Review Paper:

Role of Circadian Disruptions and Epigenetic Modifications in Cancer and Neuro Developmental Disorders

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Abstract

Circadian rhythms are physiological processes which regulate the sleep-wake cycle, metabolism and hormonal functions driven by clock genes. Suprachiasmatic nucleus (SCN), located in the anterior hypothalamus, regulates the mammalian circadian clock. Disruptions in circadian rhythms are associated with various metabolic disorders, neurodevelopmental conditions and cancers. Melatonin is crucial for synchronizing circadian rhythms, it maintains physiological homeostasis and particularly stabilizing the circadian clock under conditions of inflammation and stress. Circadian rhythms misalignments such as sleep disturbances and melatonin deficiencies lead to the pathogenesis of Autism Spectrum Disorder (ASD).

Time restricting feeding (TRF) is an effective strategy for improving metabolic health and in reducing obesity related risks. Epigenetic regulation, including DNA methylation and histone modifications influence the circadian machinery, with dysregulation of these processes contributing to cancer and neurodevelopmental disorders. Therapeutic strategies targeting circadian rhythm alignment, such as melatonin supplementation and epigenetic modifications, offer promising avenues for reducing circadian related pathologies including metabolic syndrome, ASD and cancer.

Keywords: Circadian rhythms, Neurodevelopmental disorders, Time restricted feeding, Clock genes, DNA methylation, Cancer.

Introduction

Mammalian sleep-wake cycles also known as circadian rhythms are clock controlled. The internal biological clock can anticipate 24-hours day-night cycle. The hypothalamic suprachiasmatic nucleus (SCN) functions as a master circadian oscillator, which controls behavior[71]. Melatonin plays a critical role in synchronizing circadian rhythms. It is primarily produced by the pineal gland at night and is influenced by the length of the dark period. The hormone is involved in regulating body weight and energy balance, potentially impacting the development of obesity[6]. Melatonin helps in stabilizing circadian rhythm disruptions caused by inflammation by upregulating clock genes like BMAL1, PER1 and CLOCK[26,65] and also helps to maintain the excitation balance in the brain which is critical for normal function and potentially disrupted in ASD, suggesting that melatonin deficiency could contribute to ASD pathogenesis[96]. It can mitigate circadian disruptions and can promote healthy aging.

The research suggests melatonin supplementation as a potential therapeutic strategy for age-related circadian rhythm disturbances[88]. At the molecular level, the discovery of circadian rhythm genes began with the identification of the period (per) gene in drosophila. Subsequent research in mammals, particularly mice, revealed the clock gene (or Npas2 in neuronal tissue) through chemical mutagenesis. Mutations in the clock gene were associated with a prolonged rest-activity cycle. Further investigations unveiled additional clock genes involved in circadian regulation in mice, including brain and muscle ARNT-like protein 1 (BMALL), Per1, Per2 and Cryptochrime-1 (Cry1) and Cry2 genes[16,28,53,70,78].

The SCN express genes related to stem cell are typical for mature brain regions. These genes may play a role in neural plasticity. 25 genes highly expressed in the SCN, suggesting their potential involvement in maintaining circadian timing and adapting to environment changes[7].

Molecular and Cellular network of Suprachiasmatic Nucleus: The Suprachiasmatic nucleus is crucial for maintaining daily physiological rhythms. It prepares the body for activity by regulating heart rate, glucose levels and cortisol levels while melatonin decreases. The SCN targets mainly midline regions of the hypothalamus and specific neurons involved in autonomic and endocrine systems[12]. The SCN can be divided into two distinct sub regions: a ventrolateral core region and dorsomedial shell region, the core of the SCN transfers regulatory information to the shell through vasoactive intestinal peptide (VIP), while the shell projects sparsely back to the core. Peripheral clock gene expression is rhythmically regulated by glucocorticoids from the HPA axis[58].

The SCN anticipates events by adjusting its phase relative to the core and external cues. This is facilitated by the core – shell organization where the shell can lead the core in

activity peaks, enabling preparation for changes in downstream organs[34]. Three dimensional rendering of the six neurons revealed extensive soma, soma plate like contact sites within the SCN core, glial membrane intercalations were present in most soma, soma contact sites, although devoid of cytoplasm, suggest structural support[22]. Neuronal density was slightly higher in the shell compared to the core, non – myelinated axons, particularly mRGCs, formed a dense dendro – dendritic network and exhibited distinct morphological characteristics in boutons[13].

The researchers find SCN – enriched genes such as Avp, Nms, Prok2 and Vipr2 are considered important for signaling and rhythmicity in SCN[10]. Calbindin (CalB) cells interact with other peptidergic cell types such as vasoactive intestinal peptide (VIP), arginine vasopressin (AVP), cholecystokinin (CCK) and gastrin – releasing peptide (GRP). VIP fibers extensively innervate CalB cells, while AVP and CCK show limited interaction. GRP and CalB cells located in the same region demonstrate dense connectivity with some colocalization. Neuropeptide Y (NPY) and sparse serotonin fibers are also in contact with CalB cells[57]. VIP, AVP, GRP and CalB show distinct overlapping patterns within the SCN. VIP and AVP form extensive projections throughout the hypothalamus. Neuropeptide Y (NPY) and serotonin (5-HT) fibers surround and penetrate the SCN, with NPY concentrated in the central caudal region and 5-HT fibers dispersed ventrally and throughout the hypothalamus[21].

Feeding Cycles as modulators of Circadian rhythms and metabolic health: Disruption in Circadian rhythms is linked to metabolic disorders such as obesity and diabetes[29]. BMAL1 is traditionally seen as a crucial component of the mammalian circadian clock; its deletion disrupts circadian behavior, sleep – wake cycles and other physiological processes. The observation of 24-hour molecular oscillations in skin fibroblasts and liver tissues of Bmal1 knockout mice by deletion of Bmal1 suggests that Bmal1 is not indispensable for circadian rhythms[73]. The liver and skeletal muscle maintain their own circadian rhythms, with Bmal1 being the key gene driving these processes.

Muscle specific reconstructions of Bmal1 partially restore glucose metabolism, but full systemic glucose tolerance requires the interplay between clocks in both liver and muscle, emphasizing the importance of cross – tissue circadian synchronization. Feeding – fasting rhythms enhance circadian gene expression in both liver and muscle, leading to improve glucose metabolism[39,81]. Bmal1's pivotal function is maintaining the circadian clock by binding to core oscillator genes and regulatory elements like E- boxes and tandem E1-E2 elements. Bmal1 in metabolic regulations has implications for diseases like diabetes, obesity and cancer, where circadian dysfunction is often observed[74].

Clock gene expression exhibited robust rhythmicity in the SCN and HPA axis, with tissue – specific patterns. In the SCN, per1, per2 and bmal displays strong rhythmic expression, with per1 and per2 peaking during light period and bmal peaking during the dark period. HPA axis components showed rhythmic expression of clock genes, even though with tissue – specific phase relationships and responses to restricted feeding[33]. Circadian regulation of triglyceride (TAG) metabolism in the mouse liver has shown that TAG levels and the expression of key metabolic enzymes follow daily oscillations. Feeding – fasting cycles play a crucial role in modulating TAG oscillations[2].

Time – restricted feeding (TRF) prevented body weight gain, reduced fat accumulation and improved metabolic health compared to ad-libitum feeding, it also induced diurnal rhythms in fuel utilization, enhancing energy expenditure during feeding without changing total caloric intake[14,15]. TRF altered gene network linked to BMI, insulin and fatty acid levels, highlighting its potential to improve metabolic health and reduce obesity – related complications.

These findings suggest TRF as an effective dietary strategy for enhancing circadian regulation and overall metabolic functions in individuals with obesity[98]. TRF enriched metabolic pathways related to amino acid metabolism in skeletal muscle and fatty acid metabolism in serum, demonstrating its ability to modulate peripheral metabolic regulators independently of the central clock[64]. Upon fasting for more than 24 hours, mammals switch from glucose to ketone bodies as a primary energy source. Intermittent and periodic fasting can enhance disease prevention and treatment[62].

A 10- hour time –restricted eating regimen in patients with metabolic syndrome led to weight loss, improved body composition and reductions in lipid levels and blood pressure, though it did not significantly affect thyroid function or blood cell counts and it enhances the purine cycle in diet – induced obesity models and AMPK signaling pathways in genetic induced obesity models. AMPK acts as a central censor of energy status, maintaining energy balance by regulating metabolic and catabolic pathways. AMPK activation improves glucose uptake and insulin sensitivity and enhances mitochondrial biogenesis[61,69,86].

Neurodevelopmental impact of Circadian Dysregulation in Autism Spectrum Disorder: Autism spectrum disorder (ASD) is prevalent neurodevelopmental condition diagnosed in early childhood, characterized by communication deficits, behavioral challenges and comorbidities like intellectual disability and epilepsy. ASD is linked to genetic, environmental and neural factors including abnormalities in the frontal cortex, hippocampus and cerebellum.

Complicating treatment is due to its heterogeneous nature[9,25]. Disruption in circadian rhythms may play a significant role in the etiology and pathogenesis of Autism spectrum disorder (ASD). Fundamental processes related to synaptic functions such as ribosome maturation and mRNA

regulation, were identified as regulated by specific genes associated with ASD.

Changes in these processes could potentially serve as causative factor for ASD occurs within the synaptic region. Mutations in specific circadian genes can impact circadian regulation and potentially contributes to ASD pathogenesis[27,97]. The researchers identify significant links between circadian rhythm disruption and ASD, emphasizing that sleep issues, hormonal imbalances and genetic mutations in circadian genes are prominent in ASD patients. It highlights sleep disturbances, abnormal melatonin levels and circadian gene mutations as key factors influencing ASD symptoms[60].

Ubiquitin – Conjugating Enzyme (UBE2O) promotes degradation of BMAL1, a core circadian transcription factor, affecting circadian rhythm regulation. Knockdown of UBE2O increases Bmal1 stability, enhancing circadian clock amplitude[18]. Dim light at night (DLaN) disrupted these rhythms by increasing the total amount of sleep and eliminating the usual day/night differences in sleep duration. DLaN exposure led to altered molecular circadian rhythms in various tissues including SCN, hippocampus and liver[89]. Children and adolescents with ASD show delayed melatonin onset and earlier age, related melatonin decline, contributing to pronounced sleep disruptions and less stable circadian rhythms[67]. Lower morning light exposure and differences in motor activity further exacerbate these circadian misalignments in ASD.

Maternal immune activation (MIA) can disrupt fetal brain development, leading to long-term neuropsychiatric disorders like ASD in offspring. Neurodegenerative diseases like Alzheimer's (AD) and Parkinson's disease (PD) are increasingly recognized as having significant neuroinflammatory components. Neuroinflammation contributes to the progression of these diseases by altering the gene expression of neurons and glial cells, leading to neurodegeneration[55,72]. SCN neurons interconnected via dendro- dendritic chemical synapses (DDCSs), ipRGCs displays different branching patterns and synaptic features across the brain regions. ipRGCs preferentially form synapses with SCN neurons that are part of this DDCS network, potentially enhancing the synchronization of circadian rhythms[51].

ASD-related genes expressed in the human amygdala are also expressed in mouse amygdala, particularly in regions like the basolateral complex and the medial amygdala nucleus which have known roles in social behavior and emotional processing.

The genes were also enriched in other cell types such as fibrous astrocytes, oligodendrocyte precursor cells and inter neurons[41,90]. miRNAs, which regulate gene expression, have emerged as key players in linking circadian rhythm and ASD. Many miRNAs, such as miR-219, miR-132 and miR-146 are involved in regulating genes linked to both circadian rhythm and ASD, dysregulation of these miRNAs can influence ASD pathology through the control of circadian clock genes[50]. Valproic acid (VPA) in utero showed significant circadian disruptions, including extended active phases and delayed activity offset. Abnormal expression of the core clock gene Bmal1 was observed in the SCN of VPA- exposed animals[30]. The 16p11.2 deletion and cntnap2 null mouse models both displayed hyperactivity and some motor deficits, but only cntnap2 null mice showed altered responses to social stimuli, enhanced cognitive flexibility and improved sensorimotor gating. Neither model exhibited significant social deficits nor increased behavioral variability compared to controls, highlighting distinct but overlapping behavioral phenotypes[11]. The CNTNAP2 gene, encoding the Contactin –associated protein-like 2 (Caspr2), has been linked to conditions such as autism, intellectual disability and epilepsy.

Mutations in this gene exhibit Pitt-Hopkins –like syndrome (PTHLS), characterized by severe developmental and neurological impairments. Successful treatment with stiripentol illustrates a significant advancement in the application of genetic analysis and precision medicine for treating complex epileptic syndrome[31,76]. The central nervous system (CNS) triggers specific epigenetic changes that influence both neurons and glial cells. Epigenetic mechanisms including DNA methylation, histone modifications and non-coding- RNA involvement regulate gene expression in response to injury. These mechanisms either promote or inhibit axon regeneration depending on the specific context and cell type[79]. The research identifies that the aggravation of brain injury due to circadian disruption is associated with increased oxidative stress, inflammation and apoptosis in the brain.

Specifically, the study points out the upregulation of pro-apoptotic proteins and down regulation of anti- apoptotic ones indicating a shift towards cell death pathways that contributes to worsened outcomes[56]. Axon regeneration is critical process following nerve injury, particularly in the peripheral nervous system. Bmal1 was found to inhibit axon regeneration by interacting with the epigenetic factor Tet3, which is crucial for DNA demethylation and regenerative gene expression. Bmal1 acts as a gatekeeper of neuroepigenetics responses to axon injury by limiting Tet3 expression and restricting 5-hydroxymethylcytosine (5hmC) modifications. Tet3 is a crucial epigenetic regulator of axon regeneration[24].

The primary treatments for ASD with psychopharmacological options are like typical antipsychotics, stimulants and melatonin that address sleep issues and N-acetyl cysteine. Emerging targeted treatments for ASD include Oxytocin, Bumetanide, Metformin, Lovastatin, Cannabidiol, Arbaclofen, Trofinetide, Phosphordiasterase 4D inhibitors, Anavex 2-73 and gene therapy[3].

Epigenetic Link between Circadian Clock Disruptions and Cancer: Epigenetics as heritable changes in phenotype do not involve alterations in the DNA sequence. Traditionally, this concept has been associated with chromatin modifications such as DNA methylation and histone modifications[32,40]. By the early 1980s, it was discovered that DNA methylation within the 5′ promoter regions inhibits transcription, highlighting its role in gene silencing. Later research in the 1990s and 2000s linked DNA methylation to histone modifications and 3D genome organization, furthering our understanding of its regulatory function[45,68]. DNA methylation is the process of adding a methyl group to specific DNA bases, mainly cytosines (Cs), in eukaryotes, in mammals and insects. Most DNA methylation occurs at CG sites[4,5].

Changes in DNA methylation including both hypo and hypermethylations, play crucial role in carcinogenesis by altering cellular processes like the cell cycle, DNA repair and cell proliferation. These methylation changes can silence tumor suppressor genes and activate oncogenes, making them key epigenetic drivers of cancer. Current methods for analyzing DNA methylation patterns such as whole- genome bisulfite sequencing (WGBS) and other targeted approaches, are essential for understanding these modifications[94, 54]. Epigenetic clocks, based on changes in DNA methylation at specific CpG sites, can accurately predict chronological age. These clocks have been linked to biological aging, showing correlations with diseases like Alzheimer's, cancer and cardiovascular conditions [87].

Disruption of circadian rhythms has been linked to an increased risk of cancer, whether due to genetic mutations or lifestyle factors such as irregular sleep patterns. The circadian clock regulates the expression of genes involved in cell cycle control, apoptosis and DNA repair, suggesting that clock dysfunction can contribute to tumorigenesis[17,19,44,66,80] and is strongly linked to various cancers including breast, colorectal, prostate and lung cancer, as well as leukemia. Factors like light exposure at night and shift work can increase cancer risk by altering clock gene expression and melatonin suppression. The circadian clock regulates the expression nearly 50% protein-coding genes in mammals. In breast cancer, hypermethylation of these genes plays a role while in colorectal cancer, Bmal1 expression influences chemotherapy sensitivity.

Circadian gene disruption is also observed in leukemia and short sleep is associated with a higher risk of fatal prostate and lung cancers[77,92]. A significant fraction of genes in any given tissue shows circadian oscillations at the mRNA level. However, this regulation extends beyond steady-state mRNA rhythms to multiple stages of gene expression including transcription, splicing, termination, polyadenylation, nuclear export, microRNA regulation, translation and RNA degradation[1,83]. Histone methylation, regulated by histone methyltransferases (HMTs) and demethylases (HDMs), plays a crucial role in circadian rhythms by modulating chromatin states and gene expression. Various histone marks such as H3K4me3, H3K9me2/3 and H3K27me3, are implicated in circadian regulation, interacting with core clock components like Bmal1-CLOCK. The dynamic and complex interplay between histone methylation and demethylation suggests a sophisticated regulatory mechanism that is essential for maintaining circadian rhythms[35,37,48].

A genome wide mRNA screen identified the miR-183/96/182 cluster as a key regulator of circadian rhythms, miR-96 within the cluster directly targets and represses the core circadian gene PER2, affecting its expression at both mRNA and protein levels[99]. DNA methyltransferases (DNMT1 and DNMT3A) revealed that these enzymes play non-redundant roles in circadian gene regulation, further supporting the importance of epigenetic regulation in circadian rhythm control[36,59]. lncRNAs (Long non-coding RNAs) are emerging as key regulators of circadian rhythms. They modulate gene expression, interact with chromatin-modifying proteins and affect the stability of the mRNAs.

Specific lncRNAs exhibit rhythmic expression in tissues like pineal gland, linking them directly to circadian control[8]. Analysis of chicken hypothalamus samples across a 24-hour cycle identified two gene sets related to circadian rhythms and retinal metabolism, highlighting several lncRNAs, circRNAs and 200 genes within a regulatory network.

Key molecules including three lncRNAs (MSTRG.16890.1, ENSGALT00000098661 and ENSGALT00000100816) and one circRNA (novel_circ_010168) were found to regulate the gene AOX1 (Aldehyde oxidase 1), linking it to circadian regulation and retinal metabolism[82,84]. The repeated intake of cocaine and sucrose influences DNA methylation, circadian rhythms and gene expression in rat brain structure.

The changes in DNA methylation are linked to chromatin remodeling, indicating a complex interaction between epigenetic regulation and behavioral outcomes related to addiction[63,75]. Misalignment of the circadian clock might be a risk factor for breast cancer development, disrupted circadian rhythms or changes in gene expression related to the circadian clock in breast cancer cells[52].

Bmal1 plays a crucial role in the development and functionality of reproductive organs by regulating reactive oxygen species (ROS) and influencing gonadotropin secretion, making it a potential therapeutic target for reproductive endocrine disorders. Bmal1 knockout (KO) mice show disrupted gonadotropin secretion, with female mice lacking the luteinizing hormone (LH) surge and males exhibiting altered Follicle stimulating hormone (FSH) levels, while Bmal1 also regulates Gonadotropin releasing hormone (GnRh) receptor expression, enhancing pituitary sensitivity to kisspeptin and GnRH. Additionally, Bmal1 influences the rhythmic secretion of GnRH, impacting

downstream hormones like LH and FSH, which are essential for reproductive function.

Mutations in Bmal1 lead to impaired fertility in both male and females due to altered hormone levels and disrupted reproductive cycles[46,47]. Bmal1 influences histone acetylation, which in turn affects the expression of genes involved in wound healing and tissue repair[23]. Bmal1 also acts as tumor suppressor gene that is epigenetically silenced in ovarian cancer through promoter hypermethylation and repressive histone modifications. The restoration of Bmal1 expression leads to tumor-suppressive effects including reduced cell proliferation, enhanced chemosensitivity and restoration of normal circadian rhythms in cancer cells[43,85,95]. Melatonin was shown to improve nuclear and cytoplasmic maturation in mouse oocytes during *in vitro* maturation (IVM).

Melatonin, known for its antioxidant properties, protects oocytes from oxidative damage by reducing ROS. This protection enhances oocyte developmental competence during IVM, indicating melatonin's potential in improving fertility preservation techniques[49]. EpiTOC (Epigenetic Timer of Cancer) is a novel tool that uses DNA methylation patterns to create a mitotic clock for predicting cancer risk by linking increased methylation at specific loci to heightened stem cell mitotic activity. EpiTOC provides insights into cancer susceptibility, integrating biological knowledge and bioinformatics for tissue specific analysis. It identifies age- related hypermethylation at polycomb target loci as potential biomarkers for assessing cancer risk[20,38,42,93].

Conclusion

In this review we focus on the important role of circadian rhythms in regulating a wide range of physiological processes including metabolism, neurodevelopmental conditions and cancer progression and specifically focused on genes involved in circadian rhythms such as BMAL1, CLOCK, PER and other important genes. Circadian rhythms disruptions are associated with metabolic disorders such as obesity and diabetes and also autism spectrum disorder (ASD) and various cancers. The epigenetic regulation of circadian rhythms reveals how DNA methylation and histone modifications play a crucial role in regulating neurological disorders and cancers.

More research is needed to evaluate potential therapeutic targeting circadian rhythms, such as melatonin supplementation, time restricting feeding (TRF) and epigenetic modifications. These approaches show the way in improving metabolic health, stabilizing circadian rhythms disruptions and providing potential strategies to reduce the pathogenesis of ASD and cancers. In conclusion, synchronizing circadian rhythms through therapeutic approaches will find the way to cure a variety of health conditions, from metabolic disorders to neurodevelopmental and oncological diseases.

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