalakes E, Lydall D, Connolly BA. The telomere binding protein cdc13 and the single-stranded DNA binding protein rpa protect telomeric DNA from resection by exonucleases. J Mol Biol. 2015;427(19):3023-30. doi:10.1016/j.jmb.2015.08.002

64. Robin JD, Ludlow AT, Batten K, Magdinier F, Stadler G, Wagner KR, et al. Telomere position effect: Regulation of gene expression with progressive telomere shortening over long distances. Genes Dev. 2014;28(22):2464-76. doi:10.1101/gad.251041.114

65. Mukherjee AK, Sharma S, Sengupta S, Saha D, Kumar P, Hussain T, et al. Telomere length-dependent transcription and epigenetic modifications in promoters remote from telomere ends. PLoS Genet 2018;14(11):e1007782. doi:10.1371/journal.pgen.1007782

66. O'Sullivan RJ, Karlseder J. Telomeres: Protecting chromosomes against genome instability. Nat Rev Mol Cell Biol. 2010;11(3):171-81. doi:10.1038/nrm2848

67. Baird DM. Mechanisms of telomeric instability. Cytogenet Genome Res 2008;122(3-4):308-14. doi:10.1159/000167817

68. Bailey SM, Cornforth MN, Ullrich RL, Goodwin EH. Dysfunctional mammalian telomeres join with DNA double-strand breaks. DNA Repair (Amst) 2004;3(4):349-57. doi:10.1016/j.dnarep.2003.11.007

69. Montpetit AJ, Alhareeri AA, Montpetit M, Starkweather AR, Elmore LW, Filler K, et al. Telomere length: A review of methods for measurement. Nurs Res. 2014;63(4):289. doi:10.1097/NNR.0000000000000037

70. Wurtman RJ, Axelrod J. The pineal gland.

Sci Am. 1965;213:50-60. doi:10.1038/scientificamerican0765-50

71. Macchi MM, Bruce JN. Human pineal physiology and functional significance of melatonin. Fron Neuroendocrinol. 2004;25(3-4):177-95. doi:10.1016/j.yfrne.2004.08.001

72. Reiter RJ. Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. Endocrine Rev. 1991;12(2):151-80.

73. Karasek M, Winczyk K. Melatonin in humans. Physiol Pharmacol. 2006;57:19.

74. Ackermann K, Stehle JH. Melatonin synthesis in the human pineal gland: Advantages, implications, and difficulties. Chronobiol Int. 2006;23(1-2):369-79. doi:10.1080/07420520500464379

75. Foulkes NS, Whitmore D, Sassone-Corsi P. Rhythmic transcription: The molecular basis of circadian melatonin synthesis. Biol Cell. 1997;89(8):487-94. doi:10.1016/s0248-4900(98)80004-x

76. Zawilska JB, Skene DJ, Arendt J. Physiology and pharmacology of melatonin in relation to biological rhythms. Pharmacol Rep. 2009;61(3):383-410. doi:10.1016/s1734-1140(09)70081-7

77. Farhud D, Tahavorgar A. Melatonin hormone, metabolism and its clinical effects: A review. Iran J Endocrinol Metab. 2013;15(2):211-23.

78. Brzezinski A. Melatonin in humans. N Engl J Med. 1997;336(3):186-95. doi:10.1056/NEJM199701163360306

79. Claustrat B, Brun J, Chazot G. The basic physiology and pathophysiology of melatonin. Sleep Med Rev. 2005;9(1):11-24. doi:10.1016/j.smrv.2004.08.001

80. Carrillo-Vico A, Lardone PJ, Álvarez-Sánchez N, Rodríguez-Rodríguez A, Guerrero JM. Melatonin: Buffering the immune system. Int J Mol Sci. 2013;14(4):8638-83.

81. Claustrat B, Leston J. Melatonin: Physiological effects in humans. Neurochirurgie. 2015;61(2-3):77-84. doi:10.1016/j.neuchi.2015.03.002

82. Cagnacci A. Melatonin in relation to physiology in adult humans: Mini review. J Pineal Res. 1996;21(4):200-13. doi:10.1111/j.1600-079x.1996.tb00287.x

83. viviD D, Bentley GE. Seasonal reproduction in vertebrates: Melatonin synthesis, binding, and functionality using tinbergen's four questions. Molecules. 2018;23(3):652. doi:10.3390/molecules23030652

84. Karasek M, Lerchl A. Melatonin and magnetic fields. Neuro Endocrinol Lett. 2002;23 Suppl 1:84-7.

85. Fournier I, Ploye F, Cottet-Emard J-M, Brun J, Claustrat B. Folate deficiency alters melatonin secretion in rats. J Nutr. 2002;132(9):2781-4. doi:10.1093/jn/132.9.2781

86. Munoz-Hoyos A, Amoros-Rodriguez I, Molina-Carballo A, Uberos-Fernández J, Acuña-Castroviejo D. Pineal response after pyridoxine test in children. J Neural Transm 1996;103(7):833-42. doi:10.1007/BF01273361.

87. Luboshitzky R, Ophir U, Nave R, Epstein R, Shen-Orr Z, Herer P. The effect of pyridoxine administration on melatonin secretion in normal men. Neuroendocrinol Lett. 2002;23(3):213-8.

88. Zimmermann R, McDougle C, Schumacher M, Olcese J, Mason J, Heninger G, et al. Effects of acute tryptophan depletion on nocturnal melatonin secretion in humans. Clin Endocrinol Metab. 1993;76(5):1160-4. doi:10.1210/jcem.76.5.8496306

89. Crooke A, Huete-Toral F, Colligris B, Pintor J. The role and therapeutic potential of melatonin in age-related ocular diseases. J Pineal Res. 2017;63(2):e12430. doi:10.1111/jpi.12430

90. Bonmati-Carrion M-A, Tomas-Loba A. Melatonin and cancer: A polyhedral network where the source matters. Antioxidants. 2021;10(2):210. doi:10.3390/antiox10020210

91. White AJ, Weinberg CR, Park YM, D'Aloisio AA, Vogtmann E, Nichols HB, et al. Sleep characteristics, light at night and breast cancer risk in a prospective cohort. Int J Cancer. 2017;141(11):2204-14. doi:10.1002/ijc.30920

92. Naji L, Carrillo-Vico A, Guerrero JM, Calvo JR. Expression of membrane and nuclear melatonin receptors in mouse peripheral organs. Life Sci. 2004;74(18):2227-36. doi:10.1016/j.lfs.2003.08.046.

93. Slominski RM, Reiter RJ, Schlabritz-Loutsevitch N, Ostrom RS, Slominski AT. Melatonin membrane receptors in peripheral tissues: Distribution and functions. Mol Cell Endocrinol. 2012;351(2):152-66. doi:10.1016/j.mce.2012.01.004

94. von Gall C, Stehle JH, Weaver DR. Mammalian melatonin receptors: Molecular biology and signal transduction. Cell Tissue Res. 2002;309(1):151-62. doi:10.1007/s00441-002-0581-4

95. Carlson LL, Weaver DR, Reppert SM. Melatonin signal transduction in hamster brain: Inhibition of adenylyl cyclase by a pertussis toxin-sensitive g protein. Endocrinology. 1989;125(5):2670-6. doi:10.1210/endo-125-5-2670

96. Reppert SM, Godson C, Mahle CD, Weaver DR, Slaugenhaupt SA, Gusella JF. Molecular characterization of a second melatonin receptor expressed in human retina and brain: The mel1b melatonin receptor. Proc Natl Acad Sci U S A. 1995;92(19):8734-8. doi:10.1073/pnas.92.19.8734

97. Morgan PJ, Barrett P, Howell HE, Helliwell R. Melatonin receptors: Localization, molecular pharmacology and physiological significance. Neurochem Int. 1994;24(2):101-46. doi:10.1016/0197-0186(94)90100-7

98. Barrett P, Conway S, Morgan PJ. Digging deep–structure–function relationships in the melatonin receptor family. J Pineal Res. 2003;35(4):221-30. doi:10.1034/j.1600-079x.2003.00090.x

99. Carrillo-Vico A, Garcia-Perganeda A, Naji L, Calvo J, Romero M, Guerrero J. Expression of membrane

and nuclear melatonin receptor mrna and protein in the mouse immune system. Cell Mol Life Sci. 2003;60(10):2272-8. doi:10.1007/s00018-003-3207-4

100. Srinivasan V, Spence DW, Trakht I, Pandi-Perumal SR, Cardinali DP, Maestroni GJ. Immunomodulation by melatonin: Its significance for seasonally occurring diseases. Neuroimmunomodulation 2008;15(2):93-101. doi:10.1159/000148191

101. Miller SC, Pandi PS, Esquifino AI, Cardinali DP, Maestroni GJ. The role of melatonin in immuno-enhancement: Potential application in cancer. Int J Exp Pathol. 2006;87(2):81-7. doi:10.1111/j.0959-9673.2006.00474.x

102. Cajochen C, Kräuchi K, Wirz-Justice A. Role of melatonin in the regulation of human circadian rhythms and sleep. J Neuroendocrinol. 2003;15(4):432-7. doi:10.1046/j.1365-2826.2003.00989.x

103. Bubenik GA. Gastrointestinal melatonin: Localization, function, and clinical relevance. J Dig Dis. 2002;47(10):2336-48. doi:10.1023/a:1020107915919

104. Bubenik G. Thirty four years since the discovery of gastrointestinal melatonin. J Physiol Pharmacol. 2008;59(2):33-51.

105. Reiter RJ. Melatonin and human reproduction. Ann Med 1998;30(1):103-8. doi:10.3109/07853899808999391

106. Reiter RJ, Tan D-X, Manchester LC, Paredes SD, Mayo JC, Sainz RM. Melatonin and reproduction revisited. Biol Reprod. 2009;81(3):445-56. doi:10.1095/biolreprod.108.075655

107. Cardinali DP, Ladizesky MG, Boggio V, Cutrera RA, Mautalen C. Melatonin effects on bone: Experimental facts and clinical perspectives. J Pineal Res. 2003;34(2):81-7. doi:10.1034/j.1600-079x.2003.00028.x

108. Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ. A review of the multiple actions of melatonin on the immune system. Endocrine. 2005;27(2):189-200. doi:10.1385/ENDO:27:2:189

109. Talaee N, Ebrahimpour S, Sfandbod M, Majedi H, Jafarzadeh Kohneloo A, Gholami K, et al. Effect of melatonin on paclitaxel-associated acute and chronic pain: a randomized, double-blind, placebo-controlled clinical trial. Pharm Sci. 2022;28(4):579-88. doi:10.34172/PS.2021.81

110. Peschke E. Melatonin, endocrine pancreas and diabetes. J Pineal Res. 2008;44(1):26-40. doi:10.1111/j.1600-079X.2007.00519.x

111. Sharma S, Singh H, Ahmad N, Mishra P, Tiwari A. The role of melatonin in diabetes: Therapeutic implications. Arch Endocrinol Metab. 2015;59(5):391-9. doi:10.1590/2359-3997000000098

112. Shukla M, Govitrapong P, Boontem P, Reiter RJ, Satayavivad J. Mechanisms of melatonin in alleviating alzheimer's disease. Curr Neuropharmacol. 2017;15(7):1010-31. doi:10.2174/1570159X15666170313123454

113. Srinivasan V, Smits M, Spence W, Lowe AD, Kayumov L, Pandi-Perumal SR, et al. Melatonin in mood disorders. World J Biol Psychiatry. 2006;7(3):138-51. doi:10.1080/15622970600571822

114. Papantoniou K, Castaño-Vinyals G, Espinosa A, Turner MC, Alonso-Aguado MH, Martin V, et al. Shift work and colorectal cancer risk in the mcc-spain case-control study. Scand J Work Environ Health. 2017;43(3):250-9. doi:10.5271/sjweh.3626

115. Schernhammer E, Schulmeister K. Melatonin and cancer risk: Does light at night compromise physiologic cancer protection by lowering serum melatonin levels? Br J Cancer. 2004;90(5):941-3. doi:10.1038/sj.bjc.6601626

116. González-González A, Mediavilla MD, Sánchez-Barceló EJ. Melatonin: A molecule for reducing breast cancer risk. Molecules. 2018;23(2):336. doi:10.3390/molecules23020336

117. Schernhammer ES, Hankinson SE. Urinary melatonin levels and breast cancer risk. J Natl Cancer Inst. 2005;97(14):1084-7. doi:10.1093/jnci/dji190

118. Vijayalaxmi, Thomas Jr CR, Reiter RJ, Herman TS. Melatonin: From basic research to cancer treatment clinics. J Clin Oncol. 2002;20(10):2575-601. doi:10.1200/JCO.2002.11.004

119. Jung B, Ahmad N. Melatonin in cancer management: Progress and promise. Cancer Res. 2006;66(20):9789-93. doi:10.1158/0008-5472.CAN-06-1776

120. Karaaslan C, Suzen S. Antioxidant properties of melatonin and its potential action in diseases. Curr Top Med Chem. 2015;15(9):894-903. doi:10.2174/1568026615666150220120946

121. Suzen S. Recent developments of melatonin related antioxidant compounds. Comb Chem High Throughput Screen. 2006;9(6):409-19. doi:10.2174/138620706777698553

122. Mao L, Dauchy RT, Blask DE, Dauchy EM, Slakey LM, Brimer S, et al. Melatonin suppression of aerobic glycolysis (warburg effect), survival signalling and metastasis in human leiomyosarcoma. J Pineal Res. 2016;60(2):167-77. doi:10.1111/jpi.12298

123. Mehdipour P, Pirouzpanah S, Sarafnejad A, Atri M, Shahrestani ST, Haidari M. Prognostic implication of cdc25a and cyclin e expression on primary breast cancer patients. Cell biol int. 2009;33(10):1050-6. doi:10.1016/j.cellbi.2009.06.016

124. Mehdipour P, Pirouzpanah S, Kheirollahi M, Atri M. Androgen receptor gene cag repeat polymorphism and breast cancer risk in iranian women: A case-control study. Breast J. 2011;17(1):39-46. doi:10.1111/j.1524-4741.2010.01031.x

125. Kim HS, Kim T-J, Yoo Y-M. Melatonin combined with endoplasmic reticulum stress induces cell death via the pi3k/akt/mtor pathway in b16f10 melanoma cells. PLoS One. 2014;9(3):e92627. doi:10.1371/journal.pone.0092627

126. Shen YQ, Guerra-Librero A, Fernandez-Gil BI, Florido J, García-López S, Martinez-Ruiz L, et al.

Combination of melatonin and rapamycin for head and neck cancer therapy: Suppression of akt/mtor pathway activation, and activation of mitophagy and apoptosis via mitochondrial function regulation. J Pineal Res. 2018;64(3):e12461. doi:10.1111/jpi.12461

127. Mortezaee K, Potes Y, Mirtavoos-Mahyari H, Motevaseli E, Shabeeb D, Musa AE, et al. Boosting immune system against cancer by melatonin: A mechanistic viewpoint. Life Sci. 2019;238:116960. doi:10.1016/j.lfs.2019.116960

128. Kim KJ, Choi JS, Kang I, Kim KW, Jeong CH, Jeong JW. Melatonin suppresses tumor progression by reducing angiogenesis stimulated by hif-1 in a mouse tumor model. J Pineal Res 2013;54(3):264-70. doi:10.1111/j.1600-079X.2012.01030.x

129. Park JW, Hwang MS, Suh SI, Baek WK. Melatonin down-regulates hif-1 alpha expression through inhibition of protein translation in prostate cancer cells. J Pineal Res. 2009;46(4):415-21. doi:10.1111/j.1600-079X.2009.00678.x

130. Lissoni P, Rovelli F, Malugani F, Bucovec R, Conti A, Maestroni G. Anti-angiogenic activity of melatonin in advanced cancer patients. Neuroendocrinol Lett 2001;22(1):45-8.

131. Cheng J, Yang HL, Gu CJ, Liu YK, Shao J, Zhu R, et al. Melatonin restricts the viability and angiogenesis of vascular endothelial cells by suppressing hif-1α/ros/vegf. Int J Mol Med. 2019;43(2):945-55. doi: 10.3892/ijmm.2018.4021

132. Cos S, Blask DE, Lemus-Wilson A, Hill AB. Effects of melatonin on the cell cycle kinetics and "estrogen-rescue" of mcf-7 human breast cancer cells in culture. J Pineal Res. 1991;10(1):36-42. doi: 10.1111/j.1600-079x.1991.tb00007.x

133. Martín-Renedo J, Mauriz JL, Jorquera F, Ruiz-Andrés O, González P, González-Gallego J. Melatonin induces cell cycle arrest and apoptosis in hepatocarcinoma hepg2 cell line. J Pineal Res. 2008;45(4):532-40. doi: 10.1111/j.1600-079X.2008.00641.x

134. Zwirner NW, Ziblat A. Regulation of nk cell activation and effector functions by the il-12 family of cytokines: The case of il-27. Front Immunol. 2017;8:25. doi:10.3389/fimmu.2017.00025

135. Reiter RJ, Sharma R, Ma Q, Rosales-Corral S, Acuna-Castroviejo D, Escames G. Inhibition of mitochondrial pyruvate dehydrogenase kinase: A proposed mechanism by which melatonin causes cancer cells to overcome cytosolic glycolysis, reduce tumor biomass and reverse insensitivity to chemotherapy. Melatonin Res. 2019;2(3):105-19.

136. Zhang S, Li W, Gao Q, Wei T. Effect of melatonin on the generation of nitric oxide in murine macrophages. Eur J Pharmacol. 2004;501(1-3):25-30. doi:10.1016/j.ejphar.2004.08.015

137. Chuffa LGA, Fioruci-Fontanelli BA, Mendes LO, Seiva FRF, Martinez M, Fávaro WJ, et al. Melatonin attenuates the tlr4-mediated inflammatory response through myd88-and trif-dependent signaling pathways in an in vivo model of ovarian cancer. BMC Cancer. 2015;15:34. doi:10.1186/s12885-015-1032-4

138. Najafi M, Shirazi A, Motevaseli E, Rezaeyan A, Salajegheh A, Rezapoor S. Melatonin as an anti-inflammatory agent in radiotherapy. Inflammopharmacology 2017;25(4):403-13. doi:10.1007/s10787-017-0332-5

139. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer. 1997;33(5):787-91. doi:10.1016/S0959-8049(97)00062-2

140. Artandi SE. Complex roles for telomeres and telomerase in breast carcinogenesis. Breast Cancer Res. 2002;5:37. doi:10.1186/bcr553

141. Korkmaz A, Sanchez-Barcelo EJ, Tan D-X, Reiter RJ. Role of melatonin in the epigenetic regulation of breast cancer. Breast Cancer Res Treat. 2009;115(1):13-27. doi:10.1007/s10549-008-0103-5

142. Pirouzpanah S, Taleban F-A, Mehdipour P, Sabour S, Atri M. Hypermethylation pattern of esr and pgr genes and lacking estrogen and progesterone receptors in human breast cancer tumors: Er/pr subtypes. Cancer Biomark. 2018;21(3):621-38. doi:10.3233/CBM-170697

143. Travis RC, Key TJ. Oestrogen exposure and breast cancer risk. Breast Cancer Res. 2003;5(5):239. doi:10.1186/bcr628

144. Ackerman GE, Smith ME, Mendelson CR, Macdonald PC, Simpson ER. Aromatization of androstenedione by human adipose tissue stromal cells in monolayer culture. J Clin Endocrinol Metab. 1981;53(2):412-7. doi:10.1210/jcem-53-2-412

145. Bayne S, Liu J-P. Hormones and growth factors regulate telomerase activity in ageing and cancer. Mol Cell Endocrinol. 2005;240(1-2):11-22. doi:10.1016/j.mce.2005.05.009

146. Kyo S, Takakura M, Kanaya T, Zhuo W, Fujimoto K, Nishio Y, et al. Estrogen activates telomerase. Cancer Res. 1999;59(23):5917-21.

147. Misiti S, Nanni S, Fontemaggi G, Cong Y-S, Wen J, Hirte HW, et al. Induction of htert expression and telomerase activity by estrogens in human ovary epithelium cells. Mol Cell Biol. 2000;20(11):3764-71. doi:10.1128/MCB.20.11.3764-3771.2000

148. Jinbo G, Daoda C, Yuan T, Jinhui Z, Kailin C. Effect of estrogen on telomerase activity in human breast cancer cells. J Huazhong Univ Sci Technol. 2003;23(3):286-7. doi:10.1007/BF02829516

149. Sánchez-Barceló EJ, Cos S, Fernández R, Mediavilla MD. Melatonin and mammary cancer: A short review. Endocr Relat Cancer. 2003;10(2):153-9. doi:10.1677/erc.0.0100153

150. Cos S, González A, Martínez-Campa C, Mediavilla MD, Alonso-González C, Sánchez-Barceló EJ. Estrogen-signaling pathway: A link between breast cancer and melatonin oncostatic actions. Cancer Detect Prev. 2006;30(2):118-28. doi:10.1016/j.

cdp.2006.03.002

151. Ram PT, Kiefer T, Silverman M, Song Y, Brown GM, Hill SM. Estrogen receptor transactivation in mcf-7 breast cancer cells by melatonin and growth factors. Mol Cell Endocrinol. 1998;141(1-2):53-64. doi:10.1016/s0303-7207(98)00095-1

152. Rato AG, Pedrero JG, Martínez MA, Del Rio B, Lazo PS, Ramos S. Melatonin blocks the activation of estrogen receptor for DNA binding. FASEB J. 1999;13(8):857-68. doi:10.1096/fasebj.13.8.857

153. Kubatka P, Zubor P, Busselberg D, Kwon TK, Adamek M, Petrovic D, et al. Melatonin and breast cancer: Evidences from preclinical and human studies. Crit Rev Oncol Hematol. 2018;122:133-43. doi:10.1016/j.critrevonc.2017.12.018

154. Anbalagan M, Rowan BG. Estrogen receptor alpha phosphorylation and its functional impact in human breast cancer. Mol Cell Endocrinol. 2015;418 Pt 3:264-72. doi:10.1016/j.mce.2015.01.016

155. Pirouzpanah S, Taleban FA, Atri M, Abadi A-R, Mehdipour P. The effect of modifiable potentials on hypermethylation status of retinoic acid receptor-beta2 and estrogen receptor-alpha genes in primary breast cancer. Cancer Cause Cont. 2010;21(12):2101-11. doi:10.1007/s10552-010-9629-z.

156. Liu A, Zhang D, Yang X, Song Y. Estrogen receptor alpha activates mapk signaling pathway to promote the development of endometrial cancer. J Cell Biochem. 2019;120(10):17593-601. doi:10.1002/jcb.29027

157. Razavi P, Chang MT, Xu G, Bandlamudi C, Ross DS, Vasan N, et al. The genomic landscape of endocrine-resistant advanced breast cancers. Cancer Cell. 2018;34(3):427-38.e6. doi:10.1016/j.ccell.2018.08.008

158. Kiefer T, Ram P, Yuan L, Hill S. Melatonin inhibits estrogen receptor transactivation and camp levels in breast cancer cells. Breast Cancer Res Treat. 2002;71(1):37-45. doi:10.1023/a:1013301408464

159. Castoria G, Migliaccio A, D'Amato L, Di Stasio R, Ciociola A, Lombardi M, et al. Integrating signals between camp and mapk pathways in breast cancer. Front Biosci. 2008;13(1318):27. doi:10.2741/2764

160. Zivadinovic D, Gametchu B, Watson CS. Membrane estrogen receptor-alpha levels in mcf-7 breast cancer cells predict camp and proliferation responses. Breast Cancer Res. 2005;7(1):R101-12. doi:10.1186/bcr958

161. Aronica SM, Katzenellenbogen BS. Stimulation of estrogen receptor-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-i. J Mol Endocrinol. 1993;7(6):743-52. doi:10.1210/mend.7.6.7689695

162. Dai J, Inscho EW, Yuan L, Hill SM. Modulation of intracellular calcium and calmodulin by melatonin in mcf-7 human breast cancer cells. J Pineal Res. 2002;32(2):112-9. doi:10.1034/j.1600-079x.2002.1844.x

163. Li Z, Joyal JL, Sacks DB. Calmodulin enhances the stability of the estrogen receptor. J Biol Chem. 2001;276(20):17354-60. doi:10.1074/jbc.M010238200

164. Li L, Li Z, Sacks DB. Calmodulin regulates the transcriptional activity of estrogen receptors. Selective inhibition of calmodulin function in subcellular compartments. J Biol Chem. 2003;278(2):1195-200. doi:10.1074/jbc.M210708200

165. Lee DH, Asare BK, Rajnarayanan RV. Discovery at the interface: Toward novel anti-proliferative agents targeting human estrogen receptor/s100 interactions. Cell Cycle. 2016;15(20):2806-18. doi:10.1080/15384101.2016.1220460

166. García Pedrero JM, del Rio Bz, Martínez-Campa C, Muramatsu M, Lazo PS, Ramos Sa. Calmodulin is a selective modulator of estrogen receptors. J Mol Endocrinol. 2002;16(5):947-60. doi:10.1210/mend.16.5.0830

167. del Río B, Pedrero JMG, Martínez-Campa C, Zuazua P, Lazo PS, Ramos S. Melatonin, an endogenous-specific inhibitor of estrogen receptor α via calmodulin. J Biol Chem. 2004;279(37):38294-302. doi:10.1074/jbc.M403140200

168. Molis TM, Walters MR, Hill SM. Melatonin modulation of estrogen-receptor expression in mcf-7 human breast-cancer cells. Int J Oncol 1993;3(4):687-94. doi:10.3892/ijo.3.4.687

169. Martínez-Campa C, Alonso-González C, Mediavilla MD, Cos S, González A, Ramos S, et al. Melatonin inhibits both er alpha activation and breast cancer cell proliferation induced by a metalloestrogen, cadmium. J Pineal Res. 2006;40(4):291-6. doi:10.1111/j.1600-079X.2006.00315.x

170. Martínez-Campa CM, Alonso-González C, Mediavilla MD, Cos S, González A, Sanchez-Barcelo EJ. Melatonin down-regulates htert expression induced by either natural estrogens (17β-estradiol) or metalloestrogens (cadmium) in MCF-7 human breast cancer cells. Cancer lett. 2008;268(2):272-7.

171. Silva N, Peiris-John R, Wickremasinghe R, Senanayake H, Sathiakumar N. Cadmium a metalloestrogen: Are we convinced? J Appl Toxicol. 2012;32(5):318-32.

172. Moon IK, Jarstfer MB. The human telomere and its relationship to human disease, therapy, and tissue engineering. Front Biosci. 2007;12(1):4595-620. doi:10.2741/2412

173. Stoica A, Katzenellenbogen BS, Martin MB. Activation of estrogen receptor-alpha by the heavy metal cadmium. Mol Endocrinol. 2000;14(4):545-53. doi:10.1210/mend.14.4.0441

174. Nelson LR, Bulun SE. Estrogen production and action. J Am Acad Dermatol. 2001;45(3 Suppl):S116-24. doi:10.1067/mjd.2001.117432

175. Gonzalez A, Cos S, Martinez-Campa C, Alonso-Gonzalez C, Sanchez-Mateos S, Mediavilla M, et al. Selective estrogen enzyme modulator actions of melatonin in human breast cancer cells. J

Pineal Res. 2008;45(1):86-92. doi:10.1111/j.1600-079X.2008.00559.x

176. Cos S, Martínez-Campa C, Mediavilla MD, Sánchez-Barceló EJ. Melatonin modulates aromatase activity in mcf-7 human breast cancer cells. J Pineal Res. 2005;38(2):136-42. doi:10.1111/j.1600-079X.2004.00186.x

177. Wang T, Liu B, Guan Y, Gong M, Zhang W, Pan J, et al. Melatonin inhibits the proliferation of breast cancer cells induced by bisphenol a via targeting estrogen receptor-related pathways. Thorac Cancer. 2018;9(3):368-75. doi:10.1111/1759-7714.12587

178. Zhu X, Kumar R, Mandal M, Sharma N, Sharma HW, Dhingra U, et al. Cell cycle-dependent modulation of telomerase activity in tumor cells. Proc Natl Acad Sci U S A. 1996;93(12):6091-5. doi:10.1073/pnas.93.12.6091

179. Tomlinson RL, Ziegler TD, Supakorndej T, Terns RM, Terns MP. Cell cycle-regulated trafficking of human telomerase to telomeres. Mol Biol Cell. 2006;17(2):955-65.

180. Ten Hagen KG, Gilbert DM, Willard HF, Cohen SN. Replication timing of DNA sequences associated with human centromeres and telomeres. Mol Cell Biol. 1990;10(12):6348-55. doi:10.1128/mcb.10.12.6348-6355.1990.

181. Woodfine K, Fiegler H, Beare DM, Collins JE, McCann OT, Young BD, et al. Replication timing of the human genome. Hum Mol Genet. 2004;13(2):191-202. doi:10.1093/hmg/ddh016

182. Bertoli C, Skotheim JM, De Bruin RA. Control of cell cycle transcription during g1 and s phases. Nat Rev Mol Cell Biol. 2013;14(8):518-28. doi:10.1038/nrm3629

183. Osborne CK, Boldt DH, Estrada P. Human breast cancer cell cycle synchronization by estrogens and antiestrogens in culture. Cancer Res. 1984;44(4):1433-9.

184. Cos S, Blask DE, Lemus-Wilson A, Hill AB. Effects of melatonin on the cell cycle kinetics and "estrogen-rescue" of mcf-7 human breast cancer cells in culture. J Pineal Res. 1991;10(1):36-42.

185. Blask D, Hill S. Effects of melatonin on cancer: Studies on mcf-7 human breast cancer cells in culture. J Neural Transm Suppl. 1986;21:433-49.

186. Talib WH, Alsayed AR, Abuawad A, Daoud S, Mahmod AI. Melatonin in Cancer Treatment: Current Knowledge and Future Opportunities. Molecules. 2021;26(9):2506. doi:10.3390/molecules26092506

187. Hill S. The antiproliferative effect of the pineal hormone, melatonin, on human breast cancer cells in vitro [dissertation]. Tucson : University of Arizona; 1986.

188. Mediavilla M, Cos S, Sanchez-Barcelo E. Melatonin increases p53 and p21wafl expression in mcf-7 human breast cancer cells in vitro. Life Sci. 1999;65(4):415-20. doi:10.1016/S0024-3205(99)00262-3

189. Shaw PH. The role of p53 in cell cycle regulation.

190. Moverare-Skrtic S, Svensson J, Karlsson MK, Orwoll E, Ljunggren O, Mellstrom D, et al. Serum insulin-like growth factor-i concentration is associated with leukocyte telomere length in a population-based cohort of elderly men. Clin Endocrinol Metab. 2009;94(12):5078-84. doi:10.1210/jc.2009-1450

191. Tu W, Zhang DK, Cheung PT, Tsao SW, Lau YL. Effect of insulin-like growth factor 1 on pha-stimulated cord blood mononuclear cell telomerase activity. Br J Haematol. 1999;104(4):785-94. doi:10.1046/j.1365-2141.1999.01272.x.

192. You H, Zheng H, Murray SA, Yu Q, Uchida T, Fan D, et al. Igf-1 induces pin1 expression in promoting cell cycle s-phase entry. J Cell Biochem. 2002;84(2):211-6. doi:10.1002/jcb.10037

193. Vriend J, Sheppard M, Borer K. Melatonin increases serum growth hormone and insulin-like growth factor i (igf-i) levels in male syrian hamsters via hypothalamic neurotransmitters. Growth Dev Aging. 1990;54(4):165-71.

194. Schaeffer H-J, Sirotkin A. Melatonin and serotonin regulate the release of insulin-like growth factor-i, oxytocin and progesterone by cultured human granulosa cells. Exp Clin Endocrinol. 1997;105(02):109-12. doi:10.1055/s-0029-1211736

195. Kos-Kudła B, Zwirska-Korczala K, Marek B, Kajdaniuk D, Ostrowska Z, Buntner B, et al. Does the negative correlation found in breast cancer patients between plasma melatonin and insulin-like growth factor-i concentrations imply the existence of an additional mechanism of oncostatic melatonin influence involved in defense? Med Sci Monit. 2002;8(6):CR457-61.

196. Ishido M. Transient inhibition of synergistically insulin-like growth factor-1-and bisphenol a-induced poliferation of estrogen receptor alpha (ERalpha)-positive human breast cancer MCF-7 cells by melatonin. Environ Sci. 2004;11(3):163-70.

197. Yu Y-F, Zhang Y, Shen N, Zhang R-Y, Lu X-Q. Effect of vegf, p53 and telomerase on angiogenesis of gastric carcinoma tissue. Asian Pac J Trop Dis. 2014;7(4):293-6. doi:10.1016/S1995-7645(14)60041-9

198. Bermudez Y, Yang H, Saunders BO, Cheng JQ, Nicosia SV, Kruk PA. Vegf-and lpa-induced telomerase in human ovarian cancer cells is sp1-dependent. Gynecol Oncol. 2007;106(3):526-37. doi:10.1016/j.ygyno.2007.05.005

199. Zhou L, Zheng D, Wang M, Cong Y-S. Telomerase reverse transcriptase activates the expression of vascular endothelial growth factor independent of telomerase activity. Biochem Biophys Res Commun. 2009;386(4):739-43. doi:10.1016/j.bbrc.2009.06.116

200. Alvarez-García V, González A, Alonso-González C, Martínez-Campa C, Cos S. Regulation of vascular endothelial growth factor by melatonin in human breast cancer cells. J Pineal Res. 2013;54(4):373-80.

Pathol Res Pract. 1996;192(7):669-75. doi:10.1016/S0344-0338(96)80088-4

doi:10.1111/jpi.12007

201. Shokri A, Pirouzpanah S, Foroutan-Ghaznavi M, Montazeri V, Fakhrjou A, Nozad-Charoudeh H, et al. Dietary protein sources and tumoral overexpression of RhoA, VEGF-A and VEGFR2 genes among breast cancer patients. Genes Nutr. 2019;14:22. doi:10.1186/s12263-019-0645-7

202. Pirouzpanah S, Varshosaz P, Fakhrjou A, Montazeri V. The contribution of dietary and plasma folate and cobalamin to levels of angiopoietin-1, angiopoietin-2 and tie-2 receptors depend on vascular endothelial growth factor status of primary breast cancer patients. Sci Rep. 2019;9:14851. doi:10.1038/s41598-019-51050-x

203. Abdi S, Montazeri V, Garjani A, Shayanfar A, Pirouzpanah S. Coenzyme q10 in association with metabolism-related ampk/pfkfb3 and angiogenic VEGF/VEGFR2 genes in breast cancer patients. Mol Biol Cell. 2020;47(4):2459-73. doi:10.1007/s11033-020-05310-z

204. Park SY, Jang WJ, Yi EY, Jang JY, Jung Y, Jeong JW, et al. Melatonin suppresses tumor angiogenesis by inhibiting hif-1α stabilization under hypoxia. J Pineal Res. 2010;48(2):178-84. doi:10.1111/j.1600-079x.2009.00742.x

205. Jorissen RN, Walker F, Pouliot N, Garrett TP, Ward CW, Burgess AW. Epidermal growth factor receptor: Mechanisms of activation and signalling. Exp Cell Res. 2003284(1):31-53. doi:10.1016/s0014-4827(02)00098-8

206. Masuda H, Zhang D, Bartholomeusz C, Doihara H, Hortobagyi GN, Ueno NT. Role of epidermal growth factor receptor in breast cancer. Breast Cancer Res Treat. 2012;136(2):331-45. doi:10.1007/s10549-012-2289-9

207. Augustine T, Maitra R, Goel S. Telomere length regulation through epidermal growth factor receptor signaling in cancer. Genes Cancer. 2017;8(5-6):550. doi:10.18632/genesandcancer.140

208. Dznelashvili N, Kasradze D, Tavartkiladze A. Expression of epidermal growth factor receptor and concentrations of epidermal growth factor and melatonin in endometrial carcinoma. Georgian Med News. 2014;235:17-24.

209. Cos S, Blask DE. Melatonin modulates growth factor activity in mcf-7 human breast cancer cells. J Pineal Res. 1994;17(1):25-32. doi:10.1111/j.1600-079x.1994.tb00110.x

210. Papanikolaou V, Iliopoulos D, Dimou I, Dubos S, Tsougos I, Theodorou K, et al. The involvement of her2 and p53 status in the regulation of telomerase in irradiated breast cancer cells. Int J Oncol. 2009;35(5):1141-9. doi:10.3892/ijo_00000430

211. Papanikolaou V, Athanassiou E, Dubos S, Dimou I, Papathanasiou I, Kitsiou-Tzeli S, et al. Htert regulation by nf-κb and c-myc in irradiated her2-positive breast cancer cells. Int J Radiat Biol. 2011;87(6):609-21. doi:1

212. Ferreira GM, Martinez M, Camargo IC, Domeniconi RF, Martinez FE, Chuffa LG. Melatonin attenuates her-2, p38 mapk, p-akt, and mtor levels in ovarian carcinoma of ethanol-preferring rats. J Cancer. 2014;5(9):728-35. doi:10.7150/jca.10196

213. Baturin DA, Alimova IN, Anisimov VN, Popovich IG, Zabezhinski MA, Provinciali M, et al. The effect of light regimen and melatonin on the development of spontaneous mammary tumors in her-2/neu transgenic mice is related to a downregulation of her-2/neu gene expression. Neuro Endocrinol Lett. 2001;22(6):441-7.

214. Blackburn EH. Structure and function of telomeres. Nature. 1991;350(6319):569-73. doi:10.1038/350569a0

215. von Zglinicki T, Saretzki G, Döcke W, Lotze C. Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: A model for senescence? Exp Cell Res. 1995;220(1):186-93. doi:10.1006/excr.1995.1305

216. Tarry-Adkins JL, Ozanne SE, Norden A, Cherif H, Hales CN. Lower antioxidant capacity and elevated p53 and p21 may be a link between gender disparity in renal telomere shortening, albuminuria, and longevity. Am J Physiol Renal Physiol. 2006;290(2):F509-F16. doi:10.1152/ajprenal.00215.2005

217. Saretzki G, von Zglinicki T. Replicative aging, telomeres, and oxidative stress. Ann N Y Acad Sci. 2002;959(1):24-9. doi:10.1111/j.1749-6632.2002.tb02079.x

218. Kawanishi S, Oikawa S. Mechanism of telomere shortening by oxidative stress. Ann N Y Acad Sci. 2004;1019(1):278-84. doi:10.1196/annals.1297.047

219. Grahame TJ, Schlesinger RB. Oxidative stress-induced telomeric erosion as a mechanism underlying airborne particulate matter-related cardiovascular disease. Particle Fibre Toxicol. 2012;9(1):21. doi:10.1186/1743-8977-9-21

220. Mazidi M, Kengne AP, Cheskin LJ, Banach M. Serum lipophilic antioxidants levels are associated with leucocyte telomere length among us adults. Lipids Health Dis. 2018;17(1):164. doi:10.1186/s12944-018-0781-x

221. Shen J, Gammon MD, Terry MB, Wang Q, Bradshaw P, Teitelbaum SL, et al. Telomere length, oxidative damage, antioxidants and breast cancer risk. Int J Cancer. 2009;124(7):1637-43. doi:10.1002/ijc.24105

222. Rastmanesh R. Potential of melatonin to treat or prevent age-related macular degeneration through stimulation of telomerase activity. Med Hypotheses. 2011;76(1):79-85. doi:10.1016/j.mehy.2010.08.036

223. Reiter RJ, Tan D-X, Qi W, Manchester LC, Karbownik M, Calvo JR. Pharmacology and physiology of melatonin in the reduction of oxidative stress in vivo. Neurosignal. 2000;9(3-4):160-71. doi:10.1159/000014636

224. Gozzo A, Lesieur D, Duriez P, Fruchart J-C, Teissier E. Structure-activity relationships in a series of melatonin

analogues with the low-density lipoprotein oxidation model. Free Radic Biol Med. 1999;26(11-12):1538-43. doi:10.1016/s0891-5849(99)00020-9

225. Millán-Plano S, Piedrafita E, Miana-Mena FJ, Fuentes-Broto L, Martínez-Ballarín E, López-Pingarrón L, et al. Melatonin and structurally-related compounds protect synaptosomal membranes from free radical damage. Int J Mol Sci. 2010;11(1):312-28. doi:10.3390/ijms11010312

226. Matuszak Z, Reszka KJ, Chignell CF. Reaction of melatonin and related indoles with hydroxyl radicals: Epr and spin trapping investigations. Free Radic Biol Med. 1997;23(3):367-72. doi:10.1016/s0891-5849(96)00614-4

227. Kryston TB, Georgiev AB, Pissis P, Georgakilas AG. Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res Genet Toxicol Environ Mutagen. 2011;711(1-2):193-201. doi:10.1016/j.mrfmmm.2010.12.016

228. Tan D-x, Reiter RJ, Chen L-d, Poeggeler B, Manchester LC, Barlow-Walden LR. Both physiological and pharmacological levels of melatonin reduce DNA adduct formation induced by the carcinogen safrole. Carcinogenesis. 1994;15(2):215-8. doi:10.1093/carcin/15.2.215

229. Reddy MV, Randerath K. A comparison of DNA adduct formation in white blood cells and internal organs of mice exposed to benzo [a] pyrene, dibenzo [c, g] carbazole, safrole and cigarette smoke condensate. Mutat Res Genet Toxicol Environ Mutagen. 1990;241(1):37-48.

230. Paradies G, Petrosillo G, Paradies V, Reiter RJ, Ruggiero FM. Melatonin, cardiolipin and mitochondrial bioenergetics in health and disease. J Pineal Res. 2010;48(4):297-310. doi:10.1111/j.1600-079X.2010.00759.x

231. Manchester LC, Vijayalaxmi EK, Kilic Ü, Wójcikowski MK, Daniel WaaA, Drelewska SaJC, et al. Review–pharmacological utility of melatonin in reducing oxidative cellular and molecular. Pol J Pharmacol. 2004;56(2):159-70.

232. Hardeland R, Reiter RJ, Poeggeler B, Tan D-X. The significance of the metabolism of the neurohormone melatonin: Antioxidative protection and formation of bioactive substances. Neurosci Biobehav Rev. 1993;17(3):347-57. doi:10.1016/s0149-7634(05)80016-8

233. Anderson G. Breast cancer: Occluded role of mitochondria n-acetylserotonin/melatonin ratio in co-ordinating pathophysiology. Biochem Pharmacol 2019;168:259-68. doi:10.1016/j.bcp.2019.07.014

234. Baydas G, Gursu MF, Yilmaz S, Canpolat S, Yasar A, Cikim G, et al. Daily rhythm of glutathione peroxidase activity, lipid peroxidation and glutathione levels in tissues of pinealectomized rats. Neurosci Lett. 2002;323(3):195-8. doi:10.1016/s0304-3940(02)00144-1

235. Rodriguez C, Mayo JC, Sainz RM, Antolín I, Herrera F, Martín V, et al. Regulation of antioxidant enzymes: A significant role for melatonin. J Pineal Res. 2004;36(1):1-9.

236. Albarran M, Lopez-Burillo S, Pablos M, Reiter RJ, Agapito MT. Endogenous rhythms of melatonin, total antioxidant status and superoxide dismutase activity in several tissues of chick and their inhibition by light. J Pineal Res. 2001;30(4):227-33. doi:10.1034/j.1600-079x.2001.300406.x

237. Pablos MI, Reiter RJ, Ortiz GG, Guerrero JM, Agapito MT, Chuang J-I, et al. Rhythms of glutathione peroxidase and glutathione reductase in brain of chick and their inhibition by light. Neurochem Int. 1998;32(1):69-75. doi:10.1016/s0197-0186(97)00043-0

238. Sewerynek E, Abe M, Reiter RJ, Barlow-Walden LR, Chen L, McCabe TJ, et al. Melatonin administration prevents lipopolysaccharide-induced oxidative damage in phenobarbital-treated animals: J Cell Biochem. 1995;58(4):436-44. doi:10.1002/jcb.240580406.

239. Mayo J, Sainz R, Antolin I, Herrera F, Martin V, Rodriguez C. Melatonin regulation of antioxidant enzyme gene expression. Cell Mol Life Sci. 2002;59(10):1706-13. doi:10.1007/pl00012498

240. Sugden D. Melatonin biosynthesis in the mammalian pineal gland. Experientia. 1989;45(10):922-32. doi:10.1007/BF01953049

241. Reiter RJ, Tan D-x, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. J Biomed Sci. 2000;7(6):444-58. doi:10.1007/BF02253360

242. Reina M, Martínez A. A new free radical scavenging cascade involving melatonin and three of its metabolites (3OHM, AFMK and AMK). Comput Theor Chem. 2018;1123:111-8. doi:10.1016/j.comptc.2017.11.017

243. Galano A, Tan DX, Reiter RJ. On the free radical scavenging activities of melatonin's metabolites, AFMK and AMK. J Pineal Res. 2013;54(3):245-57. doi:10.1111/jpi.12010

244. Galano A, Tan DX, Reiter RJ. Cyclic 3-hydroxymelatonin, a key metabolite enhancing the peroxyl radical scavenging activity of melatonin. RSC Adv. 2014;4(10):5220-7. doi:10.1039/C3RA44604B