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Melatonin Therapy for Circadian Disturbances and Its Inhibitory Effects on Breast Cancer Development

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Abstract. Breast cancer is a disease that poses a danger to women's health and survival rate in the modern world. Because of the wide range of elements that influence it, breast cancer is a very valuable and intriguing subject to research. Melatonin is one of the medicines for circadian rhythm disorders that are used by many people in modern society. Researchers have been interested in the connection between circadian rhythms and several diseases in recent years. In particular, the treatment of disorders involving circadian rhythms has revealed new avenues for the creation of therapeutic medications. This led to an investigation into whether melatonin is effective in treating breast cancer and what its specific mechanism of action is. In addition to providing clear evidence of melatonin's therapeutic benefits for breast cancer, the finding that it interacts with the REV-ERB target in this disease may also open the door to the development of more potent, side-effect-free breast cancer medications by revealing a novel molecular pathway. Also, several other mechanisms including BMAL1, CLOCK genes, DNA repair, and the cell cycle have been identified that can also be affected by melatonin and REV- ERB through circadian rhythms.

Keywords: Breast cancer, Circadian rhythm, Melatonin.

1. Introduction

The alterations that occur in an organism's physiology, mind, and behaviour during the course of a day are known as circadian rhythms. While light and dark are the primary elements influencing circadian rhythms, temperature, food consumption, stress, physical activity, and social situations can all have an effect. Internal clock genes in the SCN (suprachiasmatic nucleus) release signals throughout the day to regulate bodily functions. Light sensitivity affects the SCN. Light affects the messages that the SCN uses to coordinate your body's circadian cycles. Because almost every human tissue and organ has a circadian rhythm of its own, it is possible to synchronize it with the day-night cycle. Numerous essential biological functions, such as hormone release, sleep cycles, appetite, digestion, and body temperature, are impacted by circadian rhythms. The pineal gland is the main producer of melatonin, a hormone that is essential for controlling the circadian cycles of the body. In the absence of light, the body releases more melatonin, which signals the body to sleep. The body responds to light by producing less melatonin, which indicates wakefulness. Melatonin functions as a temporal cue, or signal of darkness, to a number of organs, including the SCN. When light is lacking, it can also synchronize the neuroendocrine and sleep-wake cycles to the 24-hour cycle.

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Breast cancer is a disease with breast cells abnormal developing. And tumors might spread throughout the body and become fatal. In 2022, 670,000 individuals lost their lives to breast cancer, accounting for 2.3 million diagnoses among women globally. With such a high incidence, lethality and growth rate of breast cancer, research on breast cancer has attracted the attention of researchers all over the world, and in this paper, circadian rhythms are used as a new entry point to investigate the breast cancer development. An higher risk of developing breast cancer was associated with disruption of the normal circadian melatonin rhythm, which is frequently brought on by things like nighttime light exposure [1,2]. Melatonin may also have strong anticancer effects, according to new research, especially when it comes to breast cancer.

One component of the circadian clock that represses transcription is called REV-ERBα (NR1D1). Because it directly regulates some genes about metabolism. For example, REV-ERBα is regarded as an integrator of cell metabolism which through the circadian clock.

Therefore, the aim of this study was to demonstrate that a definite improvement in circadian dysregulation has a direct benefit on the development of breast cancer. Melatonin is a highly promising drug for treating breast tumor. With an emphasis on the underlying mechanisms of action, it seeks to present a thorough summary of the possible inhibitory effects of melatonin through the REV-ERB agonist treatment on breast cancer. Particular attention will be paid to the ways in which melatonin can modulate key signaling pathways, cellular processes, and molecular targets involved in breast cancer progression [1-3].

It is essential to comprehend the therapeutic potential of melatonin in relation to circadian disruptions and the onset of breast cancer, as this might facilitate the creation of innovative techniques for therapy and prevention that focus on this hormone-based modality. We shall summarize the present level of knowledge in this review.

2. Circadian Disturbance, melatonin and breast cancer

There is evidence connecting circadian disruptions—such as tampering with the sleep-wake cycle—and a higher risk of breast cancer. Melatonin, a hormone regulated by circadian rhythms, plays a key role in maintaining these cycles and exhibits anti-cancer properties. Reduced melatonin levels, often associated with circadian misalignment, may promote cancer progression by affecting cell growth and tumor suppression mechanisms. Knowing how melatonin, circadian disruptions, and breast cancer are related may help develop preventative and therapeutic approaches.

2.1. Circadian disturbance

Any deviations or anomalies in the body's normal 24-hour circadian rhythm are referred to as circadian disturbances. Numerous physiological processes and behaviours, including as hormone production, digestion, body temperature, sleep-wake cycles, and body temperature, are regulated by an internal biological clock called the circadian rhythm. A higher risk of breast cancer is correlated with disturbance of the circadian rhythm [4,5]. A significant proportion of breast tumor patients have circadian rhythm disturbances. According to Kelly D'cunha's system evaluation from 2023, behaviours that interfere with circadian rhythm may have an impact on the prognosis of breast cancer in women.

Disruption of the circadian clock can lead to dysregulation of cell cycle, DNA repair, apoptosis, and other processes that are important for cancer development and progression. It can also create an immunosuppressive tumor microenvironment, promoting tumor growth and metastasis [4].

Disruption of core circadian clock genes like CLOCK, PER, CRY, and BAML1 directly increases breast cancer development.[5]

The BMAL1 and CLOCK create heterodimers. Target genes' E-box regions are bound by the CLOCK and BMAL1 complex throughout the day, which encourages the production of those genes. Among these genes are negative regulators that affect the circadian rhythm, such CRY gene and PER gene. Translocation to the nucleus, heterodimer formation between PER and CRY, and inhibition of CLOCK-BMAL1 activity at night cause transcription to stop. After then, post-translational changes

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cause PER and CRY to degrade, which permits the cycle to resume when their levels are quickly slow down.

The CLOCK and BMAL1 complex binds orphan nuclear receptors REV-ERBα and REV-ERBβ in addition to PER and CRY. These receptors play a role in the formation of a RORα, RORβ, and RORγ secondary feedback loop. Target gene promoters and enhancers have RRE elements, and REV-ERB and ROR proteins fight with one another to bind to these elements to control transcription and ensure the rhythmic expression of BMAL1. A group of circadian genes, that are closely linked to the developing of breast cancer, control circadian rhythms. Important transcription factors like CLOCK and BMAL1 are essential to the development of breast cancer. Increased invasion and proliferation of tumor cells are correlated with their overexpression.

2.2. Circadian disturbance and melatonin

The hormone melatonin is essential for controlling circadian cycles. Decreased melatonin levels due to circadian disruption have been linked to a higher risk of breast cancer development. Through a variety of processes, melatonin's oncostatic (tumor-suppressive) qualities can prevent breast cancer cells from proliferating and growing [6].

2.3. Melatonin and cancer

Melatonin is a hormone that is naturally produced by the pineal gland in the brain. It is necessary to regulate the body's circadian rhythms and sleep-wake cycles. Melatonin levels rise in the evening as darkness falls, signalling the body that it is time to go to sleep. Typically, melatonin supplements are administered orally as pills or capsules. The main uses of melatonin as a drug are treating sleep disorders like insomnia, delayed sleep phase syndrome, and jet lag. Research indicates that melatonin may possess anti-cancer properties, particularly in relation to breast cancer. It has demonstrated oncostatic effects, meaning it can inhibit the growth and metastasis of cancer cells. Animal studies and clinical data by Stephen G Grant in 2006 showed that melatonin reduced the incidence of tumour cell-induced cancer in vitro [7]. Additionally, studies conducted in 2015 by Steven M. Hill revealed that it greatly slowed the development of several human breast tumors [8]. In several methods, melatonin inhibits the growth and triggers apoptosis in the breast cancer cells. In the MCF-7 cell-induced transfection paradigm, melatonin's antiproliferative effect is mediated via activation of the MT1 receptors. In breast tumor cells that express ER-α, melatonin receptors impede the transcriptional activity mediated by ER-α.

Inhibitory effects on Ca2+ signalling and CaM expression, as well as activation of p53 expression and p21 gene transcription, mediate cell cycle arrest and proliferation. In MCF-7 cells, blocking MT1/MT2 receptors has been shown to inhibit p53-mediated DNA damage repair. By suppressing aromatase activity, indomethacin stops the growth of the breast tumor cells in gap phase 1 (G1) and oestrogen production. Melatonin also prevents tumor cells from absorbing linoleic acid (LA).

3. REV-ERB agonist therapy

REV-ERB agonist therapy works by activating REV-ERB proteins, which play a role in regulating circadian rhythms, metabolism, and inflammation. Melatonin can activate REV-ERB proteins within the circadian system, helping to synchronize biological rhythms. Additionally, synthetic REV-ERB agonists have shown potential in treating cancer by inhibiting tumor growth through their effects on the cell cycle and metabolic pathways. This indicates that REV-ERB agonists not only regulate circadian rhythms but also hold promise as a therapeutic approach for cancer.

3.1. Melatonin can activate the REV-ERB proteins in circadian rhythms

REV-ERB agonist therapy refers to the use of synthetic compounds that activate or agonize the REV-ERB nuclear receptor. Some key points about REV-ERB agonist therapy: The nuclear receptor known as REV-ERB is crucial for controlling inflammation, metabolism, and the circadian rhythm. The natural ligand for REV-ERB is heme; however, synthetic agonists, such as STL1267, SR9009, and GSK4112, have been created to more precisely target and activate REV-ERB. [9,10] REV-ERB agonists have been

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shown to enhance the recruitment of the transcriptional co-repressor NCoR to REV-ERB, leading to increased transcriptional repression of REV-ERB target genes. This allows REV-ERB agonists to modulate circadian rhythms, metabolism, and inflammatory processes. [9] The circadian clock's component proteins, the REV-ERB proteins, can in fact be activated by melatonin. The transcriptional activators BMAL1 and CLOCK, as well as the transcriptional repressors CRY, PER, and REV-ERB proteins, control the basic molecular machinery that makes up the circadian clock. The pineal gland secretes melatonin, which is a key player in controlling the circadian clock. Melatonin influences the expression of fundamental clock genes, such as REV-ERB proteins, and so plays a major role in controlling circadian rhythms. Circadian oscillations in gene expression are produced by transcriptional-translational feedback loops, both positive and negative, which include the REV-ERB proteins (REV-ERBα and REV-ERBβ). As repressors, REV-ERBs prevent the production of clock gene Bmal1, a crucial circadian clock activator.

Melatonin can activate REV-ERB proteins through several mechanisms, including: Direct binding and activation: Melatonin can directly bind to and activate REV-ERBα, enhancing its repressive effects on Bmal1 transcription. This contributes to strengthening the circadian clock feedback loop's negative limb. Indirect regulation via RORs: Melatonin can also indirectly modulate REV-ERB activity by regulating the expression of retinoid-related orphan nuclear receptors (RORs). RORs act as activators of Bmal1 transcription. Melatonin suppresses ROR expression, which then allows REV-ERBs to more effectively repress Bmal1.Interaction with clock proteins: Melatonin can also influence the stability and nuclear localization of important clock proteins like PER and CRY, which then interact with and modulate REV-ERB activity.

By promoting the rhythmic production of REV-ERB proteins, melatonin helps synchronize and entrain the circadian clock through several processes. Melatonin can thereby synchronize daily physiological and behavioural cycles with the light/dark cycle outside. In summary, the evidence indicates that melatonin can help entrain and regulate the core circadian clock proteins, including the REV-ERB proteins, which are part of the negative feedback loop that controls circadian rhythms.[11]

3.2. REV-ERB agonist therapy can treat cancer

Cell core receptors REV-ERBα and REV-ERBβ are repressors which depend on the ligand. Although heme is these receptors' natural ligand, several artificial agonists and antagonists have recently been created. In 2016, Wang and colleagues investigated the impact of SR9011, a synthetic REV-ERB agonist, on many cell lines of breast cancer that were ER+, ER−, HER2+, HER2−, and triple negative. Regardless of the ER or HER2 status of the breast cancer cell lines, they discovered that SR9011 inhibited their growth. The proliferation of MCF10A cells was unaffected by SR9011. It seems that SR9011 stops the breast cancer cells' cell cycle before they enter the M phase. Since cyclin A (CCNA2) was shown to be a real target gene of REV-ERB, it is possible that SR9011's reduction of this cyclin's expression caused the cell cycle. These findings suggest that REV-ERB ligands may be useful in the treatment of conditions like cancer that are linked to unchecked cell proliferation [12].

Circadian rhythms regulate many important cancer-related processes like cell division, metabolism, and immune function. Disruption of circadian rhythms can promote cancer development. Targeting components of the circadian clock machinery is emerging as a new approach for cancer treatment. Compounds that activate the REV-ERB proteins, which are key regulators of the circadian clock, have been shown to kill various cancer cells in lab experiments and slow tumor growth in mouse models.

The REV-ERB agonist compounds work by blocking two key pathways that cancer cells rely on heavily - lipid synthesis and autophagy (cellular recycling). Healthy cells don't depend on these pathways as much, so the compounds selectively target cancer cells. The REV-ERB agonists were also found to kill senescent (pre-cancerous) cells that can promote tumor growth and cancer relapse. This suggests these compounds could have applications beyond just treating active cancers [13]. According to research, melatonin can activate REV-ERB proteins, which then inhibit the expression of genes linked to tumor development and cell division [5]. This helps to inhibit the growth and progression of breast cancer cells.

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Melatonin's anti-cancer effects on breast cancer seem to be mediated in part by the circadian clock, REV-ERB proteins, and melatonin interactions. Modulating this pathway may represent a promising therapeutic approach for breast cancer. In summary, melatonin can treat breast cancer by activating REV-ERB proteins, which are key components of the circadian rhythm regulatory network. This helps to suppress tumor growth and progression through effects on cell proliferation and other cancer-related pathways [13].

4. Other pathways to treat breast cancer through circadian rhythms

Other circadian rhythm pathways, such as those involving the core circadian genes BMAL1 and CLOCK, are important in the advancement of breast cancer in addition to REV-ERB agonist treatment. These genes are included in the genesis and spread of cancer because mutations in them can impact DNA repair, cell cycle control, and tumor metabolism. Understanding and targeting these circadian pathways offers additional therapeutic opportunities for improving breast cancer treatment and outcomes.

4.1. The Role of Circadian Rhythm Genes BMAL1 and CLOCK in the Breast Cancer Progression

The circadian rhythms of the body are primarily regulated by the circadian clock genes BMAL1 and CLOCK. The BMAL1: CLOCK heterodimer binds to E-box DNA sequences in these genes, forming a positive limb of the core circadian clock network that stimulates the transcription of additional clock genes such as CRY and PER. The search results for "breast cancer" show that dysregulation of BMAL1 and CLOCK, along with other circadian clock disruptions, can have a substantial effect on the biology, invasiveness, and prognosis of breast cancer. This is because BMAL1 expression maintains circadian amplitude, facilitates the hypoxia response, controls fatty acid oxidation, and declines with cellular senescence, all of which can affect the progression of breast cancer. CLOCK is linked to an upper incidence and risk of breast cancer. It regulates circadian acetylation, promotes the proto-oncogene c-MYC, and suppresses the cell cycle regulator WEE1, impacting G2/M cell cycle transitions. When compared to normal breast tissue, breast cancers exhibit subtype-specific alterations in the circadian structure and expression of BMAL1, CLOCK, and other core clock genes.

In summary, the circadian clock genes BMAL1 and CLOCK appear to be intimately linked to breast cancer biology. Dysregulation of these core clock components can promote aggressive tumor phenotypes, metastasis, and poor prognosis, particularly in luminal A breast cancers. Targeting circadian pathways may represent a promising avenue for improving breast cancer outcomes. After that, the circadian clock proteins BMAL1 and CLOCK can directly influence breast cancer progression by modulating cell cycle control, EMT, and metastasis-related pathways like MMP9 expression. Breast cancers frequently exhibit dysregulation of the molecular clock, with subtype-specific alterations affecting prognoses. One intriguing option for breast cancer prevention and treatment might be to target circadian clock processes.

4.2. Circadian Regulation of DNA Repair Mechanisms in Breast Cancer

Breast cancer diagnosis, prognosis, and therapy are all heavily influenced by DNA. Patients who are at a great risk of getting breast cancer can be identified as the use of genetic testing for mutations in important genes such as BRCA1, BRCA2, PALB2, CHEK2, and others. Improved screening and preventative interventions for these high-risk patients are made possible by this knowledge.

In breast cancer patients, analysis of the tumor's DNA provides important insights. Breast cancer is a heterogeneous disease, and the genetic makeup of the tumor can influence its behaviour and response to treatment. Circulating tumor DNA (ctDNA) found in the patient's bloodstream can be analysed to detect specific mutations, monitor disease progression, and identify resistance mechanisms. This liquid biopsy approach is less invasive than traditional tissue biopsies and can provide real-time information about the tumor's evolution.

Histone alterations and other epigenetic alterations, such as methylation of DNA, are important factors in the initiation and spread of breast tumor. Furthermore, microRNAs (miRNAs) that regulate gene expression are also being explored as biomarkers and therapeutic targets in breast cancer.

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Modulating the expression of specific miRNAs, such as miR-34 and anti-miR-10b, has shown promise in inhibiting tumor growth and metastasis. Notably, it has been demonstrated that clock genes like CLOCK affect DNA repair mechanisms. These genes play a role in regulating the effectiveness and timing of several DNA repair pass way, such as homologous recombination (HR), which are necessary to repair DNA damage brought on by environmental exposure and regular biological functions.

Disruptions in circadian rhythms can lead to impaired DNA repair, allowing mutations to accumulate over time, which can promote cancer initiation and progression. Studies suggest that the circadian clock regulates the expression of key DNA repair genes, and when the clock is misaligned, such as through shift work or disrupted sleep patterns, the body's ability to repair DNA becomes compromised. In breast cancer, this can contribute to the accumulation of genetic mutations, driving tumor growth and increasing resistance to treatments like chemotherapy, which rely on functional DNA repair mechanisms.

4.3. Tumor microenvironment immune regulation

The intricate and ever-changing terrain of the tumor microenvironment in breast cancer is a critical factor in the advancement of the illness and its reaction to treatment. Treating breast cancer by focusing on the TME, the immune regulatory pathways, has shown promise. The circadian rhythm controls metabolism, cellular communication, and a few immunological functions that are essential to the tumor microenvironment. A disturbed immune response, altered metabolism, and elevated inflammation can result from circadian rhythm disruption, all of which can encourage the development of tumors and treatment resistance [14].

Tumor-associated macrophages (TAMs) and cancer-associated fibroblasts are two examples of circadian-controlled immune cells that are crucial components of the tumor microenvironment (TME). Studies have shown that the infiltration and activity of these cells follow circadian patterns, and that disruptions in circadian rhythms can amplify their pro-tumorigenic effects. For example, TAMs' capacity to produce a potent anti-tumor immune response may be compromised by disturbances in their circadian control, which might enable cancer cells to elude immune monitoring.

Additionally, the circadian clock influences metabolic pathways in the TME, such as glycolysis and oxidative phosphorylation, which cancer cells heavily rely on for growth and survival. Disruption of circadian control over these metabolic processes can enhance the Warburg effect, where cancer cells switch to anaerobic glycolysis even in the presence of oxygen, promoting rapid tumor progression.

By targeting circadian rhythm pathways in the TME, it may be possible to modulate immune cell activity, reprogram the metabolic environment, and ultimately improve the effectiveness of breast cancer therapies.

5. Conclusion

Breast cancer growth is significantly influenced by a number of biological processes that are fundamentally regulated by circadian rhythms, such as immune response, DNA repair, and tumor microenvironment dynamics. Aside from impairing the body's innate capacity for DNA repair, disruptions in important circadian genes such as BMAL1 and CLOCK also encourage the growth and invasion of tumors by changing the immunological and metabolic milieu around the tumor. By focusing on circadian rhythm pathways, therapeutic approaches that increase DNA repair processes, modify the tumor immune response, and improve breast cancer treatment appear promising. Better patient outcomes and more effective medicines may result from incorporating circadian-based techniques into current treatment regimens as research into the complex relationships between circadian cycles and breast cancer progresses.

References

[1] Das NK and Samanta, S. 2022 The potential anti-cancer effects of melatonin on breast cancer. *Explor Med.* 112 127

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[2] Hill, S. M., Belancio, V. P., Dauchy, R. T., Xiang, S., Brimer, S., Mao, L., Hauch, A., Lundberg, P. W., Summers, W., Yuan, L., Frasch, T. and Blask, D. E. 2015 Melatonin: an inhibitor of breast cancer Endocrine-*related cancer* 22(3) R183–R204

[3] Li, Y., Li, S., Zhou, Y., Meng, X., Zhang, J. J., Xu, D. P. and Li, H. B. 2017 Melatonin for the prevention and treatment of cancer Oncotarget 8(24) 39896–39921

[4] Fu, L. and Kettner, N. M. 2013 The circadian clock in cancer development and therapy Progress *in molecular biology and translational science* 119 221–282

[5] Lee Y. 2021 Roles of circadian clocks in cancer pathogenesis and treatment Experimental *& molecular medicine* 53(10) 1529–1538

[6] Das NK and Samanta, S. 2022 The potential anti-cancer effects of melatonin on breast cancer. *Explor Med.* 112 127

[7] Grant, S. G., Melan, M. A., Latimer, J. J. and Witt-Enderby, P. A. 2009 Melatonin and breast cancer: cellular mechanisms, clinical studies and future perspectives *Expert reviews in molecular medicine* 11 e5

[8] Hill, S. M., Belancio, V. P., Dauchy, R. T., Xiang, S., Brimer, S., Mao, L., Hauch, A., Lundberg, P. W., Summers, W., Yuan, L., Frasch, T. and Blask, D. E. 2015 Melatonin: an inhibitor of breast cancer Endocrine-*related cancer* 22(3) R183–R204

[9] Murray, M. H., Valfort, A. C., Koelblen, T., Ronin, C., Ciesielski, F., Chatterjee, A., Veerakanellore, G. B., Elgendy, B., Walker, J. K., Hegazy, L. and Burris, T. P. 2022 Structural basis of synthetic agonist activation of the nuclear receptor REV-ERB Nature *communications* 13(1) 7131

[10] Wolff, S. E. C., Wang, X. L., Jiao, H., Sun, J., Kalsbeek, A., Yi, C. X. and Gao, Y. 2020 The Effect of Rev-Erbα Agonist SR9011 on the Immune Response and Cell Metabolism of Microglia *Frontiers in immunology* 11 550145

[11] Logan, R. W., & McClung, C. A. 2019 Rhythms of life: circadian disruption and brain disorders across the lifespan *Nature reviews Neuroscience* 20(1) 49–65

[12] Wang, Y., Kojetin, D. and Burris, T. P. 2015 Anti-proliferative actions of a synthetic REV-ERBα/β agonist in breast cancer cells Biochemical *pharmacology* 96(4) 315–322

[13] Munteanu, C., Turti, S., Achim, L., Muresan, R., Souca, M., Prifti, E., Mârza, S. M. and Papuc, I. 2024 The Relationship between Circadian Rhythm and Cancer Disease International *journal of molecular sciences* 25(11) 5846

[14] Affinito, A., Quintavalle, C., Chianese, R. V., Roscigno, G., Fiore, D., D'Argenio, V., Thomas, G., Savarese, A., Ingenito, F., Cocca, L., Nuzzo, S., Berezovski, M. V., Stoppelli, M. P. and Condorelli, G. 2024 MCT4-driven CAF-mediated metabolic reprogramming in breast cancer microenvironment is a vulnerability targetable by miR-425-5p Cell *death discovery* 10(1) 140