Postprint: Causal Relationship between Circadian Rhythm Disruption and Lung Cancer Risk and Prediction of Interventional Traditional Chinese Medicines

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Abstract

Background Lung cancer, as a malignant tumor with high incidence and mortality rates, has become a major research focus in the academic community. Circadian rhythm disruption (CRD) is considered an important risk factor for lung cancer occurrence, but the causal association between the two remains unclear. Objective To investigate the causal relationship between circadian rhythm disruption and lung cancer risk and the potential mechanisms, and to predict potential intervening Chinese medicines. Methods Genome-wide association study (GWAS) data for daytime napping, daytime sleepiness, short sleep duration, long sleep duration, chronotype, insomnia, and early rising were obtained from UKBiobank and other websites, and GWAS data for overall lung cancer, lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and small cell lung cancer (SCLC) were obtained from the IEU OpenGWAS database. Mendelian randomization (MR) analysis was performed, with the inverse variance weighted (IVW) method used as the primary analytical approach to evaluate the causal relationship between circadian rhythm disruption and lung cancer occurrence. Single nucleotide polymorphisms (SNPs) were mapped, and core genes were screened through protein-protein interaction (PPI) network analysis, followed by functional enrichment analysis and survival analysis. The core genes were uploaded to the Coremine database to search for Chinese medicines with potential intervention effects, and the properties, flavors, and meridian tropism of the medicines were analyzed, with core Chinese medicines screened out. The key components of the core Chinese medicines were obtained using the TCMSP and BATMAN-TCM databases, and molecular docking was employed to verify the binding capacity of the key components of the potential Chinese medicines with

the core genes. Results Insomnia (OR=1.149, 95%CI=1.074-1.232, P=0.013) and short sleep duration (OR=1.462, 95%CI=1.033-2.061, P=0.031) showed causal associations with lung cancer; insomnia (OR=1.181, 95%CI=1.061-1.322, P=0.001), short sleep duration (OR=1.563, 95%CI=1.024-2.401, P=0.038), and daytime sleepiness (OR=4.033, 95%CI=1.062-15.434, P=0.042) with LUAD; insomnia (OR=1.152, 95%CI=1.028-1.281, P=0.001) with LUSC; and short sleep duration (OR=1.952, 95%CI=1.120-3.383, P=0.017) with SCLC. A total of 139 core genes were screened, mainly enriched in G protein-coupled receptor (GPCR), mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt) pathways. The top 5 core genes included: histone acetyltransferase p300 (EP300), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), G protein subunit beta 1 (GNB1), G protein subunit gamma 13 (GNG13), and tumor necrosis factor (TNF). Among them, EP300 expression level was positively correlated with survival time in LUAD, LUSC, and SCLC patients (P<0.05); CNB1 expression level was negatively correlated with survival time in LUAD patients and positively correlated with survival time in SCLC patients (P<0.05); GAPDH, GNG13, and TNF expression levels were negatively correlated with survival time in LUAD patients (P<0.05). A total of 40 Chinese medicines were screened, including buffalo horn, Salvia miltiorrhiza, fish brain stone, Scutellaria baicalensis, Curcuma aromatica, Astragalus membranaceus, Ganoderma lucidum, Panax ginseng, etc. The medicinal flavors were mainly sweet, followed by bitter and pungent; the medicinal properties were mainly cold, followed by warm and neutral; the meridian tropism mainly belonged to liver, spleen, lung, stomach, heart, and kidney meridians; and the efficacies were mainly tonifying qi, activating blood and resolving stasis, and clearing heat and detoxifying. Eight core Chinese medicines were screened: buffalo horn, Salvia miltiorrhiza, Panax notoginseng, Scutellaria baicalensis, Curcuma aromatica, Curcuma longa, corn silk, and Ganoderma lucidum. Molecular docking showed that the key components had good binding efficacy with the core target genes (binding energy <-4kcal/mol). Conclusion Circadian rhythm disruption has a causal relationship with lung cancer, and its mechanism of action is related to GPCR, MAPK, PI3K/Akt pathways. The predicted Chinese medicines can provide a reference basis for the prevention and treatment of lung cancer by regulating circadian rhythm with traditional Chinese medicine.

Full Text

Exploring the Causal Relationship between Circadian Rhythm Disorders and Lung Cancer and Potential Interventional Traditional Chinese Medicine

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Abstract

Background Lung cancer is a leading cause of morbidity and mortality, making its prevention and treatment a key focus of research. Circadian rhythm disruption (CRD) is considered an important risk factor for lung cancer, but the causal relationship between CRD and lung cancer remains unclear.

Objective To investigate the causal relationship and potential mechanisms between circadian rhythm disruption and lung cancer risk, and to predict potential Traditional Chinese Medicine (TCM) interventions.

Methods GWAS data on daytime napping, daytime sleepiness, short and long sleep duration, chronotype, insomnia, and early waking were obtained from UK Biobank and other sources, while lung cancer-related data, including overall lung cancer, lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and small cell lung cancer (SCLC), were collected from the IEU Open GWAS database. Mendelian randomization (MR) analysis was conducted using the inverse-variance weighted (IVW) method to evaluate the causal relationship between circadian rhythm disruption and lung cancer, with supplementary methods used to confirm result robustness. Core genes were identified through SNPs localization and PPI network analysis, followed by functional enrichment and survival analysis. Core genes were then uploaded to the Coremine database to identify TCMs with potential intervention effects. The properties, flavors, meridian tropism, and efficacy of the TCMs were cataloged. Core TCMs were then selected, and their key components were obtained from the TCMSP and BATMAN-TCM databases. Molecular docking was used to validate the binding ability of the key components of the core TCMs to the core genes.

Results Insomnia (OR=1.149, 95%CI=1.074-1.232, P=0.013) and short sleep duration (OR=1.462, 95%CI=1.033-2.061, P=0.031) showed a causal relationship with lung cancer. Insomnia (OR=1.181, 95%CI=1.061-1.322, P=0.001), short sleep duration (OR=1.563, 95%CI=1.024-2.401, P=0.038), and daytime sleepiness (OR=4.033, 95%CI=1.062-15.434, P=0.042) were causally linked to LUAD, while insomnia (OR=1.152, 95%CI=1.028-1.281, P=0.001) was linked to LUSC and short sleep duration (OR=1.952, 95%CI=1.120-3.383, P=0.017) to SCLC. A total of 139 core genes were identified, enriched in pathways such as GPCR, MAPK, and PI3K/Akt. The top five core genes included EP300, GAPDH, GNB1, GNG13, and TNF. EP300 expression correlated positively with survival in LUAD, LUSC, and SCLC patients (P<0.05). GNB1 expres-

sion negatively correlated with survival in LUAD patients but positively with survival in SCLC patients (P<0.05). GAPDH, GNG13, and TNF expressions negatively correlated with survival in LUAD patients (P<0.05).

Forty TCMs, including Shui Niu Jiao, Dan Shen, Yu Nao Shi, Huang Qin, Yu Jin, Huang Qi, Ling Zhi, and Ren Shen were predicted and screened, with sweet flavor being predominant, followed by bitter and pungent, and cold nature being dominant, followed by warm and neutral. The TCMs mainly targeted the liver, spleen, lungs, stomach, heart, and kidney meridians, with functions focused on tonifying qi, promoting blood circulation, and clearing heat and toxins. Eight key herbs (Shui Niu Jiao, Dan Shen, San Qi, Huang Qin, Yu Jin, Jiang Huang, Yu Mi Xu, Ling Zhi) showed excellent molecular docking affinity (binding energy<-4 kcal/mol) with core target genes.

Conclusion The findings suggest a causal relationship between circadian rhythm disruption and lung cancer, with potential mechanisms involving pathways such as GPCR, MAPK, and PI3K/Akt. The predicted TCMs offer new insights for using Chinese medicine to regulate circadian rhythms and prevent lung cancer.

Keywords Circadian rhythm disorders; Lung cancer; Causal relationship; Mendelian randomization; Bioinformatics; Traditional Chinese medicine prediction

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Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide. According to the International Agency for Research on Cancer (IARC), there were 2.481 million new lung cancer cases and 1.817 million lung cancer deaths globally in 2022. Early prevention represents an effective strategy to save lives and reduce healthcare costs. While smoking has been established as a primary risk factor for lung cancer and smoking cessation effectively reduces risk, the increasing incidence of lung cancer among non-smokers has prompted researchers to examine other potential risk factors, particularly lifestyle modifications. Circadian rhythm disruption (CRD), widely prevalent in modern society, has emerged as an important risk factor for lung cancer development.

Circadian rhythms are 24-hour periodic physiological processes that are closely linked to immune function and metabolism. Research has shown that circadian rhythms influence the secretion of cortisol, insulin, and growth hormone, maintain normal immune responses and metabolic processes, regulate immune cell activity and inflammatory factor expression patterns, and affect immune responses and chronic inflammation. Clock genes (Clock, Bmal1, Per, etc.) that are closely associated with circadian rhythms play essential roles in immune cell lineage development and differentiation. Multiple studies have demonstrated a strong association between circadian rhythm disruption and lung cancer in-

cidence, with epidemiological research indicating that night shift workers face higher lung cancer risk and poorer prognosis. Animal studies have further confirmed that disrupting circadian behavior in mice promotes lung tumorigenesis and progression. Therefore, investigating the causal relationship between CRD and lung cancer is crucial for understanding lung cancer pathogenesis and developing prevention strategies.

Although previous research has established correlations between CRD and lung cancer, direct evidence of causality remains limited. Mendelian randomization (MR), based on genome-wide association studies (GWAS), uses genetic variants closely associated with exposure factors as instrumental variables to assess causal relationships between modifiable risk factors and disease outcomes. This approach is less susceptible to confounding factors and reverse causation than observational studies. Traditional Chinese medicine has demonstrated unique advantages in regulating circadian rhythms and preventing lung cancer, but its potential for lung cancer prevention through circadian rhythm modulation remains uncertain. This study employs MR to investigate the causal relationship between CRD and lung cancer, explores underlying mechanisms through bioinformatics analysis, and predicts potential interventional TCMs to provide novel insights for TCM-based lung cancer prevention strategies.

Methods

Study Design This study utilized a two-sample Mendelian randomization design, using single nucleotide polymorphisms (SNPs) associated with chronotype (sleep-wake preference), early rising, insomnia, daytime napping, daytime sleepiness, short sleep duration ($ 6*h*), *andlongsleepduration*($9 h) as instrumental variables (IVs) to analyze genetic causal relationships with four outcome variables: overall lung cancer, lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and small cell lung cancer (SCLC). Genes located near the instrumental variables were identified to predict TCMs that might regulate these genes, with molecular docking used for validation.

GWAS Data Collection GWAS data for daytime napping, daytime sleepiness, short sleep duration, long sleep duration, chronotype, and early rising were obtained from the Sleep Disorder Genetics Portal (http://sleepdisordergenetics.org/) and UK Biobank (https://www.ukbiobank.ac.uk/). The daytime napping dataset included 452,633 participants; long and short sleep duration datasets included 441,934 and 339,926 participants, respectively; daytime sleepiness data included 452,071 participants, all from UK Biobank. Chronotype GWAS data included 697,834 participants from both UK Biobank and 23andMe. Insomnia GWAS data were obtained from the Amsterdam Neurogenetics and Cognitive Research Center (https://cncr.nl/ctg/), comprising 593,724 insomnia cases and 1,171,286 controls.

GWAS data for overall lung cancer, LUAD, LUSC, and SCLC were obtained from the IEU Open GWAS database (https://gwas.mrcieu.ac.uk/). The overall

lung cancer group included 27,209 participants (11,348 cases and 15,861 controls); the LUAD group included 18,316 participants (3,422 cases and 14,894 controls); the LUSC group included 17,295 participants (2,275 cases and 15,038 controls); and the SCLC group included 23,910 participants (2,466 cases and 21,444 controls).

Instrumental Variable Selection Instrumental variables were selected using the following criteria: (1) SNPs significantly associated with exposure factors at P<5$×10^{-8};(2)removaloflinkagedisequilibrium(LD)withparametersr^{2}$<0.1 and kb=1000; (3) calculation of F-statistics for all SNPs, with F>10 used to select strong instrumental variables.

Statistical Analysis and Bioinformatics Methods MR Analysis: Statistical analysis was performed using the "TwoSampleMR" package in R Studio 4.4.1. The inverse-variance weighted (IVW) method was used as the primary approach for genetic causal effect assessment, with P<0.05 considered statistically significant. Supplementary methods including MR-Egger regression, simple mode (SM), weighted median (WM), and weighted mode (WME) were used to evaluate result robustness. Mendelian Randomization Pleiotropy Residual Sum and Outlier (MR-PRESSO) was used to assess horizontal pleiotropy, with outliers removed and MR analysis repeated to correct for pleiotropy when detected. Cochran' s Q test was used to detect heterogeneity among instrumental variables; if P<0.05 indicated heterogeneity, IVW with multiplicative random effects (IVW mre) was employed. Scatter plots and leave-one-out (LOO) analysis were used to evaluate the validity of IVW results.

Core Gene Screening: Using the "vautils" and "dplyr" packages in R Studio 4.4.1 with flanking=100, genes located within 100kb of SNPs were identified based on SNP IDs and chromosomal positions. The STRING 12.0 online tool was used to construct protein-protein interaction (PPI) networks, which were then imported into Cytoscape for topological analysis using NetworkAnalyzer. Genes with degree values >15 were selected as core genes.

Functional Enrichment and Survival Analysis: The "clusterProfiler" package in R Studio 4.4.1 was used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of core genes, with P<0.01 considered significant. Survival analysis for the top 5 core genes by degree value was performed using the Kaplan-Meier Plotter database (https://kmplot.com/) for LUAD and LUSC, and data from GEORGE et al. for SCLC. P<0.05 indicated correlation between gene expression and survival time.

Potential TCM Prediction: Core genes were submitted to the Coremine database (https://coremine.com/medical/) to identify significantly associated TCMs (P<0.05). Herbs appearing $ $5 times were selected as key medicines. Properties, flavors, meridian tropism, and efficacy were cataloged based on the Chinese Pharmacopoeia, Dictionary of Chinese Medicine, Chinese Materia Medica, and Traditional Chinese Medicine textbooks. Key herbs and genes were im-

ported into Cytoscape to construct a "herb-gene" network, and cytoNCA was used to calculate degree, betweenness centrality (BC), and closeness centrality (CC). Herbs with all three parameters above average values were selected as core medicines.

Molecular Docking: Core medicines were uploaded to TCMSP (https://www.tcmsp-e.com/) and BATMAN-TCM (http://bionet.ncpsb.org.cn/batman-tcm/) databases. Active ingredients were screened using oral bioavailability (OB) $ $30% and drug-likeness (DL) $ $0.18, with molecular weight (MW), Caco-2 permeability, and fractional absorption surface area (FASA-) used to select three key active components per herb. Two-dimensional structures were obtained from PubChem and converted to three-dimensional structures using ChemBio 3D 22.0.0 with energy minimization. The top 5 core genes by degree value were selected as receptors, with three-dimensional structures downloaded from the PDB database (https://www.rcsb.org/). Water molecules and small ligands were removed using PyMol, and AutoDockTools 1.5.7 was used to convert proteins and ligands to pdbqt format and identify active pockets. AutoDock4 was used for molecular docking and binding energy calculation, with GraphPad Prism 8 used to generate binding energy heatmaps and PyMOL for visualization of selected docking results.

Results

Instrumental Variables A total of 847 eligible SNPs were selected as instrumental variables, including 194 SNPs associated with chronotype, 240 with insomnia, 7 with long sleep duration, 141 with daytime napping, 26 with short sleep duration, 53 with daytime sleepiness, and 186 with early rising.

MR Analysis of Circadian Rhythm Disorders and Lung Cancer MR analysis with overall lung cancer revealed that insomnia (OR=1.149, 95%CI=1.074-1.232, P=0.013) and short sleep duration (OR=1.462, 95%CI=1.033-2.061, P=0.031) were positively associated with lung cancer risk. Chronotype, early rising, daytime sleepiness, daytime napping, and long sleep duration showed no association (P>0.05) [Figure 1: see original paper].

For LUAD, insomnia (OR=1.181, 95%CI=1.061-1.322, P=0.001), short sleep duration (OR=1.563, 95%CI=1.024-2.401, P=0.038), and daytime sleepiness (OR=4.033, 95%CI=1.062-15.434, P=0.042) were positively associated with risk. Daytime napping, chronotype, early rising, and long sleep duration showed no association (P>0.05) [Figure 1: see original paper].

For LUSC, insomnia (OR=1.152, 95%CI=1.028-1.281, P=0.001) was positively associated with risk, while short sleep duration, long sleep duration, daytime sleepiness, daytime napping, chronotype, and early rising showed no association (P>0.05) [Figure 1: see original paper].

For SCLC, short sleep duration (OR=1.952, 95%CI=1.120-3.383, P=0.017) was

positively associated with risk, while insomnia, daytime sleepiness, daytime napping, long sleep duration, and chronotype showed no association (P>0.05) [Figure 1: see original paper].

Sensitivity, Heterogeneity, and Pleiotropy Analysis MR-PRESSO detected horizontal pleiotropy with outliers including rs73079014 and rs76145129 (insomnia), rs5757675 (short sleep duration), and rs174541 (daytime napping) in overall lung cancer analysis, and rs45453598 (insomnia) in LUSC analysis. These outliers were removed and MR analysis repeated. Cochran' s Q test revealed heterogeneity in insomnia IVs for LUAD, LUSC, and SCLC (P<0.05); daytime napping for lung cancer, LUAD, and SCLC (P<0.05); short sleep duration and daytime sleepiness for LUAD and SCLC (P<0.05); and chronotype and early rising for LUAD and SCLC (P<0.05). IVW mre analysis of heterogeneous phenotypes showed statistically significant results for insomnia in lung cancer and LUSC, and short sleep duration in lung cancer and SCLC (P<0.05) .

Scatter plots showed consistent directionality across analytical methods, indicating robust results [Figure 2: see original paper]. LOO sensitivity analysis revealed that remaining SNPs consistently fell to the right of zero without disproportionately influential SNPs, confirming good sensitivity and robustness [Figure 3: see original paper].

Core Gene Screening for CRD-Mediated Lung Cancer Based on SNP IDs and chromosomal positions, 1,520 genes were located within 100kb of SNPs associated with CRD phenotypes showing causal relationships with lung cancer subtypes. PPI network analysis identified 139 core genes with degree values >15 [Figure 4: see original paper].

Functional Enrichment Analysis of Core Genes GO analysis revealed that core genes were primarily enriched in biological processes including translation, positive regulation of c-Jun N-terminal kinase (JNK) cascade, and cellular senescence. Cellular component (CC) enrichment showed plasma membrane, cytosolic ribosome, and heterotrimeric G-protein complex. Molecular function (MF) enrichment was significant in structural constituent of ribosome and G protein-coupled receptor (GPCR) activity [Figure 5: see original paper]A. KEGG pathway analysis showed enrichment in neurotransmission-related pathways including cholinergic synapse, dopaminergic synapse, and glutamatergic synapse, as well as hormone regulation pathways such as GnRH signaling, estrogen signaling, relaxin signaling, and growth hormone synthesis, secretion and action [Figure 5: see original paper]B.

Survival Analysis of Key Genes The top 5 core genes by degree value were EP300, GAPDH, GNB1, GNG13, and TNF. Survival analysis showed EP300 expression positively correlated with survival in LUAD, LUSC, and SCLC patients (P<0.05). GNB1 expression negatively correlated with LUAD survival

but positively correlated with SCLC survival (P<0.05). GAPDH, GNG13, and TNF expressions negatively correlated with LUAD survival (P<0.05) [Figure 6: see original paper][Figure 7: see original paper][Figure 8: see original paper].

Potential TCM Prediction Core genes were uploaded to Coremine database (P<0.05), yielding 661 TCMs with 1,383 occurrences. Statistical analysis identified 40 key herbs appearing $ $5 times, including Shui Niu Jiao, Dan Shen, Yu Nao Shi, Huang Qin, Yu Jin, Huang Qi, Ling Zhi, and Ren Shen . The "herb-core gene" network was constructed in Cytoscape [Figure 9: see original paper]. Topological analysis using cytoNCA identified eight core herbs with degree $ $6.763, BC $ $333.105, and CC $ $0.366: Shui Niu Jiao, Dan Shen, San Qi, Huang Qin, Yu Jin, Jiang Huang, Yu Mi Xu, and Ling Zhi .

Analysis of properties, flavors, meridian tropism, and efficacy (based on major TCM references) revealed that predicted herbs were predominantly sweet (21 occurrences), followed by bitter (14) and pungent (12); primarily cold (10), followed by warm (10) and neutral (9) in nature; and mainly targeted liver (17), spleen (17), lung (14), heart (13), stomach (12), and kidney (9) meridians. Primary functions included tonifying qi (7), promoting blood circulation and removing stasis (4), and clearing heat and toxins (4) [Figure 10: see original paper].

Active Components and Molecular Docking Twenty-three key active components were identified from the eight core herbs using TCMSP and BATMAN-TCM databases . Molecular docking of these components with the top 5 core genes showed binding energies below -4 kcal/mol for all pairs, indicating good binding affinity [Figure 11: see original paper]. Visualization of selected docking results confirmed favorable binding conformations [Figure 12: see original paper].

Discussion

The association between CRD and lung cancer risk has been extensively studied, but most research has remained at the correlation level without establishing direct causality or identifying effective intervention targets and treatment strategies. This study explored potential causal associations between CRD phenotypes and lung cancer subtypes using Mendelian randomization based on large-scale GWAS data, providing novel evidence for molecular mechanisms underlying CRD-mediated lung carcinogenesis. By integrating bioinformatics approaches, we constructed a "CRD-core gene-TCM intervention" pathway and proposed new strategies for CRD-targeted regulation, offering innovative perspectives for using TCM to modulate circadian rhythms and prevent lung cancer.

Previous studies have suggested that CRD may increase lung cancer risk. For example, XIE et al. found a U-shaped relationship between sleep duration and lung cancer risk in a UK Biobank prospective cohort, with both short and long

sleep associated with higher risk. However, a 7.5-year follow-up study of 21,026 US male physicians found no significant association. Such discrepancies likely stem from confounding factors, small sample sizes, and inability to establish causality in observational studies. Our MR approach overcame these limitations by examining genetic causal relationships. We found significant genetic causal associations between insomnia, short sleep duration, and increased lung cancer risk. Subtype analysis revealed that insomnia, short sleep duration, and daytime sleepiness increased LUAD risk; insomnia elevated LUSC risk; and short sleep duration increased SCLC risk. These findings demonstrate that CRD plays important roles in different lung cancer types, particularly insomnia and short sleep duration across multiple subtypes, providing important direction for lung cancer prevention through circadian rhythm adjustment.

Elucidating molecular mechanisms of CRD-mediated lung carcinogenesis is crucial for identifying new prevention targets. Our SNP localization and PPI network analysis identified core genes closely associated with lung cancer. GO analysis revealed enrichment in GPCR and JNK pathways. GPCR abnormalities can promote lung cancer development through downstream MAPK/ERK and PI3K/Akt pathways, leading to uncontrolled proliferation and apoptosis inhibition. The JNK pathway, a major MAPK branch, can be persistently activated by CRD-induced oxidative stress and inflammation, promoting c-Jun phosphorylation and upregulating proliferation-related genes while disrupting apoptosis, consistent with our findings. KEGG analysis also revealed enrichment in relaxin and growth hormone-related pathways, which CRD can disrupt, thereby promoting cell proliferation via MAPK and PI3K/Akt pathways and increasing mutation risk. Survival analysis demonstrated that core genes were clinically significant and correlated with prognosis across lung cancer subtypes, suggesting involvement in both tumorigenesis and disease progression.

TCM has shown significant advantages in delaying lung cancer progression and improving quality of life. Notably, modern chronobiology theory aligns closely with TCM chronomedicine, both emphasizing the intimate connection between physiological activities and circadian rhythms. TCM chronomedicine, based on "correspondence between human and nature" and "holism," posits that diurnal yin-yang changes directly affect qi-blood circulation. Pathologically, CRD manifests as yin-yang imbalance and ying-wei disharmony, with symptoms like insomnia representing yang failing to enter yin, potentially leading to tumor formation. Ying-wei dysfunction can cause fluid distribution disorders and decreased defense, providing a microenvironment for tumor development. Thus, from a TCM chronomedicine perspective, CRD permeates all stages of lung cancer development, revealing unique advantages of TCM in rhythm regulation and cancer prevention.

Our core gene-based TCM prediction identified high-frequency herbs such as Dan Shen, San Qi, Huang Qin, Yu Jin, Jiang Huang, and Ling Zhi, which are clinically used to improve sleep and regulate circadian rhythms. Previous studies have shown that Ling Zhi and Huang Qi can improve insomnia and early

waking by regulating melatonin and cortisol secretion. Importantly, these herbs also possess anti-lung cancer activity, delaying progression, reducing symptoms, inhibiting metastasis, and improving prognosis. Pharmacological studies demonstrate that representative active components like ginsenosides, tanshinone IIA, and curcumin exert anti-tumor effects through PI3K/Akt, MAPK, and JNK pathways—precisely the core pathways identified in our CRD-mediated lung cancer analysis. For example, baicalin, a major active component of Huang Qin, inhibits lung cancer cell proliferation and migration through PI3K/Akt pathway modulation. Ginsenosides, primary active components of Ren Shen and San Qi, target MAPK pathways by reducing ROS levels to inhibit ERK and JNK pro-proliferation pathways while promoting autophagy and apoptosis via AMPK/mTOR and JNK pathways. Molecular docking confirmed good binding affinity between selected herbs and key genes affecting survival across lung cancer subtypes, suggesting their potential to delay progression and improve prognosis.

This study has several limitations. First, both exposure and outcome GWAS data were derived from European populations, potentially limiting generalizability and requiring validation in other populations. Second, the original data lacked key information such as sex and smoking status, preventing subgroup analyses to examine heterogeneity across populations. Third, this study relied on public databases for two-sample MR analysis and TCM prediction; the revealed mechanisms require experimental validation, and the efficacy and safety of predicted herbs need further verification through cellular experiments, animal models, and clinical studies.

In conclusion, based on large-scale GWAS data, our two-sample MR study identified causal effects between CRD characteristics (insomnia, short sleep duration, daytime sleepiness) and lung cancer incidence. We further predicted potential TCMs that may intervene in lung cancer development and progression by regulating CRD. These findings provide strong evidence for exploring lung cancer mechanisms from a CRD perspective and offer important references for using TCM to regulate circadian rhythms for lung cancer prevention, delaying disease progression, and developing new drugs. This study opens new perspectives for TCM in lung cancer prevention and treatment, emphasizing the importance of circadian rhythm restoration as a lung cancer control strategy.

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Note: Figure translations are in progress. See original paper for figures.