Advances in the Study of Circadian Genes in Non-Small Cell Lung Cancer

Hao Zhang, MMed1*, Renwang Liu, MD1*, Bo Zhang, MD1, Huandong Huo, MMed1, and Zuoqing Song, MD1

Abstract
Circadian genes regulate several physiological functions such as circadian rhythm and metabolism and participate in the cytogenesis and progression of various malignancies. The abnormal expression of these genes in non-small cell lung cancer (NSCLC) is closely related to the clinicopathological features of NSCLC and may promote or inhibit NSCLC progression. Circadian rhythm disorders and clock gene abnormalities may increase the risk of lung cancer in some populations. We collected 15 circadian genes in NSCLC, namely PER1, PER2, PER3, TIMELESS, Cry1, Cry2, CLOCK, BMAL1/ARNTL-1, ARNTL2, NPAS2, NR1D1 (REV-ERB), DECI, DEC2, RORα, and RORγ, and determined their relationships with the clinicopathological features of patients and the potential mechanisms promoting or inhibiting NSCLC progression. We also summarized the studies on circadian rhythm disorders and circadian genes associated with lung cancer risk. The present study aimed to provide theoretical support for the future exploration of new therapeutic targets and for the primary prevention of NSCLC from the perspective of circadian genes. Interpretation of circadian rhythms in lung cancer could guide further lung cancer mechanism research and drug development that could lead to more effective treatments and improve patient outcomes.

Keywords
circadian gene, non-small cell lung cancer, gene expression variability, prognosis, mechanism

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Introduction
Circadian genes are widespread in living organisms. Circadian genes, such as TIMELESS, were discovered in Drosophila by Michael W. Young et al. Subsequently, approximately 20 circadian genes have been found to be interrelated and coordinated, forming the circadian gene system.1 This system is made up of 2 parts: a core circadian system found in the suprachiasmatic nucleus and a peripheral circadian system found in nearly all peripheral tissues. As a master pacemaker, the circadian clock system synchronizes or drives the peripheral circadian systems distributed throughout the body, which are closely linked to regulating circadian rhythms.2 Simultaneously, the circadian clock genetic system controls nearly all physiological and pathological functions, including basal metabolism, body temperature, blood pressure, hormone secretion, and immunity, enabling organisms to anticipate environmental changes and modify their behavior and physiological functions efficiently.3

Circadian genes have powerful regulatory functions on circadian rhythms and physiological metabolism and are closely associated with tumor progression. Numerous studies have presented that circadian genes such as PER, CLOCK, BMAL1, and TIMELESS are closely related to the progression and prognosis of breast, pancreatic, colon, and kidney cancers.4-14 In breast cancer, patients with a high expression of circadian genes CLOCK, PER1, PER2, PER3, CRY2, NPAS2, and RORγ have longer metastasis-free survival (MFS), those with a high expression of PER3 and RORγ have longer disease-free survival (DFS).8 Additionally, the downregulation of PER2 gene expression in vitro promotes the level of cyclin D and cyclin E as well as the proliferation of breast cancer cells.9 Low BMAL1 expression is related to pancreatic cancer progression and

*These authors have contributed equally to this work.

Corresponding Author:
Professor Zuoqing Song, Department of Lung Cancer Surgery, Lung Cancer Institute, Tianjin Medical University General Hospital, 154 Anshan Road, Heping, Tianjin 300052, P.R. China.
Email: thoracic_expert@aliyun.com
poor prognosis. In vitro, overexpression of BMAL1 significantly inhibits pancreatic cancer cell proliferation and invasion by activating the P53 pathway, which is consistent with the clinical findings. In colon cancer, high CLOCK expression is associated with better overall survival (OS); however, it promotes tumor cell proliferation by regulating iron metabolism in colon cancer cells. Furthermore, high CLOCK expression inhibits the apoptosis of colon cancer cells in vitro. It is common to find such inconsistencies between in vitro experiments and clinical observations in studies of circadian genes, suggesting that they may have a complex action mechanism in tumor progression.

The relationship between circadian genes and lung cancer progression and associated mechanisms has rarely been studied in lung cancers. Lung cancer has the highest morbidity and mortality rates among malignant tumors, and has increased significantly over the last 40 years. In 2015, lung cancer caused 25% of all cancer deaths in the United States and 30% of deaths in China. Lung cancer ranks second in the United States and first in China in terms of morbidity. Smoking and environmental pollution are high-risk factors for lung cancer, while circadian rhythm disorders might also be closely connected with lung cancer progression. Although circadian rhythm changes and alterations in circadian genes have been extensively studied in the field of oncology, their roles in lung cancer are poorly studied. Hence, we searched the published literature and summarized 15 genes in the circadian gene family, including PER1, PER2, PER3, TIMELESS, CRY1, CRY2, CLOCK, BMAL1/ARNTL-1, ARNTL2, NPAS2, NR1D1 (REV-ERB), DEC1, DEC2, RORA, and RORC. RORA/RORB activates transcription of ARNTL, whereas NR1D1 and NR1D2 repress it, which further increases the regulatory level of CLOCK/NPAS2 activity. Heterodimers of PER and CRY proteins activate a negative feedback loop that acts directly on CLOCK and NPAS2.

Figure 1. Hypothesized models of circadian rhythm genes in mammals. The CLOCK and NPAS2 form heterodimers with BMAL1. These heterodimers act as enhancer e-box elements upstream of transcription factors binding target genes to activate transcription of other core circadian genes like the PER family (PER1, PER2, PER3) and CRY family (CRY1, CRY2). CLOCK and NPAS2 can also trans-activate the expression of other pathway components, such as NR1D1, NR1D2 (also known as Rev-ERB), RORA, RORB, and RORC. RORA/RORB activates transcription of ARNTL, whereas NR1D1 and NR1D2 repress it, which further increases the regulatory level of CLOCK/NPAS2 activity.

Relationships Between Circadian Genes and the Clinicopathological Features of Lung Cancer Patients

Circadian genes might be closely related to clinicopathological features, tumor-node-metastasis (TNM) staging, and lung cancer patients’ prognosis. TIMELESS, PER1, PER2, PER3, DEC1, and ARNTL-2 are associated with the degree of differentiation in non-small cell lung cancer (NSCLC). Zhang et al compared the clinical data of 72 NSCLC patients and revealed that lung cancer patients with high TIMELESS expression had a low degree of differentiation. Liu et al discovered that PER1, PER2, and PER3-deficient NSCLC patients had a low degree of differentiation.
Giartromanolaki et al. collected data from 115 NSCLC patients and presented that patients with low $DECI$ expression had a low degree of differentiation. In the same way, Brady et al. found that in lung adenocarcinoma, $ARNTL-2$ expression was higher in poorly differentiated lung cancer tissues. The associations between circadian genes and the degree of differentiation of lung cancer tissues are presented in Table 1.

Circadian genes are also closely related to TNM staging in lung cancer patients. Patients with TNM stage III NSCLC express significantly more $TIMELESS$ than those with TNM stage I and II. Additionally, $TIMELESS$ expression in lung adenocarcinoma tissues with more than 3 cm tumor size was significantly higher than that in lung cancer tissue sizes smaller than 3 cm. However, it has also been shown that $TIMELESS$ does not correlate with the TNM stage significantly. A clinical study of 130 NSCLC cases reported that $PER1$, $PER2$, and $PER3$ were less prevalent in lung cancer tissues than in the adjacent normal lung tissues, and the TNM staging was relatively late in patients with $PER1$, $PER2$, and $PER3$ deletion. Furthermore, Liu et al. found that $DEC1$-deficient NSCLC patients had later TNM staging. However, some studies show that the circadian clock genes have no association with the TNM staging of NSCLC. The associations between circadian genes and the TNM staging of NSCLC patients are presented in Table 1.

Prognostically, there is also a relation between circadian genes and lung cancer. It has been established that $TIMELESS$, $PER1$, $PER2$, $PER3$, $DECI$, $BMAL1$, $ARNTL-2$, $NPAS2$, $CRY2$, $RORα$, and $RORγ$ are associated with patient prognosis. Patients with high $TIMELESS$ expression have a considerably shorter OS and poorer prognosis, and patients with $PER1$, $PER2$, and $PER3$ deletion also show lower OS. However, it has been revealed that the expression of $PER$ family does not correlate with lung cancer patients’ prognosis significantly. A database-based study presented that high $DECI$ expression corresponded with a poor prognosis in NSCLC patients. However, it has also been shown that the expression of the $DEC$ family does not correlate with lung cancer prognosis significantly. Thus, whether the $PER$ family proteins and $DECI$ can predict NSCLC prognosis must be investigated further. Another study discovered that OS was significantly longer in lung adenocarcinoma patients with a high expression of $BMAL1$, $RORα$, and $NPAS2$. The relationships between each circadian gene and the prognosis of NSCLC patients are presented in Table 1.

### Abnormal Circadian Gene Expression Is Closely Associated With Lung Cancer Progression

Several studies have shown that the abnormal expression of circadian genes might be closely related to the progression of lung cancer. The role of the aberrant expression of 15 circadian genes, including $TIMELESS$ and $BMAL-1$, in lung cancer progression and the underlying mechanisms, has been summarized below (Figure 2).

#### TIMELESS

$TIMELESS$ ($TIM$) gene is located on 12q13.3. Several retrospective clinical analyses have reported high $TIMELESS$ expression in lung cancer suggests hypodifferentiation, late-stage, and poor prognosis. In cellular experiments, however, $TIM$ deletion may promote the cytogensis and progression of lung cancer by significantly accelerating the proliferation of lung cancer cells and by inhibiting their apoptosis. The underlying mechanism by which $TIM$ deletion increases the proliferative capacity of lung cancer cells remains unclear.

Smith et al. discovered that the complex makeup of $TIMELESS$ and $TIMELESS$ interacting protein Tipin (Tim- Tipin) played an important role in DNA replication and genome stabilization; Tim-Tipin deletion can result in the abnormal aggregation of single-stranded DNA at replication forks and affect normal DNA replication. At this time, the cell can maintain the DNA replication process only via the activation of the ATR-Chk1 signaling pathway. When both Tim-Tipin and ATR are absent, phosphorylation of histone (H2AX) phosphorylation increases, resulting in DNA double-strand breaks, thus blocking DNA replication in the S-phase. Tim and Tipin can inhibit DNA replication by suppressing excessive fork rotation and also prevent DNA damage during DNA replication by inhibiting excessive fork rotation and DNA precatenation, thus maintaining stability of DNA replication. Simultaneously, $TIM$ deletion can also increase the sister chromatid exchange by 3- to 4-fold during DNA synthesis, signifying the role of $TIM$ in maintaining genomic stability during DNA replication. Hence, we hypothesized that $TIM$ deletion might result in a higher probability of damage and sister chromatid exchange during DNA replication and lower stability, promoting carcinogenesis.

$TIMELESS$ is required to ligate the CMG helicase complex (CDC45/MCM2–7/GINS helicase complex) to DNA polymerase, and $TIMELESS$ deletion can lead to the aggregation of abnormal CMG helicase complexes, affecting DNA synthesis. $TIMELESS$ deletion inhibits the stable chromatin binding. Tipin was identified as a substrate for cyclin E/cytosolic protein-dependent kinase 2 in African clawed toads. Additionally, poly(ADP-ribose)polymerase1 (PARP1) binding to certain substrates and its complementation of DNA damage is impaired by $TIMELESS$ knockdown, and $TIMELESS$ silencing significantly impairs DNA double-strand break repair. The deletion of $TIMELESS$ genes might affect DNA synthesis, stimulating tumorigenesis.
| Gene name    | Author                  | Object of study                  | Gene expression | Tumor specimens | Differentiation | T stage | LN involvement | Distant metastatic status | TNM stage | Prognosis |
|--------------|-------------------------|----------------------------------|-----------------|-----------------|----------------|---------|-----------------|----------------------------|------------|-----------|
| TIMELESS     | Zhang et al[25]         | Human tumor specimens            | N/A             | H               | Poorer         | Positive | Positive        | Positive                    | N/A        | Poorer    |
|              | Yoshida et al[27]       | Human tumor specimens            | L               | L               | Poorer         | Positive | Positive        | Positive                    | N/A        | Poorer    |
| PER1         | Qiu et al[10]           | TCGA                             | H (in ADC cancer) | H (in ADC cancer) | Poorer         | Positive | Positive        | Positive                    | N/A        | Poorer    |
|              | Liu et al[24]           | Human tumor specimens            | N/A             | L               | Poorer         | Negative | Negative        | Negative                    | N/A        | Poorer    |
| PER2         | Qiu et al[10]           | TCGA                             | H (in SCC cancer) | H (in SCC cancer) | Poorer         | Negative | Negative        | Negative                    | N/A        | Poorer    |
|              | Liu et al[24]           | Human tumor specimens            | N/A             | L               | Poorer         | Negative | Negative        | Negative                    | N/A        | Poorer    |
| PER3         | Liu et al[26]           | Human tumor specimens            | N/A             | L               | Poorer         | Negative | Negative        | Negative                    | N/A        | Poorer    |
| CLOCK        | Qiu et al[10]           | TCGA                             | No significance | No significance | N/A            | N/A     | N/A             | N/A                        | N/A        | N/A       |
| BMAL1        | Qiu et al[10]           | TCGA                             | No significance | No significance | N/A            | N/A     | N/A             | N/A                        | N/A        | N/A       |
| ARNTL2       | Brady et al[28]         | Human tumor specimens/animal experiment | No significance | No significance | N/A            | N/A     | N/A             | N/A                        | N/A        | Poorer    |
| NPAS2        | Qiu et al[10]           | TCGA                             | L               | L               | N/A            | N/A     | N/A             | N/A                        | N/A        | N/A       |
|              | Gao et al[32]           | Animal experiments               | N/A             | L               | N/A            | N/A     | N/A             | N/A                        | N/A        | N/A       |
| DEC1         | Giatromanolaki et al[27] | Human tumor specimens            | N/A             | L               | Positive       | N/A     | N/A             | Positive                    | N/A        | Better    |
|              | Liu et al[51]           | Human tumor specimens            | N/A             | L               | N/A            | N/A     | N/A             | Positive                    | N/A        | Better    |
| DEC2         | Qiu et al[10]           | TCGA                             | No significance | No significance | N/A            | N/A     | N/A             | N/A                        | N/A        | Poorer    |
| RORγ2        | Qiu et al[10]           | TCGA                             | H (in ADC cancer) | H (in ADC cancer) | N/A            | N/A     | N/A             | N/A                        | N/A        | Poorer    |
| RORγ1        | Huang et al[33]         | Human tumor specimens            | L               | L               | N/A            | N/A     | N/A             | Negative                    | N/A        | Better    |
| CRY2         | Qiu et al[10]           | TCGA                             | H               | L               | N/A            | N/A     | N/A             | Negative                    | N/A        | Better    |
| CRY1         | Qiu et al[10]           | TCGA                             | H               | H               | No significance | N/A     | N/A             | Negative                    | N/A        | Better    |
| NR1D1        | Qiu et al[10]           | TCGA                             | L               | L               | N/A            | N/A     | N/A             | Negative                    | N/A        | Better    |

Abbreviations: ADC, adenocarcinoma; SCC, squamous cell carcinoma; BMAL1 = ARNTL1; L, low expression; H, high expression; N/A, not applicable.
Conversely, increased levels of TIMELESS expression can protect lung cancer cells from oncogene-directed replicative stress and inhibit their intrinsic negative feedback mechanisms, thus promoting cancer progression. \(^{40}\) TIMELESS knockdown reduces the cancer cell proliferation rate significantly and may lead to apoptosis caused by impaired intra-S checkpoints as well as induced apoptosis, which inhibits the proliferation and clonal growth of H157 and H460 cells. \(^{29}\) The TIMELESS expression shows contradictory effects on lung cancer, suggesting more precise research to determine the relationship between their abnormal alterations and lung carcinogenesis and the underlying mechanisms.

BMAL1

BMAL1 is also recognized as brain and muscle ARNT-like protein 1 (BMAL1/ARNTL-1), and its gene is located at 11p15.3. The protein BMAL1 always forms heterodimers with CLOCK and NPAS2 (CLOCK-BMAL1, NPAS2-CLOCK), working as 1 heterodimer. A controlled study of 409 lung cancer patients and 417 normal subjects in a northeastern Chinese population reported that BMAL1 single-nucleotide polymorphisms were strongly related to lung cancer. The rs3816360 heterozygous CT genotype and variant pure CC genotype in BMAL1 are connected with a significantly increased risk of lung cancer versus the wild-type pure TT genotype. For rs2290035, an increased risk of lung adenocarcinoma was associated with those carrying the AA genotype, considering the TT genotype as the reference group. \(^{41}\) Meanwhile, intracellular studies have suggested that BMAL1 may inhibit the ability of the Bcl-w oncogene to activate the PI3K-Akt-MMP-2 pathway and attenuate the ability of Bcl-w to promote MMP-2 aggregation and A549 cell invasion, thereby inhibiting lung cancer cell growth and invasion. \(^{42}\) BMAL1/PER2 can synergize with KRAS and P53 mutations, promoting lung carcinogenesis. For simultaneous mutations in KRAS and P53, a simple alteration of the photoperiod to physiologically disrupt circadian rhythms can accelerate lung cancer development. The numbers of tumor cells are significantly increased in animals with BMAL1 knockdown. In Kras\(^{L/A2}\) lung cancer cells, BMAL1 mutations increase the number of lung cancer cells and decrease survival. Furthermore, the loss of BMAL1 in K-ras\(^{LSL-G12D}\) tumor cells accelerates lung cancer cell proliferation. In KP (K-ras\(^{LSL-G12D}\) and p53\(^{lox/lox}\)) tumor cells, that is, cells with KRAS mutation and P53 deletion, tumor load is not increased after BMAL1 knockdown, signifying a possible P53-dependent role of BMAL1 deletion in promoting lung cancer. \(^{43}\) BMAL1 deletion can also increase

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**Figure 2.** The association between circadian genes and lung cancer.

Abbreviations: (−), gene downregulation or gene deletion; PI3K-Akt-mTOR, phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mechanistic target of rapamycin (mTOR) signaling pathway; PI3K-Akt-MMP-2, phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/matrix metalloproteinase-2 (MMP-2) signaling pathway; VEGF, vascular endothelial growth factor; VEGF-C, vascular endothelial growth factor-c; PARP1, poly (ADP-ribose) polymerase 1; CGM, CDC45/MCM2-7/GINS; RHOA-ROCK-CFL, Ras homolog family member A (ROHA)-Rho-associated coiled-coil containing kinase (ROCK)-Actin-depolymerizing factor (CFL); GSK3β, glycogen synthase kinase 3β; FBXW7, F-box and WD40 repeat domain-containing 7.
c-myc transcriptional output, thus promoting lung cancer cell proliferation. Therefore, BMAL1 deletion may affect or synergize with KRAS and cancer regulatory genes such as P53, c-myc, and Bcl-w to promote lung carcinogenesis.

**PERIOD Family**

The PERIOD (PER) family comprises 3 genes, PER1, PER2, and PER3, located at 17p13.1, 2q37.3, and 1p36.22. The PER2 gene is positively correlated. PER1 inhibits the growth of NSCLC cells and their clonal proliferation ability, and DNA hypermethylation and histone H3 acetylation. Deletion of PER2 function, an anti-oncogene, accelerates tumor progression and reduces tumor DNA damage repair. c-myc expression in tumor cells increases after PER2 knockdown, enhancing lung cancer proliferation. PER2 also decreased the activity of PI3K/AKT/mTOR signaling pathway, promoting apoptosis in lung adenocarcinoma cells. Overexpression of PER3 inhibits the proliferation of NSCLC, induces apoptosis, and suppresses the migration and invasion abilities of cells. Furthermore, PER3 single nucleotide polymorphisms are strongly related to lung cancer, and the risk of lung cancer is higher in T/T pure individuals with single nucleotide polymorphism (SNP) (rs228729). Hence, deletion of the PER family genes may increase the proliferation and invasion abilities of lung cancer cells and inhibit their apoptosis. Single-nucleotide polymorphisms in the PER gene may be associated with a high risk of lung cancer.

**CLOCK**

The Circadian locomotor output cycles kaput (CLOCK) encoding gene is located at 4q12. The CLOCK forms heterodimers with BMAL1, and CLOCK-BMAL1 heterodimers drive the positive component of transcriptional oscillations. The deletion of CLOCK may hinder the proliferation, migration, and invasion abilities of lung cancer cells. Jiang et al. found that after CLOCK knockdown in A549 and H1299 spherical cells, the Wnt/β-catenin protein pathway was significantly activated, and the expression levels of the proteins β-catenin and GSK-3β reduced, thereby inhibiting the proliferation of lung cancer cells. Meanwhile, the number of lung tumor stem cells was reduced significantly, and the sphere-forming ability of lung cancer A549 and H1299 cell lines was also decreased.

**NPAS2**

The gene encoding neuronal PAS domain protein 2 (NPAS2)—also identified as MOP4—is located on 2q11.2. NPAS2 and BMAL1 polypeptides also form heterodimeric transcription factor to regulate positively clock gene expression. The role of NPAS2 in lung cancer is still not clear. NPAS2 expression and BAML-1-related signaling pathways are significantly controlled by indole-3-carbinol (I3C) and/or silibinin (Sil) + I3C. Contrary to NNK 4-(methylnitroso)-1-(3-pyridyl)-1-butanone-induced lung cancer cells, inflammation-driven lung adenocarcinoma cells show a significantly low expression of NPAS2. This might play a role in promoting inflammation-driven lung tumorigenesis; however, its role and mechanism remain unclear. Studies with breast cancer cell line MCF-7 and colon cancer cell line HCT-15 have reported that reduced NPAS2 expression can affect DNA repair capacity, cell cycle checkpoints, and inhibit the DNA damage response to cell proliferation, making it easier to enter the next cycle to promote tumor proliferation. Therefore, NPAS2 deletion might promote...
tumor cell proliferation via its effect on the cell cycle. Conversely, NSCLC patients with low Npas2 expression had a better prognosis in clinical studies.\(^{30,32}\) Such contradictory outcomes indicate that the role of Npas2 in lung cancer is unclear, and the mechanisms behind it require further exploration.

**DEC Family**

The differentiated embryo-chondrocyte expressed gene (DEC) family contains genes expressed by differentiated embryonic chondrocyte cells, with DEC1 located on 3p26.1 and DEC2 on 12p12.1. DEC1, a negative transcriptional regulator of DEC2, mainly binds via the E-box of DEC2 proximal promoter to negatively regulate DEC2 expression.\(^{53}\) DEC2 displays low expression in lung cancer, whereas DEC1 expression is significantly increased in cancer.\(^{53}\) DEC1 may downregulate hypoxia-inducible factor-1α (HIF1α) in A549 in response to hypoxia. In lung cancer cells, the gene and protein levels of HIF1α in A549 might be downregulated during hypoxia and act as an inhibitor of apoptosis.\(^{27,54}\) However, Liu et al\(^{31}\) and Giatromanolaki et al\(^{27}\) showed contradictory results with DEC1 showing low expression in lung cancer. After DEC1 knockdown, the proliferation of lung cancer cells was reduced, significantly increasing after overexpression. Loss of DEC1 can lead to the upregulation of cyclin D1, while upregulating cyclin D1 can stimulate tumorogenesis progression in NSCLC.\(^{31,55,56}\)

**CRY Family**

The cryptochrome (CRY) family genes include CRY1 and CRY2 at 12q23.3 and 11p11.2, respectively. CRY2 stimulates c-myc degradation and synergizes with F-box and leucine-rich repeat protein 3 to co-promote c-myc degradation.\(^{57}\) Downregulation of CRY2 increases c-myc expression to promote lung cancer cell growth.\(^{58}\) However, the underlying mechanism is still not clear. As a result of CRY2 knockdown in osteosarcoma, the S-phase cell population increases, the G1-phase cell population decreases, P53 expression decreases, c-myc and cyclin D1 expression increases, and the phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 increases without altering the phosphorylation of c-Jun N-terminal kinase (JNK) and P38. CRY2 knockdown enhances the expression of matrix metalloproteinase (MMP)-2 and β-catenin and increases the proliferation and migration of osteosarcoma cells by promoting cell cycle progression and inducing mitogen-activated protein kinase (MAPK) and Wnt/β-catenin signaling pathways.\(^{59}\) CRY2 deletion might reduce the degradation of c-myc and accordingly promote tumor cell proliferation by inducing cell cycle progression, which may be a potential mechanism for its deletion to promote lung cancer cell proliferation.

**RORα/RORγ**

ROR family genes encode the retinoic acid receptor-related orphan receptors, with RORα (RORA) located on 15q22.2 and RORγ (RORC) located on 1q21.2–22. In lung cancer, RORγ expression is increased and is positively correlated with IL-17 expression. Its relation with lung cancer progression might be associated with the upregulation of Th17 cells,\(^{33,60}\) which secrete IL-17A that activates tumor-associated macrophages in NSCLC and drives tumor progression by inducing angiogenesis and lymphangiogenesis and directly inducing tumor cell growth. The Th17 effector cytokine IL-22 might also directly stimulate the proliferation of NSCLC cells.\(^{61}\) Studies on the role of RORα in lung cancer are limited, with some indicating that RORα may induce apoptosis in lung epithelial cells,\(^{62}\) and its high expression may offer a better prognosis.\(^{30}\)

**Other Genes**

The role of other circadian genes in lung cancer is not well understood. Some genes, such as CSNK1D (CK1δ) (encoding casein kinase 1δ), located on 17q25.3, have defective CK1δ-mediated phosphorylation, leading to the disruption of the associated CK1δ/GSK3β/FBXW7α axis-regulated ZNF322A oncoprotein, which can result in ZNF322A overexpression and stimulate lung cancer progression.\(^{53}\)

**Conclusions and Future Directions**

Circadian genes are essential components of the multi-feedback loop of the regulatory system of organisms and are associated with the clinicopathological features of NSCLC, playing an important role in its growth, invasion, and metastasis. Circadian genes can promote lung carcinogenesis via numerous pathways, comprising the c-myc and regulation of metastatic factors, immune cells, and cell cycle proteins. Thus, they may serve as potential biomarkers and therapeutic targets for lung cancer. Targeting of circadian genes has also been reported in relation to other tumors; for example, inhibitors to suppress RORγ can hinder tumor growth and improve the survival of pancreatic cancer patients.\(^{64}\) There is still a need for further study in order to determine whether targeting circadian genes in lung cancer can improve the therapeutic effect.

As a consequence of irregular circadian gene expression and circadian rhythm disorders, there is a higher incidence of lung cancer among smokers who have circadian rhythm disorders. Further studies using a larger sample size and more detailed stratified analysis are required to validate whether circadian rhythm disorders and circadian genetic abnormalities increase the risk of lung cancer, find specific pathways of biological clock genes acting on lung cancer, as well as for the exploration of the underlying mechanisms.
to provide effective theoretical support for the primary prevention of lung cancer.

In conclusion, circadian genes are closely connected to physiological functions like sleep and metabolism in humans, and their abnormal expression might promote lung cancer progression. Circadian rhythm disorders and abnormal circadian genes could contribute to lung development in a population. Further research on circadian clock genes will result in new targets for lung cancer treatment and theoretical support for the primary prevention of lung cancer.

Author Contributions
Hao Zhang: conceptualization, data selection, project administration, writing—original draft, writing—review and editing. Renwang Liu: conceptualization, data selection, writing—original draft. Bo Zhang: conceptualization. Huandong Huo: conceptualization. Zuoqing Song: writing—review and editing.

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ORCID iD
Hao Zhang https://orcid.org/0000-0003-4732-8049

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