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BRIGHT FACE OF MELATONIN AGAINST BREAST CANCER PROGRESSION AND METASTASIS

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ABSTRACT
Cancer remains one of the most devastating diseases in the world. Breast cancer is considered the second most widely common among all types of cancers. Melatonin (N-acetyl-5-methoxytryptamine) is a natural hormone secreted by the pineal gland, acting on regulation of several physiological processes such as circadian clocks, acting as antioxidant, anti-inflamatory, and apoptosis regulation. During the recent decades the level of melatonin in patients has been linked to cancer progression and inhibition. In circadian cancer biology, the question how melatonin level plays a role in regulation of cancer progression and metastasis, including breast cancer is a major question. In this review, the molecular actions of melatonin and its prospective role in regulating breast cancer progression and metastasis have been discussed.

KEYWORDS: Melatonin, breast cancer, metastasis.

1- INTRODUCTION
Melatonin is a natural hormone secreted by the Pineal gland.[1-4] Also, melatonin could be observed in several extrapineal tissues such as lens, cochlea, Harderian gland, brain, retina, airway epithelium, skin, kidney, thyroid, gastrointestinal tract, liver, pancreas, thymus, reproductive tract, spleen, immune system cells, carotid body, and endothelial cells.[5, 6] Melatonin has wide physiological effects as an antioxidant, anti-iflamatory, regulation of aging, rhythmic functions, anti-cancer and several clinical applications.[7-11] The anticancer activities of melatonin against several types of cancers, including breast, prostate, colon and other kinds of cancers have been demonstrated.[12-17]

Ample data linked and found a reverse relationship between melatonin and cancer progression and metastasis, including breast cancer.[18-21] Through this review, the action of melatonin as antioxidant and a regulator of circadian rhythm in relation to inhibit cancer progression and metastasis particularly breast cancer was discussed.

2- CANCER
Cancer remains one of the most devastating diseases in the world. Tumor is the uncontrolled development and spreading of cells that may affect almost any tissue of the body. In this regard, more than 10 million individuals every year are determined to have cancer around the world. Around the world, every year cancer causes six million passings (12% of all deaths). Carcinogenesis is a multistep process regulated by genetic and epigenetic changes; disrupting several signaling pathways regulating cellular growth, apoptosis, differentiation and angiogenesis.[22, 23] As a normal routine, mammalian cells grow, divide, and die in an orderly fashion. However, sometimes the normal regulation of growth, division and death of cell is disturbed that leads to a rapid and uncontrolled proliferation of cells ultimately resulting into the development of Cancer. The development of tumor is a multistep process that can be initiated by different natural cancer-causing agents (carcinogens). These carcinogens regulates apoptotic-inducing proteins (Caspases, PARP), several transcription factors (NFκB, AP-1, STAT3), anti-apoptotic proteins (Akt, Bcl-2, Bcl-XL), protein kinases (IKK, JNK, MAP kinase), cell cycle regulatory proteins (cyclins, cyclin-dependent kinases), cell adhesion molecules, cyclooxygenase-2, and growth factor signaling pathways.[24, 25]

2.1- Cancer Metastasis
Cancer metastasis means spreading of cancer cells from its original place to another destination in the body. A tumor formed by this way is called metastatic tumor. The

newly formed metastatic cancer carries the same name and type of original cancer cells in the primary site. For instance, breast cancers that spread to the lung and form a new metastatic tumor is called metastatic breast cancer. Furthermore, metastatic cancer cells resemble the cells of the original cancer in their molecular features, including the expression pattern of some proteins and the presence of specific chromosomal changes.

Tumor cells pull in endothelial cells to advance angiogenesis; separate from the essential tumor mass and move towards endothelial cells; intravasate through endothelial cells and the encompassing network; enter the intra-tumoral vasculature; leave the vasculature at the optional destinations. Penetrate the new environment; and build up developing tumor inside another organ.[26] These events are probably going to be driven in huge part by changes in transcriptional programs that influence gene expression required for these procedures (for instance, genes-related angiogenesis, cell attachment and cell matrix proteolysis). These transcriptional changes are interceded by modifications in the tumor microenvironment[27] and additionally by hereditary genetic alterations in some oncogenes and tumor suppressors.[28] For instance, several studies have demonstrated that oncogenic transformations in the tumor suppressor functions of H-Ras or loss of p53 initiate transcriptional programs that advance metastasis through modifications in the expression of genes required for the migration and invasion of cancer cells into the encompassing tissues.[29, 30, 26] Transcription factors that advance the loss of epithelial features, for example, (cell-to-cell) attachment, have likewise proposed to assume a key part in the progression of metastasis.

Metastasis, a sequence of steps starting with epithelial-to-mesenchymal transition, is the primary driver of growth related mortality.[31] Formation of metastasis is a complex cellular process that includes cell spreading, migration and relocation.[32] The movement of cells is promoted by changes in cytoskeletal organization, presenting a highly polarized cell shape and the structure of focal adhesion complexes.[33, 34]

Metastatic cells are highly invasive and migratory cells have and inadequately organized cytoskeleton and scarce attachment to their substratum. while low invasive cells connect to the substrate via focal adhesion formed by actin fibers and are connected together by adhesion proteins including integrins and cadherins.[35] Epithelial-to-mesenchymal transition (EMT) is One of the most critical process in metastasis cascade, during which epithelial cells lose their polarity and cell–cell junctions. This process involves changes in cell shape and in the cytoskeletal organization and gaining mesenchymal features, including fibroblast-like cell morphology and promoting cell invasion and migration. EMT cascade occurs not only in embryonic development but also in several pathological conditions.[36]

EMT includes the loss of epithelial markers, for example, the tight junction proteins occludins and claudins, E-cadherin, α and β-catenin, and cytokeratins. Associatively, various mesenchymal markers are known to be increased, including N-cadherin, vimentin, fibronectin, matrix metalloproteinase, integrins α and β, and actin proteins.[37-39] The recent experiments of gene expression profile analyses in several cellular systems of EMT recommended hundreds if not thousands of genes significantly changed in their expression during EMT.[40] EMT might be transient, as saw in colorectal growth, where cells at the beginning of the invasive stage lose E-cadherin expression and induced nuclear β-catenin loalization, while liver metastatic cells have re-differentiation into epithelial cells and morphologically resemble the original tumor cells.[41] Subsequently, the occurrence of EMT in malignancy patients is still faced off regarding and better markers to screen tumor cells during metastatic spread are urgently needed to understand the order of events in patients.[42]

3.1- Breast cancer

Breast cancer (BC) is the most well-known neoplastic disorder diagnosed in women[43] and is considered the second most successive reason of death in women, after lung malignancy.[43] The incidence of breast cancer worldwide, is rising up in the female population, but the growth rate is higher in young women in comparison to older ones.[44] Substantial progress has been made in the management of breast cancer, both from clinical and new supportive point of view[45] and this, together with the results obtained in the early diagnosis, has been reflected in substantial improvements in breast cancer mortality over the last decades.[46] Despite recent advances in early diagnosis and effective treatment, it is estimated that up to one-third of patients having been diagnosed with breast cancer will develop metastatic disease.[47]

In contrast, BC in men, male breast cancer (MBC), is rare and accounts for less than 1% of total neoplastic cases in male population and about 1% of all breast cancer diagnoses.[48, 49] Male breast cancer's incidence is increasing over the years, due to lack of awareness of male population regarding this disease. As a result, male patients are diagnosed in a more advanced stage of the disease.[50]

3.2- Breast Cancer Metastasis

Before breast cancer cells get access into the vascular lumina, they have to detach themselves from the growing tumor cell mass, followed by invasion and migration through the extracellular matrix (ECM) towards the vascular walls.[51] Malignant cells infiltrate the surrounding frontline of ECM as single cells or as clusters of malignant cells. BC cells on the tumor boundaries upregulate genes like SNAIL, SLUG and ZEB1, and other transcriptional factors that accelerate potential transformation of the malignant epithelial cells to the mesenchymal-like phase through repression of E-

cadherin and other cell-cell adhesion molecules, which, hence, acquire more motility.[52]

4.1- Melatonin biosynthesis and pharmacology

Melatonin (N-acetyl-5-methoxytryptamine) was initially isolated from the bovine pineal gland in 1958.[53] Melatonin in animal cells is directly synthesized from tryptophan, while other organisms through the shikimic acid pathway.[54] The biosynthesis of melatonin in humans and some other organisms occurs in four enzymatic steps from amino acid tryptophan. During the first two steps, L-tryptophan is converted to 5-hydroxy-L-tryptophan (5-HTP) by an enzyme, tryptophan 5-hydroxylase. 5-HTP is then decarboxylated (CO_2 removal) by 5-hydroxytryptophan decarboxylase to produce serotonin. This point is the rate limiting stage, is determined by light-dark conditions. The key enzyme, aralkylamine N-acetyltransferase (AANAT) is activated only in darkness and converts serotonin to N-acetyl serotonin. Serotonin is ultimately converted to melatonin by acetylserotonin O-methyltransferase enzyme. It is the key regulator of melatonin synthesis from tryptophan, as its gene AANAT is directly influenced by photoperiod.[55, 56]

In plants, bacteria, fungi, and protists, melatonin is produced indirectly though shikimic acid pathway. In these cells, synthesis of melatonin begins with d-erythrose-4-phosphate and phosphoenol pyruvate and in photosynthetic cells with carbon dioxide.[57, 58]

Melatonin mainly produces at the onset of darkness from the pineal gland and other organs as well: skin, retina and bone marrow.[59] Melatonin, being highly lipophilic, diffuse easily through most cell membranes, into the circulatory system to be delivered to target organs and also into the cerebrospinal fluid to bathe the hypothalamus and central nervous system. Its half-life time is about 30 minutes, and it is mostly cleared via the liver and subsequently removed in the urine as urinary 6-sulfatoxymelatonin.[60]

4.2- Melatonin and Circadian rhythms

Historically, our planet and as a result of the arrangement of the moon, the Earth has rotated around its own axis once in around 24 h.[61] The periodic organization of cycles, for example, alertness rest. Bolstering fasting, and excitement-resting are among these adaptations. Underlying these occasional exercises was the development of an endogenous planning framework that gives on most life forms the ability to show ~24-h changes in physiological parameters. These day by day variations are known as circadian rhythms (from circa, approximately, and dies, day) as mentioned by Hut *et al*.[62]

There are three segments constituting the circadian rhythms: 1- the molecular clock that has the ability to quantify time and is formed of a panel of genes/proteins controlled by feedback loops; 2- synchronizing components that permit the molecular clock to be entrained bynatural promts; and 3- output signals that communicate the oscillatory activity of the molecular clock from metabolic to behavioral activities inside any living being.[63] The circadian planning framework in mammals includes a master clock located in the hypothalamic suprachiasmatic core (SCC) and an arrangement of peripheral oscillators that are facilitated by the SCC.[64] Most of the time, the circadian planning framework is synchronized by a light boost.[65]

The decreased inhibitory neuronal activity in the SCC during nighttime drives the pineal organ to produce high concentrations of melatonin. On the contrary of that, the secretion of melatonin by pineal gland during the daytime is almostly insignificant.[66] High level of melatonin in the blood at nighttime are in charge of telling all the body cells, including cancer cells, that it is nighttime.[67] Fluctuations in either the length of the day time or the timing of light exposure can affect SCC activity and consequently the secretion of melatonin in pineal gland, a phenomenon referred to as circadian interruption. Another part of circadian interruption due to the capability of an organism for exposure to light at night to suppress the nighttime circadian melatonin signal.[68]

Circadian disturbance caused by expanding the exposure duration of daily light suppressed the nocturnal circadian signal of melatonin, promoting the development and growth of tumors.[69] The temporal expression of a multitude of processes controlling cancer initiation, growth, progression, and invasion/metastasis is governed by the circadian rhythmic outputs from the central circadian pacemaker in the SCC.[70] A large portion of the chronobiotic and sleep inducing impact of melatonin are intervened through two receptors: MT1 and MT2. Both subtypes are highly expressed in the SCC, but they present also throughout other sites in the brain and other organs, referring that melatonin may affect other biological systems. therefore, it is not surprising that melatonin has a number of effects on human biology that have not been clearly demonstrated, such as regulating the sleep-wake cycle and acting as a neurogenic/neuroprotective agent.[71]

4.3- Melatonin and Redox activity

Melatonin has unique physiological properties, for example, scavenging of superoxide (O_2^-), hydrogen peroxide (H_2O_2), water-soluble peroxyl radical ($RO_2^\cdot$), hydroxyl ($^\cdot OH$), singlet oxygen, and antioxidative enzymes (glutathione peroxidase, superoxide dismutase etc.).[72, 73] Besides, meltonin can also inhibit nitric oxide synthase.[74]

In vivo study show the antioxidant role of melatonin in neutralization the oxidative stress resulted from atrazine-induced damage of erythrocyte in adult male albino rats.[75] The study of[76] showed that beside the role of melatonin as scavenger of free radicles and its

antioxidant action because of its ability to enters cells, the administration of melatonin *in vivo* induces the mitochondrial respiratory complexes I and IV time dependently. Further examination by[77] explain the possible protective action of melatonin on the toxic effects of pro oxidant antitumor drugs on normal and neoplastic such as oxidative stress-inducing damage in mice liver induced by the adriamycin.

It has been improved that melatonin inhibited the mitochondrial cell-death pathway in cultured cells via inhibition of Rip2/Caspase-1 signaling pathway.[78] González *et al.*[79] reported that melatonin and its metabolites have protective role, preventing oxidative damage induced by several toxic agents and metabolic processes of tissue. They found that, melatonin treatment blockedthe decrease of biliary flow in rats with hepatectomy and attenuated the oxidative stress induced by partial hepatectomy and normalized the Na^+/K^+ ATPase activity.

It has been confirmed that melatonin has a protective role against hepatic oxidative stress induced by paraquat and showed the cytoprotective effect of melatonin against oxidative stress induced by xenobiotics.[80] Also, melatonin showed an extra ability as an anti-inflammatory agent.[81] Popov *et al.*[82] reported that melatonin exerted effective enhancement in inhibition of decompensation of the function of glutathione antioxidant system. The antioxidative effects of most molecules are regulated and controlled by their intercellular localization. Anti-oxidative effects of melatonin involve the protection of plasma membrane lipids, cytosolic proteins and DNA. Besides, melatonin has the ability to crossover all morphophysiological barriers; entering all cells in the organism.[83]

4.4- Melatonin and cancer therapy

Melatonin has wide physiological effects, regulates not only circadian rhythm, but also plays a role as antioxidant, anti-ageing and Immunomodulatory hormone.[84] Melatonin has a wide range of actions; includes but not all, its ability to inhibit tumor growth. This tumor inhibition by melatonin involves one or more of the following that melatonin exerts its action through: a- antioxidant effects; b- strogen receptor regulation; c- attenuation of the enzymes involved in estrogen synthesis; d- modulation of cell cycle and apoptosis; e- inhibition of telomerase activity; f- inhibition of metastasis; g- prevention of circadian disruption; h- anti-angiogenesis; i- epigenetic effects; j- stimulation of cell differentiation; and k- activation of the immune system.[85, 86] Melatonin has a multiple effect and plays an important regulatory effect in cell adhesion, migration and apoptosis of cancer cells.[87]

4.5- Melatonin and breast cancer

A few types of malignancy, especially breast tumor, have been observed to be markedly increased in night shift workers, who contain no less than 20% of our workforce.[88] Melatonin applies oncostatic impacts on various types of tumors, particularly on hormone-subordinate breast tumor. Generally, melatonin diminishes the occurrence and development of mammary tumors in rodents *in vivo*, and represses the viability and invasion of human breast tumor *in vitro*. Other studies support the suggestion that melatonin hinders the development of breast tumor by interacting with estrogen-signaling pathways.[89] Studies demonstrated an inverse correlation between ER alpha and MT1 receptor expression in primary human breast cancers. Through activation of melatonin MT1 receptor, melatonin suppressed the development and growth of breast tumor via regulation of growth factors, gene expression, clock genes, inhibition of tumor cell invasion and metastasis, and even direction of mammary organ development.[60]

It has been reported that physiological doses of melatonin at daytime is 10^{-11} M, and at night-time 10^{-9}M in human serum. 10^{-9} M markedly inhibited the proliferation of both estrogen receptor alpha-positive (T47D, MCF-7, ZR-75-1) and estrogen receptor alpha-negative (MDA-MB-468) breast cancer cells.[90, 91, 60]

Furthermore, melatonin suppressed estrogen-induced transcriptional activity of the estrogen receptor alpha, resulting in downregulation of a number of mitogenic proteins and pathways such as the anti-apoptotic Bcl-2 protein, while induced the expression of growth-inhibitory and apoptotic pathways such as TGF-b and Bax.[92] Studies of Dai *et al*.[93] showed that melatonin suppressed the transcriptional activity of the glucocorticoid receptor while inducing the transcription of the retinoic acid receptor alpha in human breast cancer cells. Besides, it has been observed that melatonin is the nocturnal anticancer signal that directly linked to the central circadian clock with tumor cell linoleic acid uptake/important metabolism in human breast carcinogenesis.[69] Additionally, it is stated that, melatonin effectively inhibited breast cancer stem cells viability.[94] It is reported also that, MT1 receptor is the main transducer of melatonin's actions in the breast, inhibited mammary gland development and mediating the anticancer effects of melatonin via multiple pathways.[60]

4.6- Melatonin and breast cancer metastasis

Several studies reported that the plasma level of melatonin is markedly decreased in patients with metastatic cancer when compared with those without metastases.[95, 96] It has been suggested that melatonin negatively regulated breast cancer invasion and metastasis. Therefore, the current review focused on the different mechanisms by which melatonin inhibits breast cancer metastasis. Recently the mechanisms of action of melatonin against cancer metastasis have been reviewed by Su et al.[97]

Melatonin cause inhibition of breast cancer metastasis via different signaling pathways: The E-cadherin

expression increased in MCF-7 cells when treated with the nocturnal physiological dose (1nM) of melatonin. Also, the number of E-Cadherin–expressing cells increased markedly after treatment with melatonin.[98] Melatonin with (1nM) not only was observed to increase the adhesive capacity of MCF-7 to the basement membrane; inhibiting EMT but also lowering its chemotactic migration.[98]

The anti-metastatic activity of melatonin was promoted by overexpression of the MT1 receptor and inhibited by adding MT1 /MT2 receptor antagonist; luzidole.[60] Breast cancer invasion and metastasis are driven in many cases by activation of the P38 MAPK signaling pathway, inducing matrix metalloproteinase expression (MMP2) and MMP9.[60] In this regard, addition of melatonin inhibited P38 phosphorylation and subsequently decreased MMP2 and MMP9 expression and consequently inhibition of breast cancer cell invasion.[60]

Melatonin also activated glycogen synthase kinase 3β (GSK3β), an enzyme critical in metabolism and cell proliferation and survival, exhibits a circadian rhythm of phosphorylation in human breast cancers by decreasing serine-threonine kinase Akt phosphorylation.[99] This inhibition induced degradation of β-catenin and inhibition of epithelial-to-mesenchymal transition.[99] Melatonin administration reduced tumor growth and cell proliferation, as well as the inhibition of angiogenesis in breast cancer. So, MLT is retarded metastasis via inhibition of neovascularization formation which is important for cancer growth and metastasis.[100] Melatonin inhibited cancer invasion and migration by producing a well-organized cytoskeleton through Rho-associated protein kinase-regulated migration/anchorage switch.[101] This inhibition supports the suggestion that the cytoskeleton can be a target for melatonin to inhibit invasion and metastasis in cancer patient.[101]

In MCF-7 cells, it is reported that, 1 nM melatonin inhibited invasion and migration through increasing the expression of E-cadherin and beta(1)- integrin.[101] Melatonin inhibited Metastasis in Her2 positive human breast cancer cells via suppressing RSK2 expression and inhibiting other mechanisms that promote metastasis; disruption of the melatonin signal may promote metastatic progression in breast cancer.[19] Also, melatonin inhibited breast cancer viability and invasion as well as modulating the expression of EMT-related proteins, referring to its potential anti-metastatic effect human breast cancer cell lines.[102] It is stated also that, melatonin inhibited breast cancer metastasis through modulating the expression of Rho-associated kinase protein-1.[103] On the contrary of that, another study showed that melatonin had no influence on the invasive capabilities of any of three breast cancer cell lines; MCF-7, MDA-MB-231and MDA-MB-435.[104]

5- Future remarks

Melatonin has a wide spectrum of biological effects. In this review, we discussed the action and usefulness of melatonin against cancer progression and metastasis treatment. However, the available data refer to the lack of full coverage of the mechanisms behind the actions of melatonin to inhibit cancer growth and metastasis. Therefore, further studies on the action and the signaling mechanisms of melatonin to inhibit cancer progression, including breast cancer are still required. In the same time, the available studies showed that, melatonin can be a potential candidate for clinical trials to be used against breast cancer metastasis and progression and other types of cancers.

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