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

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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), DEC1, 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

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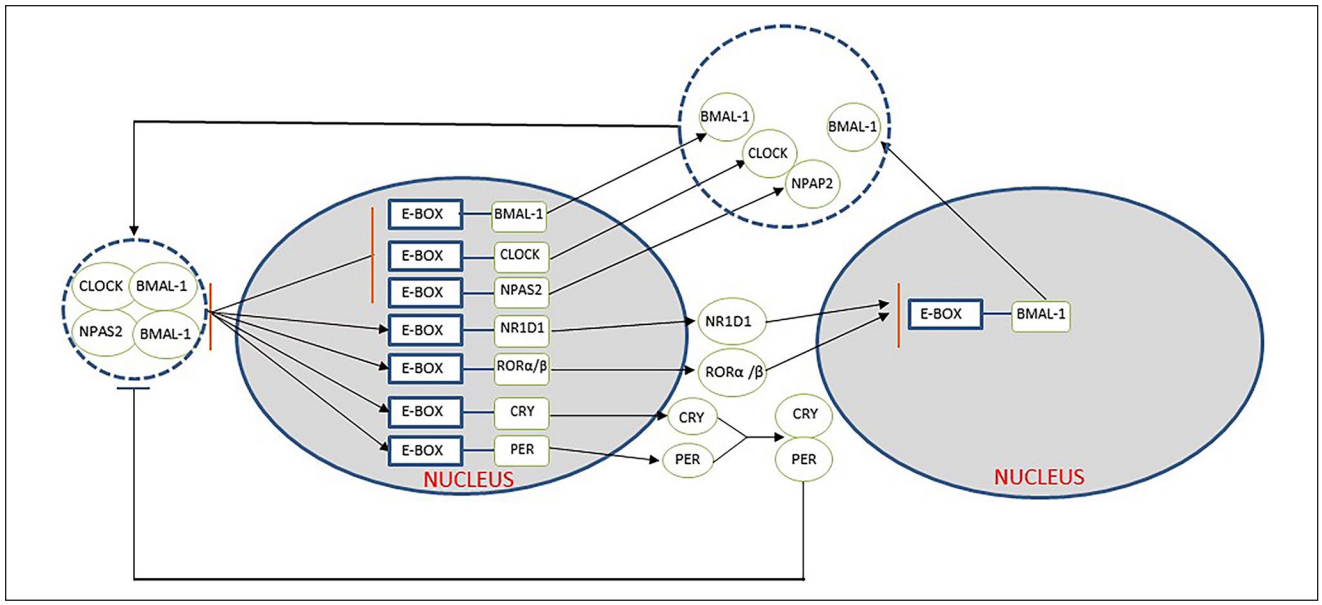

Figure 1. Hypothesized models of circadian rhythm genes in mammals. The *CLOCK* and *NPAS2* form heterodimers with *BMAL1*. These heterodimers act as enhancer e-box elements upstream of transcription factors binding target genes to activate transcription of other core circadian genes like the *PER* family (*PER1, PER2, PER3*) and *CRY* family (*CRY1, CRY2*).[20,21] *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.[22,23] Heterodimers of *PER* and *CRY* proteins activate a negative feedback loop that acts directly on *CLOCK* and *NPAS2*[22,24]

poor prognosis.[13] 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.[15] In colon cancer, high *CLOCK* expression is associated with better overall survival (OS)[12]; however, it promotes tumor cell proliferation by regulating iron metabolism in colon cancer cells.[10] Furthermore, high *CLOCK* expression inhibits the apoptosis of colon cancer cells in vitro.[16] 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.[17] 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.[18,19] 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, RORα*, and *RORγ*. We also observed the current status of their research in lung cancer to provide directions and ideas for future studies.

Circadian clock genes interact with each other forming a circadian gene network, and the circadian system is based on an autonomous transcriptional autoregulatory feedback loop within an activating unit (*CLOCK, NPAS2*, and *ARNTL [BMAL1]*) and a repressing unit (*PER* and *CRY*) (Figure 1).

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[25] 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[26] discovered that *PER1, PER2*, and *PER3*-deficient NSCLC patients had a low degree of differentiation.

Giatromanolaki et al[27] collected data from 115 NSCLC patients and presented that patients with low *DEC1* expression had a low degree of differentiation. In the same way, Brady et al[28] 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.[25] However, it has also been shown that *TIMELESS* does not correlate with the TNM stage significantly.[29,30] 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.[26] Furthermore, Liu et al[31] 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.[27,30] 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, DEC1, BMAL1, ARNTL-2, NPAS2, CRY2, RORα*, and *RORγ* are associated with patient prognosis.[25-33] Patients with high *TIMELESS* expression have a considerably shorter OS and poorer prognosis,[29,30] and patients with *PER1, PER2*, and *PER3* deletion also show lower OS.[26] However, it has been revealed that the expression of *PER* family does not correlate with lung cancer patients' prognosis significantly.[30] A database-based study presented that high *DEC1* expression corresponded with a poor prognosis in NSCLC patients.[30] However, it has also been shown that the expression of the *DEC* family does not correlate with lung cancer prognosis significantly.[27] Thus, whether the *PER* family proteins and *DEC1* 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*.[30] 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 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 that high *TIMELESS* expression in lung cancer suggests hypodifferentiation, late-stage, and poor prognosis.[25,29,30] In cellular experiments, however, *TIM* deletion may promote the cytogenesis and progression of lung cancer by significantly accelerating the proliferation of lung cancer cells and by inhibiting their apoptosis.[29] The underlying mechanism by which *TIM* deletion increases the proliferative capacity of lung cancer cells remains unclear.

Smith et al.[34] 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 sustaining the stability of DNA replication.[35] 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.[36] 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.[37,38] *TIMELESS* deletion inhibits the stable chromatin binding.[38] Tipin was identified as a substrate for cyclin E/cytosolic protein-dependent kinase 2 in African clawed toads.[38] 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.[39]

Table 1. Associations Between Circadian Genes and the Degree of Differentiation of Lung Cancer Tissues.

Gene name	Author	Object of study	Gene expression				Differentiation	T stage	LN involvement	Distant metastatic status	TNM stage	Prognosis
			Adjacent normal tissues		Tumor specimens							
			mRNA	Protein	mRNA	Protein						
TIMELESS	Zhang et al[25]	Human tumor specimens	N/A	N/A	H	H	Poorer	Positive correlation	Positive correlation	N/A	Positive correlation	Poorer
	Yoshida et al[29]	Human tumor specimens	L	L	H	H	N/A	N/A	N/A	N/A	No significance	Poorer
	Qiu et al [30]	TCGA	L	L	H	H	N/A	N/A	N/A	N/A	No significance	Poorer
PER1	Qiu et al[30]	TCGA	H (in ADC cancer)	H (in ADC cancer)	H (in ADC cancer)	H (in ADC cancer)	N/A	N/A	N/A	N/A	N/A	No significance
	Liu et al[26]	Human tumor specimens	N/A	N/A	L	L	Poorer	Negative correlation	Negative correlation	N/A	Negative correlation	Poorer
PER2	Qiu et al[30]	TCGA	H (in SCC cancer)	H (in SCC cancer)	H (in SCC cancer)	H (in SCC cancer)	N/A	No significance	No significance	No significance	N/A	No significance
	Liu et al[26]	Human tumor specimens	N/A	N/A	L	L	Poorer	Negative correlation	Negative correlation	N/A	Negative correlation	Poorer
PER3	Liu et al[26]	Human tumor specimens	N/A	N/A	L	L	Poorer	Negative correlation	Negative correlation	N/A	Negative correlation	Poorer
	Qiu et al[30]	TCGA	No significance	No significance	No significance	No significance	N/A	N/A	N/A	N/A	N/A	No significance
CLOCK	Qiu et al[30]	TCGA	H (in SCC cancer)	H (in SCC cancer)	H (in SCC cancer)	H (in SCC cancer)	N/A	N/A	N/A	N/A	N/A	No significance
BMAL1	Qiu et al[30]	TCGA	No significance	No significance	No significance	No significance	N/A	N/A	N/A	N/A	No significance	Poorer
ARNTL2	Brady et al[28]	Human tumor specimens/ animal experiment	H	H	H	H	Negative correlation	N/A	Positive correlation	Positive correlation	N/A	Poorer
NPAS2	Qiu et al[30]	TCGA	L	L	L	N/A	N/A	N/A	N/A	N/A	No significance	Better
	Gao et al[32]	Animal experiments	N/A	N/A	L	L	N/A	N/A	N/A	N/A	N/A	Better
DEC1	Giatromanolaki et al [27]	Human tumor specimens	N/A	N/A	L	L	Positive correlation	N/A	N/A	N/A	No significance	No significance
	Liu et al[31]	Human tumor specimens	N/A	N/A	L	L	Poorer	No significance	No significance	N/A	Negative correlation	N/A
	Qiu et al[30]	TCGA	No significance	No significance	No significance	No significance	N/A	N/A	N/A	N/A	No significance	Poorer
DEC2	Qiu et al[30]	TCGA	No significance	No significance	No significance	No significance	N/A	N/A	N/A	N/A	N/A	No significance
RORα	Qiu et al[30]	TCGA	H (in ADC cancer)	H (in ADC cancer)	H (in ADC cancer)	H (in ADC cancer)	N/A	N/A	N/A	N/A	Negative correlation	Better
RORγ2	Huang et al[33]	Human tumor specimens	L	L	H	H	N/A	No significance	Negative correlation	No significance	No significance	Poorer
CRY2	Qiu et al[30]	TCGA	H	H	L	L	N/A	N/A	N/A	N/A	Negative correlation	Better
CRY1	Qiu et al[30]	TCGA	H	H	H	H	No significance	N/A	N/A	N/A	N/A	No significance
NR1D1	Qiu et al[30]	TCGA	L	L	L	L	N/A	N/A	N/A	N/A	N/A	Better

Abbreviations: ADC, adenocarcinoma; SCC, squamous cell carcinoma; BMAL1 = ARNTL1; L, low expression; H, high expression; N/A, not applicable.

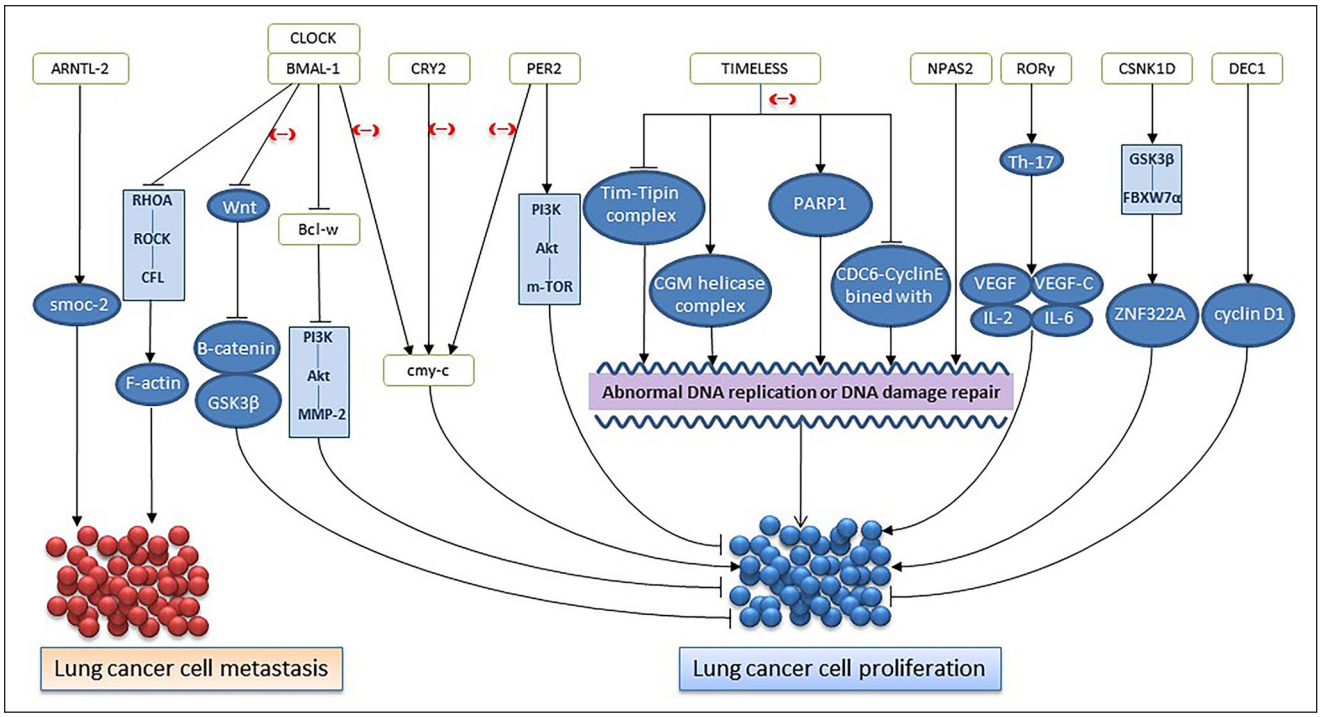

Figure 2. The association between circadian genes and lung cancer.
Abbreviations: (–), gene downregulation or gene deletion; PI3K-Akt-mTOR, phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mechanistic target of rapamycin (mTOR) signaling pathway; PI3K-Akt-MMP-2, phosphoinositide 3-kinase (PI3K)/protein 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.

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 $Kras^{LA2/+}$ 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 $p53^{flox/flox}$) 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

c-myc transcriptional output, thus promoting lung cancer cell proliferation.[43] 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.[26] *PER1* inhibits the growth of NSCLC cells and their clonal proliferation ability, and DNA hypermethylation and histone H3 acetylation.[44] Deletion of *PER2* function, an anti-oncogene, accelerates tumor progression and reduces tumor DNA damage repair.[45] *c-myc* expression in tumor cells increases after *PER2* knockdown, enhancing lung cancer proliferation.[43] *PER2* also decreased the activity of PI3K/AKT/mTOR signaling pathway, promoting apoptosis in lung adenocarcinoma cells.[46] Overexpression of *PER3* inhibits the proliferation of NSCLC, induces apoptosis, and suppresses the migration and invasion abilities of cells.[47] 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).[48] 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.[20] 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 inactivated, 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.[49] CLOCK-BMAL1 also medicates the downregulation of RHOA-ROCK-CFL pathway expression, alters F-actin/G-actin conversion, causes F-actin aggregation, and promotes cancer cell proliferation, migration, and invasion.[50] The specific mechanisms by which *CLOCK* might influence lung cancer cell proliferation and invasion remain unclear.

ARNTL-2

Brain and muscle ARNT-like protein 2 (*ARNTL-2*) encoding gene is located on 12p11.23 and is likely related to the metastatic capacity of lung cancer. Brady et al[23] reported that *ARNTL-2* was highly expressed in metastatic lymph nodes and distant metastatic lung cancer tissues, and those *ARNTL-2*-regulated secretory factors like Wnt5a, IL-11, and Cxcl5 were more highly expressed in the cell lines of metastatic origin. For that reason, *ARNTL-2* might be closely associated with metastasis in lung cancer. This study also knocked down *ARNTL-2* in lung adenocarcinoma H1792 cells and discovered that it could significantly reduce their ability to form colonies. Meanwhile, Smoc2, a member of the secreted protein acidic and rich in cysteine family, is a potential regulator of metastatic capacity promoting lung cancer cell metastasis in vivo. In cells with *ARNTL-2* knockdown, its transcript levels are significantly downregulated. Additionally, it is found in animal experiments that lung and liver metastases were significantly reduced in *ARNTL-2*-deficient homozygous immunoreactive receptor mice. Hence, it was hypothesized that *ARNTL-2* aberrant expression might promote the distant metastasis of lung cancer. *ARNTL-2* could also synergize with *CLOCK* to stimulate the distant metastasis of lung cancer. *CLOCK* knockdown resulted in a significant decrease in the mRNA transcription of *ARNTL-2*, thereby decreasing the protein expression in cells. Lung cancer cell colonies are significantly reduced after simultaneous knockdown of *CLOCK* and *ARNTL-2*, suggesting that the 2 may act synergistically to promote the distant metastasis of lung cancer.[28]

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-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung cancer cells, inflammation-driven lung adenocarcinoma cells show a significantly low expression of *NPAS2*.[51] 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.[52] 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 tumorigenesis 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, *RORγ2* 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 low *CK1*δ-mediated phosphorylation, leading to the disruption of the associated *CK1*δ/GSK3β/FBXW7α axis-regulated ZNF322A oncoprotein, which can result in ZNF322A overexpression and stimulate lung cancer progression.[63]

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, supervision.

Declaration of Conflicting Interests

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