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Circadian Clock-Related Genes and Their Impact on Glioma Prognosis: Creation of a Risk Scoring System and Treatment Insights

Lingdi Guo[1,2], Ruiguo Li[1], Xingyun Mao[1], Xueyuan Heng[1,3]*

[1]Linyi People's Hospital Postgraduate Training Base of Guangzhou University of Traditional Chinese Medicine, Linyi 276000, Shandong, China

[2]Department of Obstetrics and Gynecology, Linyi People's Hospital, Linyi 276003, Shandong, China

[3] Department of Neurosurgery, Linyi People's Hospital, Linyi 276003, Shandong, China

**Author to whom correspondence should be addressed.*

Abstract: Glioma, a common and aggressive type of brain tumor, poses considerable difficulties in terms of prognosis and treatment. This research seeks to clarify the influence of circadian clock-related genes on glioma prognosis and to establish a reliable risk scoring system. We obtained RNA sequencing data from the TCGA-GBM and GSE15209. A total of 297 circadian clock-related genes were sourced from the MSigDB database. The DESeq2 algorithm was assigned to detect differentially expressed genes, resulting in the discovery of 16 co-expressed genes through Venn diagram analysis. A risk scoring system was developed using LASSO regression analyses, pinpointing eight crucial predictive genes. Their prognostic significance was evaluated through Kaplan-Meier survival analysis and ROC curve validation. A nomogram was created to estimate overall survival probabilities at 1, 3, and 5 years, showing strong predictive accuracy. This study offers a comprehensive framework for understanding the prognostic significance of circadian clock-associated genes in glioma and suggests potential therapeutic strategies.

Keywords: Glioma; Circadian rhythm; Bioinformatics; Prognosis

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1. Introduction

Glioma represents a common and highly aggressive category of primary brain tumors, making up nearly 30%of central nervous system(CNS)tumors and accounting for 80%of all malignant brain tumors[1]. The prevalence of gliomas has been increasing, with an approximated occurrence rate of 5. 26 cases per 100, 000 citizens each year across the United States[2]. Unfortunately, the prognosis for individuals diagnosed with gliomas, particularly glioblastoma—the most aggressive variant—is disheartening, with average survival rate of merely 15 months[3]. The current therapeutic strategies primarily encompass surgical intervention, radiation therapy, and chemotherapy; nevertheless, these approaches frequently

exhibit limited efficacy due to the infiltrative nature of the tumors and their inherent resistance to conventional treatment modalities[4]. Despite advancements in elucidating the molecular underpinnings of glioma, effective management of this malignancy remains a formidable challenge, underscoring the urgent need for novel biomarkers and therapeutic targets to improve prognostic precision and treatment efficacy.

Circadian rhythms, which are governed by a set of circadian clock-associated genes, are essential for maintaining physiological homeostasis and influencing various biological processes, including cellular proliferation, programmed cell death, and metabolic regulation[5]. These rhythms are orchestrated by a sophisticated network of transcriptional and translational feedback loops that involve critical clock genes such as CLOCK, BMAL1, PER, and CRY. Disturbances in circadian rhythms have been associated with numerous diseases, particularly cancer, where alterations in the expression of circadian genes can facilitate tumorigenesis and progression[6]. In the context of gliomas, a notably aggressive form of brain neoplasm, investigations have revealed that dysregulated expression of circadian clock genes correlates with unfavorable clinical outcomes and aggressive tumor phenotypes[7]. For instance, aberrant expression levels of BMAL1 and PER proteins have been associated with enhanced cell proliferation and increased resistance to apoptosis in glioma cells[8]. Although several studies have suggested possible therapeutic targets within this gene network, the clinical implications of these discoveries are still in nascent stages, highlighting the need for additional research into the therapeutic potential of circadian rhythm modulation in glioma treatment paradigms.

2. Results

2.1. Identification of Circadian Rhythm-Associated Genes in GBM Patients

A thorough examination identified 59, 428 genes linked to glioma prognosis, with 1, 262 genes upregulated and 1, 784 downregulated. Further analysis of genes in the GSE15209 dataset revealed 297 genes related to circadian rhythms, with 16 of these genes found to be co-expressed (Figure 1A and 1B). Subsequent validation of the expression of these 16 genes was conducted using the TCGA-GBM dataset (Figure 1C). Figure 1D and 1E showcase the significant enrichment of these genes in biological processes associated with rhythmic activities, circadian rhythms, and circadian regulation of gene expression, according to Gene Ontology (GO) analysis. Moreover, these genes were closely linked to cellular components such as the transcription regulator complex, NuRD complex, and Sin3 complex. They also exhibited molecular functions including RNA polymerase II-specific DNA-binding transcription factor activity, promoter-specific chromatin binding, and DNA-binding transcription factor binding. The KEGG pathway analysis results presented in Figure 1E highlighted significant enrichment in pathways connected to Amphetamine addiction, Thyroid hormone signaling, and Circadian rhythm.

2.2. Construction and Testing of the Risk Scoring Methodology

A risk scoring system was developed and evaluated in this study. Initially, a univariate Cox regression analysis was applied to assess the correlation between the expression profiles of 16 circadian rhythm-associated genes and overall survival (OS) in TCGA-GBM cohort. Twelve genes were identified as having potential predictive capabilities for OS based on a Cox regression P-value cutoff below 0. 05. To further refine this gene set, LASSO regression analysis was utilized, leading to the identification of eight key predictive genes (Figure 2A, B). The RhythmicDB was then used to confirm that these eight genes had been experimentally validated as oscillating genes across various species[9]. To explore the functions of these eight genes, a gene-gene interaction network was constructed using the GeneMANIA database. This network identified the eight genes as central hub nodes and included 20 surrounding nodes that represented significantly associated genes. The top seven functions related to these genes were also highlighted in the network (Figure 2C). The outcomes of this research underscore the important contribution of these eight genes in predicting overall survival in patients with glioblastoma (GBM). This risk scoring system provides a foundation for further investigations into the biological roles of these genes and their prospective therapeutic uses.

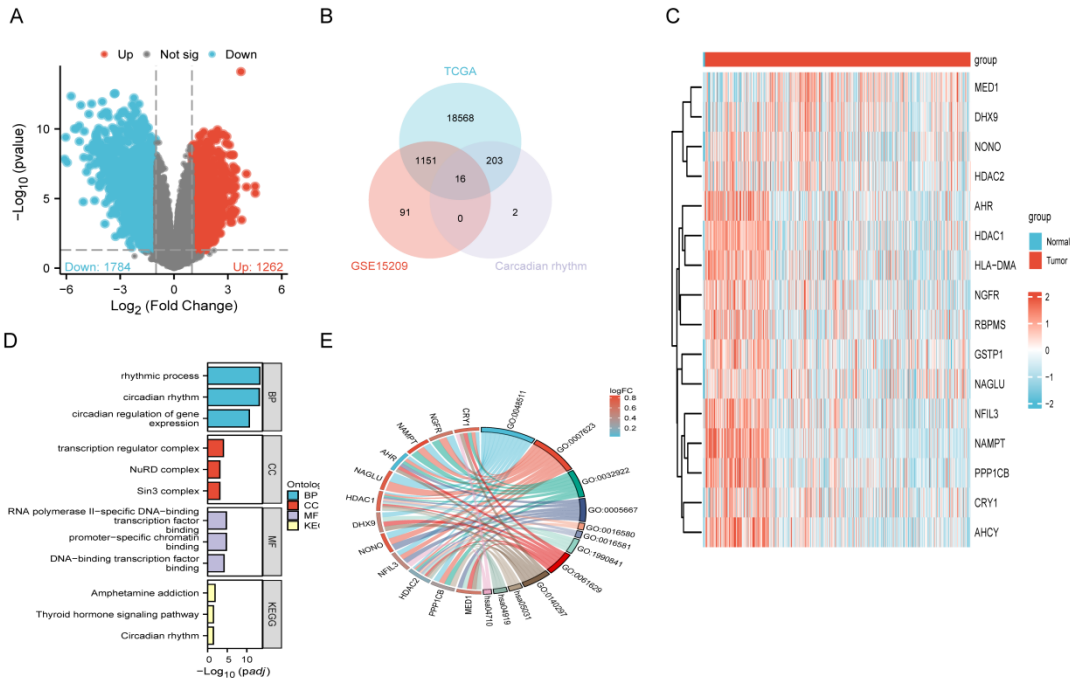

Figure 1. Detection and analysis of functional enrichment analysis of circadian clock-associated genes in the TCGA-GSE15209-GBM cohort compared to normal brain tissue.

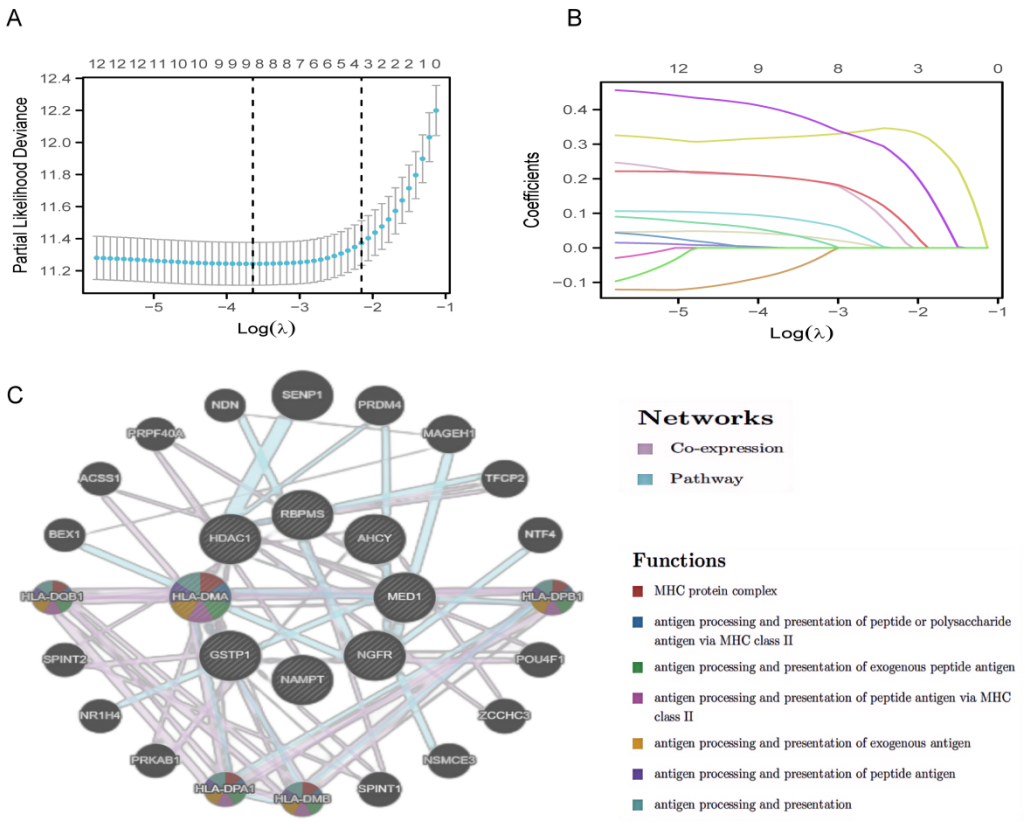

Figure 2. Illustration of DEGs with p-values less than 0. 05 in univariate Cox regression analysis.

Our research delved into the relationship between eight specific genes and various clinical factors, as depicted in Figure 3 Through our analysis, we unearthed notable disparities in the levels of “NGFR” and “GSTP1” across different grades and types of gliomas. Additionally, significant statistical variances were observed in the expression of “NGFR,””GSTP1,””AHCY,””NAMPT,””HDAC1,””MED1,””RBPMS,” and “HLA-DMA” concerning IDH status, age, and overall survival (OS) classification. Particularly noteworthy was the heightened expression of “HLA-DMA” in male patients, while the other genes displayed no prominent gender-based differences. These findings emphasize the intimate connection between the expression patterns of these genes and clinical parameters, suggesting their potential as valuable biomarkers for aiding clinical decisions in the management of gliomas.

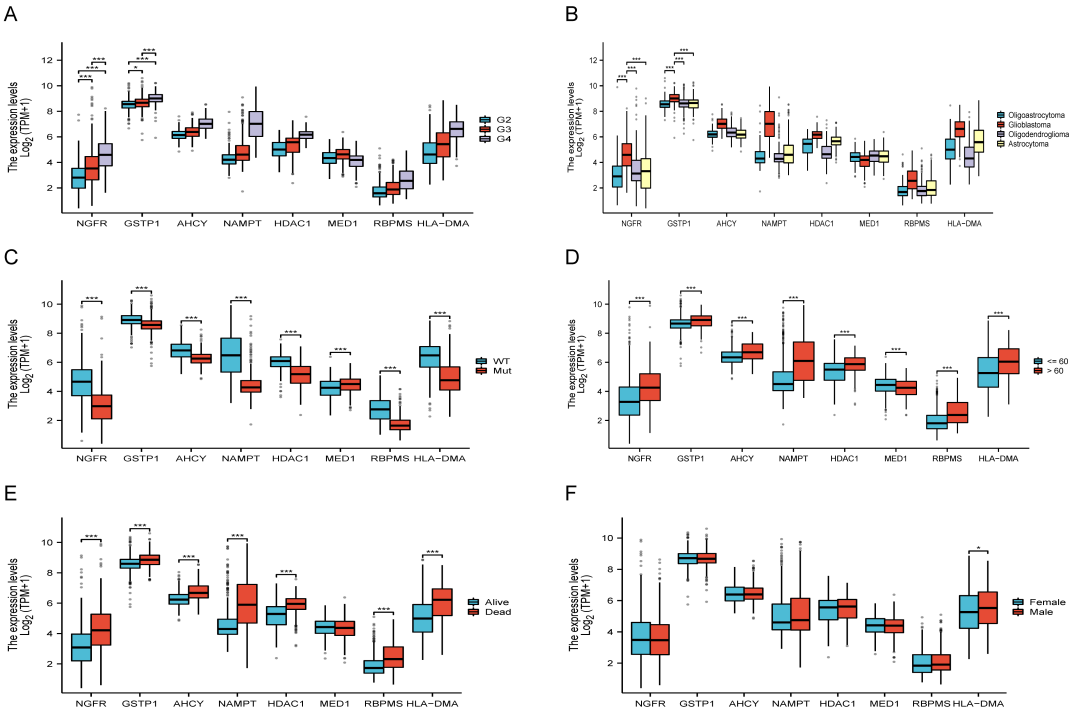

Figure 3. The association between eight distinct genes and a range of clinical parameters.

The researchers calculated risk scores for all patient by analyzing the expression and regression coefficients of eight genes associated with circadian rhythms. The spread of these risk scores, along with the gene expression, is depicted graphically in Figure 4A. Individuals were separated into high and low risk cohorts in line with their respective median risk scores. An analysis of survival times indicated a strong correlation between risk scores and prognosis, with higher risk scores linked to poorer outcomes ($P < 0.001$; Figure 4B). Moreover, the study findings suggested that the expression levels of the eight circadian rhythm genes (NGFR, GSTP1, AHCY, NAMPT, HDAC1, MED1, RBPMS, and HLA-DMA) were positively associated with overall survival. Among these genes, NAMPT, HDAC1, and RBPMS were determined to be independent prognostic indicators for glioma. The researchers evaluated the performance of the risk scoring system by generating ROC curves for survival prognosis at 1, 3, and 5 years, resulting in area under the curve (AUC) values of 0.866, 0.891, and 0.823, respectively (4C). The risk scoring system established using circadian rhythm-related gene expression demonstrated significant predictive ability for determining glioma patient prognosis.

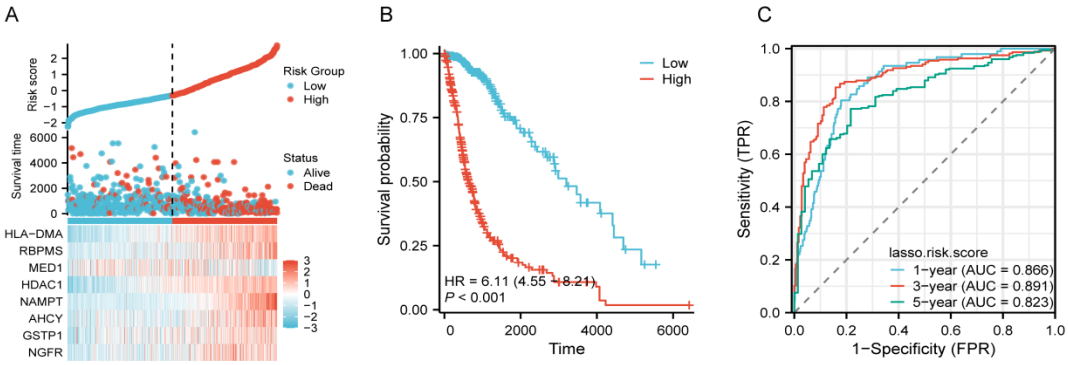

Figure 4. Analysis of the risk score, predictive capability, and survival outcomes of a risk scoring model derived from the differential expression of eight circadian clock-related genes in TCGA-GBM patients.

2.3. Development and Validation of the Nomogram

In our comprehensive study, we delved into the intricate world of circadian-related genes, specifically focusing on NGFR, GSTP1, AHCY, NAMPT, HDAC1, MED1, RBPMS, and HLA-DMA, to investigate their potential prognostic significance in glioma patients. Through a meticulous analysis involving both univariate and multivariate Cox regression (Table 1), we discovered that NAMPT, HDAC1, and RBPMS emerge as strong, independent predictors of overall survival (OS) among those grappling with glioma. The significance of these findings was underscored by the development of a sophisticated nomogram model, beautifully encapsulated in Figure 5A. To evaluate the predictive abilities of this nomogram, we turned to ROC analysis, which yielded impressive AUC values of 0.862, 0.892, and 0.825 for 1-year, 3-year, and 5-year OS within the TCGA-GBM cohort, respectively (Figure 5B). Seeking further validation, we looked to the GSE43378 dataset, which provided additional AUC statistics of 0.752, 0.830, and 0.740 for 1-year, 3-year, and 5-year OS, respectively (Figure 5C). To ensure the reliability of nomogram model, we meticulously scrutinized calibration curves, finding that it displayed noteworthy alignment with an ideal model across multiple time frames-1-year, 3-year, and 5-year OS rates (Figure 5D). Further validation through the use of calibration curves solidified the robustness of our model (Figure 5E).

Table 1. Univariate and multivariate Cox regression analysis between the genes'expression level and OS in the TCGA-GBM cohort. HR Hazard ratio, 95% CI 95% confidence interval.

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		HR(95%CI)	P value	HR(95%CI)	P value
NGFR	698	1. 294(1. 220-1. 373)	< 0. 001	1. 047(0. 962-1. 139)	0. 288
GSTP1	698	2. 094(1. 692-2. 590)	< 0. 001	1. 111(0. 840-1. 469)	0. 459
AHCY	698	2. 632(2. 196-3. 154)	< 0. 001	1. 307(0. 980-1. 744)	0. 069
NAMPT	698	1. 795(1. 672-1. 928)	< 0. 001	1. 402(1. 209-1. 626)	< 0. 001
HDAC1	698	2. 842(2. 387-3. 384)	< 0. 001	1. 605(1. 262-2. 041)	< 0. 001
MED1	698	0. 714(0. 591-0. 864)	< 0. 001	0. 888(0. 700-1. 127)	0. 328
RBPMS	698	2. 106(1. 850-2. 398)	< 0. 001	1. 251(1. 051-1. 490)	0. 012
HLA-DMA	698	1. 754(1. 590-1. 934)	< 0. 001	1. 114(0. 954-1. 300)	0. 171

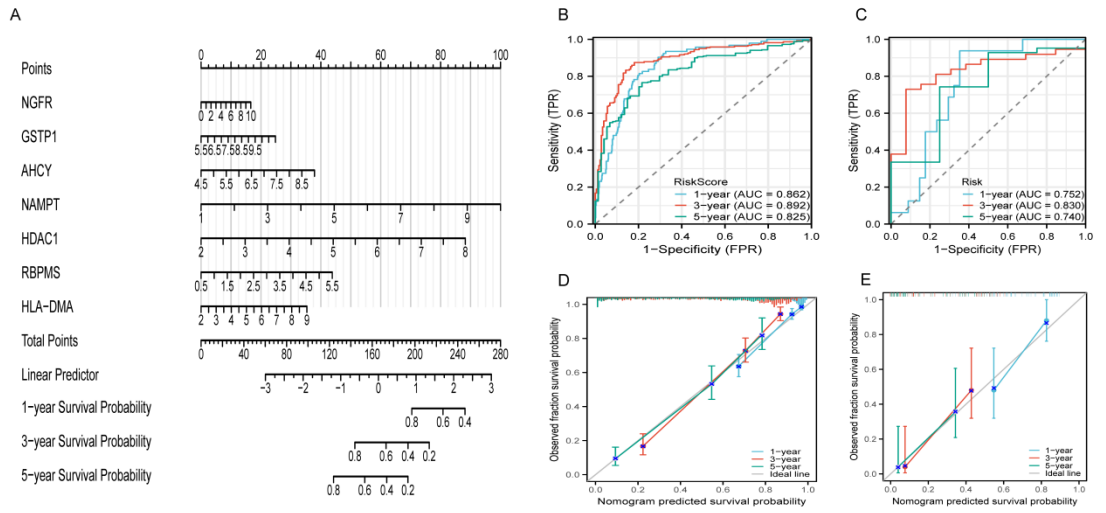

Figure 5. Prognostic nomogram for predicting 1-year, 3-year, and 5-year overall survival(OS)in glioblastoma(GBM)patients.

3. Discussion

Gliomas represent a constitute a diverse collection of primary brain tumors, characterized by their aggressive behavior and poor prognosis[10], particularly in the context of glioblastoma multiforme(GBM). These tumors originate from glial cells and are classified based on both their histological and molecular characteristics[11]. Despite progress in surgical techniques, radiotherapy, and chemotherapy, the survival rates for patients diagnosed with gliomas remain alarmingly low, highlighting the urgent need for innovative therapeutic approaches. The current state of cancer prognosis and treatment remains inadequate, emphasizing the urgent requirement for innovative prognostic biomarkers and therapeutic targets[12].

Research has established that genes related to circadian rhythms(CCRGs)display unique expression patterns across various diseases, notably in cancer. Yang's investigation revealed that the PARP1 inhibitor Olaparib boosts the level of circadian clock genes, which may lower the risk of breast cancer, indicating that PARP1 inhibitors could have antitumor properties in breast cancers with heightened PRMT6 expression[13]. Moreover, studies suggest that both systemic and cellular disruptions of circadian rhythms can promote lung cancer progression[14]. Liang's research indicates that an mRNA signature derived from essential circadian clock genes could serve as a prognostic biomarker and therapeutic approach, significantly correlating with the aggressive nature of hepatocellular carcinoma[15]. We identified NAMPT, HDAC1 and RBPMS as independent prognostic factors for overall survival in this cohort. Furthermore, we constructed a nomogram integrating these genes to forecast glioma patient survival, which was validated through external datasets, showcasing strong predictive accuracy and calibration across independent groups.

Notably, NGFR is significant for its diverse roles in cellular activities including survival, differentiation, and apoptosis. Its expression levels have been associated with tumor progression and patient outcomes in various cancers[16]. In glioma, our study suggest that NGFR may represent a vital predictive biomarker due to its strong correlation with clinical parameters, including tumor grade. This supports earlier studies that have recognized NGFR as a promising therapeutic target, highlighting the importance of further investigating its role in glioma pathophysiology.

Another critical gene in our study is NAMPT, which has surfaced as a significant factor. NAMPT is integral to the synthesis of NAD+, a compound essential for cellular metabolism and energy balance. Recent research indicates that NAMPT is overexpressed in numerous tumors and correlates with adverse clinical outcomes[17]. Our results align with these observations, as NAMPT expression was significantly associated with glioma grades and patient survival. The identification of NAMPT as a prognostic factor highlights its potential as a treatment target, especially for strategies intended for regulating NAD+levels in glioma treatment.

Recent progress in HDAC1 research has shed light on its crucial roles in cancer progression, with Eichner

demonstrating that HDAC3 is essential for lung cancer tumor growth in a genetically modified mouse model[18]. The findings from this study corroborate prior research, illustrating a significant link between HDAC1 expression, tumor grades, and patient prognosis. The identification of HDAC1 as a prognostic biomarker emphasizes its potential as a therapeutic target, particularly in developing interventions that focus on histone deacetylation and gene expression modulation in cancer therapy.

4. Conclusions

In summary, our research offers significant perspectives on the involvement of circadian-associated genes in glioma prognosis and their promise as therapeutic targets. The nomogram formulated in this research may be a useful reference for medical professionals to evaluate survival outcomes in glioma patients and support treatment planning. Future studies should concentrate on confirming the predictive value of these genes in larger patient populations and exploring the mechanisms through which they influence glioma development.

4.1. Materials and Methods

4.1.1. Data Acquisition and Processing

The data for our research were obtained from the TCGA dataset through the TCGA-GBM and TCGA-LGG projects. These datasets, formatted in transcript per million(TPM), alongside clinical information, provided a foundation for our analysis. Additionally, we derived differentially expressed genes connected to glioma from the GSE15209 data series archived at GEO. The selection criteria were stringent, with an adjusted p-value of less than 0. 05 and a fold change either exceeding 1 or dipping below-1, as outlined by the GPL570 platform. To complement this, a comprehensive listing of 297 genes linked to circadian rhythms was retrieved from the MSigDB database. For the examination of gene expression patterns, the DESeq2 method was employed. Notably, since both TCGA and GEO data are openly accessible, our study did not require ethical approval, adhering strictly to the respective data access and dissemination protocols.

4.1.2. Detection of Genes with Altered Expression and Circadian Rhythm-Associated Genes

We identified differential expression genes between tumor versus normal samples utilizing the DESeq2 package, applying stringent criteria of an adjusted p-value under 0.05 and a fold change more than 1 or less than-1. To determine genes critical for glioma prognosis, we utilized the R package “survival” (v3.3.1) within R(v4.2.1). A Venn diagram analysis compared these genes with 297 circadian clock-related genes, yielding 16 co-expressed genes. A gene expression heatmap for these 16 genes was generated using the“ComplexHeatmap”package. Functional enrichment analysis and visualization were conducted on the 16 genes employing the “clusterProfiler”, “org. Hs. eg. db”, and “GOplot” packages to elucidate their biological significance.

4.1.3. Creating and Confirming a Risk Classification Framework

After univariate Cox regression analysis of the 16 circadian rhythm-associated genes, eight genes emerged with p-values below the 0.05 threshold. These genes were further evaluated using LASSO cross-validation. GENEMANIA software was utilized to create a protein-protein interaction (PPI) network for the selected genes. A risk assessment model was then constructed utilizing normalized gene expression values and their corresponding coefficients. Visualizations of risk factors and Receiver Operating Characteristic (ROC) curves were created with the “ggplot2” and “timeROC” packages, respectively. Individuals were categorized into high and low risk cohorts in line with the median risk score, and survival curves were generated to evaluate the connection between risk scores and clinical features by employing the “survminer” and “ggplot2” packages.

4.2. Creation and Assessment of the Nomogram

Univariate as well as multivariate Cox regression analyses were carried out utilizing the”survival”package, examining the expression levels of the eight genes. The”rms”package was used to develop a nomogram for forecasting overall survival (OS) at 1, 3, and 5 years. To assess the nomogram’s predictive capabilities, ROC and calibration analyses were conducted. Furthermore, the dataset from GSE43378 was included as a supplementary validation cohort, evaluating the effectiveness of our clinical prediction model.

4.3. Statistical Analysis

Statistical evaluations were performed using R software. Survival rates were analyzed via Kaplan-Meier with log-rank testing. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using regression. Continuous variables were compared using independent t-tests or Mann-Whitney U tests for non-normal distributions. Categorical variables were analyzed by chi-square or Fisher’s exact tests. Univariate and multivariate Cox analyses were conducted to determine independent prognostic factors, with significance set at $p < 0.05$.

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Disclosure statement

The author declares no conflict of interest.

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