Genomics and Prognosis Analysis of Circadian clock genes in Hepatocellular Carcinoma

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Abstract

Background: Circadian clock genes have been reported to exhibit a regulatory effect on the carcinogenesis and progression of numerous cancers. Nevertheless, the specific relationship between hepatocellular carcinoma (HCC) and circadian rhythm associated genes still remain to be clarified. Therefore, we evaluate the prognosis function of circadian clock genes in HCC with the online datasets of The Cancer Genome Atlas (TCGA) and the international cancer genome consortium (ICGC).

Methods: In our research, the RNA-seq of the selected core circadian genes in HCC patients and their relevant clinical data were acquired from the online TCGA database and the ICGC database. R software and cBioPortal website were performed.

Results: As consequence, among the 22 typical circadian clock genes, 16 genes were statistically expressed between HCC and adjacent normal tissues. Accordingly, 11 clock genes with regression coefficients were used to constitute a new risk score formula, which was related to the prognosis in HCC. Moreover, the new nomogram, which consisting risk score and several clinical traits, could be applied for the purpose of accurate prediction of the overall survival (OS) time for the patients. Finally, we identified a novel nomogram related with OS in HCC patients with a comprehensive analysis of circadian clock genes and other clinical characteristics profiles. It was also the first time we systematically demonstrated the relationship between clock genes and the HCC prognosis, which would contribute to the treatment of HCC.

Conclusions: The current study demonstrated the potential of circadian clock genes as clinically associated biomarkers for prognosis prediction in HCC, which may make a significant contribution to the further investigations of HCC progression.

Keywords: Circadian clock genes, Hepatocellular carcinoma, Biomarker, Prognosis

Background

Hepatocellular carcinoma (HCC) is one of the most frequent reasons of human abdominal malignancies-associated mortality worldwide(1). As the most prevalent kind of primary liver cancers, HCC accounts for over 70% of liver carcinomas(2), with its incidence increasing over the past several decades(3), which essentially results from hepatitis, alcohol abuse and several metabolic syndromes(4). The present tactics to HCC consist of radical treatments such as curative resection, liver transplantation for local lesions(5), and palliative therapies including ablation, chemotherapy et cetera(6). However, owing to the limited existing medical examinations and the asymptomatic progression, the patients tend to be diagnosed during middle or even advanced period, which will inevitably affect the prognosis of HCC. Previous researches have demonstrated several markers related with the development of HCC(7-9), it helped clinicians to make reasonable treatment choices and evaluate the prognosis among patients. Nevertheless, owing to the sample size and selection bias, marker targeted treatment may be affected to a certain degree. Therefore, it is imperative to figure out

reliable biomarkers to develop a more accurate model for early diagnosis and prognosis prediction for the patients.

As the endogenous oscillator, circadian clock is located at the suprachiasmatic nucleus of the hypothalamus and it is controlled by a couple of transcription-translation feedback loops(10, 11). The mentioned feedback loops coded genes are the so-called circadian clock genes, and the expression of circadian clock genes vary during the 24-hour basis oscillation(12). Core circadian clock genes contain *CLOCK*, *ARNTL*, *PER1*, *PER2*, *PER3*, *CRY1*, and *CRY2*; however the core components of the mammalian circadian clock are more extensive and contain 22 genes, including the core clock genes plus *BTRC*, *CSNK1D*, *CSNK1E*, *CUL1*, *DBP*, *FBXL21*, *FBXL3*, *NFIL3*, *NR1D1*, *NR1D2*, *PRKAA1*, *PRKAA2*, *RORA*, *RORB*, and *SKP1*(13, 14), which were involved in various physiological processes (such as wakefulness, sleep, hormone secretion and biological metabolism etc.) and dynamic internal environment(15). Currently, compelling lines of evidence has revealed the significance of clock genes in terms of oncology. Genetic mutation or environment disturbance could lead to perturbations of circadian genes expression, which result in the progression of various cancers, including tongue squamous cell carcinoma (TSCC)(16), non-small cell lung cancer (NSLC)(17), colorectal cancer (CRC)(18), kidney cancer(19), pancreatic cancer(20), gastric cancer(21). Circadian clock gene *Bmal1* was demonstrated to inhibit tumorigenesis and enhance the paclitaxel sensitivity in TSCC treatment via the *EZH2* recruitment to the transcription promotor of *TERT*(16). Xiang et al(17) reported that circadian clock gene *PER2* could promote the expression of anti-oncogenes and suppress the pro-oncogenes expression underlying the tumorigenesis in NSLC. *CRY2* expression was upregulated in the colorectal cancer, which was closely related with chemoresistance(18). Thus, *CRY2* could be utilized as a novel biomarker for the prognosis prediction and therapy selection in CRC patients. In spite of that, the association between circadian clock genes and HCC still remains to be clarified so as to monitor the onset, development and prognosis among the patients.

In the current study, we identified 22 circadian clock genes in HCC from the online The TCGA database. Performed functional enrichment analysis to reveal Gene Oncology terms and several related signal pathways. Subsequently, with the method of least absolute shrinkage and selection operator (LASSO) regression, 11 circadian clock genes with regression coefficients were applied to establish a risk scale for HCC. According to the risk score, the risk scale was able to distinguish high-risk group patients from low-risk group ones in terms of survival time. Combined with other clinical characteristics, a novel nomogram was constructed to precisely predict the probability of survival time, which potentially provided clinical value in the management of HCC.

Material and methods

Tissue and data acquisition

In our research, the RNA-seq of the selected core circadian genes(22) in HCC

patients and their relevant clinical data were acquired from the online TCGA database (<https://cancergenome.nih.gov/>) and the International Cancer Genome Consortium (ICGC, ([LIRI-JP] Liver Cancer-RIKEN, JP, Oct-1-2019)) (<https://dcc.icgc.org/>). The TCGA database normalized as log2 (TPM+1) were selected as the training set and the ICGC dataset log2 (RNA-seq+1) transformation was used as validation set. A total of 664 specimens were analyzed in our research. The selected core circadian clock genes expression analysis was carried out between 374 liver cancer tissues and 50 adjacent normal tissues in TCGA database as well as 240 primary liver cancer tissues in ICGC database. The Basic clinical characteristics of HCC were shown in Table 1. All the data analyzed in this research were online publicly available.

Functional enrichment analysis of circadian clock genes

The functional enrichment analysis was performed to identify the specific functions (biological process, cellular component, molecular function) of the circadian clock genes by *clusterProfiler* package in R. The main enrichment terms were visualized with the packages named *ggplot2* and *GOplot* in R. It was viewed statistically significant while p.adjust was no more than 0.05.

The cBioPortal

Almost 30 different kinds of cancers(23) related RNA sequencing and pathological data could be obtained from The Cancer Genome Atlas datasets. The hepatocellular carcinoma (TCGA, PanCancer Atlas) and (AMC, hepatology 2014) datasets with pathological features were chosen for next investigation into the 22 circadian clock genes with cBioPortal (www.cbioportal.org)(24). Progression-free survival (PFS) and overall survival (OS) were estimated in accordance with the online cBioPortal's instructions.

Construction of the risk score

Then we performed the LASSO-Cox analysis with the package *survival* and *glmnet* in R. Then, lambda value of the significant circadian clock genes was generated on the basis of overall survival of TCGA HCC patients. The expression of circadian clock genes and their corresponding lambda value made up the risk score of each patient. And the prognostic value of the rhythm related genes was attached in the Supplementary Table 1.

Prognostic and predictive analysis of the risk score construction

The clustering ability of circadian clock genes-based risk score was confirmed by principal component analysis (PCA) and visualized via *ggplot2* package in R. Then *survival* package was used to analyze the survival time between the two groups. Subsequently, to evaluate the specificity of the risk score construction, we performed the receiver operating characteristic (ROC) analysis with the *survivalROC* package in R. The prognostic or predictive accuracy was described by the specific area under the curve (AUC). P value < 0.05 was regarded to be statistically significant.

Construction of the nomogram

The nomogram was conducted with the package *rms* in R software. Based on TCGA database, the age, gender, tumor grade, tumor stage, and the risk score were selected as risk elements for prognosis prediction of HCC. The discrimination of the nomogram was measured by C-index (concordance index). The value of C-index ranged from 0.5 (no discrimination) to 1 (perfect discrimination), and higher C-index showed better discrimination of the prognostic model.

Statistical analyses

Statistical analysis and drawings were accomplished by R software (<https://www.r-project.org/>, v3.6.2). Lambda values of rhythm related genes were indicated with GraphPad Prism software. The other statistical calculations and figures were produced by a couple of R packages. When the p value < 0.05, it was thought to be statistically significant. All the statistical examinations were two-sided.

Results

Rhythm related genes were differentially expressed in HCC.

In order to explore the expression of circadian clock genes in HCC, we selected 22 core clock genes according to the current researches(25, 26) and downloaded the patient samples from the TCGA dataset. As illustrated in the heat map in Figure 1A, 16 statistically differentially expressed genes (*ARNTL*, *BTRC*, *CLOCK*, *CRY1*, *CRY2*, *CSNK1D*, *CSNK1E*, *CUL1*, *DBP*, *NFIL3*, *NR1D2*, *PER1*, *PRKAA2*, *RORA*, *RORB*, *SKP1*) were identified between liver cancers and the normal tissues. Notably, except for *NFIL3*, *PER1* and *RORA*, most of the 16 circadian clock genes exhibited higher expressions in HCC samples than normal ones (Figure 1B). Additionally, as showed in the correlation matrix (Figure 1C), with the method of *corrplot* package in R, there existed several internal correlations between the core circadian clock genes (*CRY2*, *PER1*, *PER2*, *PER3*, *CLOCK*, *ARNTL*, *CRY1*).

Functional enrichment analysis results

Subsequently, functional enrichment analysis was conducted to assess the 22 circadian clock genes specified activities, which demonstrated significant enriched Gene Oncology (GO) terms. Specifically, the GO biological processes contained the biological process (BP), cellular component (CC) and molecular function (MF), including circadian rhythm, rhythm process, SCF ubiquitin ligase complex, cullin-RING ubiquitin ligase complex, transcription corepressor binding, transcription cofactor binding (Figure 2A). And several significant biological processes statistically associated with the circadian rhythm related genes were shown in Figure 2B and C.

The mutations of circadian clock genes should have influenced the prognosis in HCC patients.

The hepatocellular carcinoma (TCGA, PanCancer Atlas) and (AMC, hepatology 2014) datasets were utilized to explore the effect of the alterations in the 22 rhythm related genes on the patient prognosis. In Figure 3A, it could be seen that the alterations

of circadian clock genes occurred in 130 samples out of 603 HCC patients (22%). With the survival analysis, the alterations in circadian clock genes was notably related with OS (logrank p value = $7.863e-4$), whereas not with PFS (logrank p value = 0.216) in HCC patients (Figure 3B and C).

The prospect of current risk scores and clinicopathologic characteristics in HCC.

Among the 22 genes related with HCC, 11 most significant genes with their matching lambda values were generated via the LASSO-Cox dimensional evaluation (Figure 4A and B). In accordance with the lambda values and the expression of the mentioned 11 genes, a novel risk score construction was developed for each HCC patient (Figure 4C). Separated the patients into low-risk group and high-risk group according to the medium of the risk score. Make comparison of the circadian clock genes' transcription profiles of low-risk and high-risk subgroups by using the principal component analysis (PCA). Consequently, the results displayed significant differences between two groups (Figure 4D). The relationship between clinicopathological traits and risk scores was proved as follows. As illustrated in the heat map, the risk scores revealed asymmetric distributions on the clinicopathological features (Figure 5A). Particularly, in both TCGA and ICGC datasets, tumor stage and grade showed a remarkably positive association with the risk score. However, as for the part of gender and age showed no statistical relationship with the risk score (Figure 5B and C).

Patients suffered from unsatisfied survival time with elevated risk scores.

To further confirm the credibility of the risk score construction, we studied the prognostic value of the risk score. Based on the TCGA database, risk score was an independent prognostic factor for overall survival. Patients in the low-risk group showed a relatively considerably favorable overall survival ($p = 1.79e-06$) than those in high-risk group (Figure 6A and B). What's more, the ROC analysis also testified that the risk score had a higher prediction accuracy in comparison with clinical characteristics, with its AUC values equaled to 0.754 for one year, 0.691 for three years and 0.639 for five years' survival predictive accuracy (Figure 6C). Intriguingly, the ICGC dataset verified the above results as well. As presented in the Figure 6D - F, increased risk score indicated poor prognosis in HCC patients ($p = 4.232e-06$) and the AUC value (0.760 for one year, 0.723 for three year and 0.718 for five year's survival predictive accuracy) of the ROC curve also showed the consistent credibility. Above all, the risk score was obviously negatively associated with the survival time in HCC patients.

The nomogram was able to predict the survival probability of the HCC patients at different time periods.

Finally, a new nomogram was generated combined risk score and clinicopathological characteristics. As displayed in the Figure 7A, the fracture map was composed of two sections. The upper section suggested the point scoring and lower section implied prognosis prediction for the patients. Importantly, the nomogram points were based on the age, gender, tumor grade, tumor stage and risk score. The total point of nomogram

could assist physicians to predict the survival probability for patients who were diagnosed after one year, two years and three years. The C-index values of the TCGA database and ICGC database were 0.702 (SD=0.053) and 0.770 (SD=0.078), respectively. And the actual observation and prediction values of the present nomogram were highly consistent with the training as well as the validation sets (Figure 7B and C).

Discussion

Hepatocellular carcinoma (HCC) ranks as one of the most aggressive and fatal malignancies, with its incidence increasing yearly worldwide. As the development in the current management of HCC, surgical resection and liver transplantation still remain to be the mainstream treatment for patients. In spite of that, over 500,000 new patients are diagnosed each year(27), due to its tumor heterogeneity and asymptomatic progression(28, 29). Therefore, unraveling the pathological mechanism of HCC would be of great benefit to establish precious medical options for the patients. During the past few decades, substantial evidence has demonstrated that circadian clock genes exhibited determinant parts in different types of carcinomas with significant enhancements in RNA-seq analysis. Accordingly, Castrucci et al(30) indicated a high bulk expression of *BMAL1* in metastatic melanoma, which could be utilized as an indicator for immunotherapy response. As the typical individual gene of circadian clock, *PER2* could handle the downstream related regulators (*p53*, *Ki-67*, *c-Myc*, *Bcl-2*, *Bax*, *MMP2*) in breast cancer cell proliferation, differentiation, metastasis and apoptosis, making it the new effective molecular therapy target(31). With that in mind, disruption in timekeeping system could facilitate tumor progression(32), and circadian clock genes have also been reported to be involved in the carcinogenesis and prognosis of carcinomas(33), which may in turn contributes to the survival prognosis prediction and individualized medical treatment for patients. However, the interrelationship between HCC and circadian clock genes still remains to be ambiguous. Thus, there is an urgent need to identify novel circadian clock related biomarkers for prognosis prediction and personalized therapy selection in patients.

In our study, it was our first time tried to explore the robust prognosis prediction power of the circadian rhythm associated genes' expression in HCC. Dimensionality analysis on the circadian clock genes was performed in a cohort of HCC specimens and other clinical characteristics from the online TCGA and ICGC database, whose data was free to download publicly. First of all, among the prevailing circadian rhythm related genes, altered genes were verified with the original data conversion. Consequently, according to the 22 circadian clock genes, 16 statistically differential genes were figured out in HCC and normal adjacent samples among 364 patients in TCGA dataset. Additionally, some interrelations are detected among the significant genes, especially with the correlation coefficients of *CRY2* & *PER1* 0.59, *CRY2* & *PER3* 0.50, *PER2* & *PER3* 0.59, *PER2* & *CLOCK* 0.56, *PER2* & *CRY1* 0.50, *PER3* & *CLOCK* 0.52, *CLOCK* & *ARNTL* 0.56, *CLOCK* & *CRY1* 0.59, *ARNTL* & *CRY1* 0.67, some of which were reported to be fairly relevant to pathological process and tumor

development(34-37). Therefore, the internal coordinated regulation between circadian genes may help to get unique insight into HCC progression. The functional enrichment analyses further verified their significance in GO terms and several related signal pathways, most of which were associated with circadian rhythm adjustment. Moreover, the effect of mutation status of the above 22 genes in HCC patients were explored as well. Unsurprisingly, any mutation type in clock genes could bring about an obvious decrease in patients' survival time, either OS or PFS.

Secondly, the LASSO regression was applied to define the clock genes signature, which identified 11 genes with regression coefficients: *CRY2*, -0.121157237; *CSNK1D*, 0.362944001; *CSNK1E*, 0.084595845; *CUL1*, 0.165897507; *FBXL21*, -0.128683295; *NR1D1*, 0.052093038; *PER1*, -0.144513835; *PRKAA1*, 0.030019524; *PRKAA2*, 0.007940802; *RORA*, -0.040614728; *SKP1*, 0.023270767. Within the 11 powerful genes, most of them were reported to participate in tumorigenesis(38-44). Accordingly, *CRY2* was reported to be a potential anti-oncogene in osteosarcoma. What's more, silencing of *CRY2* could activate osteosarcoma cell proliferation and metastasis via cell cycle and MAPK signal pathway(38). Liakos et al(39) investigated that *CSNK1D* could enhance EPO secretion with the phosphorylation of HIF-2 α at Thr528 and Ser383, which promoted HCC cells *in vitro*. As an indispensable element in the complex of SCF E3 ubiquitin ligase, *CUL1* was demonstrated to be positively related with breast cancer progression and disadvantageous survival, whether *in vivo* or *in vitro*(42). Likewise, *PER2* exhibited its remarkably proapoptotic function in squamous cell carcinoma (OSCC) via induction of autophagy(44). Above all, the significant oncogenic or anti-tumor effect in the 11 current genes was almost the same as previous mentioned. A highlight of our study was assembled a brand-new risk score formula via the 11 genes with their corresponding regression coefficients, which separated the patients into low risk group and high-risk group according to the medium of the risk score. Subsequently, the PCA suggested that there were significant differences between two groups.

In addition, several vital clinical characteristics were found to be related with the risk score. Specifically, the correlation analysis between risk score and clinical traits explained that tumor grade and tumor stage classification were positively connected with risk score, whereas null correlation was existed within risk score and age or sex. It was well known that tumor stage and grade were related with patient survival, which further confirmed our analysis. As expected, risk score was an independent prognostic factor for overall survival and increased risk score indicted poor prognosis. What's more, the ROC analysis also testified that the risk score had a higher prediction accuracy in comparison with clinical characteristics.

Finally, combined risk score with age, gender, tumor grade and stage, a novel nomogram was developed to assess the prognosis in the diagnosed HCC patients. The total point of each patient was summed up using the point scale in the nomogram. Utilized the C-index in order to evaluate discrimination ability, on the other hand, compared the consistency of the calibration curve of the nomogram with the actual

observation curve to evaluate the calibration capability. The calibration curve was highly consistent with the actual observation curve in both the training and validation sets implied the ideal reliability and repeatability of the nomogram. According to nomogram, the total point could reliably predict individual's survival prognosis, which was another focal point of our research. However, some deficiencies do exist in the current study. Since several results were restricted to the mRNA expression of circadian clock genes and the data were acquired from the public online datasets. Hence, more experiments and protein level expression analyses are required to validate the relationship between clock genes and HCC in our following researches.

Conclusion

Herein, we first took a glance with respect at the effect of circadian clock genes on HCC. Accordingly, with the several significant clock genes, a new formula was developed to calculate the risk score in HCC patients. Additionally, the risk score was statistically related with other clinical characteristics. Taken together, we constructed a reliable nomogram to predict the prognosis for the patients. With this line, the current study demonstrated the potential of circadian clock genes as clinically associated biomarkers for prognosis prediction in HCC, which may make a significant contribution to the further investigations of HCC progression.

Abbreviations

HCC: Hepatocellular carcinoma; TCGA: The cancer genome atlas; LASSO: Least absolute shrinkage and selection operator; TPM: transcripts per million; ICGC: The international cancer genome consortium; OS: Overall survival; PFS: Progression-free survival; PCA: principal component analysis; KM: Kaplan-Meier; ROC: Receiver operating characteristic; AUC: Area under the curve; GO: Gene oncology; BP: Biological process; CC: Cellular component; TSCC: Tongue squamous cell carcinoma; NSLC: Non-small cell lung cancer; CRC: Colorectal cancer; C-index: Concordance index.

Ethics approval and consent to participate

Due to the dataset in the current study was downloaded from TCGA and ICGC, and data acquiring and application complied with the TCGA and ICGC publication guidelines and data access policies, additional approval by an ethics committee and consent to participate were not needed.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the TCGA Network: <https://cancergenome.nih.gov/> and ICGC Network: <https://dcc.icgc.org/>

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YKX designed the study and revised the manuscript, QKH wrote the first draft of the manuscript, PYG and YNL organized the database, YSL and ZJZ performed the statistical analysis. All authors contributed to manuscript revision, read and approved the submitted version.

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Figure legends

Figure 1 Differential expression of circadian clock genes between liver cancers and the normal tissues. (A) Heat map of rhythm related genes between liver cancers and the normal tissues. (B) The statistically differentially expressed circadian clock genes between liver cancers and the normal tissues. (C) Several intercorrelations were studied between the core circadian clock genes with the regard to transcription data. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 2 Functional enrichment analysis results. (A) Circadian clock genes showed several significant enriched GO terms. (B and C) The connection between significant biological processes and the circadian rhythm related genes.

Figure 3 Mutations of circadian clock genes in HCC patients. (A) Oncoprint in cBioPortal indicated the scale and distribution of the HCC samples with alterations in clock genes. Kaplan-Meier plots comparing OS (B) and DFS (C) in cases with/without clock genes alterations.

Figure 4 Construction of risk score based on circadian clock genes in HCC. (A and B) The most typical genes were acquired by LASSO-COX dimensional evaluation of the circadian clock genes. (C) The lambda value of 11 most typical genes. (D) The PCA demonstrated significant difference between the low risk group and high risk group.

Figure 5 The relationship between clinicopathologic characteristics and risk score of HCC. (A) The heatmap presented clinicopathologic features and 11 typical clock genes for each HCC patient with the ascending order of risk score. The relationship between risk scores and clinicopathologic characteristics in TCGA database (B) and ICGC database (C). ** $p < 0.01$, *** $p < 0.001$.

Figure 6 Patients suffered from unsatisfied survival time with elevated risk scores. The constitution of the multi-variable cox model demonstrated as a forest plot based on TCGA database (A) and ICGC database (D). Kaplan-Meier plots comparing OS in patients with low risk and high risk in TCGA database (B) and ICGC database (E). ROC analysis of risk scores and clinical characteristics at 1 year, 3 years and 5 years in TCGA database (C) and ICGC database (F). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 7 The quantitative risk evaluation and C-index values for survival prediction in HCC patients. (A) The nomogram was the quantitative risk evaluation for OS prediction in HCC at 1 year, 2 years and 3 years. The C-index of TCGA database (B) and ICGC database (C) in HCC patients at 1 year, 2 years and 3 years.

553 **Figure 1**

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Figure 7

Table 1 Basic clinical characteristics of HCC

Clinicopathological variables	TCGA database	ICGC database
	n = 364	n = 231
Age (years)		
< 60	165 (45.33%)	44 (19.05%)
≥ 60	199 (54.67%)	187 (80.95%)
Gender		
Female	119 (32.69%)	61 (26.41%)
Male	245 (67.31%)	170 (73.59%)
Tumor grade		
G1 + G2	230 (63.19%)	155 (67.10%)
G3 + G4	129 (35.44%)	56 (24.24%)
Pathological stage		
I + II	253 (69.51%)	139 (60.17%)
III + IV	87 (23.90%)	92 (39.83%)
Pathologic T		
T1 + T2	270 (74.18%)	-
T3 + T4	91 (25.00%)	-
Pathologic N		
N0	247 (67.86%)	-
N1	4 (1.10%)	-
Pathologic M		
M0	262 (71.98%)	-
M1	3 (0.82%)	-
Vital status		
Alive	234 (64.29%)	189 (81.82%)
Dead	130 (35.71%)	42 (18.18%)

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Supplementary Table 1. Univariate analysis of overall survival of circadian rhythm-related genes in HCC

Gene	TCGA database		ICGC database	
	HR (95% CI)	p Value	HR (95% CI)	p Value
ARNTL	1.037 (0.843-1.277)	0.729	0.850 (0.544-1.329)	0.476
BTRC	1.250 (0.934-1.673)	0.133	0.823 (0.348-1.942)	0.656
CLOCK	1.206 (0.924-1.576)	0.168	0.950 (0.564-1.600)	0.846
CRY1	1.141 (0.927-1.404)	0.213	1.080 (0.616-1.895)	0.788
CRY2	0.796 (0.653-0.970)	0.024	0.441 (0.277-0.701)	0.001
CSNK1D	1.840 (1.383-2.449)	< 0.001	2.617 (1.433-4.777)	0.002
CSNK1E	1.437 (1.163-1.777)	0.001	1.238 (0.806-1.903)	0.330
CUL1	1.541 (1.139-2.085)	0.005	1.671 (0.886-3.152)	0.113
DBP	1.072 (0.872-1.317)	0.508	0.678 (0.443-1.039)	0.074
FBXL21	0.865 (0.668-1.122)	0.274	0.893 (0.567-1.407)	0.627
FBXL3	1.054 (0.826-1.345)	0.671	1.015 (0.586-1.758)	0.957
NFIL3	1.050 (0.878-1.256)	0.592	1.308 (0.915-1.868)	0.141
NR1D1	1.193 (1.007-1.413)	0.041	1.171 (0.788-1.739)	0.436
NR1D2	1.102 (0.883-1.377)	0.391	0.827 (0.478-1.430)	0.496
PER1	0.783 (0.654-0.937)	0.008	0.587 (0.406-0.848)	0.005
PER2	1.057 (0.841-1.329)	0.633	0.900 (0.484-1.672)	0.738
PER3	1.010 (0.851-1.199)	0.910	0.689 (0.443-1.072)	0.099
PRKAA1	1.296 (1.028-1.634)	0.029	1.115 (0.617-2.012)	0.719
PRKAA2	1.187 (1.041-1.355)	0.011	1.445 (1.016-2.055)	0.041
RORA	0.817 (0.672-0.993)	0.043	0.621 (0.413-0.935)	0.022
RORB	1.021 (0.431-2.418)	0.963	5.217 (1.019-26.702)	0.047
SKP1	1.636 (1.130-2.370)	0.009	1.309 (0.759-2.259)	0.333

Figures

Figure 1

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Figure 2

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Supplementary Files

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