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REVIEW

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It's About Time: Advances in Understanding the Circadian Regulation of DNA Damage and Repair in Carcinogenesis and Cancer Treatment Outcomes

Prasanna V. Ashok Kumar[a,1], Panshak P. Dakup[a,1], Soumyadeep Sarkar[a], Jinita B. Modasia[a], Madison S. Motzner[a], and Shobhan Gaddameedhi[a,b,*]

[a]*Department of Pharmaceutical Sciences, College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane, WA;* [b]*Sleep and Performance Research Center, Washington State University, Spokane, WA*

The circadian rhythm is established by a coordinated network of peripheral clocks interlocked and regulated by a central pacemaker. This network is maintained by the rhythmic expression of core clock genes, which in turn generate oscillatory expression patterns of different sets of target proteins in a tissue-specific manner. Precise regulation of biological processes driven by the body's circadian network in response to periodic changes in the environment determines healthy life. The delicate balance in the cycling of enzymes, metabolites, cofactors, and immune regulators is essential to achieve cellular homeostasis. Disruption of this circadian homeostasis has been linked with the development and progression of various diseases including cancer. Over the years, circadian regulation of drug metabolism and processing has been employed in the treatment of diabetes, hypertension, peptic ulcers, and allergic rhinitis. Although time dictated drug administration was demonstrated many decades ago, its application in cancer treatment is limited due to insufficient mechanistic data supporting experimental results and inconsistency between clinical trials. However, timed administration of anti-cancer drugs is rapidly gaining attention as studies with animal and human models unveil molecular intricacies involved in the circadian control of biological pathways. In this regard, striking a balance between maximizing tumor responsiveness and minimizing side effects is crucial to achieve positive patient outcomes. This review focuses on regulation of the circadian clock in carcinogenesis outcomes through DNA damage and repair mechanisms and its application in therapy with specific emphasis on skin and breast cancers.

*To whom all correspondence should be addressed: Shobhan Gaddameedhi, Department of Pharmaceutical Sciences, College of Pharmacy and Pharmaceutical Sciences, Washington State University, PO Box 1495, Spokane, WA 99210-1495; Tel: 509-368-6570; Fax: 509-368-6561; Email: shobhan.gaddameedhi@wsu.edu.

†Abbreviations: CCGs, clock-controlled genes; RT, radiation therapy; SCN, suprachiasmatic nucleus; TTFL, transcription-translation feedback loop; UVR, ultraviolet radiation; NER, nucleotide excision repair; DDR, DNA damage response.

Keywords: circadian clock, chronotherapy, chemotherapy, radiation therapy, immunotherapy, nucleotide excision repair, breast cancer

[1]These authors contributed equally to this work.

INTRODUCTION

Our daily intrinsic cues for wakefulness, hunger, and sleepiness repeat in about 24-hour cycles driven by our body's internal rhythms. This circadian rhythmicity evolved as a survival strategy in living organisms and is generated through a genetically-encoded central master clock and unique peripheral clocks in different tissues of the body. Most cellular processes are coupled to this circadian network, which modulates their functions appropriately in response to light-dark and temperature cycles caused by the earth's rotation in order to sustain life in a best-fit manner [1-5].

However, circadian clocks are subject to disruption, either environmentally from modern living conditions and behaviors such as chronic jetlag, rotating night shift work, and excessive exposure to artificial light at night, or genetically through mutations or altered function in the clock genetic circuit [3,6]. Given that the circadian clock regulates various biological processes, it is not surprising that disruption of the clock may be a plausible cause for the development of various diseases including sleep and metabolic disorders, cardiovascular and Alzheimer's diseases, and several cancer types [6-9]. Cancer is one of the most studied diseases with respect to circadian disruption. Growing scientific evidence confirms the altered expression patterns of core clock proteins and clock-controlled genes (CCGs†) involved in cell cycle, DNA replication and repair, post-translational modifications, and metabolic pathways in different cancer types, while understanding the implications of clock mutations in healthy cells [10-12]. Yet, establishing complete circadian rhythm profiles and their function within tumor cells remains a prodigious task due to the intrinsic heterogenicity and mutagenic nature of malignant cells in a tumor microenvironment.

Cancer treatment often involves a combination of regimens which includes surgery, chemotherapy, radiation therapy (RT), and immunotherapy. The effectiveness of a treatment regimen depends on drug efficacy towards a specific target and desirable pharmacokinetic profile [13]. Since most biomolecular drugs target receptors, enzymes, or proteins, involved in cell signaling pathways, which have timed expressions governed by the circadian clock, it is crucial that medications and treatment therapies are delivered accordingly. Notably, more than 170 drug targets, including targets of 56 of the top 100 best-selling drugs in the United States as of 2014, are controlled by the circadian clock [14]. More so, a recent study showed that up to a thousand rhythmically expressed genes in at least one of 13 human tissues are drug targets, or encode for proteins involved in drug transportation or metabolism [15]. Chronotherapy is a treatment strategy that utilizes the body's circadian cycles to achieve optimal efficacy and minimize side effects for a particular therapy [16]. This strategy has been in use for many decades for the treatment of various sleep-related disorders and has been successful in the treatment of arthritis, asthma, and hypertension [17-19]. However, chronotherapy treatment regimens for cancer are currently scarce mainly due to inadequate understanding of the molecular basis of circadian regulation in different cancers multifaceted nature of tumor development and progression. This review encompasses recent advancements in understanding the role of the circadian clock in DNA damage and repair, carcinogenesis and the up-and-coming treatment strategies, which utilize the body's clock to achieve better prognosis in cancer patients.

THE CIRCADIAN CLOCK AND ITS FUNCTION IN CELLULAR HOMEOSTASIS

Circadian Clock

In mammals, the suprachiasmatic nucleus (SCN), which houses the master clock, in the anterior hypothalamus perceives signals through visual photoreception and synchronizes peripheral clocks present in other organs of the body including heart, liver, lungs, breast, and skin. This master clock is periodically calibrated through light perception in the morning. One of the vital features of circadian clocks is that they are endogenously driven without any environmental cues due to the self-sustained transcription-translation feedback loop (TTFL) [3,20,21]. As shown in Figure 1, the TTFL is subdivided into the primary feedback loop which is comprised of proteins, namely: brain and muscle ARNT-like protein-1 (BMAL1), circadian locomotor output cycles kaput (CLOCK), cryptochrome (CRY1, CRY2), and period (PER1, PER2, PER3), and the secondary feedback loop made up of retinoic acid orphan receptor alpha (RORA) and reverse-erb receptor alpha (REV-ERBA) proteins [1]. CLOCK and BMAL1 dimerize to form a functional transcriptional complex, which binds to specific E/E'-box sequences (CACGTG/CACGTT) in the promoter regions of various target CCGs. The primary transcriptional targets of CLOCK/BMAL1 are *PER* and *CRY* genes, which upon transcription and translation, form a heterodimer and shuttle back into the nucleus to bind to BMAL1/CLOCK heterodimeric complex, in order to inhibit its activity [1,22-24]. Simultaneously, the secondary feedback loop components regulate the levels of BMAL1, with RORA increasing and REV-ERBA decreasing transcription of *ARNTL* gene (which encodes BMAL1 protein), thereby maintaining a circadian rhythm of about 24 hours [25]. This 24-hour circadian rhythm influences about 43 percent and 50 percent of protein-coding genes at transcript levels in mice and humans respectively. This influential

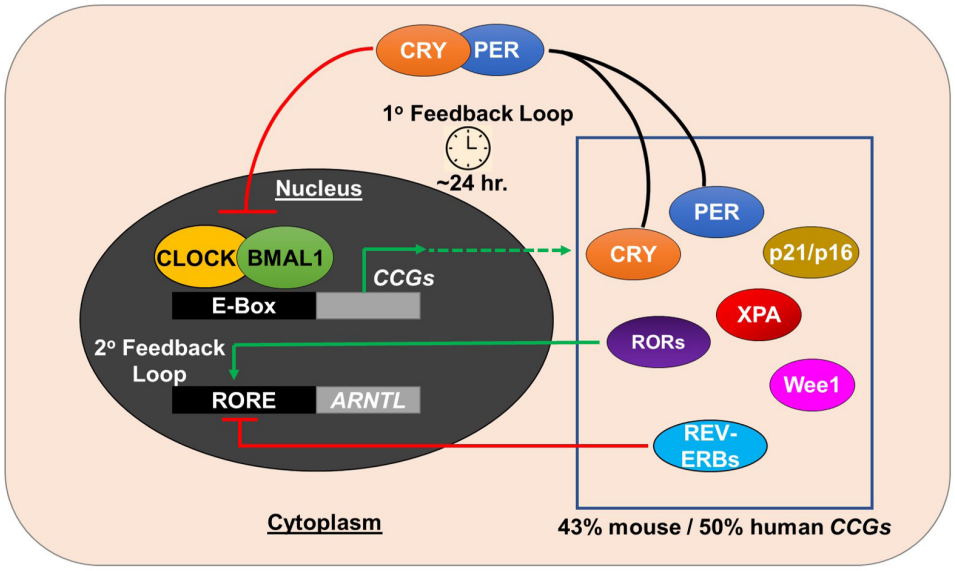

Figure 1. The mammalian circadian TTFL system. In the primary feedback loop, the heterodimeric transcription factor CLOCK-BMAL1 activates transcription of target clock genes *CRY1/2* and *PER1/2,* which in turn suppress CLOCK-BMAL1 post-translationally. In the secondary feedback loop, CLOCK-BMAL1 drives the expression of REV-ERB proteins, which in turn bind to RORE sequences in *ARNTL* (encoding gene for BMAL1) promoter and inhibit its transcription. The coordinated function of these two loops results in the circadian expression of clock-controlled genes (*CCGs*) in both mice and humans.

rhythmicity of protein-coding genes is collectively from different tissues in the body, in a tissue-specific manner [14,15]. Importantly, different sets of CCGs have been found to exhibit phase variability in different tissues correlating to their tissue-specific functions [26]. Thus, the circadian system regulates various cellular processes such as metabolism, signaling networks, DNA repair, cell cycle, and proliferation. Two of such cellular processes, cell cycle and nucleotide excision repair, will be briefly discussed in this review.

Circadian Control of Cell Cycle and DNA Damage Checkpoint Signaling

The cell cycle is a core cellular mechanism that malfunctions in many cancer types leading to uncontrolled cellular proliferation [27]. Timed progression through various checkpoints in the cell cycle is crucial for maintaining genome stability in normal cells [10,28]. The circadian clock influences the cell cycle either transcriptionally or by protein-protein interactions. For instance, WEE1 kinase, which acts at the G2/M checkpoint, is transcriptionally activated by BMAL1/CLOCK and repressed by PERs or CRYs [29]. Similarly, expression of *p21*, which inhibits the entry of cells from G1 into S phase, is regulated by BMAL1, and also by PER1 through p53-independent mechanisms, likely through c-MYC stabilization [30,31]. Moreover, PER1 promotes cell cycle arrest during DNA damage by directly interacting with ataxia telangiectasia mutated (ATM) at a protein level, which is responsible for phosphorylation of checkpoint kinase 2 and activation of p53 [32-34]. Also, PER2 helps to stabilize p53 by modulating its ubiquitination through E3 ubiquitin-protein ligase mouse double minute 2 homolog (MDM2) [35]. These interactions are likely mediated by key regulatory factors of the cell cycle and clock, which regulate the coupling or uncoupling of these two systems.

In vivo studies on mouse models have provided physiological relevance to elucidate the mechanistic role of the circadian clock in regulating the cell cycle of the skin and its susceptibility to DNA damage. Our group showed DNA replication to be time-of-day dependent in mouse skin epidermis, with higher BrdU incorporation during DNA synthesis in the morning compared to the evening. To confirm that replication is under the control of the circadian clock, the time-of-day variation of replication was lost in Cry1/2$^{-/-}$ mice [36]. Geyfman *et al*. further demonstrated that time-of-day dependent cell proliferation in the interfollicular epidermis and upper hair follicles is controlled by BMAL1 intrinsic to keratinocytes. Transcriptome analysis identified distinct circadian genes in anagen (growing) and telogen (resting) skin with the latter exhibiting higher number of oscillating genes. Specifically, the majority of cell cycle- and metabolism-associated genes were elevated in the early morning and early evening respectively. Interestingly, *Bmal1*$^{-/-}$ mice not only lost circadian control of cellular proliferation but also exhibited a constant elevated cell

proliferation rate indicating a suppressive role of the core clock protein BMAL1 on cell cycle in the interfollicular epidermis [37]. Another intriguing study which utilized abnormal food intake (restricted feeding) as a skin clock disruption strategy observed decreased proportion of cells in S phase, though the shift in clock phase did not alter the phase of DNA synthesis in proliferating interfollicular progenitor cells [38]. This finding suggests that the peripheral clock may not be solely responsible for determining the phase of cell cycle in skin epidermis, even though it is a crucial regulator of cell proliferation [38]. Nevertheless, the importance of the clock control on the cell cycle has also been demonstrated through increased BMAL1-dependent susceptibility of the skin to reactive oxygen species- and UVR-induced DNA damage when the cells are in S-phase [37,38]. These findings collectively demonstrate that the circadian control of skin proliferation is a protective measure against endogenous and UVR-induced genomic instability.

Circadian Regulation of Nucleotide Excision Repair

Nucleotide excision repair (NER) is an exclusive repair mechanism that acts on bulky genomic DNA lesions caused by UV radiation (UVR), cigarette smoke, and platinating agents including cisplatin [13,36]. UVR induces DNA damage through the formation of adjacent bonds between cytosine (C) and thymine (T) residues to create two types of bulky photoproducts, namely: cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine-pyrimidone photoproducts ((6-4) PPs). Consequently, DNA damage response (DDR) signaling gets activated to halt cellular processes such as DNA replication, and initiates DNA repair and/or apoptosis [39-41]. The repair of bulky DNA adducts requires the coordinated activities of numerous repair factors including XPA, XPC, RPA, TFIIH complex, XPG, and XPF-ERCC1. This sequence of events will excise and seal the damaged oligonucleotide containing the lesion. The NER machinery operates through two sub-pathways: transcriptional coupled repair (TC-NER) repairs lesions of actively transcribed genes recognized by RNA polymerase II; global genomic repair (GG-NER) repairs global damage in the genome. These sub-pathways differ in the way the DNA damage is recognized [39,42,43].

The effect of time-of-day is particularly evident in excision repair of UVR and cisplatin-induced DNA damage. Rhythmic NER activity has been observed in brain, liver, and skin tissues–except testis of mice [36,44,45]. Clock control of the NER pathway is executed through direct binding of BMAL1 to E-box sequences in the promoter region of *Xpa* [46]. This results in rhythmic expression of Xpa protein, which is rate limiting in the NER process [44]. In mouse skin, it was shown that DNA replication and excision repair are counter-phased. NER peaks in the evening and slows down in the morning; replication activity peaks in the morning and slows down in the evening [36,37,47]. Consequently, this circadian connection between DNA replication and NER has been exploited in understanding the initiation and progression of UVR-induced skin cancer. UVR-induced DNA damage is the primary causative agent for skin carcinogenesis and since NER activity is directly controlled by the clock, we previously reported up to a five-fold increase in invasive squamous cell carcinomas, when mice were exposed to solar UVR in the morning than in the evening, corresponding to times of decreased and increased NER activity respectively [36]. Since humans are behaviorally anti-phased to mice, humans are expected to have a higher NER rate in the morning when there is higher exposure to UVR compared to the evening [48]. Interestingly, a recent study confirmed this proof-of-concept by showing less sunburn erythema in human subjects exposed UVR in the morning compared to the evening [49,50].

CIRCADIAN DISRUPTION AS A DRIVING FACTOR IN CARCINOGENESIS

In the modern competitive world, shift work, particularly at night and across different time zones, is part of the work culture in many industries including law enforcement, healthcare, and technology [6]. These conditions render excessive exposure to artificial light at night and disrupted feeding patterns, which result in circadian misalignment and potential disruption of the CCGs expression pattern [6,51,52]. Several studies conducted with shift workers have shown strong positive associations between shift work and increased risk of various cancers, including skin and breast cancers [53,54]. Based on the available epidemiological evidence, the International Agency for Research on Cancer (IARC) declared that “shiftwork that involves circadian disruption” is a probable carcinogenic factor in humans [55].

Changes in the sleep-wake cycles and feeding routine trigger incongruence in metabolic pathways leading to an imbalance in cellular homeostasis [56,57]. When these changes are chronic, it increases the propensity of metabolic disorders and cancer [56]. Melatonin, a pleiotropic hormone that regulates the sleep-wake cycle and is impacted by light exposure, exerts anti-cancerous effects through multiple pathways in breast cancer by binding to its receptor MT1 [58,59]. It was shown that increased levels of melatonin protect the skin from UVR damage [58]. Furthermore, reduced levels of the nocturnal melatonin in rodents by exposure to artificial light at night enhanced breast tumor development and increased intrinsic resistance to the chemotherapeutic drug tamoxifen compared to the animals under normal 12-hour light/dark cycles [59]. Using genetic mouse models to under-

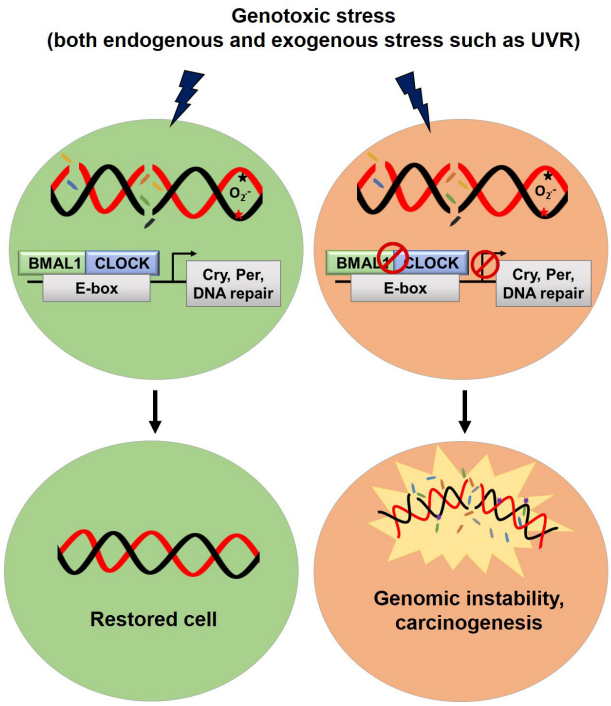

Figure 2. Circadian dysregulation drives carcinogenesis events. In response to genotoxic stress agents, a healthy circadian clock protects the genome through regulating cell cycle and DNA repair mechanisms to restore the cell to normal function. However, with circadian disruption, both cell cycle and DNA repair pathways are compromised leading to increased DNA replication errors and genomic instability ultimately causing carcinogenesis.

stand how disruption of the core clock elements would influence cancer development, it was shown that the loss of *Cry1/2*$^{-/-}$ do not predispose mice to spontaneous and ionizing radiation-induced cancers [60]. Another study also looked at the loss of *Per1*$^{-/-}$ and *Per2*$^{-/-}$ independently and made similar findings [61], even though it was contrary to earlier published reports of *Per2*-deficient mice being cancer-prone [62]. A different approach using a chronic jet lag model saw onset of hepatocellular carcinoma in wild-type mice, a phenotype that was exacerbated in genetic clock-disrupted mice. The chronic jet lag condition disrupts the liver clock and dysregulates liver metabolism thereby initiating non-alcoholic fatty acid liver disease (NAFLD), which subsequently leads to hepatocarcinogenesis through activation of constitutive androstane receptor (CAR), a CCG [63]. These findings, though contradictory, suggests that specific pathways deregulated by clock disruption are crucial to determining carcinogenesis outcomes, and *Per2* gene demonstrates a tumor-suppressive role. In studies that have utilized genetically-engineered cancer-prone mouse models, the results have so far shown a clear distinction in the effect of clock disruption in carcinogenesis. A genetically-engineered *Kras*^{G12D} mouse model subjected to jet lag as well as loss of clock genes *Per2* or *Arntl* (Bmal1) conditions saw accelerated initiation and progression of lung tumorigenesis compared to mice without circadian disruption. On a cellular level in tumors, clock-disrupted mice demonstrated enhanced proliferation and increased glucose and glutamine metabolism through increased levels of c-Myc [64]. When circadian disruption was employed in breast cancer-prone *p53*$^{R270H©/+}$*WAPCre* mice through chronically alternating light/dark cycles, decreased tumor suppression, increased body weight, and modest desynchronization in clock genes, such as *Arntl*, and cell-cycle control gene, *c-Myc* was observed, showing experimental proof that clock disruption increases breast cancer development [65]. Collectively, these findings suggest that the circadian clock regulates sleep-wake cycles, cell cycle, DNA repair, apoptosis, and metabolism in preventing genomic instability and carcinogenesis as summarized in Figure 2.

Clock Dysregulation in Breast Cancer

Breast cancer has been long implicated in circadian disruption outcomes. In the United States, breast cancer is the most common cancer, apart from skin cancers, and is the second leading cause of death in women [66]. The increased incidence of breast cancer is attributed partly to lifestyle changes in the modern world that are often associated with circadian clock dysregulation.

Indeed, several epidemiological studies have demonstrated a strong association between clock disruption and malignancy of breast cancer [54,67]. Two independent research groups reported the absence of rhythmic transcriptional oscillations in canonical circadian genes of breast cancer cells. Interestingly though, these clock genes rhythmicity was reset by the host's circadian clock or serum shock [68,69]. A detailed study on the expression pattern of the core clock genes revealed a distorted expression profile of *ARNTL* and *PER2* genes in malignant breast cancer cells irrespective of their receptor status [70]. Based on these studies, it is clear that aberrant or broken circadian rhythm is a common feature of many breast cancer cell lines and tissues, specifically the aggressive metastatic carcinomas. Further, the disruption of circadian rhythm is often associated with poor prognosis of breast cancer.

Circadian dysregulation has been associated with breast cancer mainly through the estrogen receptor alpha (ERα, which is encoded by *ESR1* gene) signaling network. ERα plays a pivotal role in mammary cell proliferation and mediates hormonal response during puberty [71]. PER2 suppresses transcriptional activation and induces degradation of ERα, but is in turn inducible by estrogen [71]. In breast cancer cells, PER2 functions as a tumor suppressor as the overexpression of *PER2* hinders cell proliferation and promotes apoptosis while its knockdown increases ERα -related cell proliferation [72]. Numerous studies also reported low transcript levels of *PER2* in breast cancer cells compared to normal breast cells [73-76], suggesting a critical role for *PER2* as a tumor suppressor in breast cancers.

HARNESSING THE CLOCK AS A STRATEGY FOR CANCER TREATMENT

The concept of "chronotherapy"–the timed administration of treatment–was documented over three decades ago. This treatment strategy harnesses the endogenous circadian cycles to achieve optimal efficacy and minimize side effects for a particular therapy [16]. Given that two of the major limitations with cancer therapy are tumor resistance and toxicity in healthy cells, which can range from mild to life-threatening, chronotherapy provides a promising tool to naturally exploit the specific time of the day, when critical cellular processes of DNA replication, targeted gene expression, and DNA repair are highly vulnerable in tumor versus normal cells [4,16]. Chemo- and radiation-therapies are widely used in the treatment of most cancers either solely or in combination with other regimens as adjuvant or neoadjuvant options [13]. Importantly, the knowledge of circadian biology has been applied to improve these treatment strategies. Despite the promising prospects of chronotherapy, its application is not yet broadly applied in the clinic. There are a number of successes and failures in studies involving chronotherapy (further discussed below). The failures can be attributed to interindividual variability (including genetics) and hierarchical complexities from molecules to systems in humans. The effectiveness of chronotherapy will depend on a broad range of factors which include uptake/efflux, distribution, metabolism, and elimination [77], which are not yet fully accounted for in chrono-administration of therapy. Though a lot remains unknown, significant progress has been made in the area of DNA repair from the mechanistic to clinical applications of cancer therapies, especially with chronochemotherapy.

Chronochemotherapy: Emerging Molecular Connections in Cisplatin-induced Adduct Repair

Cisplatin or c*is*-Diamminedichloroplatinum (II) is a very commonly used chemotherapeutic drug. It acts in cells by forming direct crosslinks with purine bases in DNA resulting in bulky adducts, thereby affecting cellular processes of DNA replication, transcription, and DNA repair [78]. Over 30 years ago, a few clinical and *in vivo* animal studies observed significant advantages of timed dosages of chemotherapeutic agents such as cisplatin and oxaliplatin. One of the premier findings was made in advanced ovarian cancer patients where the administration of cisplatin in the morning resulted in greater delays, reduction, modification, and attenuation of treatments due to toxicity compared to the evening administration [79]. Another similar study in rodents which used oxaliplatin for the treatment of colorectal cancer found encouraging results with evening administration resulting in a threefold increase in tolerability compared to morning administration [80]. This observation was later successfully translated to patients [81]. Some of the well documented clinical studies in renal cell carcinoma, breast and lung cancers that employed circadian timings in treatment administration registered lower toxicity levels at certain times [82-84]. Significantly, more than 40 chemotherapeutic drugs showed dramatic variations in efficacy or tolerance concerning the time of administration in rodent models [85].

Recently, our group has found a mechanistic explanation for the observed clinical outcomes from the previous studies. Using melanoma mouse models, we demonstrated that the time-of-day of administration of cisplatin influenced renal and blood toxicities. There was an enhanced rate of removal of cisplatin-DNA adducts through NER in wild-type mice treated in the evening, and subsequently less toxicity compared to mice treated in the morning, a variation that was lost in genetically clock disrupted (*Per1/2* mutated) mice. Interestingly, observations from blood cells, treated *ex vivo* with cisplatin, from human subjects simulated to day and night sched-

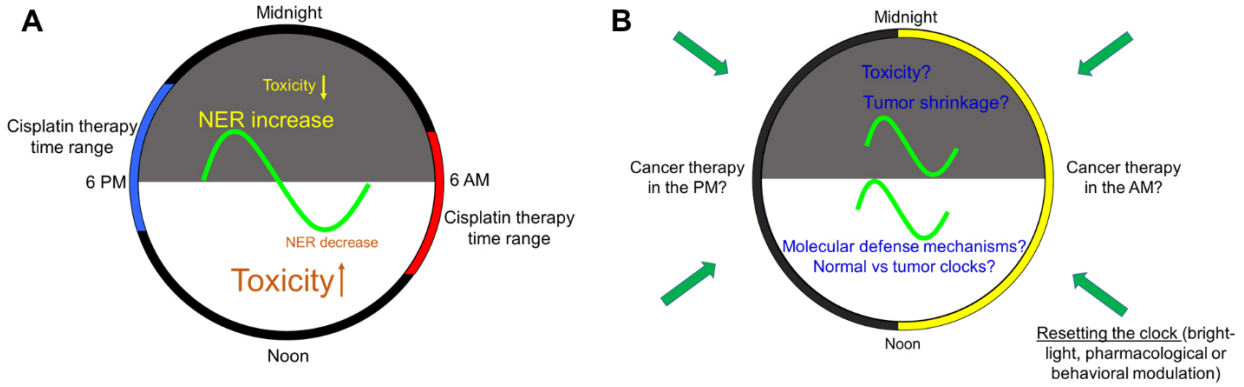

Figure 3. Circadian clock implications for cancer treatment strategies. (**A**) Circadian clock regulation of NER activity is responsible for modulation of cisplatin toxicity. Chronotherapeutic cisplatin treatment regimens can be developed based on this model. This image was reproduced from [46]. (**B**) The possibility of chronomodulation of currently existing cancer therapies to target cancerous cells in their most sensitive phase while protecting normal cells.

ules corroborated our view of the circadian regulation of cisplatin toxicity. Mechanistically, we demonstrated that *XPA* was regulated by the clock to influence NER of cisplatin-DNA adducts in mice and humans (Figure 3A) [46]. A recent study by the Sancar group in mouse tissues, including kidney and liver, revealed two distinct circadian repair patterns. Authors mapped the genome-wide repair of cisplatin-induced DNA adducts by eXcision Repair-sequencing (XR-seq) assay where excision repair of the transcribed strand of active CCGs was governed by each gene transcription status, while the global repair involving the non-transcribed strand of all genes, intergenic DNA, and transcribed strand of silent genes, was highest in the late afternoon/early evening period, giving a validation to the time-of-day-dependent Xpa levels and NER activity [86]. Collectively, these basic studies delineate an underlying mechanism to explain the previously observed chronotherapeutic outcomes with cisplatin administration in rodent models and human subjects [79,85,87-90]. Furthermore, as circadian regulation of cisplatin-induced DNA damage repair is mediated through XPA, it has the potential of being a biomarker for the development of personalized chronotherapeutic regimens [46]. These molecular studies connecting the circadian clock to chemotherapeutic outcomes have laid a solid foundation for further mechanistic studies into the use of circadian clock manipulation as a novel strategy for effective cancer treatments.

Chronoradiotherapy: Effect of the Circadian Clock on Radiation Therapy

Over 50 years ago, it was demonstrated that the circadian rhythm was an important factor in dictating hematopoietic stem cell response to ionizing radiation in rats [91,92]. A few decades later, a handful of studies have investigated the effect of circadian timing on radiation therapy (RT) in humans. Patients with non-small cell lung carcinoma (NSCLC), head and neck, cervical, prostate, and breast cancers have been subjected to RT by time-of-day (morning versus evening) and unfortunately, most of the results to date are conflicting [93-100].

Nevertheless, findings have been made using mouse models on the role of the clock in radiation-induced DNA damage response. One of the common side effects of radiation treatment is hair loss and subsequent dermatitis. Plikus *et al.* identified that mice hair possessed a robust autonomous circadian clock machinery, which translated to periods of maximum hair follicle growth (mornings) and maximum hair follicle repair or restoration (evenings) [101]. Notably, after intense RT there may be no hair regrowth due to permanent damage to hair cells or regrowth may take as long as 6 months. Studies in mice demonstrated increased hair loss upon exposure to γ radiation in the morning when the percentage of mitotic cells were high and Xpa levels were low compared to the evening [101]. This is because radiation severely affects actively proliferating cells in their M phase and low repair rate causing accumulation of DNA damage that is not effectively repaired leading to excess hair loss. The circadian influence on the time-of-day sensitivity of growing hairs to radiation was further demonstrated in wild-type mice, and *Cry1/2*$^{-/-}$ mice – which did not exhibit rhythmic radioprotective effect [101]. Furthermore, Fu *et al*. have shown that the inactivation of *Per2* gene by mutation sensitized mice to radiation by delaying p53 accumulation

[62]. The implications of the hair circadian system can be translated into future chronotherapy treatment modules aiming at minimizing RT-associated side effects.

Two clinical studies have investigated the circadian regulation of RT in breast cancer patients. The first study published by Noh *et al*. treated 395 patients in the early morning and early evening times and observed that the early evening treated group experienced significantly higher acute skin reactions compared to the morning treated group [100]. However, a second study by Ishaq *et al*. involving 140 breast cancer patients who received radiotherapy in the morning, afternoon, and evening time periods showed no statistically significant differences in the incidence of radiation-associated fatigue or dermatitis [102]. The inconsistencies in these studies mean that further clinical and mechanistic investigation is warranted.

Chronoimmunotherapy: Enhancing Immune Responses via Circadian Clock Regulation

Immunotherapy is a very promising approach in the future of cancer treatment because it seeks to use the body's immune system to fight cancer cells. Hence, the possibility of bolstering the immune system to fight off tumor cells will be a critical area of interest moving forward. Remarkably, different studies have demonstrated circadian control of the immune system, which will be discussed.

Time-of-day plays a role in lymphocyte trafficking in and out of the lymph nodes, which in turn regulates the adaptive immune responses. Rhythmic oscillations in pro-migratory factors CCR7 which peaks in the evening and S1P1 which troughs during the day causes lymphocytes ($CD4^+$ T cells, $CD8^+$ T cells, and B cells) to be retained in the lymph nodes during night time and migrate out into the bloodstream during daytime [103].

In understanding the role of the clock in immune response to tumors, our group demonstrated that circadian clock disruption, by the loss of *Per1/2* genes function, enhanced the immune response in melanoma tumors. There were significantly higher levels of $CD4^+$ and $CD8^+$ T cells in circulation and infiltrating the tumors, which rendered cisplatin treatment to be more effective in these mice compared with circadian efficient wild-type group [46]. This finding paves the way for potentially modulating the clock to enhance the adaptive immune response to tumors for immunotherapeutic applications, as well as in combination with other therapies.

In addition to the adaptive immune system, the innate immune system has also been shown to be under circadian control. Pro-inflammatory cytokines and chemokines such as *Il-6* and *Ccl2* respectively secreted by the immune cells were shown to have a rhythmic secretory pattern and recruitment to tissues [104,105]. This circadian control of cytokine and chemokine expression was abolished in myeloid cells of *Bmal1* knockout mice after lipopolysaccharide (LPS) induction. Furthermore, $Clock^{\Delta 19/\Delta 19}$ mutant and $Cry1/2^{-/-}$ mice showed reduced and increased expressions of cytokines respectively in response to LPS [106,107]. On the secondary arm of the circadian clock feedback loop mechanism, pro-inflammatory cytokines such as Il-6 are strongly regulated by Rev-erba at a molecular level, and in a *Nr1d1* knockout mice, the circadian rhythmicity in the release of cytokines by macrophages was lost [104]. A mechanistic link between the clock and time-of-day expressions of macrophages was shown in myeloid cells, where Bmal1 directly controls *Nrf2* transcriptional activity and antioxidant capacity by regulating Il-1β levels [108]. Further studies are still required to elucidate how the circadian system selectively regulates various immune components.

Potential for Circadian Influence in Breast Cancer Treatment

Everolimus (EV), a selector inhibitor of the mammalian target of rapamycin mTOR1 complex, is currently used along with anti-estrogens in the treatment of ER-positive breast cancer. Its mode of action is through cell cycle arrest by inhibiting mTORC1 downstream signaling which plays a crucial role in cell cycle progression and transcription [109]. Zhang *et al*. reported that the efficacy of G0/G1 cell cycle arrest varied up to 4-fold with different times of EV administration in MCF-7 breast cancer cells [110]. The authors showed that although serum shock did not synchronize core clock genes, it induced rhythmicity in mTOR activity, which in turn promoted circadian regulation of the key G1 phase progression proteins (Cyclin D1 and retinoblastoma protein). This study provides mechanistic proof that modulating the circadian clock and clock-controlled mechanisms may be harnessed in targeted therapy for optimal breast cancer treatments. This proof-of-concept can be applied to other forms of cancer, such as melanoma, where the mTOR pathway is also dysregulated [111].

CONCLUSION: FROM BENCH TO CLINICAL PRACTICE

The field of chronopharmacology holds significant promise. It deals with applying an endogenous remedy to reducing cancer therapy-associated toxicity while at the same time improving efficacy. However, the pipeline from bench findings to clinical translation or from clinical findings to mechanistic understanding remains quite hollow. Additional knowledge is essential to overcome hurdles in the applicability of chronomodulation in combination therapies, which is often mandatory for the treatment of most cancers. Currently, circadian timing of cancer treatment is not widely used in clinical practice mainly due

to inconsistencies between trials and lack of molecular mechanisms behind the clinical observations in chronotherapy trials. Nevertheless, the little we know so far is very significant and holds promise for the future. DNA damage response mechanisms, particularly XPA regulation by the circadian clock, can be a useful biomarker for personalizing chemotherapy in the clinical setting. Also, the modulation of the clock through pharmacological agents, behavior, and bright light therapy can be powerful tools to improve patient outcomes (Figure 3B). Additionally, the fact that the molecular basis for any cancer therapy rests upon the resistance or sensitivity of cells, which hinge on cellular proliferation and double-strand break repair, means that profiling the circadian clocks and CCGs in normal versus tumor cells is essential. Furthermore, recent advances in targeted gene delivery and integrated "omics" techniques will deepen our understanding of the circadian network in normal and cancerous tissues. Most of the understanding of clock-related genes is at the transcript level, but with newer advances in the field of quantitative proteomics and targeted gene delivery, our understanding of circadian-regulated biological processes can be expanded.

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REFERENCES

1. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. Nature. 2002;418(6901):935-41.
2. Duguay D, Cermakian N. The crosstalk between physiology and circadian clock proteins. Chronobiol Int. 2009;26(8):1479-513.
3. Buhr ED, Takahashi JS. Molecular components of the Mammalian circadian clock. Handb Exp Pharmacol. 2013(217):3-27.
4. Sancar A, Lindsey-Boltz LA, Gaddameedhi S, Selby CP, Ye R, Chiou YY, et al. Circadian clock, cancer, and chemotherapy. Biochemistry. 2015;54(2):110-23.
5. Bass J, Lazar MA. Circadian time signatures of fitness and disease. Science. 2016;354(6315):994-9.
6. James SM, Honn KA, Gaddameedhi S, Van Dongen HPA. Shift Work: Disrupted Circadian Rhythms and Sleep-Implications for Health and Well-Being. Curr Sleep Med Rep. 2017;3(2):104-12.
7. Panda S. Circadian physiology of metabolism. Science. 2016;354(6315):1008-15.
8. Marcheva B, Ramsey KM, Buhr ED, Kobayashi Y, Su H, Ko CH, et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature. 2010;466(7306):627-31.
9. Yu EA, Weaver DR. Disrupting the circadian clock: gene-specific effects on aging, cancer, and other phenotypes. Aging (Albany NY). 2011;3(5):479-93.
10. Lamia KA. Ticking time bombs: connections between circadian clocks and cancer. F1000Res. 2017;6:1910.
11. Gaddameedhi S, Reardon JT, Ye R, Ozturk N, Sancar A. Effect of circadian clock mutations on DNA damage response in mammalian cells. Cell Cycle. 2012;11(18):3481-91.
12. Padmanabhan K, Billaud M. Desynchronization of Circadian Clocks in Cancer: A Metabolic and Epigenetic Connection. Front Endocrinol (Lausanne). 2017;8:136.
13. Kelland L. The resurgence of platinum-based cancer chemotherapy. Nat Rev Cancer. 2007;7(8):573-84.
14. Zhang R, Lahens NF, Ballance HI, Hughes ME, Hogenesch JB. A circadian gene expression atlas in mammals: implications for biology and medicine. Proc Natl Acad Sci U S A. 2014;111(45):16219-24.
15. Ruben MD, Wu G, Smith DF, Schmidt RE, Francey LJ, Lee YY, et al. A database of tissue-specific rhythmically expressed human genes has potential applications in circadian medicine. Sci Transl Med. 2018;10(458).
16. Levi F. Circadian chronotherapy for human cancers. Lancet Oncol. 2001;2(5):307-15.
17. Buttgereit F, Smolen JS, Coogan AN, Cajochen C. Clocking in: chronobiology in rheumatoid arthritis. Nat Rev Rheumatol. 2015;11:349.
18. Pincus DJ, Szefler SJ, Ackerson LM, Martin RJ. Chronotherapy of asthma with inhaled steroids: the effect of dosage timing on drug efficacy. J Allergy Clin Immunol. 1995;95(6):1172-8.
19. Hermida RC, Smolensky MH. Chronotherapy of hypertension. Curr Opin Nephrol Hypertens. 2004;13(5):501-5.
20. Sancar A. The intelligent clock and the Rube Goldberg clock. Nat Struct Mol Biol. 2008;15(1):23-4.
21. Takahashi JS. Transcriptional architecture of the mammalian circadian clock. Nat Rev Genet. 2017;18(3):164-79.
22. Shearman LP, Sriram S, Weaver DR, Maywood ES, Chaves I, Zheng B, et al. Interacting molecular loops in the mammalian circadian clock. Science. 2000;288(5468):1013-9.
23. Partch CL, Green CB, Takahashi JS. Molecular architecture of the mammalian circadian clock. Trends Cell Biol. 2014;24(2):90-9.
24. Hughes ME, DiTacchio L, Hayes KR, Vollmers C, Pulivarthy S, Baggs JE, et al. Harmonics of circadian gene transcription in mammals. PLoS Genet. 2009;5(4):e1000442.
25. Cho H, Zhao X, Hatori M, Yu RT, Barish GD, Lam MT, et al. Regulation of circadian behaviour and metabolism by REV-ERB-alpha and REV-ERB-beta. Nature. 2012;485(7396):123-7.
26. Korenčič A, Košir R, Bordyugov G, Lehmann R, Rozman D, Herzel H. Timing of circadian genes in mammalian tissues. Sci Rep. 2014;4:5782.
27. Foster I. Cancer: A cell cycle defect. Radiography. 2008;14(2):144-9.
28. Bieler J, Cannavo R, Gustafson K, Gobet C, Gatfield D, Naef F. Robust synchronization of coupled circadian and cell cycle oscillators in single mammalian cells. Mol Syst Biol. 2014;10:739.
29. Matsuo T, Yamaguchi S, Mitsui S, Emi A, Shimoda

F, Okamura H. Control mechanism of the circadian clock for timing of cell division in vivo. Science. 2003;302(5643):255-9.
30. Grechez-Cassiau A, Rayet B, Guillaumond F, Teboul M, Delaunay F. The circadian clock component BMAL1 is a critical regulator of p21WAF1/CIP1 expression and hepatocyte proliferation. J Biol Chem. 2008;283(8):4535-42.
31. Gery S, Komatsu N, Baldjyan L, Yu A, Koo D, Koeffler HP. The circadian gene per1 plays an important role in cell growth and DNA damage control in human cancer cells. Mol Cell. 2006;22(3):375-82.
32. Unsal-Kacmaz K, Mullen TE, Kaufmann WK, Sancar A. Coupling of human circadian and cell cycles by the timeless protein. Mol Cell Biol. 2005;25(8):3109-16.
33. Yang X, Wood PA, Hrushesky WJ. Mammalian TIMELESS is required for ATM-dependent CHK2 activation and G2/M checkpoint control. J Biol Chem. 2010;285(5):3030-4.
34. Kondratov RV, Antoch MP. Circadian proteins in the regulation of cell cycle and genotoxic stress responses. Trends Cell Biol. 2007;17(7):311-7.
35. Gotoh T, Vila-Caballer M, Santos CS, Liu J, Yang J, Finkielstein CV. The circadian factor Period 2 modulates p53 stability and transcriptional activity in unstressed cells. Mol Biol Cell. 2014;25(19):3081-93.
36. Gaddameedhi S, Selby CP, Kaufmann WK, Smart RC, Sancar A. Control of skin cancer by the circadian rhythm. Proc Natl Acad Sci U S A. 2011;108(46):18790-5.
37. Geyfman M, Kumar V, Liu Q, Ruiz R, Gordon W, Espitia F, et al. Brain and muscle Arnt-like protein-1 (BMAL1) controls circadian cell proliferation and susceptibility to UVB-induced DNA damage in the epidermis. Proc Natl Acad Sci U S A. 2012;109(29):11758-63.
38. Wang H, van Spyk E, Liu Q, Geyfman M, Salmans ML, Kumar V, et al. Time-Restricted Feeding Shifts the Skin Circadian Clock and Alters UVB-Induced DNA Damage. Cell Rep. 2017;20(5):1061-72.
39. Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem. 2004;73:39-85.
40. Chen H, Weng QY, Fisher DE. UV signaling pathways within the skin. J Invest Dermatol. 2014;134(8):2080-5.
41. Khan AQ, Travers JB, Kemp MG. Roles of UVA radiation and DNA damage responses in melanoma pathogenesis. Environ Mol Mutagen. 2018;59(5):438-60.
42. Mu D, Park CH, Matsunaga T, Hsu DS, Reardon JT, Sancar A. Reconstitution of human DNA repair excision nuclease in a highly defined system. J Biol Chem. 1995;270(6):2415-8.
43. Reardon JT, Sancar A. Nucleotide excision repair. Prog Nucleic Acid Res Mol Biol. 2005;79:183-235.
44. Kang TH, Lindsey-Boltz LA, Reardon JT, Sancar A. Circadian control of XPA and excision repair of cisplatin-DNA damage by cryptochrome and HERC2 ubiquitin ligase. Proc Natl Acad Sci U S A. 2010;107(11):4890-5.
45. Kang TH, Reardon JT, Kemp M, Sancar A. Circadian oscillation of nucleotide excision repair in mammalian brain. Proc Natl Acad Sci U S A. 2009;106(8):2864-7.
46. Dakup PP, Porter KI, Little AA, Gajula RP, Zhang H, Skornyakov E, et al. The circadian clock regulates cisplatin-induced toxicity and tumor regression in melanoma mouse and human models. Oncotarget. 2018;9(18):14524-38.
47. Gaddameedhi S, Selby CP, Kemp MG, Ye R, Sancar A. The circadian clock controls sunburn apoptosis and erythema in mouse skin. J Invest Dermatol. 2015;135(4):1119-27.
48. Dakup P, Gaddameedhi S. Impact of the Circadian Clock on UV-Induced DNA Damage Response and Photocarcinogenesis. Photochem Photobiol. 2017;93(1):296-303.
49. Nikkola V, Gronroos M, Huotari-Orava R, Kautiainen H, Ylianttila L, Karppinen T, et al. Circadian Time Effects on NB-UVB-Induced Erythema in Human Skin In Vivo. J Invest Dermatol. 2018;138(2):464-7.
50. Sarkar S, Gaddameedhi S. UV-B-Induced Erythema in Human Skin: The Circadian Clock Is Ticking. J Invest Dermatol. 2018;138(2):248-51.
51. Kogi K. International research needs for improving sleep and health of workers. Ind Health. 2005;43(1):71-9.
52. Haus EL, Smolensky MH. Shift work and cancer risk: potential mechanistic roles of circadian disruption, light at night, and sleep deprivation. Sleep Med Rev. 2013;17(4):273-84.
53. Pukkala E, Aspholm R, Auvinen A, Eliasch H, Gundestrup M, Haldorsen T, et al. Cancer incidence among 10,211 airline pilots: a Nordic study. Aviat Space Environ Med. 2003;74(7):699-706.
54. Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, et al. Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. J Natl Cancer Inst. 2001;93(20):1563-8.
55. Straif K, Baan R, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, et al. Carcinogenicity of shift-work, painting, and fire-fighting. Lancet Oncol. 2007;8(12):1065-6.
56. Altman BJ. Cancer Clocks Out for Lunch: Disruption of Circadian Rhythm and Metabolic Oscillation in Cancer. Front Cell Dev Biol. 2016;4:62.
57. Skene DJ, Skornyakov E, Chowdhury NR, Gajula RP, Middleton B, Satterfield BC, et al. Separation of circadian- and behavior-driven metabolite rhythms in humans provides a window on peripheral oscillators and metabolism. Proc Natl Acad Sci U S A. 2018;115(30):7825-30.
58. Fischer TW, Kleszczynski K, Hardkop LH, Kruse N, Zillikens D. Melatonin enhances antioxidative enzyme gene expression (CAT, GPx, SOD), prevents their UVR-induced depletion, and protects against the formation of DNA damage (8-hydroxy-2'-deoxyguanosine) in ex vivo human skin. J Pineal Res. 2013;54(3):303-12.
59. Dauchy RT, Xiang S, Mao L, Brimer S, Wren MA, Yuan L, et al. Circadian and melatonin disruption by exposure to light at night drives intrinsic resistance to tamoxifen therapy in breast cancer. Cancer Res. 2014;74(15):4099-110.
60. Gauger MA, Sancar A. Cryptochrome, circadian cycle, cell cycle checkpoints, and cancer. Cancer Res. 2005;65(15):6828-34.
61. Antoch MP, Toshkov I, Kuropatwinski KK, Jackson M. Deficiency in PER proteins has no effect on the rate of spontaneous and radiation-induced carcinogenesis. Cell Cycle. 2013;12(23):3673-80.
62. Fu L, Pelicano H, Liu J, Huang P, Lee C. The circadian

gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. Cell. 2002;111(1):41-50. Epub 2002/10/10. doi: S0092867402009613 [pii].
63. Kettner NM, Voicu H, Finegold MJ, Coarfa C, Sreekumar A, Putluri N, et al. Circadian Homeostasis of Liver Metabolism Suppresses Hepatocarcinogenesis. Cancer Cell. 2016;30(6):909-24.
64. Papagiannakopoulos T, Bauer MR, Davidson SM, Heimann M, Subbaraj L, Bhutkar A, et al. Circadian Rhythm Disruption Promotes Lung Tumorigenesis. Cell Metab. 2016;24(2):324-31.
65. Van Dycke KC, Rodenburg W, van Oostrom CT, van Kerkhof LW, Pennings JL, Roenneberg T, et al. Chronically Alternating Light Cycles Increase Breast Cancer Risk in Mice. Curr Biol. 2015;25(14):1932-7.
66. Noone AM, Howlader N, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2015, National Cancer Institute. Bethesda, MD; https://seer.cancer.gov/csr/1975_2015/.
67. Megdal SP, Kroenke CH, Laden F, Pukkala E, Schernhammer ES. Night work and breast cancer risk: a systematic review and meta-analysis. Eur J Cancer. 2005;41(13):2023-32.
68. Xiang S, Mao L, Duplessis T, Yuan L, Dauchy R, Dauchy E, et al. Oscillation of clock and clock controlled genes induced by serum shock in human breast epithelial and breast cancer cells: regulation by melatonin. Breast Cancer (Auckl). 2012;6:137-50.
69. Rossetti S, Esposito J, Corlazzoli F, Gregorski A, Sacchi N. Entrainment of breast (cancer) epithelial cells detects distinct circadian oscillation patterns for clock and hormone receptor genes. Cell Cycle. 2012;11(2):350-60.
70. Gutierrez-Monreal MA, Trevino V, Moreno-Cuevas JE, Scott SP. Identification of circadian-related gene expression profiles in entrained breast cancer cell lines. Chronobiol Int. 2016;33(4):392-405.
71. Gery S, Virk RK, Chumakov K, Yu A, Koeffler HP. The clock gene Per2 links the circadian system to the estrogen receptor. Oncogene. 2007;26(57):7916-20.
72. Xiang S, Coffelt SB, Mao L, Yuan L, Cheng Q, Hill SM. Period-2: a tumor suppressor gene in breast cancer. J Circadian Rhythms. 2008;6:4.
73. Kuo SJ, Chen ST, Yeh KT, Hou MF, Chang YS, Hsu NC, et al. Disturbance of circadian gene expression in breast cancer. Virchows Arch. 2009;454(4):467-74.
74. Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, et al. The consensus coding sequences of human breast and colorectal cancers. Science. 2006;314(5797):268-74.
75. Chen ST, Choo KB, Hou MF, Yeh KT, Kuo SJ, Chang JG. Deregulated expression of the PER1, PER2 and PER3 genes in breast cancers. Carcinogenesis. 2005;26(7):1241-6.
76. Winter SL, Bosnoyan-Collins L, Pinnaduwage D, Andrulis IL. Expression of the circadian clock genes Per1 and Per2 in sporadic and familial breast tumors. Neoplasia. 2007;9(10):797-800.
77. Selfridge JM, Gotoh T, Schiffhauer S, Liu J, Stauffer PE, Li A, et al. Chronotherapy: Intuitive, Sound, Founded...But Not Broadly Applied. Drugs. 2016;76(16):1507-21.
78. Jamieson ER, Lippard SJ. Structure, Recognition, and Processing of Cisplatin-DNA Adducts. Chem Rev. 1999;99(9):2467-98.
79. Hrushesky WJ. Circadian timing of cancer chemotherapy. Science. 1985;228(4695):73-5.
80. Boughattas NA, Levi F, Fournier C, Lemaigre G, Roulon A, Hecquet B, et al. Circadian rhythm in toxicities and tissue uptake of 1,2-diamminocyclohexane(trans-1)oxalatoplatinum(II) in mice. Cancer Res. 1989;49(12):3362-8.
81. Levi F, Perpoint B, Garufi C, Focan C, Chollet P, Depres-Brummer P, et al. Oxaliplatin activity against metastatic colorectal cancer. A phase II study of 5-day continuous venous infusion at circadian rhythm modulated rate. Eur J Cancer. 1993;29A(9):1280-4.
82. Depres-Brummer P, Berthault-Cvitkovic F, Levi F, Brienza S, Vannetzel JM, Jasmin C, et al. Circadian rhythm-modulated (CRM) chemotherapy of metastatic breast cancer with mitoxantrone, 5-fluorouracil, and folinic acid: preliminary results of a phase I trial. J Infus Chemother. 1995;5(3 Suppl 1):144-7.
83. Focan C, Denis B, Kreutz F, Focan-Henrard D, Levi F. Ambulatory chronotherapy with 5-fluorouracil, folinic acid, and carboplatin for advanced non-small cell lung cancer. A phase II feasibility trial. J Infus Chemother. 1995;5(3 Suppl 1):148-52.
84. Hrushesky WJ, von Roemeling R, Lanning RM, Rabatin JT. Circadian-shaped infusions of floxuridine for progressive metastatic renal cell carcinoma. J Clin Oncol. 1990;8(9):1504-13.
85. Levi F, Okyar A, Dulong S, Innominato PF, Clairambault J. Circadian timing in cancer treatments. Annu Rev Pharmacol Toxicol. 2010;50:377-421.
86. Yang Y, Adebali O, Wu G, Selby CP, Chiou YY, Rashid N, et al. Cisplatin-DNA adduct repair of transcribed genes is controlled by two circadian programs in mouse tissues. Proc Natl Acad Sci U S A. 2018;115(21):E4777-E85.
87. Hrushesky WJ, Borch R, Levi F. Circadian time dependence of cisplatin urinary kinetics. Clin Pharmacol Ther. 1982;32(3):330-9.
88. Lin HX, Hua YJ, Chen QY, Luo DH, Sun R, Qiu F, et al. Randomized study of sinusoidal chronomodulated versus flat intermittent induction chemotherapy with cisplatin and 5-fluorouracil followed by traditional radiotherapy for locoregionally advanced nasopharyngeal carcinoma. Chin J Cancer. 2013;32(9):502-11.
89. Li J, Chen R, Ji M, Zou SL, Zhu LN. Cisplatin-based chronotherapy for advanced non-small cell lung cancer patients: a randomized controlled study and its pharmacokinetics analysis. Cancer Chemother Pharmacol. 2015;76(3):651-5.
90. Verma Y, Chauhan A, Singh H, Sabharwal R, Bharti M, Kaur P. Chronomodulated chemotherapy and concomitant radiotherapy for the management of locally advanced head and neck squamous cell carcinoma. Intl J Pharm Sci Res. 2014;5(3):1015-22.
91. Pizzarello DJ, Isaak D, Chua KE, Rhyne AL. Circadian Rhythmicity in the Sensitivity of Two Strains of Mice to Whole-Body Radiation. Science. 1964;145(3629):286-91.
92. Lappenbusch WL. Effect of circadian rhythm on the radiation response of the Chinese hamster (Cricetulus griseus).

Radiat Res. 1972;50(3):600-10.
93. Chan S, Rowbottom L, McDonald R, Bjarnason GA, Tsao M, Danjoux C, et al. Does the Time of Radiotherapy Affect Treatment Outcomes? A Review of the Literature. Clin Oncol (R Coll Radiol). 2017;29(4):231-8.
94. Bjarnason GA, Mackenzie RG, Nabid A, Hodson ID, El-Sayed S, Grimard L, et al. Comparison of toxicity associated with early morning versus late afternoon radiotherapy in patients with head-and-neck cancer: a prospective randomized trial of the National Cancer Institute of Canada Clinical Trials Group (HN3). Int J Radiat Oncol Biol Phys. 2009;73(1):166-72.
95. Rahn DA, 3rd, Ray DK, Schlesinger DJ, Steiner L, Sheehan JP, O'Quigley JM, et al. Gamma knife radiosurgery for brain metastasis of nonsmall cell lung cancer: is there a difference in outcome between morning and afternoon treatment? Cancer. 2011;117(2):414-20.
96. Badiyan SN, Ferraro DJ, Yaddanapudi S, Drzymala RE, Lee AY, Silver SA, et al. Impact of time of day on outcomes after stereotactic radiosurgery for non-small cell lung cancer brain metastases. Cancer. 2013;119(19):3563-9.
97. Goyal M, Shukla P, Gupta D, Bisht SS, Dhawan A, Gupta S, et al. Oral mucositis in morning vs. evening irradiated patients: a randomised prospective study. Int J Radiat Biol. 2009;85(6):504-9.
98. Shukla P, Gupta D, Bisht SS, Pant MC, Bhatt ML, Gupta R, et al. Circadian variation in radiation-induced intestinal mucositis in patients with cervical carcinoma. Cancer. 2010;116(8):2031-5.
99. Hsu FM, Hou WH, Huang CY, Wang CC, Tsai CL, Tsai YC, et al. Differences in toxicity and outcome associated with circadian variations between patients undergoing daytime and evening radiotherapy for prostate adenocarcinoma. Chronobiol Int. 2016;33(2):210-9.
100. Noh JM, Choi DH, Park H, Huh SJ, Park W, Seol SW, et al. Comparison of acute skin reaction following morning versus late afternoon radiotherapy in patients with breast cancer who have undergone curative surgical resection. J Radiat Res. 2014;55(3):553-8.
101. Plikus MV, Vollmers C, de la Cruz D, Chaix A, Ramos R, Panda S, et al. Local circadian clock gates cell cycle progression of transient amplifying cells during regenerative hair cycling. Proc Natl Acad Sci U S A. 2013;110(23):E2106-15.
102. Ishaq O, Valdimarsdottir H, Cooper BT, Modrek A, Redd W. Effect of Treatment Time of Day on Radiation Fatigue and Toxicity in Early-Stage Breast Cancer Patients After Breasr Conserving Surgery. Int J of Radiat Onc Biol Phys. 2015;93(3):E54.
103. Druzd D, Matveeva O, Ince L, Harrison U, He W, Schmal C, et al. Lymphocyte Circadian Clocks Control Lymph Node Trafficking and Adaptive Immune Responses. Immunity. 2017;46(1):120-32.
104. Gibbs JE, Blaikley J, Beesley S, Matthews L, Simpson KD, Boyce SH, et al. The nuclear receptor REV-ERBalpha mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines. Proc Natl Acad Sci U S A. 2012;109(2):582-7.
105. Labrecque N, Cermakian N. Circadian Clocks in the Immune System. J Biol Rhythms. 2015;30(4):277-90.
106. Bellet MM, Deriu E, Liu JZ, Grimaldi B, Blaschitz C, Zeller M, et al. Circadian clock regulates the host response to Salmonella. Proc Natl Acad Sci U S A. 2013;110(24):9897-902.
107. Narasimamurthy R, Hatori M, Nayak SK, Liu F, Panda S, Verma IM. Circadian clock protein cryptochrome regulates the expression of proinflammatory cytokines. Proc Natl Acad Sci U S A. 2012;109(31):12662-7.
108. Early JO, Menon D, Wyse CA, Cervantes-Silva MP, Zaslona Z, Carroll RG, et al. Circadian clock protein BMAL1 regulates IL-1beta in macrophages via NRF2. Proc Natl Acad Sci U S A. 2018;115(36):E8460-E8.
109. Mita MM, Mita A, Rowinsky EK. Mammalian target of rapamycin: a new molecular target for breast cancer. Clin Breast Cancer. 2003;4(2):126-37.
110. Zhang Y, Giacchetti S, Parouchev A, Hadadi E, Li X, Dallmann R, et al. Dosing time dependent in vitro pharmacodynamics of Everolimus despite a defective circadian clock. Cell Cycle. 2018;17(1):33-42.
111. Populo H, Soares P, Faustino A, Rocha AS, Silva P, Azevedo F, et al. mTOR pathway activation in cutaneous melanoma is associated with poorer prognosis characteristics. Pigment Cell Melanoma Res. 2011;24(1):254-7.