Proceedings of ICBioMed 2024 Workshop: Computational Proteomics in Drug Discovery and Development from Medicinal Plants
DOI: 10.54254/2753-8818/74/2024.LA19347

Impact of Circadian Rhythm Disruption on Breast Cancer: Mechanisms and Therapeutic Approaches

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Abstract. Circadian rhythm disruption plays a critical role in breast cancer pathogenesis, influencing key biological processes such as cell cycle regulation, DNA repair, and apoptosis. This review investigates the molecular mechanisms linking circadian misalignment, often caused by factors such as shift work and irregular light exposure, with increased cancer risk. Dysregulation of circadian genes, including PER and BMAL1, compromises cellular homeostasis, contributing to tumorigenesis. Through a comprehensive secondary analysis of existing literature and experimental data, this review explores therapeutic strategies that harness circadian biology. Hormonal treatments, such as melatonin and aromatase inhibitors, alongside light therapy aimed at realigning circadian rhythms, represent promising interventions. Chronotherapy, which involves synchronizing treatment with the body's intrinsic biological clock, has demonstrated potential to enhance therapeutic efficacy and reduce adverse effects. This review underscores the significance of circadian regulation in breast cancer and highlights the potential of circadian-based therapeutic strategies to optimize treatment outcomes and improve patient care.

Keywords: Circadian rhythm, breast cancer, circadian genes, treatment.

1. Introduction

Circadian rhythms, which operate on approximately 24-hour cycles, regulate numerous physiological and behavioral processes in organisms, including hormone secretion, metabolism, and the sleep-wake cycle. In mammals, these rhythms are governed by the central clock located in the suprachiasmatic nuclei (SCN) of the brain and are regulated by core clock genes such as CLOCK, Period (PER), Cryptochrome (CRY), and Brain and Muscle ARNT-Like 1 (BMAL1) [1]. These genes control a transcriptional-translational feedback loop that influences the expression of downstream genes involved in metabolism, immune function, and cell cycle regulation. When these circadian rhythms are disrupted, such as by shift work or irregular light exposure, the desynchronization of internal processes can lead to various health problems, including metabolic disorders, immune dysfunction, and reduced cognitive performance. The disruption of these molecular clocks has been implicated in cancer development, particularly by affecting cell cycle checkpoints, DNA repair, and apoptosis, leading to genomic instability and uncontrolled cell growth [2].

In the context of breast cancer, circadian dysregulation plays a significant role in tumorigenesis. Disruptions in clock gene expression, such as the dysregulation of tumor suppressor genes like PER and CRY, impair the normal control of cell division and apoptosis, increasing cancer risk. Moreover,

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DOI: 10.54254/2753-8818/74/2024.LA19347

circadian misalignment can weaken the body's anti-tumor immune responses, allowing cancer cells to evade immune surveillance. Metabolic pathways regulated by circadian rhythms, such as glucose metabolism and lipid storage, are also disrupted, creating a tumor-promoting environment characterized by chronic inflammation, insulin resistance, and altered cellular signaling. Modern lifestyle factors, particularly those associated with shift work and irregular light exposure, have been linked to increased rates of breast cancer, possibly through the suppression of melatonin, a hormone critical for modulating cell proliferation and tumor suppression [3].

Breast cancer is a major global health issue, with a significantly higher incidence in industrialized regions, suggesting that aspects of modern Western lifestyles, including circadian rhythm disruption, may contribute to its onset and progression. This review aims to clarify the connection between circadian rhythm disruption and breast cancer development by examining the biological mechanisms involved. It will also explore how modern lifestyle factors exacerbate cancer risk and discuss potential therapeutic strategies, such as light therapy and chronotherapy, that aim to mitigate the impact of circadian misalignment on cancer development. By understanding these connections, we can better inform therapeutic interventions and lifestyle changes that may reduce the risk of breast cancer.

2. Circadian Rhythm and Breast Cancer

2.1. Light effect on the SCN

The circadian rhythm system relies on the suprachiasmatic nucleus (SCN) as the master clock, which synchronizes biorhythms throughout the body through light signals in the environment. Light, the strongest zeitgeber, can directly affect the activeness of SCN and thus gene expression in multiple tissues, including the breast [4].

The strongest synchronizing force for the SCN is light. Melanopsin-containing retinal ganglion cells, which are inherently photosensitive, send light information straight to the SCN via the retino hypothalamic tract. Additional indirect input to the SCN comes from rods and cones as well as intrinsically photosensitive retinal ganglion cells that carry melanopsin through the intergeniculate nucleus. In fact, melanopsin-deficient mice still exhibit photo entrainment, but triple knockouts—mice lacking rod, cone, and melanopsin function—do not. Light causes quick changes in cellular activity inside the SCN, which have been well described by looking at the expression of immediate early genes.

In contrast to peripheral tissues, which alter more slowly and in diverse ways, the SCN may quickly adapt to changes in light. The mammalian circadian system relies on light as its most powerful signal, but other elements like food availability and movement can also feedback and affect how the circadian clock works. SCN rhythms may be unaltered by these stimuli, which particularly change the expression of clock genes in peripheral tissues.

2.2. Circadian Regulation in Breast Tissue

The circadian clock plays a significant role in regulating the rhythmic expression of genes across various organs, including the breast. In breast tissue, nearly 600 genes are under circadian control, either directly or indirectly influenced by the CLOCK/BMAL1 complex and other key transcription factors. These clock-regulated genes are involved in critical biological processes such as DNA repair, apoptosis, and cell division.

Key proteins, such as Sirtuin 1 (SIRT1) and TIMELESS (Timeless Circadian Regulator), act as crucial links between the circadian clock and the cell cycle machinery. SIRT1, for instance, deacetylates the tumor suppressor protein p53, thereby regulating cell division by inhibiting its progression. TIMELESS, on the other hand, adjusts circadian rhythms in response to DNA damage [5], further demonstrating the intricate relationship between circadian rhythms and essential cellular functions.

2.3. Circadian Influence on Breast Stem Cells and Development

The stromal extracellular matrix, which surrounds the network of epithelial ducts that make up breast tissue, is rich in fibroblasts and adipocytes. Studies using real-time bioluminescent imaging of mammary

DOI: 10.54254/2753-8818/74/2024.LA19347

explants have shown that clock proteins, such as BMAL1 and PER2, exhibit rhythmic expression patterns in breast tissue. These rhythms fluctuate daily and shift during key developmental stages, such as pregnancy and lactation, with Bmal1 and PER1 mRNA levels increasing, while PER2 levels decrease in late pregnancy. Moreover, circadian rhythms are essential for controlling the behavior of mammary progenitor stem cells. Studies on BMAL1-/- mice have revealed that the absence of BMAL1 causes a disruption in stem cell regulation, which impacts the stem cells' ability to self-renew [6]. This indicates that the maintenance of the regular regeneration processes in mammary stem cells depends on a functional CLOCK/BMAL1 complex, highlighting the significance of circadian regulation in breast tissue growth and function.

2.4. Implications for Breast Cancer

Cancer is among the many illnesses that can result from circadian clock disruptions. Tissue homeostasis in breast tissue is largely dependent on circadian modulation of gene expression and cellular functions like DNA repair and cell cycle regulation. Circadian dysregulation may impede these mechanisms, hence fostering genomic instability and elevating the likelihood of tumorigenesis. The relationship between circadian genes and breast cancer emphasizes how crucial it is to preserve the integrity of the circadian rhythm in order to lower the risk of cancer[7].

3. Circadian genes and their effects of breast cancer

Tumor formation is significantly influenced by circadian disruption. Research has demonstrated that alterations in clock gene expression can affect apoptotic pathways, DNA repair systems, and cell cycle checkpoints. These alterations result in unchecked cell proliferation and genomic instability, two important components of carcinogenesis. For instance, there is evidence linking an elevated risk of cancer to the deregulation of the PER and CRY genes, which typically function as tumor suppressors by regulating cell division and death. Changes in circadian rhythms can also affect the effectiveness of immunological responses against tumors, which can help cancer cells avoid being detected by the body's immune system.

3.1. PER Gene

The central nervous system (CNS), which includes the SCN, and the peripheral nervous systems are where the Period2 (PER) gene is mostly expressed. It is a member of the PER family, which also consists of PER1, PER2, and PER3. Mutant mice, such as single knockout mice, have lately been employed by researchers in an effort to understand the role of the Period genes. PER1 and PER2 are essential for mice's circadian rhythms, however PER3 has less of an effect than those two genes, according to their findings. It's intriguing that PER2 plays a bigger role in the circadian clock than PER1 does. Compared to wild type (WT) animals, mice with PER2 mutations had longer circadian periods and lower levels of PER1 expression in the SCN, indicating that PER2 regulates PER1. As a result, PER2 is one of the primary genes of the circadian clock and helps the SCN and peripheral organs produce circadian rhythms [8-9].

In PER gene mutant mice, circadian gating of cells passing through the cell cycle has been linked. It is true that a mutant PER gene plays a function in cancer. Compared to normal breast cells, human sporadic and familial breast cancer cells express less PER genes. Methylation in particular regions of the Per promoter may be the cause of this. Mice with mutations causing PER2 loss-of-function exhibit increased tumor incidence and increased susceptibility to radiation-induced malignant lymphoma [10]. Furthermore, PER1 and PER2 promote apoptosis. In fact, they may prevent breast cancer in vivo by inducing apoptosis [11]. However, if both PER1 and PER2 are expressed at lower levels in breast cancers, then PER's ability to control growth is compromised. Furthermore, in order to reduce c-Myc transcription, they can indirectly disrupt BMAL1/Npas2's E-box-mediated transactivation. Loss of PER2 results in an accumulation of damaged cells and decreases apoptosis because it impacts p53. The discovery that PER2 overexpression results in cell cycle arrest, growth inhibition, and apoptosis in colon

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cancer lends credence to this. Furthermore, PER disruption encourages the downregulation of genes that control growth, exposing a molecular connection between cell division and the circadian rhythm.

Consequently, because of their function in tumor suppression, the PER genes, specifically PER1 and PER2, have been linked to the emergence of breast cancer. The methylation of these genes' promoter regions may be the cause of the notable decrease in PER genes expression in both sporadic and familial cases of breast cancer as compared to normal breast tissue. The tumor-suppressive effects of PER1 and PER2, which typically encourage apoptosis and prevent cell growth, are lessened by this downregulation. The downregulated expression of these genes in breast cancers diminishes their capacity to inhibit the advancement of cancer, hence augmenting the process of carcinogenesis.

3.2. BMAL1 complex

The CLOCK/BMAL1 complex can directly regulate clock target genes, or transcription factors' circadian expression can do so indirectly. Thus, a variety of tissues are given a rhythmic quality by the circadian clock. For instance, the expression of around 600 genes in the breast is regulated by the circadian cycle. BMAL1, a crucial circadian protein, is involved in preserving the regular and structured activities that make up an organism's life. Apart from regulating the operation of biological rhythms, BMAL1 is also involved in immunological disorders, cancer, aging, and cardiovascular disease [12]. According to recent study, BMAL1 regulates the cell cycle and proliferation, which raises the possibility that BMAL1 plays a significant role in carcinogenesis. Apoptosis can be induced by BMAL1, and G2/M phase arrest in a way that is dependent on p53, which prevents colorectal cancer cells from proliferating. [13] Increased apoptosis and a disruption of the cell cycle result from BMAL1 silencing. It's possible that BMAL1 prevents cancer in cases of malignant pleural mesothelioma. [14] These results imply that BMAL1 regulates cancer via a complicated mechanism. It might have opposing effects on certain malignancies and use distinct mechanisms to control the growth of tumor cells. The occurrence and advancement of breast cancer can be influenced by disruptions in the biological clock.

By employing techniques including real-time PCR, RNA extraction, Western blot, and CoIP tests. After analyzing the expression of Matrix Metalloproteinase 9 (MMP9), they concluded that BMAL1 facilitated the invasion and metastasis of breast cancer cells. The likely underlying mechanism involves recruiting CBP to raise the acetylation level of p65 (RelA protein), which further activates the NF-kB signaling pathway. These findings imply that BMAL1 encourages the spread of breast cancer and may represent a promising new prognostic indicator and therapeutic target for the disease.

One major contributing factor to breast cancer's poor prognosis is metastasis. BMAL1, a crucial core clock protein, has a direct connection to the development of tumors. Nevertheless, little is known about the molecular pathways that underlie BMAL1's function in invasion and metastasis [15]. One study from Cancer Cell International, demonstrated that overexpressing BMAL1 greatly increased cell invasion and migration.

Functionally, MMP9 mRNA and protein levels were greatly elevated and MMP9 activity was enhanced by BMAL1 overexpression. Additionally, via raising IkB phosphorylation and connecting with NF-kB p65 to enhance human MMP9 promoter activity, BMAL1 stimulated the NF-kB signaling pathway and elevated MMP9 expression. Overexpression of BMAL1 results in CBP (CREB binding protein) was enlisted to support the invasion and metastasis of breast cancer cells by increasing the activity of p65 and further triggering the NF-kB signaling pathway to control the expression of its downstream target genes, such as MMP9, TNFα, uPA, and IL8.

4. Potential Treatment

In the treatment of breast cancer, employing multiple treatments is crucial as it addresses the complexity of the disease effectively, targeting the primary tumor, potential spread, and reducing the risk of recurrence. In addition to surgery, chemotherapy and radiation, circadian rhythm regulation therapy (chronotherapy) is gaining increasing attention. offers an advantage by aligning treatment timing with the body's natural biological clock. This synchronization can potentially enhance treatment efficacy and optimize outcomes by leveraging the body's inherent physiological processes. By treating at the optimal

DOI: 10.54254/2753-8818/74/2024.LA19347

time point of the circadian rhythm, efficacy can be maximized and side effects reduced. For example, adjusting the timing of chemotherapy drug administration to synchronize with the patient's biological clock can optimize drug absorption and metabolism, thereby reducing toxic reactions.

4.1. Light therapy

Light has long been used therapeutically to treat a range of illnesses, including seasonal depression and other mental health conditions, since it is the biggest and most obvious zeitgeber of the SCN [16]. Liu et al. [17] employed actigraphy to assess the circadian activity patterns of breast cancer patients receiving treatment. Patients may have been more tired when they were exposed to less light, possibly because they spent less time outside in the sun. This result served as a catalyst for several intervention studies examining the effectiveness of exposure to light as a cure for CTRS. In order to increase the sensitivity of the circadian system, cancer patients are typically advised by protocols to use the use of light boxes or glasses that emit circadian stimulation lighting every morning for thirty to forty-five minutes after waking up for 4 weeks or during treatment. According to findings, light therapy can help cancer patients who are receiving treatment avoid feeling depressed and tired, and it can help cancer survivors who have completed main treatment feel less tired and sleep better [18-19]. Sadly, the majority of these studies lack the power to establish whether circadian rhythms mediate the effects of light therapy on CTRS. However, one study did find that bright light therapy shielded breast cancer patients from the deterioration of their circadian activity rhythms that comes with chemotherapy.

4.2. Hormone treatment

4.2.1. Aromatase inhibitor

Aromatase inhibitors are an effective therapeutic option for estrogen-dependent breast cancers. These inhibitors target the enzyme aromatase, which is responsible for converting androgens into estrogens, thereby reducing estrogen biosynthesis [20]. Third-generation aromatase inhibitors are commonly used and are classified into two types: type 1 (steroidal and irreversible, such as exemestane) and type 2 (nonsteroidal and reversible, such as anastrozole and letrozole). By inhibiting estrogen production, these drugs decrease estrogen levels, reducing the activation of estrogen receptors, which are involved in the growth of breast cancer cells. This receptor-targeted therapy helps to slow or stop tumor progression in estrogen-dependent cancers.

4.2.2. Melatonin

Because of its many physiological functions, melatonin, the pineal hormone secreted at night, has garnered a lot of interest. While its primary function is to regulate the circadian rhythm, this indoleamine is involved in various other processes such as neurogenesis, antioxidation, and inflammatory reactions. In addition to its adaptability, this molecule has earned a reputation for having tumor-suppressive properties, particularly in malignancies that are hormone-dependent. Melatonin could specifically counteract the effects of estrogen on the breast[21-22]. Melatonin's varied effects, such as its induction of apoptosis, antioxidative qualities, and anticancer immunity, underpin its oncostatic activities. It's noteworthy to point out that melatonin can protect normal cells toward ionizing radiation's (IR) cytotoxicity. Melatonin-mediated regulation of prostaglandins, Toll-like receptors (TLRs), and transcription factors reduces this non-targeted effect after IR[23-24]. Moreover, it has been proposed that melatonin may function as a radiosensitizer by enhancing the effectiveness of IR therapy [25]. Melatonin causes breast cancer cells to become more radiosensitive through a variety of mechanisms, including decreased cell division, enhanced p53 mRNA levels, enhanced cell cycle arrest, and downregulated DNA repair.

4.2.3. Estrogens

Estrogen therapy is commonly used in postmenopausal women to alleviate symptoms such as hot flashes, night sweats, and osteoporosis by restoring hormonal balance. However, in the case of estrogen-

DOI: 10.54254/2753-8818/74/2024.LA19347

dependent cancers like breast cancer, treatment strategies focus on inhibiting or reducing estrogen levels. This is often achieved through the use of aromatase inhibitors, which work by decreasing estrogen production and thereby reducing the risk of cancer progression. While estrogen therapy can significantly improve the quality of life for many women, it is important to carefully consider the potential side effects and health risks when developing a treatment plan, especially in individuals at risk for hormone-dependent cancers [26].

5. Conclusion

The complex association found between circadian rhythms and breast cancer emphasizes how crucial it is to keep our biological clocks in sync with our everyday routines. Circadian gene disruptions can impact immunological responses, DNA repair, and cell cycle regulation, which can ultimately result in cancer. Light therapy and chronotherapy offer promising strategies to enhance cancer treatment by aligning therapies with the body's natural rhythms. With more research, preventative and therapy techniques for cancer may become more effective as the function of circadian biology in the disease is understood. Wishing for the continued development of novel cancer treatment approaches in the future.

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