Polymorphism

REVIEW

Molecular underpinnings of circadian mechanism, chronotherapy and lung cancer: a mini-review

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ABSTRACT

Humans adjust to the diurnally varying light-dark cycle in nature through circadian rhythm, a phenomenon of about 24 hour oscillations in biological processes. The circadian rhythm operates at a genetic level that overts in circadian rhythm at biochemical, physiological and behavioral levels. The circadian rhythm is the output of coordinated functions of the central pacemaker suprachiasmatic nuclei (SCN) and peripheral oscillators existing in each cell of the various organ, including lungs. It is now well established that disruption in the circadian mechanism alters normal cells into cancerous cells. Lung cancer is one of the major cancers attributed to higher mortality. In this review, we have shed light on the molecular basis of the circadian rhythm, interaction between the clock genes and the cell cycle genes, association of circadian deregulation, and lung cancer pathophysiology and genetic polymorphisms linked to lung cancer. At last, the importance of chronotherapy or chronomedicine in the treatment of lung cancer to reduce the side effects and increase the efficacy for increasing survival rate in cancer patients has been discussed.

KEYWORDS: Circadian rhythm; clock genes; lung cancer; chronotherapy

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INTRODUCTION

During evolution, humans developed many subtle mechanisms like circadian mechanisms to cope with the changing natural environmental conditions. Circadian (derived from Latin word 'circa' meaning *about* and 'diem' meaning *a day*) is characterized by around 24-h oscillation in any biological processes that helps to cope and adapt to diurnal changing light-dark cycle or sunrise-sunset.

Mammals including human contain multiple oscillators viz. the central clock (Suprachiasmatic nuclei, SCN) of the basal hypothalamus in the brain and peripheral oscillators in each cell of the organs like lung, liver etc. The SCN coordinates the activities of the peripheral oscillators around 24 –h through hormones and sympathetic & parasympathetic pathways (Ueyama et al. 1999). Circadian rhythm synchronizers or time cues are known as 'zeitgeber' reset the central clock SCN, for example, the natural light-dark cycle. Feeding and fasting cycles and social timings are also the time cues that influence the circadian rhythms exhibited by peripheral clocks, such as sleep-wake rhythm (Damiola et al. 2000; Panda 2016; Roenneberg et al. 2012; Truong et al. 2016; Wright et al. 2013). The peripheral oscillators, like in lung tissues, have a wide range of entrainment towards temperature cues than SCN (Abraham et al. 2010).

In normal human beings, there are many day-to-day biological activities showing diurnal fluctuations. It is widely known that sleep-onset and wake times are regulated by circadian clock through circadian rhythm in dim light melatonin onset (DLMO) after sunset that peaks after midnight and decreases with the advent of the morning (Sletten et al. 2010). Body temperature, blood pressure, and heart rate are physiological variables with endogenous circadian rhythm with peaks seen in the evening hours (Pande et al. 2014).

The immune system cells also possess a timing system that contributes normal circadian rhythm in immune functions and imbalance in the timing of action of these immune components leading to various ailments (Scheiermann et al. 2018). Many endogenous and exogenous factors disturb the normal circadian rhythm, such as less sunlight exposure and longer exposure to light at night, shiftwork, jet lag, social timings or irregular sleep-wake timings, sleep problems, disease state etc. (Potter et al. 2016). Many diseases are considered as the outcome of circadian disruption such as cardiovascular disorders, hypertension (Morris et al. 2016), diabetes (Mason et al. 2020), respiratory diseases (Durrington et al. 2014), cancer (Li, Ao, and Wu 2013; Pati et al. 2007; Savvidis and Koutsilieris 2012), sleep disorders (Baron and Reid 2014) and many more (Sulli et al. 2018). Ailments like myocardial infarction or cardiovascular incidents occurred mostly in the morning, and temporal lobe-related epileptic seizures peak in the afternoon (Litinski, Scheer, and Shea 2009; Truong et al. 2016).

Circadian rhythm in pulmonary functions

Studies showed that pulmonary functions such as FEVC and FEV1/FEVC ratio (forced expiratory volume/forced expiratory vital capacity) are controlled by circadian system in healthy people (Spengler and Shea 2000). The circadian mutilation interrupts the normal lung functions such as airway caliber, airway resistance, respiratory symptoms, and abnormal immune-inflammatory responses (Sundar et al. 2015). Circadian impairment in pulmonary functions has been observed in respiratory diseases like asthma and chronic obstructive pulmonary disease (COPD) patients. The patients with asthma exhibit time-dependent worsening of symptoms that occurred mostly during second half of the biological night and early morning hours (Durrington et al. 2014). In these patients the circadian rhythm in air airway inflammation, airflow limitation and airways hyper-

responsiveness was disturbed (Durrington et al. 2014). In asthma patients, the concentrations of inflammatory cells in the distal airways like leukocytes, neutrophils and eosinophils peak in the early morning around 4:00 am (Kraft et al. 1996; Litinski, Scheer, and Shea 2009).

In patients with obstructive lung diseases, the circadian rhythm in clock components played a role in inflammatory responses (Truong et al. 2016). The clock genes polymorphisms (like period gene *Per3, CLOCK, RORB, BMAL1* and *Cry2*) are related to progressive lung disease with abnormal inflammation COPD modulated by age and gender (Chen et al. 2020).

The lung functions are modulated by factors like exposomes, tobacco smoke, lipopolysaccharide, hyperoxia, allergens, pathogens/ infections, and various environmental factors through altering the circadian molecular repertoire that enhances the DNA damage and inflammation (Sundar et al. 2015).

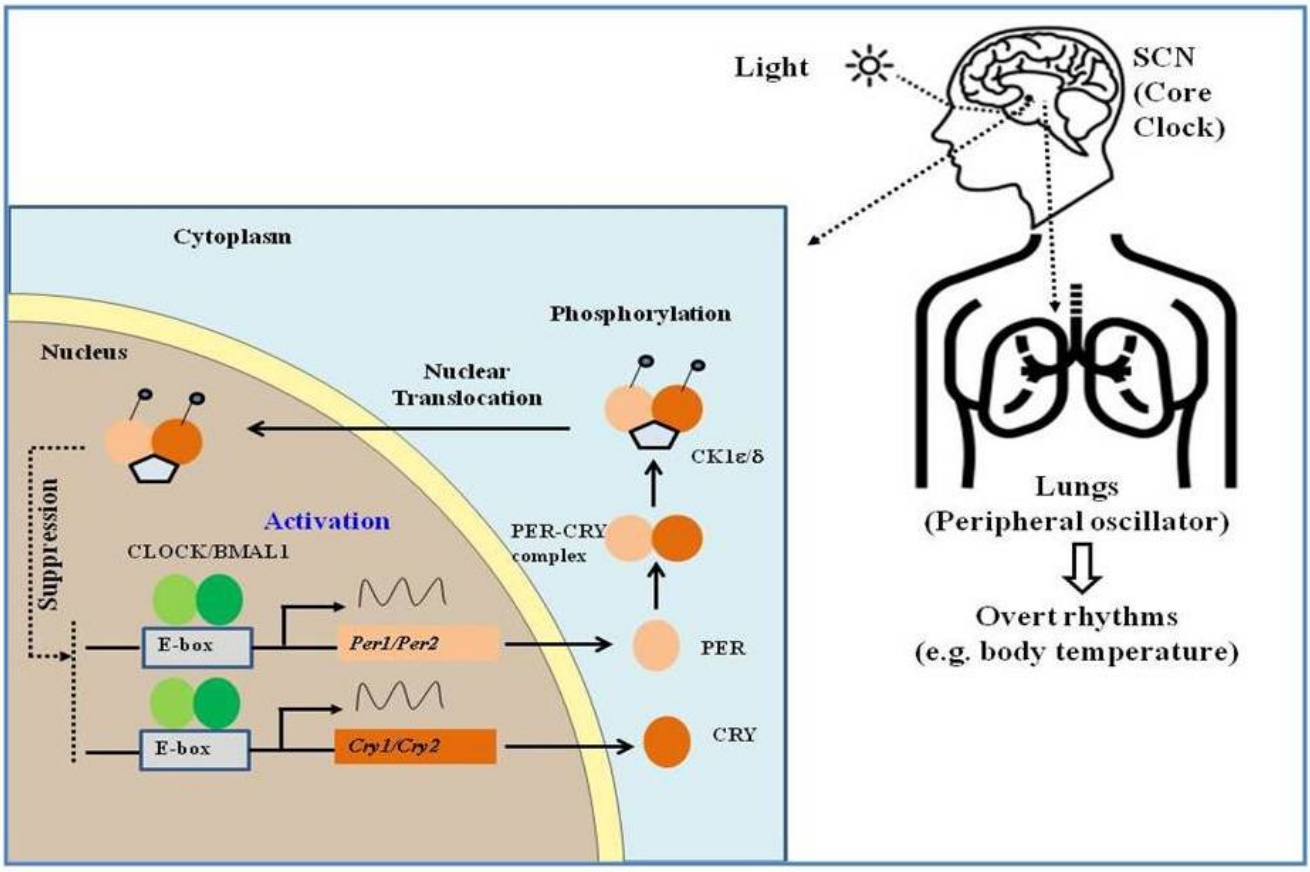

Figure 1. The basic molecular mechanism of circadian rhythm in mammals (based on a proposed model by (Mohawk, Green, and Takahashi 2012). The autoregulatory transcriptional feedback loop consists of clock genes proteins heterodimer CLOCK/BMAL1, which activates the expression of a set of clock genes called period genes *Per1, Per2, Cry1* and *Cry2*. The PER/CRY protein complex suppress their transcription through negative feedback manner after phosphorylation by kinases like casein kinase 1ε/δ (CK1ε/δ) the PER/CRY proteins.

An overview of molecular mechanism of circadian rhythm

Both the central pacemaker (SCN) and peripheral oscillators (cells in different organs of body such as lung, liver) coordinate the biological processes in circadian manner. The basis for the overt circadian rhythm is the circadian fluctuation in the underlying genetic components. This rhythm generation is due to the transcriptional and translational autoregulatory feedback loops of interlocked/coupled transcription factors known as clock genes. The clock genes expression vary according to environmental cues such as daily varying light-dark cycle and there is rhythmic formation and degradation of clock proteins complexes around 24 h (Lowrey and Takahashi 2011; Mohawk, Green, and Takahashi 2012). The main components and molecular basis of circadian rhythm have been explained using the schematic figure (Figure 1). The clock genes involved in circadian rhythm are the basic helix-loop-helix/PAS domain containing *Clock* (Circadian Locomotor Output Cycles Kaput), *Bmal1* (Brain and Muscle ARNT-Like 1), *period genes (Per1, Per2),* and cryptochrome genes (*Cry1*, *Cry2). Clock* stimulates *Bmal1* transcription, then CLOCK and BMAL1 proteins form heterodimer. This heterodimer acts as the positive activator of *Per/Cry* genes and many other downstream genes. The CLOCK/BMAL heterodimer binds to the enhancer sequences (E-box sequences) in the promoter region of *per* or *cry* genes and activates their transcription that peaks during the daytime. The CRYs and PERs protein levels are regulated by posttranslational modifications such as phosphorylation and ubiquitination. CRY and PER proteins are phosphorylated by casein kinases that cause degradation of these proteins when they are not in protein complex form; thus, kinases regulate the accumulation of the PER and CRY proteins. The PER-CRY protein complexes translocate into the nucleus. When accumulated in excess, the PER-CRY complexes inhibit CLOCK-BMAL1 mediated transcription that peaks at night, a negative feedback mechanism. Thus *Per* and *Cry* genes suppress their transcription. Additionally, there also exists secondary feedback loops in circadian system like the clock genes/nuclear receptors, for example, the retinoic acid receptor–related orphan receptor α (ROR α) that activates *Bmal1* transcription, and the REV-ERBα that inhibits *Bmal1* transcription as function of time during 24 h (Mohawk, Green, and Takahashi 2012).

Interaction of clock genes and clock-controlled genes of cell cycle in peripheral oscillators like lungs and circadian disruption

Cell cycle is one of the fundamental mechanisms that involve division of cells and control the development of cells. The circadian clock components also interact with the cell cycle components and regulate their expression (Chen-Goodspeed and Cheng 2007). Figure 2 shows a schematic overview of interaction of the circadian molecular components and the cell cycle genes. The CLOCK-BMAL1 heterodimer regulates the G2-M transition checkpoint *Wee-1* of cell cycle (Li, Ao, and Wu 2013). *Wee-1* encodes a cell-cycle protein kinase that inactivates Cyclin B1-Cdc2 kinase complex and results in arrest of the cell cycle at the G2-M, thus preventing the replication of damaged DNA (Matsuo et al. 2003).

Lungs cells also showed rhythmic expression of BMAL1 mRNA as reported in mice (Oishi et al. 1998). In addition, the involvement of *Timeless* (TIM) a clock gene has also been reported in lung morphogenesis (Xiao et al. 2003). *Timeless* also interacts with *CRY1* & cell cycle checkpoint Chk1, a DNA damage response (Ünsal-Kaçmaz et al. 2005).

Further, the CLOCK/BMAL1 complex has been reported to inhibit the expression of *c-Myc by binding to* the E-box of *c-Myc* promoter. Myc is the transcription factor that has been known for its role in cancer genesis and cell-cycle progression (G0/G1 transition), transcription, differentiation, apoptosis, and cell motility (Vita and Henriksson 2006). *c-Myc* is also expressed in circadian manner (Fu et al. 2002), and in *Per2* mutant mice, decreased

expression of *Bmal1* increase the expression of *c-Myc that* results in DNA damage, hyperplasia, and tumor formation (Fu et al. 2002). In *Per2* mutant mice, a reduced expression of p53, another important component of the G1-S checkpoint in the cell cycle has also been observed (Li, Ao, and Wu 2013). Both PER1 and PER2 are involved in cell cycle regulation through their tumor suppressor activity. Their overexpression activates the apoptosis in cancer cells and checkpoint proteins like ATM (ataxia telangiectasia mutated) and checkpoint kinase 2 or Chk2 (Fu et al. 2002; Gery et al. 2006). Thus evidence show the role of circadian disruption in the expression of clock proteins, that altered the interaction with cell cycle genes which is one of the causes for cancer development in human.

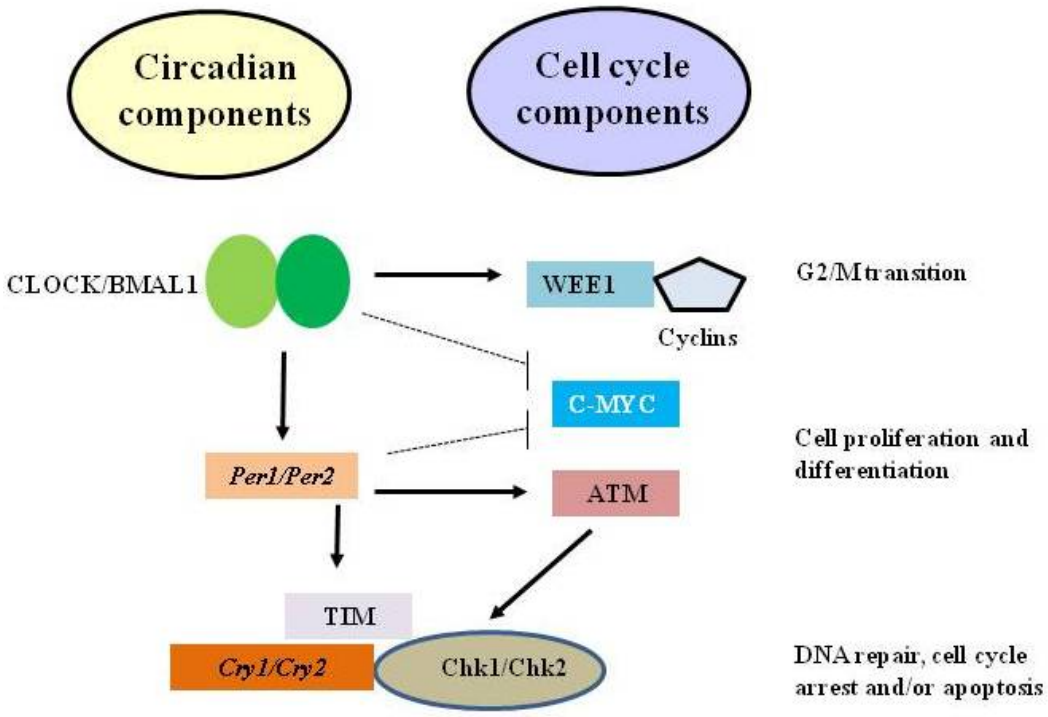

Figure 2. Essential components of the mammalian circadian clock and cell cycle and their interactions (based on proposed interaction model by (Chen-Goodspeed and Cheng 2007). The clock gene products (CLOCK/BMAL1) or PER1/PER2 regulate the transcription of cell cycle proteins Cyclins, WEE1, and c-MYC and cell cycle checkpoint pathways (ATM-Chk1/Chk2).

Circadian disruption and lung cancer

The circadian deregulation has been linked to various cancers in humans such as breast, prostate, ovary cancer, lung, oral, skin through influencing the cell cycle components (Gaddameedhi et al. 2011; Li, Ao, and Wu 2013). Among clock genes, the period (*Per*) genes have been studied much in relation to their association with various types of cancer, and in relation to apoptosis, immune response, hypoxia, and angiogenesis (Li, Ao, and Wu 2013). *Per2* expression has also been observed in Clara cells and is important in maintaining the circadian rhythm in lungs (Gibbs et al. 2009).

The healthy cells have normal circadian expression of period genes. When there is abnormal or disrupted circadian rhythm, then it has been associated with the lower expresseion of period

genes. For example, in non-small cell lung cancer (NSCLC), the decreased expression of *Per2* and *Per3* genes have been found to induce cancer progression. However, their increased expression inhibit the cell growth and increases the apoptotic rate, and lessen the lymph node metastasis as reported through sties in human or animal models (Chen-Goodspeed and Cheng 2007; Xiang et al. 2018). The downregulation in expression of *Per1* and/ or *Per2* have been observed in patients of bone acute myeloid leukemia patients, breast and lung cancers (Chen-Goodspeed and Cheng 2007; Chen et al. 2005; Gery et al. 2006). The role of *Per2* gene expression in lung metastasis has also been elucidated through using mouse model that was intravenously injected with A549 cells. *In vivo* and *in vitro* studies showd that overexpression of *Per2* in these A549 non-small cell lung cancer cells positively modulates the expression of tumour anti-oncogenes *Bax, P53* and *P21*, and suppresses the pro-oncogenes like *c-Myc* (Xiang et al. 2018). Liu and colleagues, through immunohistochemical method has found that *Per1* also associated with the poor differentiation, tumor status, and lymph node metastasis (Liu et al. 2014). Further, these researchers noted a consistent lower expression of *Per1, 2* and *3* in NSCLC compared to normal adjacent cells where the respective genes has overexpressed (Liu et al. 2014). It has been examined that jet-lag like condition impairs the normal rhythm in expression of *Per2* or *Bmal1* thereby inducing the overexpression of *c-Myc*, that ultimately promotes the tumor growth and progression in lungs (Papagiannakopoulos et al. 2016). The single nucleotide polymorphism (SNP) of *Per3* gene (rs228729) has also shown a strong link with genotype and allele frequency in NSCLC in Brazilian patients (Couto et al. 2014).

Pathophysiology of lung cancer and genetic polymorphisms

Cancer is a major disease burden worldwide, especially Gastric, pancreatic, breast, lungs, and hepatocellular cancer due to chemoresistance (Addeo et al. 2021; Verma et al. 2019; Verma, Falco, and Bhaskar 2020). Among these, Lung cancer is one of the primary causes of mortality in men and women among various cancers (Torre, Siegel, and Jemal 2016). Tumor initiation and progression process involve a series of pathologic steps which includes preneoplastic or precursor lesions with corresponding genetic and epigenetic abnormalities. The two types of lung cancers are small-cell lung carcinoma and non-small-cell lung carcinoma (NSCLC), contributing 15% and 85% among lung cancer. NSCLC is of three types viz. lung squamouscell carcinoma (LUSC), lung adenocarcinomas (LUAD), large-cell carcinoma with a prevalence of around 25-30 %, 40 % and 5-10 % (Zappa and Mousa 2016). These are found in various regions of the lungs, such airway epithelial cells in the bronchial tubes in the center (squamous-cell carcinoma), small airway epithelial and type II alveolar cells mostly in periphery (adenocarcinoma), center of the lungs (large-cell carcinoma). The squamous-cell and large-cell carcinoma are mostly detected in smokers (Kenfield et al. 2008). One of the risk factors for lung cancer is familial history. The germline TP53 mutation has been associated with lung cancer and is more likely to pass in smokers than in non-smokers progeny (Hwang et al. 2003). The different subtypes of lung cancer evolve through different molecular pathways (Goldstraw et al. 2011; Herbst, Heymach, and Lippman 2008; Wistuba and Gazdar 2006). Loss of heterozygosity (LOH) at chromosomes 3p, 9p (CDKN2A), 9q (tuberous sclerosis complex 1/TSC1), 17q, and 17p (TP53), and deletions at chromosome 3p12–23, 9p21 and 17p, are frequently seen in lung cancer (Takamochi et al. 2001; Wistuba et al. 1999, 2000; Wistuba and Gazdar 2006). Nevertheless, early steps in the molecular pathogenesis of SCLC have been poorly understood in comparison to NSCLC. Although, it has been shown that chromosome 3p14–23 deletion frequently occurred in the common SCLC (Whang-Peng et al. 1982). Human SCLCs were also shown to have known changes in *P53* and *Rb1*,

repetitive mutations in the histone modifiers CREBBP (CREB Binding Protein or cAMP response element-binding protein Binding Protein), EP300 (E1A Binding Protein P300), and MLL (mixed-lineage leukemia 1), as well as amplifications in the focal fibroblast growth factor receptor 1 (FGFR1) oncogene (Peifer et al. 2012). SOX2 (SRY-related HMG-box genes) has been discovered as a commonly amplified gene in SCLC by Rudin et al. (Rudin et al. 2012). Bi-allelic inactivation of tumor suppressor gene *P53* and *Rb1* was seen in almost all SCLC and 13% SCLCs, genomic alterations of *P73* were observed (George et al. 2015).

Table 1: Some of the important clock genes and cell cycle components in relation to cancer

Clock genes	Outcomes	References
Per1	• Over expression induces apoptosis of cancer cells • Interacts with the checkpoint proteins ATM and Chk2.	(Gery et al. 2006)
Per 2	• Disrupted expression leads to hyperplastic salivary glands due to increased cell division and reduce apoptosis; increased the sensitivity to ionizing radiation during therapy	(Fu et al. 2002)
	• In P*er2* mutant mice there is increased expression of *c-Myc* and reduce expression of *P53*	(Fu et al. 2002; Li, Ao, and Wu 2013)
	• Over expression increased the expression of tumor anti oncogenes like *Bax, P53* and *P21* and suppress the pro oncogenes like *c-Myc* • *Per2* mutation or deletion increased the tumor progression and metastasis • Over expression reduce the metastasis of NSCLC	(Xiang et al. 2018)
Per 3	• Less expressed in NSCLC and *Per3* gene polymorphism (rs228729) is considered as a risk factor for development of NSCLC	(Couto et al. 2014)
Per1, Per2 and Per3	• Lower expression in NSCLC cells compared to normal adjacent cells • Patients of NSCLC with normal expression showed increased survival compared to patients with disrupted expression	(Liu et al. 2014)
Timeless	• Interacts with cell cycle checkpoint proteins Chk1 and the ATR-ATRIP complex and plays an important role in the DNA damage checkpoint response.	(Ünsal-Kaçmaz et al. 2005)

Two of the molecular signaling pathways are involved in the genesis of NSCLC, specifically LUAD, rat sarcoma viral oncogene (k-ras) and epidermal growth factor receptor (EGFR) in smokers and non-smoker individuals, respectively. The k-ras proto-oncogene and encode K-ras G-protein. It plays an important part in the RAS/MAPK1 (Rapidly Accelerated Fibrosarcoma /Mitogen-activated

protein kinase 1) signaling pathway downstream of numerous growth factor receptors, including EGFR (found on chromosome 7p11.2, spans approximately 200 kb, and has 28 exons). Frequent deletions in exon 19 (delE746-A750, delL747-T751insS, & delL747-P753insS) and exon 21 (L861Q, L861R & L858R) of EGFR are seen, with and without non-smoking history, female gender, and East Asian background (Mounawar et al. 2007; Rudin et al. 2009; Shigematsu et al. 2005; Sun, Schiller, and Gazdar 2007). On the other hand, tobacco consumption is linked with a mutation in k-ras and development of LUAD (Le Calvez et al. 2005; Herbst, Heymach, and Lippman 2008; Ridanpaa et al. 1994). After conducting a thorough literature analysis, Yamamoto et al. identified 569 mutations in 2880 lung cancer patients (Yamamoto, Toyooka, and Mitsudomi 2009). Tumor suppressor genes such as *Rb* and p53 have also been shown inactivated in NSCLC and SCLC respectively (Harbour et al. 1988; Takahashi et al. 1989). Cyclin D1 is commonly over-expressed, with inactivation of the CDKI (p16), providing an additional method for overcoming Rb activity in cell cycle regulation (Schauer et al. 1994). There is also a loss of heterozygosity (LOH) in chromosomes 3p (3p21, 3p14, 3p22–24 and 3p12), 9p (CDKN2A), 9q (tuberous sclerosis complex 1/TSC1), 17q, and 17p (*TP53*) and at squamous preinvasive lesions, allelic abnormalities in 8p21–23, 13q14 (Rb1), and 17p13 (p53) were found in NSCLC, particularly in LUAD and LUSC (Takamochi et al. 2001; Wistuba et al. 1999, 2000; Wistuba and Gazdar 2006; Yuspa 1994).

Therapy

The non-small cell lung cancer (NSCLC) has been better prognosis than small cell lung cancer (SCLC). However the NSCLCs showed poor responsiveness towards chemotherapy (Liu et al. 2014). The patients with NSCLC and SCLC were treated through surgical removal and chemotherapy (more preferred at an advanced stage). The patients irresponsive to chemotherapy are given radiotherapy to damage the DNA of cancerous cells (Sen et al. 2019; Zappa and Mousa 2016). Immunotherapy is also a better treatment option that modulates the immune checkpoint pathways like blocking inhibitory receptors cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death-1 (PD-1) that activates tumor cells to protect themselves against the immune system and increase the tolerance & avoid side effects of treatment (Pardoll 2012). The targeted therapy like using EGFR (Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors or the tumor cell vaccines could also help to increase responsiveness towards treatment in NSCLC (Zappa and Mousa 2016). In the early stage, the tumor is removed through stereotactic body radiation therapy (SBRT), which helps in targeted radiotherapy of tumors (Grutters et al. 2010). However, the longer survival after treatment is also less. Therefore, there is need of new therapeutic approaches of diagnosis such as searching novel molecular biomarkers or small molecules targeting circadian clock components (Rahman et al. 2020) and treatment methods and chronotherapy (Sulli et al. 2018) could be suggested as one of the effective treatment technique.

Chronotherapy/chronomedicine

Human's internal body clock shows time-dependent susceptibility, resistance, tolerance, and healing capacity towards external factors like environmental, drugs, and radiation pathogens through circadian mechanisms. This property could be manipulated to treat the diseases or reduce the side effects of traditional treatment. Chronotherapy, also known as chronomedicine is time dependent intervention administration of medicine or therapy (chemo or radiation) to maximize the efficacy of treatment and reduce the side effects of treatments on patients. As it is well known that chemo and radiation therapy have many side effects, chronotherapy ensures less damage to the body at the cellular level. It helps in the fast recovery of patients. Since genetic repertoire of circadian system express in rhythmic manner and their interaction with the cell cycle components is also in a circadian manner, treatments at specific times

according to patient personal circadian behavior render the least toxicity to the body and maximize the benefits. There are also some randomized clinical trials that showed positive effects of chronotherapy in cancer like lung cancer, breast cancer, colorectal cancer that increases the survival rate among cancer patients (Gallion et al. 2003; Lévi, Zidani, and Misset 1997; Lissoni et al. 1999).

The planned drugs dosing times either directly affect the clock gene expression or their regulators like Casein Kinases, thus helps in circadian alignment (Sulli, Lam, and Panda 2019). The reports from the studies in non-human primates, rodents, or humans showed more than 80% of FDA-approved drug targets are the diurnal varying transcriptome in central or peripheral oscillators like lungs and liver (Sulli et al. 2018). *Per* gene family could be considered as molecular target because of their cancer suppression properties and activating apoptosis pathways (Liu et al. 2014). *Per2* gene has also been suggested as one of the molecules to treat nonsmall cell lung cancer (Xiang et al. 2018). It has been supposed that methylation (CpG) of promoter sequences of *Per* genes could lead to their silencing in lung cancer like silencing of tumor suppressor genes in breast cancer (Chen et al. 2005; Li, Ao, and Wu 2013). Forward genetic screen has identified miRNAs (miR-192/194 group) as the suppressor of *Per* genes at the post-transcriptional stage (Nagel, Clijsters, and Agami 2009). The *Per3* SNP is also associated with the NSCLC so that *Per* genes could be the target of treatment. Further, the synthetic drug SR9009 acts as an agonist of the REV-ERBs heterodimer involved in circadian regulation possess anticancer property against small-cell lung cancer (SCLC) cells through REV-ERB dependent inhibition of the autophagy gene Atg5 in SCLC (Shen et al. 2020).

Melatonin, a dark hormone secreted by pineal gland in the evening and a robust circadian marker has discerned its benefits by reducing the toxicity of chemotherapy through its anti-oxidant property and apoptosis of cancer cells in advance stages of metastatic non-small cell lung cancer patients treated with cisplatin (CDDP) plus etoposide or gemcitabine alone, along with its efficacy in breast cancer, gastrointestinal tract neoplasms, head and neck cancers (Lissoni et al. 1999). Melatonin (10 mg or 20 mg) administered orally in the evening (7 pm or 8 pm) has been examined to increase the survival of patients with advanced non-small-cell lung cancer without any toxicity (Hrushesky et al. 2021; Lissoni et al. 1992). Hrusheysky et al. reported that reduction in hazard ratio by 39 % against placebo when melatonin was administered in evening while no such effect was observed on morning administration of melatonin. Since cancer patients have disturbed circadian rhythm as observed through arrhythmic rest-activity, poor sleep quality & fragmented sleep so melatonin administration could adjust the patients rest-activity rhythm, improves the sleep quality and reduce the shortness of breath thus aligning the circadian system and helps in the recovery of patients (Hrushesky et al. 2021; Kurdi and Muthukalai 2016; Levin et al. 2005). Moreover, patients with non-small cell lung cancer with brain metastases showed increased survival rate when received stereotactic radiosurgery in the morning hours (*median survival of 9.5 months*) compared to afternoon (*5 months*) (Rahn et al. 2011; Chan et al. 2017). Thus these few clinical trials on lung cancer pave the way to inculcate the approach of chronotherapy specifically in developing countries where there is lack of awareness and where lung cancer is one of the leading causes of mortality. Further, chronotherapy is more relevant when the patient's circadian behavior (chronotype) is known in advance, so the dosing time could be predicted. At the appropriate dosing time/circadian phase the drugs/treatment are effective and at non-responsive circadian phase the drugs/therapy could be harmful with more side effects (Li et al. 2015; Chan et al. 2017). Li and coworkers reported lower rate of leucopenia & neutropenia in chronotherapy group compared to routine chemotherapy group in patients with advanced NSCLC taking treatment of cisplatin (Li et al. 2015). Chan et al. (2017) documented more toxic effects

and biochemical failure when treatment done in evening hours in paients with prostate cancer.

Many treatments are carried out according to availability of facilities; clinicians preferred time and patients circumstances. However, there is need that patient's biological clock should be taken into account while administrating drugs or radiation to cancer patients. Though there are administrative constraints such as making new policies and implementing them for treating patients. Nonetheless, application of chronotherapy in medicine could be an effective treatment strategy.

Conclusion

Circadian rhythm is a ubiquitous phenomenon in living organisms that is evident in each cell of the body in humans and is vital for healthy survival in accord with the diurnal changing environment. Through this current mini-review, we have given an overview of the genetic basis of circadian rhythm & its interaction with the cell cycle. Circadian rhythm disruption in normal circadian expression of clock component in lungs affects the cell cycle that ultimately may lead to lung cancer, one of the leading causes of mortality from cancer worldwide. At last, the importance of including chronotherapy/ chronomedicine in the traditional chemo/radiotherapy/surgery by citing some of the randomized clinical trials has been highlighted, which could pave the way for effective therapy and enhanced survival among cancer patients.

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Author's contribution

BP designed and conceived. BP, JPC and TS drafted the article. All authors have read and approved the final version of the manuscript for submission.

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Declaration of originality

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