



Mini Review

Circadian Rhythms in Tumor Regulation: Impacts on Tumor Progression and the Immune Microenvironment



Jinxin Li^{1#}, Peng Luo^{2#}, Ying Liu^{1#}, Yu Fang¹, Linhui Wang^{1*} and Aimin Jiang^{1*}

¹Department of Urology, Changhai Hospital, Naval Medical University (Second Military Medical University), Shanghai, China; ²The Department of Oncology, Zhujiang Hospital, Southern Medical University, Guangzhou, Guangdong, China

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Abstract

The spatial heterogeneity of tumors has long been a subject of significant interest in oncology. Recent research has revealed that tumors and their microenvironments undergo dynamic changes over time, particularly in the form of periodic circadian rhythms. Disruptions to these rhythms have been recognized as a pivotal factor in the advancement of tumorigenesis. Such disruptions not only induce dysregulation of gene expression within tumor cells, influencing tumor growth, metabolism, the cell cycle, and vascular homeostasis but also facilitate metastasis. Furthermore, they mediate the remodeling of the tumor immune microenvironment, fostering the development of an immunosuppressive milieu. Additionally, the *in vivo* metabolism and therapeutic responsiveness of tumor treatments—including chemotherapy, targeted therapy, and immunotherapy—have been shown to be modulated by circadian rhythms. This suggests that time-specific drug administration may enhance treatment efficacy, offering novel insights for precision cancer therapy. In this review, we systematically update contemporary research on the impact of circadian rhythms on tumor biology, encompassing both tumor progression and the efficacy of drug therapies. Building upon these insights, we explore the potential for a synergistic approach that integrates the targeting of rhythmic genes with current tumor treatment modalities. We also discuss the feasibility of tailoring tumor therapy to the rhythmic alterations that define *in vivo* metabolism and the efficacy of specific therapeutic agents, highlighting the significance of rhythm-based strategies in the personalized treatment of tumors and the prevention of associated diseases.

Introduction

The alternation of day and night in nature has driven living organisms to evolve strict time-maintenance mechanisms, known as circadian rhythms, to better adapt to external changes.¹ In humans, circadian rhythms are complex processes mediated by a regulatory center located in the suprachiasmatic nuclei of the hypothalamus and are regulated by a variety of rhythm-associated genes, which play a role in regulating a wide range of life activities, such as sleep/wake cycles, feeding/fasting, endocrine function, immunity,

and metabolism.²

With increasing understanding and research, it has become clear in recent years that circadian dysregulation is closely linked to the development of tumors. Although a variety of factors and pathways are involved, this effect is generally manifested in two main aspects: through the regulation of the tumor itself and through the modulation of the tumor immune microenvironment.

The effect of circadian rhythms on the biological function of tumors

The regulation of tumor biological characteristics by circadian rhythm disruption is an important mechanism promoting tumorigenesis and progression, including tumor initiation, stemness, metabolic reprogramming, and immune microenvironment remodeling (Fig. 1). Enhanced fatty acid oxidation-mediated oncogenic metabolic signaling promoting tumorigenesis has been observed in sleep deprivation-induced circadian rhythm disorders. This effect is mainly mediated by the dysregulated CLOCK gene, which over-activates long-chain fatty acyl coenzyme A synthetase 1, leading to increased production of palmitoyl coenzyme A. This, in turn, promotes CLOCK-Cys194 S-palmitoylation. This approach elimi-

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*Correspondence to: Linhui Wang and Aimin Jiang, Department of Urology, Changhai Hospital, Naval Medical University (Second Military Medical University), Shanghai 200433, China. ORCID: <https://orcid.org/0000-0002-4056-0462> (LW); <https://orcid.org/0000-0002-9563-983X> (AJ). Tel: +86-13101635510 (LW); +86-18301935531 (AJ), E-mail: wanglinhui@smmu.edu.cn (LW); czjiangaimin@smmu.edu.cn (AJ)

#These authors contributed equally to this work.

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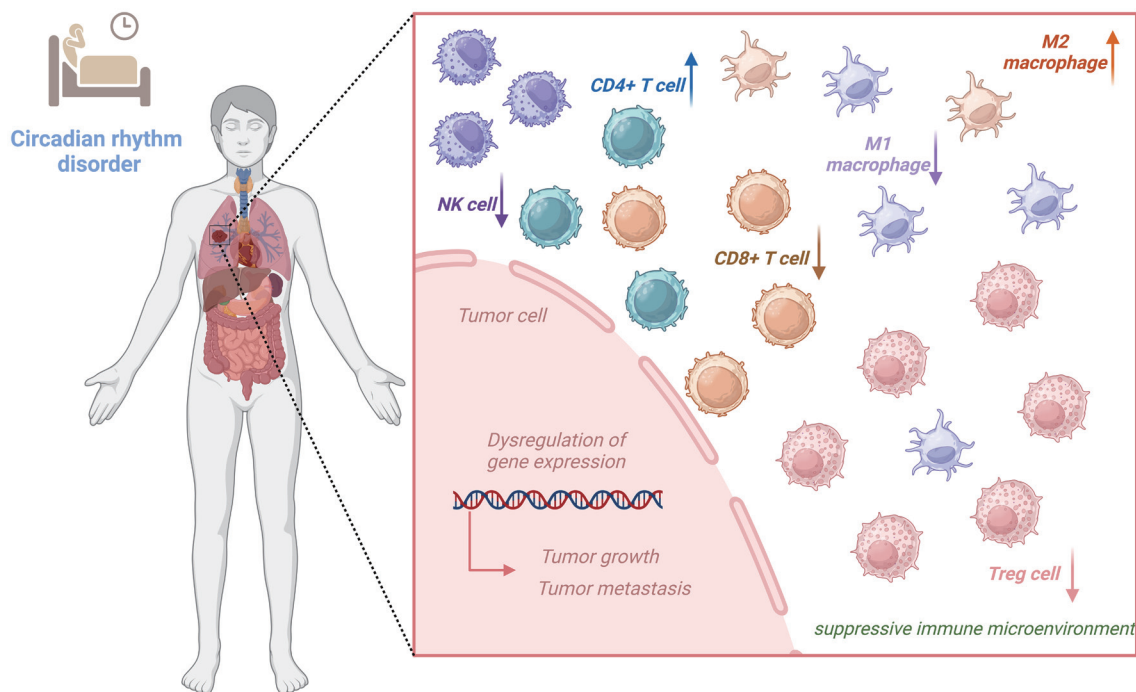


Fig. 1. Impact of circadian rhythm disorder on tumor progression and immune microenvironment. On the one hand, circadian rhythm disorder contributes to tumor growth and metastasis by influencing the dysregulation of gene expression affecting the tumor itself. On the other hand, circadian rhythm disorder can also promote the formation of a suppressive immune microenvironment, reduce the number of natural killer (NK) cells and the M1/M2 ratio, and increase the CD4/CD8 ratio and the number of regulatory T (Treg) cells, thus indirectly promoting tumor progression. This figure was created using tools provided by Biorender (www.biorender.com). CD, cluster of differentiation; M1, pro-inflammatory; M2, anti-inflammatory.

ates the CLOCK gene's ubiquitination degradation, disrupting circadian rhythms and promoting cancer stemness.³ Abnormally expressed CLOCK genes can also alter the secretion patterns of tumor cell chemokines and cytokines, promoting tumor inflammation and angiogenesis. Dysregulation of rhythm-associated genes is both a cause and an effect, serving as a link between circadian rhythm disruption and tumor development. Rhythmic genes, such as BMAL1 and CLOCK, play crucial roles in regulating signaling pathways and molecular expression related to various cellular processes, including the cell cycle, epithelial-mesenchymal transition, apoptosis, ferroptosis, cellular metabolism, and immunity. These genes exert their influence either individually or through the formation of complexes, impacting cellular vascularity, growth, metastasis, immune response, and other functions (Fig. 2). Essentially, the coordinated expression of CLOCK genes regulates the timing and order of various cellular processes, while circadian rhythm disruption induces dysregulation of CLOCK gene expression, leading to a loss of normal cellular function regulation.

The impact of rhythm-related genes on tumors is broad and direct, including tumor metastasis. Often, more attention is given to spatial variations in tumor metastasis to specify the affected tissues and organs for more targeted therapeutic decisions. However, tumor metastasis also exhibits temporal heterogeneity, as demonstrated by the significant influence of the sleep/wake cycle on tumor metastasis. Given that hematogenous dissemination due to circulating tumor cells (CTCs) is the primary mode of metastasis for most tumors, a study tracked the dynamics of CTCs and revealed the significant impact of circadian rhythms on tumor metastasis.⁴ Specifically, the production of CTCs in patients was found to be greatly disturbed by circadian rhythms, with the majority of

CTCs (78.3%) found in samples obtained during resting periods. This finding was further validated in animal experiments, where mice in the resting phase showed a six- to eight-fold increase in CTCs compared to the active phase, with a maximum increase of 278-fold. By interfering with the sleep cycle and administering rhythm-related hormones (e.g., melatonin), it was found that CTCs produced during the rest phase were not only more numerous but also exhibited a stronger metastatic capacity compared to those produced during the active phase. These results suggest that the production of CTCs is not constant but exhibits significant time-dependence and temporal heterogeneity, regulated by circadian rhythms. This also implies that dynamic regulation of drug administration schedules could be feasible during tumor therapy, with intensive resting-phase-focused therapy potentially achieving promising therapeutic outcomes.

In addition to affecting tumor function, circadian rhythms also play a crucial regulatory role in the tumor-immune microenvironment. On one hand, rhythm genes directly regulate the expression of immune checkpoints in tumor cells, indirectly affecting immune cell activation. For example, Period2 competitively binds to heat shock protein 90 via the PAS1 structural domain, reducing its interaction with inhibitors of kappa B kinase (IKKs). This leads to increased ubiquitination degradation of IKK- α/β and facilitates nuclear translocation of p65, inhibiting the IKK/NF- κ B pathway and reducing PD-L1 expression.⁵ On the other hand, the composition and percentage of immune cells fluctuate dynamically, and these fluctuations are regulated by circadian rhythms. Taking macrophages as an example, M1 and M2 macrophages are the main types in the tumor immune microenvironment. These macrophages not only play opposing roles but also exhibit opposite states

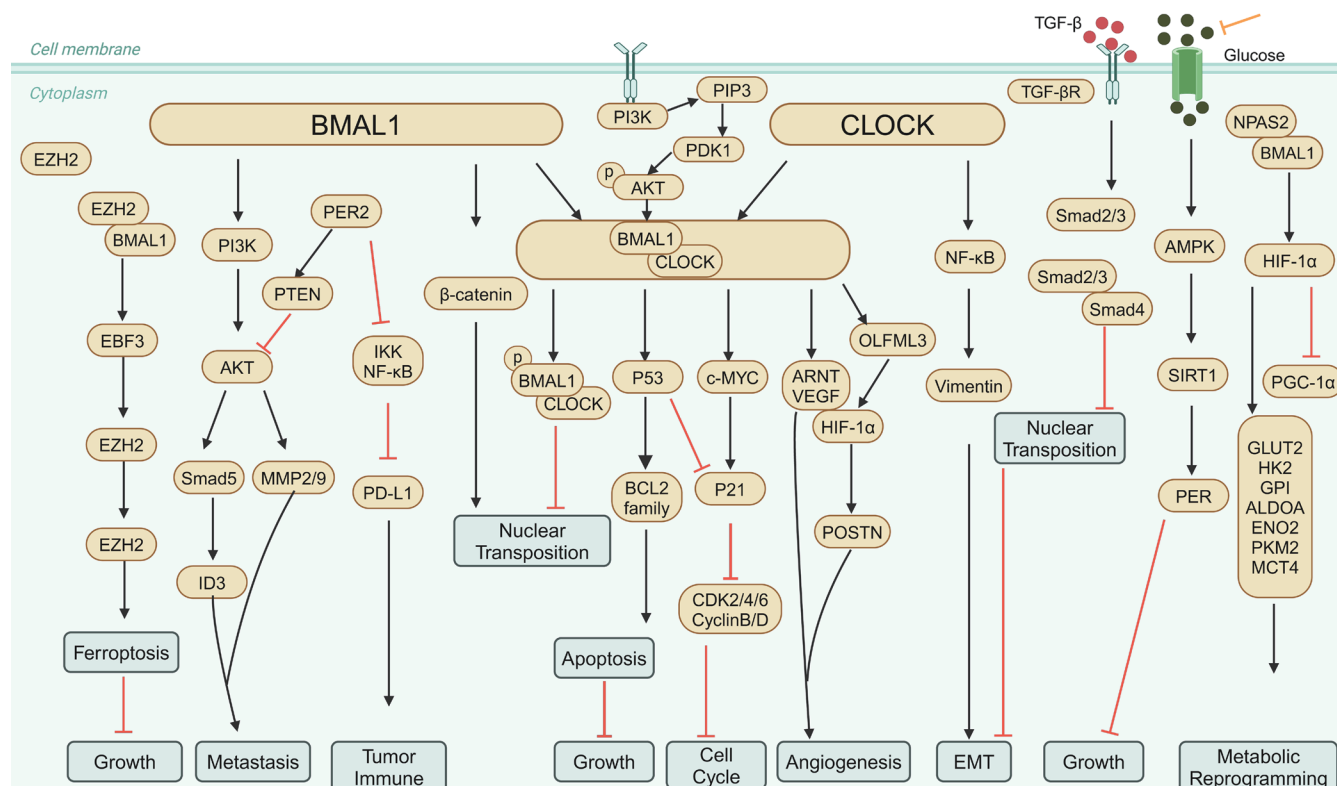


Fig. 2. Pathways and molecular regulatory networks of circadian rhythm-related genes in tumor cells. Rhythmic genes such as BMAL1, CLOCK, and others, either individually or through the formation of complexes, regulate signaling pathways and molecular expression associated with various cellular processes such as the cell cycle, epithelial-mesenchymal transition (EMT), apoptosis, ferroptosis, cellular metabolism, and immunity. These genes influence cellular angiogenesis, growth, metastasis, immune responses, and other functions. This figure was created using tools provided by Biorender (www.biorender.com).

of change during circadian fluctuations. In the normal light-dark cycle, M1 macrophages predominate at night, while M2 macrophages are more prevalent during the day, which is considered the normal macrophage daily pattern. However, circadian rhythm disruption disrupts the daily pattern of both, significantly reducing the M1/M2 ratio and resulting in the development of a suppressive immune microenvironment.⁶ In addition to macrophages, a decrease in the number of CD8⁺ T cells, an increase in the CD4/CD8 ratio, and an increase in FoxP3⁺ Treg cells, along with greater infiltration of immunosuppressive microglial cells in neurological tumors, also contribute to remodeling the tumor immune microenvironment. These changes promote the formation of metastatic niches and tumor immune escape.⁷ These studies have deepened our understanding of the relationship between circadian rhythms and tumor immunity. More importantly, by identifying the major differential genes and their mechanisms of action, we can pinpoint target genes that can inhibit the adverse effects of rhythm disruption on tumor immunity, aiding in the development of new tumor immunomodulatory drugs targeting rhythm-related genes.

The effect of circadian rhythms on the efficacy of antitumor drug therapy

Given the important role that rhythm genes play in tumor progression, it is reasonable to believe that targeting these genes will provide new options for tumor therapy. Indeed, studies have already revealed the unique role of rhythm gene modulators in controlling

circadian rhythm disorders and enhancing antitumor therapy. For example, CLK8 increases circadian rhythm amplitude by inhibiting CLOCK dimerization with BMAL1.⁸ KL001 and its derivative SHP656 can inhibit the growth of glioblastoma stem cells through the simultaneous activation of CRY1 and CRY2.⁹ Retinoic acid-related orphan receptor α agonists inhibit the growth of gastric cancer cells both *in vitro* and *in vivo*, making them promising anti-tumor agents.¹⁰ However, current research on these rhythm gene-modulating drugs is still mainly in the basic research phase, and clinical studies are needed to further confirm their effectiveness and potential for clinical application.

Moreover, the activity and antitumor effects of many antitumor agents, including chemotherapeutic agents, targeted therapies, and immunotherapies, are strongly influenced by circadian rhythms, particularly in terms of fluctuating pharmacokinetics, efficacy over time, and the mediation of drug resistance events. For example, one study evaluated the effect of circadian rhythms on the pharmacokinetics of linifanib, a novel tyrosine kinase inhibitor selective for vascular endothelial growth factor and platelet-derived growth factor receptors. The results showed that evening dosing significantly affected the oral bioavailability of linifanib, with a dose-normalized C_{max} that was 64% of that observed after morning dosing.¹¹ At specific times of the day, antitumor drugs can exert a tumor-killing effect, but their efficacy is greatly disturbed when the circadian clock is defective, suggesting that drug efficacy fluctuates with the rhythm.¹² Mechanistically, this rhythm-dependent feature is regulated by the cell cycle and is dependent on the cyclic expression of target proteins. Furthermore, given the serious chal-

lence of drug tolerance in tumor therapy, new studies have found that the development of tolerance to some drugs is also influenced by circadian rhythms.¹³ Periodic circadian expression patterns of key genes are important contributors to this phenomenon.

Chronomodulated chemotherapy regimens targeting cytotoxic drugs have also demonstrated promising results.¹⁴ A systematic review that included 18 randomized trials and 2,547 cancer patients found that the majority of studies (14/18) supported the ability of chronomodulated chemotherapy to improve outcomes while reducing drug toxicity, with potential gender differences.¹⁵ Although a small number of studies suggested that chronomodulated chemotherapy could lead to a shift in toxicity response and one study reported a worse toxicity response, no study reported a clear reduction in efficacy. This provides new insights into how tumor drug resistance arises and demonstrates that temporal therapies targeting rhythmic regulation have great potential to overcome antitumor drug resistance. Therefore, emphasizing the temporal dimension in the development of antitumor therapeutic strategies may enhance the precision and targeting of drugs while reducing the development of drug resistance, or even reversing drug resistance that has already occurred.

The strong circadian rhythmicity of the immune system also has a significant impact on the effectiveness of immunotherapy.¹⁶ Studies have shown that circadian rhythm changes drive cyclic oscillations of T cells. At the highest abundance of suppressive immune cells, CD8⁺ T cell function is severely suppressed. However, since the expression of immune checkpoints, such as PD-L1, peaks in immunosuppressive cells, administering anti-PD-L1 therapy at this time can be more effective.¹⁷ In addition, the rhythmic changes in CD8⁺ T cells were also reflected by dendritic cells, showing amplified efficacy by synchronizing tumor immunotherapy with dendritic cell function.¹⁸ Tumor immunotherapy targeting immune cells is benefiting cancer patients, but low sensitivity and high drug resistance still limit its clinical application. Immunotherapy that incorporates the circadian characteristics of immune cells may be a viable solution, whether for immune checkpoint inhibitors, tumor vaccines, or chimeric antigen receptor T-cell therapies.

In light of these findings, an increasing number of studies have focused on the impact of modulating circadian rhythms on tumor therapy. Evidence suggests that circadian rhythms significantly affect the sensitivity of tumor radiotherapy. Meanwhile, adjusting the timing of drug administration has been shown to affect its bioavailability. Additionally, adjusting circadian rhythms through phototherapy and other means can improve symptoms such as tumor-related fatigue. Several ongoing studies are also evaluating the effects of circadian rhythm disruption on tumor progression as well as tumor-associated adverse effects, which will provide valuable insights for improving tumor therapy (Table S1).

Future directions

A thorough understanding of the dynamic alterations in tumor circadian rhythms is essential for refining precision cancer therapy, shifting the focus from specific targets to a more nuanced appreciation of the therapeutic window. As research evolves, an increasing number of rhythm-associated tumor therapeutic targets are being elucidated, promising to diversify therapeutic strategies. Consequently, interventions aimed at circadian rhythm genes may emerge as a novel modality in cancer treatment, potentially synergizing with established approaches such as chemotherapy, targeted therapies, and immunotherapies. However, current research in this domain remains limited and predominantly confined to the basic

science stage; further studies are imperative to substantiate the viability of this concept.

Moreover, elucidating the cyclical expression patterns of target genes and the dynamic spatial and temporal interplay between tumor cells and immune cells is crucial for the development of more targeted cancer therapies. Tailoring drug dosing according to the metabolic and efficacy profiles of specific drugs can optimize therapeutic outcomes while minimizing adverse effects.

Given that circadian rhythm regulation is predominantly governed by central neural and hormonal mechanisms, therapies targeting these rhythms may be subject to variability among individual patients.^{19,20} Occupational and lifestyle factors that can induce circadian rhythm disorders or even inversions necessitate a more personalized approach to such treatments, requiring selection based on each patient's unique rhythmic characteristics.²¹

Considering the influence of circadian fluctuations on tumor growth, metastasis, drug resistance, and immune microenvironment formation, there is a pressing need to focus on the deleterious effects of circadian disruption on the organism, potentially exacerbating oncogenesis. This awareness is vital for prompting individuals to recognize the risks associated with unhealthy habits and to encourage behavioral changes. Future research should also consider disease prevention strategies for occupations that may contribute to circadian rhythm disruptions.

Conclusions

Disruptions in circadian rhythms have been implicated in the initiation and progression of tumorigenesis through a variety of molecular pathways. Targeting circadian rhythm-associated genes represents a potentially efficacious therapeutic strategy for cancer treatment, particularly when employed in conjunction with established tumor therapies. However, further research is warranted to substantiate this hypothesis. A deeper understanding of the temporal metabolic profiles and efficacy fluctuations of tumor therapeutics is essential for the enhancement of precision cancer therapy. This knowledge will facilitate the optimization of treatment timing and dosing, ultimately aiming to maximize therapeutic benefits while minimizing side effects.

While this review provides a comprehensive look at the effects of circadian rhythms on tumors, it still has some limitations. The findings presented are predominantly derived from the current scientific literature and thus are subject to the constraints of the quality, scope, and depth of the published research. Furthermore, the practical application of our proposed rhythm-based strategies in personalized tumor therapy and disease prevention necessitates additional empirical validation through experimental and clinical trials to ascertain their efficacy and feasibility. Nevertheless, we have delineated critical issues and challenges that warrant attention, which will serve as a roadmap for future investigative endeavors.

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Conflict of interest

The authors have declared that no competing interests exist.

Author contributions

Analyzed the literature and wrote the manuscript (JL, PL, YL), drafted the figure (JL), conceived the idea (LW, AJ), and reviewed and revised the manuscript (YF, LW, AJ). All authors gave the final approval of the submitted version.

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