January 2021

The Associations Between Circadian Genetic Factors And Cancer Survival

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The Associations Between Circadian Genetic Factors and Cancer Survival

Bingqing Li

Year Completed: 2021

Year Degree Awarded: 2021

Degree Awarded: Master of Public Health

Department: School of Public Health

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Abstract

The circadian rhythm controls a range of important biological behaviors with a 24-hour cycle. The mammalian biological clock is a layered network of vibrators with a master clock locating in the neurons of suprachiasmatic nucleus (SCN) in the hypothalamus and a set of core and related circadian genes. Several lines of evidence from previous studies suggested the relationships between tumorigenesis and circadian rhythm disruption. However, the effects of circadian genetic factors in cancer survival remains uncertain. Therefore, it is important to review and explore the circadian genetic factors which might be linked to cancer survival. This paper aimed to evaluate the potential associations between circadian genetic factors and cancer survival by summarizing 30 epidemiological studies and searching 2 databases. The circadian genetic factors identified included both gene expression and SNPs. Our results showed that 16 circadian genes, \textit{PER1, PER2, PER3, CRY1, CRY2, BMAL1, CLOCK, NPAS2, NPAS3, TIMELESS, RORA, RORC, NR1D2, CK1ε, DEC1} and TIPIN, were significantly related to cancer survival across several types of cancer. The associations between circadian genes and cancer survival differed among different genes and cancer types.

Keywords: Circadian genes; Cancer; Survival; Environmental Health Sciences.
Acknowledgements

My completion of this thesis could not have been accomplished without the support and encouragement from my advisors, Dr. Yong Zhu and Dr. Mayur Desai. I would like to express my sincere gratitude to them for their invaluable guidance and help. I would also like to thank my parents, cats and classmates for their support and understanding.
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- Figure 4. The Kaplan Meier survival curves for cancer patients with high and low circadian gene expression using Kaplan-Meier plotter database. It includes six genes: PER2, PER3, RORA, RORC, DEC1 and TIMELESS.
**Introduction**

Circadian rhythm controls a wide range of important biological behaviors in a 24-hour cycle, which responds to light and darkness, and affects many organisms, including animals, plants and microorganisms. In mammals, the biological clock controls all aspects of physiology, including sleep, metabolism and the regulation of the immune system. (Rijo-Ferreira, 2019) The mammalian biological clock is a layered network of vibrators with a master clock locating in the neurons of suprachiasmatic nucleus (SCN) of the anterior hypothalamus. SCN is entrained by the light-dark cycle and synchronizes the phases of multiple peripheral clocks in different peripheral tissues. (Reppert and Weaver, 2002)

At the molecular level, circadian clocks are cellular autonomic and involve a core set of circadian genes, which are implicated in susceptibility and prognosis of cancers by involving in the regulation of DNA damage and repair, carcinogen metabolism and detoxification, cell proliferation, apoptosis, and cell cycle. (Zmrzljak and Rozman, 2012) The primary circadian clock pathway genes in mammals are involved in transcriptional self-regulating feedback loop, including CLOCK, Brain and Muscle ARNT-Like 1 (BMAL1), period 1 (PER1), period 2 (PER2), cryptochrome 1 (CRY1), cryptochrome 2 (CRY2), REV-ERBα (also known as NR1D1), RORA, and casein kinase 1-epsilon/ delta (CK1ε/δ) (Buhr et al., 2013). CLOCK/BMAL1 heterodimer forms the positive elements of the central oscillatory loop, which is thought to be the basis of circadian rhythms. (Gekakis et al., 1998) The main driver of circadian rhythms pivots around the E-box-mediated negative feedback loop, in which Period
(PER) and Cryptochrome (CRY) proteins inhibit the complex CLOCK/BMAL1 transcription activity. (Buhr and Takahashi, 2013) CLOCK/BMAL1 also controls the transcription of Rev-ERBα to periodically suppress BMAL1 expression, creating an additional feedback loop. (Preitner et al., 2002) In summary, fluctuations in the expression of multiple genes explain the 24-hour circadian rhythms of physiological processes. (Panda and Hogenesch, 2004)

Circadian rhythms play an important role in human health. Studies have stated the role of it in affecting sleep, aging, cancer and vascular systems. (Jagannath et al., 2013; Scheiermann et al., 2018; Kondratov et al., 2006; Kettner et al., 2014; Paschos and FitzGerald, 2010) However, the increase in the amount of unnatural light during the day and at night brought about by a modern industrialized lifestyle disrupts the homeostasis, which is suggested by external circadian rhythms. For cancer, several lines of evidence from previous studies suggested the relationships between cancer risk and circadian rhythm disruption. According to several studies, an increased risk of breast cancer was found among shift workers. (Davis et al., 2001; Schernhammer et al., 2001; Lie et al., 2006) The significant associations between breast cancer and 3 single-nucleotide polymorphisms in CRY2 (rs11038689, rs7123390, and rs1401417) were identified. (Hoffman et al., 2010) Chu et al. found suggestive associations between prostate cancer risk and NPS2 variants in men using finasteride, and SNP rs746924 remained statistically significant after Bonferroni correction (OR = 1.5, P = 9.6 × 10^{-5}). (Chu et al., 2018) In a case-control study in China, gene-based analysis highlighted a significant association between RORA and colorectal cancer risk. (Gu et
al., 2018)

Since genetic polymorphisms and changes in expression of circadian genes are considered strongly related with increased disease risk, genetic variations in circadian rhythms have been deeply researched in many epidemiological studies. Nonetheless, to date, only a few epidemiological studies have investigated the relationship between circadian genetic factors and cancer survival outcomes compared to cancer susceptibility. Therefore, there is a need to timely review the latest epidemiological studies on rhythm genes and cancer survival outcomes in order to expand knowledge about candidate cancer genes.

**Methods**

I have systematically searched the PubMed database using keywords “cancer” or “tumor” or “leukemia” or “carcinoma” or “lymphoma” or “melanoma” and “circadian” and “survival” for studies published until Mar 2021. The initial search identified 653 publications in the English language. Thirty papers of them met the criteria of having an epidemiological focus on cancer and examining the associations between genetic circadian factors and cancer survival. The following data was extracted from these studies: cancer type, circadian genetic factors, related health outcome, sample size, hazard ratio and p-value, tissue type, PMID and authors.

I also searched Gene Expression database of Normal and Tumor tissues 2 (GENT2) and Kaplan-Meier plotter for public circadian gene expression data to assess the effect of gene expression on survival in different cancer types. (Nagy et al.,
By entering gene symbols of circadian genes and choosing tissue types, GENT2 and Kaplan Meier plotter will output Kaplan-Meier survival analysis and compare gene expression levels in tumor tissues. I assessed the expression level of \(\text{PER1}, \text{PER2}, \text{PER3}, \text{CRY1}, \text{CRY2}, \text{BMAL1}, \text{CLOCK}, \text{NPAS2}, \text{NPAS3}, \text{TIMELESS}, \text{RORA}, \text{RORC}, \text{NR1D2}, \text{CK1}\varepsilon, \text{DEC1} \) and \(\text{TIP1N}\) on overall survival in all cancer types, and presented the significant results. The number of patients with available clinical data for each cancer type was shown in Table 1. Patients’ expression levels of circadian genes were split by median. GENT2 is freely available at http://gent2.appex.kr, and Kaplan-Meier plotter is freely available at https://kmplot.com/analysis/.

Results

Thirty epidemiological studies on circadian genetic factors and cancer survival have been conducted since 2009, and all of them reported significant relationships. The statistically significant associations between \(\text{PERs}, \text{CRYs}, \text{BMAL1}, \text{CLOCK}, \text{NPASs}, \text{TIMELESS}, \text{RORA}, \text{RORC}, \text{NR1D2}, \text{CK1}\varepsilon, \text{DEC1} \) and \(\text{TIP1N}\) and cancer survival were indicated in 16 cancer types, including breast cancer, colorectal cancer, pancreatic cancer, gastric cancer, liver cancer, kidney cancer, endometrial cancer, lung cancer, prostate cancer, cervical cancer, diffuse large B-cell lymphoma, oral squamous cell carcinoma, head and neck squamous cell carcinoma, nasopharyngeal carcinoma, metastatic melanoma and soft tissue sarcoma (Table 2). All of the primary circadian genes were reported to play a role in cancer survival. The genetic factors investigated
included both gene expression and variants. Most of them were gene expression, and 10 were gene variants.

Among 16 cancer types, survival outcomes of colorectal cancer, breast cancer, pancreatic cancer, kidney cancer, gastric cancer and liver cancer were found associated more than 5 genetic circadian factors (Figure 1A). Colorectal cancer was researched most, by 10 papers, and its survival was linked to 9 genetic circadian factors (Figure 1A and 1B). The significant findings between expression of CLOCK, CRY2 and NPAS2 and breast cancer survival, expression of CRY1 and PER3 and colorectal cancer survival, as well as expression of CRY2 and pancreatic cancer survival were indicated more than once and showed consistent results (Table 2). Cadenas et al. reported that higher expression of CRY2 was associated with better metastasis free survival of breast cancer, and Mao et al. found it was linked to longer overall survival. (Cadenas et al., 2014; Mao et al., 2015) For patients with colorectal cancer, higher expression of CRY1 was observed to have longer overall survival and higher five-year survival rate (Yu et al., 2013; Hasakova et al., 2018). Yu et al. reported that the 5-year survival rate of the high CRY1 expression group was 74.7%, which was significantly lower than that of the low expression group (89.1%). (Yu et al., 2013) According to Relles et al. and Qiu et al., higher percent survival at 2 years and longer overall survival of pancreatic cancer were related to higher expression of CRY2. The percent survival at 2 years of patients with low CRY2 expression was 22.3%, while that of patients with high expression was 43.9%. (Relles et al., 2012)

Expression and variants of these genes were observed to have positive or negative
effect on cancer survival (Figure 2). Higher expression of \textit{PER1}, \textit{PER2}, \textit{PER3}, \textit{CRY2}, \textit{BMAL1}, \textit{CLOCK}, \textit{NPAS2}, \textit{TIMELESS}, \textit{RORA}, \textit{RORC}, \textit{NR1D2}, \textit{CK1ε} and \textit{TIPIN} showed protective effects on cancer survival. By contrast, homogeneous expression of \textit{PER2} or \textit{BMAL1}, and higher expression of \textit{CRY1}, \textit{TIMELESS} and \textit{DECI} indicated negative effects on cancer survival. Interestingly, expression of \textit{PER1}, \textit{PER2}, \textit{PER3}, \textit{CRY2}, \textit{BMAL1}, \textit{CLOCK}, \textit{NPAS2}, \textit{TIMELESS} and \textit{RORA} were associated with more than one cancer type. Besides, for the same gene, consistent directions of its effect were found across different cancer types. Higher expression of \textit{PER1} was related to better cancer survival in 6 cancer types, including breast cancer, colorectal cancer, pancreatic cancer, kidney cancer, endometrial cancer, and non-small cell lung cancer. Expression of \textit{PER2}, \textit{PER3}, and \textit{CRY2} was associated with survival of 5 different types of cancer. Expression of \textit{BMAL1}, \textit{CLOCK} and \textit{NPAS2} was related to 4 types of cancer, as well as expression of \textit{RORA} was linked to 2 types of cancer. However, significantly shorter survival was associated with higher expression of \textit{TIMELESS} in patients having breast cancer, liver cancer, kidney cancer, lung cancer, cervical cancer.

Thirteen single nucleotide polymorphisms (SNPs) were recognized in \textit{PER1}, \textit{PER2}, \textit{PER3}, \textit{CRY1}, \textit{CLOCK}, \textit{NPAS2}, \textit{NPAS3} and \textit{RORA}, indicating several statistically significant associations between circadian SNPs and cancer survival. SNP rs3027178 in \textit{PER1}, SNP rs7602358 in \textit{PER2}, SNP rs228729 in \textit{PER3}, SNP rs11133399 in \textit{CLOCK}, SNP rs2279284 in BAML1, SNP rs1053096 in \textit{NPAS2} and SNP rs2305160 in \textit{NPAS3} posed negative effects on patients’ survival with cancer. SNP rs3027178 in \textit{PER1}, SNP rs228729 in \textit{PER3}, SNP rs11133399 and SNP
rs2279284 in BAML1 in *CLOCK* were significantly related to an increased death risk in gastric cancer patients. (Qu et al., 2016; Chen et al., 2019) Epidemiological study of Yuan et al. indicated that SNP rs1053096 in *NPAS2* and SNP rs2305160 in *NPAS3* increased overall death risk of hepatocellular carcinoma patients in the recessive model and the dominant model. (Yuan et al., 2014) In soft tissue sarcoma, SNP rs7602358 in *PER2* had detrimental effects on patients’ survival. (Benna et al., 2018)

On opposite, SNP rs2640908 in *PER3*, SNP rs1056560 in *CRY1*, SNP rs76436997 in *RORA* had significant protective effect on cancer survival. In hepatocellular carcinoma, patients who carried at least one variant allele of SNP rs2640908 in *PER3* had a significantly decreased risk of death compared with those carrying homozygous wild-type alleles (11.6 months vs. 8.1 months). (Zhao et al., 2012) SNP rs1056560 in *CRY1* and SNP rs1044432 in BAML1 exhibited a significant protective effect on the overall survival in patients with gastric cancer. (Qu et al., 2016; Chen et al., 2019) For colorectal cancer patients, SNP rs3749474 and SNP rs1801260 in *CLOCK* and SNP rs76436997 in *RORA* increased their overall survival time. (Gu et al., 2018; Zhou et al., 2012)

The significant results of Kaplan Meier survival analysis using GENT2 database are shown in Fig. 3. The overall survival of patients in 7 cancer types (breast cancer, blood cancer, brain cancer, lung cancer, ovarian cancer, colon cancer and stomach cancer) were associated with circadian gene expression levels. In this analysis, expression of *PER2, PER3, CRY2, NR1D2, CLOCK* and *NPAS3* did not show
detrimental effects on cancer survival. Interestingly, expression of PER2, PER3, CRY2, NR1D2, CLOCK also only indicated protective effects on cancer survival among the types of cancer that have been studied in my review. Among these six genes, high PER2 expression levels were related to significantly better overall survival in 4 types of cancer, including breast cancer, blood cancer, brain cancer and ovarian cancer. High expression of PER3 were linked to significantly better overall survival in patients with breast cancer, blood cancer and brain cancer. PER1, CRY1, BMAL1, NPAS2, RORA, RORC, DEC1 and TIPIN showed different effects (protective or detrimental) on overall survival in different cancer types. What’s more, patients with breast cancer in the high PER1, PER2, PER3, CRY2 and RORC expression group had significant longer overall survival than those in the low expression group. The result was consistent with Cadenas et al.’s study which also indicating the protective effects of PER1, PER2, PER3, CRY2 and RORC in breast cancer survival.

I also performed the Kaplan Meier survival analysis to assess the effects of circadian genes on survival in 21 cancer types using Kaplan-Meier plotter database. The complete results were provided in the Supplemental Figures. Figure 4 showed the results of PER2, PER3, RORA, RORC, DEC1 and TIMELESS. PER2, PER3, RORA and RORC did not showed significantly negative effects on survival in these 21 types of cancer. PER2 was found associated with survival of kidney renal clear cell carcinoma and pancreatic ductal adenocarcinoma. PER3 was related to survival of kidney renal clear cell carcinoma and cervical squamous cell carcinoma. RORA had significantly protective effects on kidney renal clear cell carcinoma, kidney renal
papillary cell carcinoma and lung adenocarcinoma. Compared with low expression group, high expression levels of *RORC* indicated longer overall survival in patients with kidney renal clear cell carcinoma, esophageal adenocarcinoma, bladder Carcinoma, liver hepatocellular carcinoma, thymoma and thyroid carcinoma. On the contrary, *DEC1* only showed significantly detrimental effects in this analysis, and high expression of *DEC1* was correlated with worse overall survival in kidney renal clear cell carcinoma and lung adenocarcinoma. Besides, *TIMELESS* was observed to play a protective or detrimental role based on different cancer types. High expression of *TIMELESS* suggested significantly longer overall survival in patients with kidney renal clear cell carcinoma, liver hepatocellular carcinoma, lung adenocarcinoma or sarcoma. For cervical squamous cell carcinoma, stomach adenocarcinoma thyroid carcinoma, thymoma and esophageal squamous cell carcinoma, patients with high expression had significantly shorter overall survival than those with low levels of expression.

**Discussion**

In this study, I reviewed and investigated the associations between circadian genetic factors and cancer survival across different cancer types. According to my review of 30 papers, *PER1, PER2, PER3, CRY1, CRY2, BMAL1, CLOCK, NPAS2, NPAS3, TIMELESS, RORA, RORC, NR1D2, CK1ε, DEC1* and *TIPIN* were significantly related to patients’ survival in 16 types of cancer, including breast cancer, colorectal cancer, pancreatic cancer, gastric cancer, liver cancer, kidney
cancer, endometrial cancer, lung cancer, prostate cancer, cervical cancer, diffuse large B-cell lymphoma, oral squamous cell carcinoma, head and neck squamous cell carcinoma, nasopharyngeal carcinoma, metastatic melanoma and soft tissue sarcoma. The results of the review are consistent, indicating that circadian genes may play a role in cancer survival. Furthermore, I conducted survival analysis using GENT2 and Kaplan-Meier plotter database, which also indicated what expression level of circadian genes was significantly associated with cancer overall survival.

Our results suggested that the associations between circadian genes and cancer survival differed among different genes and cancer types. Circadian genes may act dissimilar roles in different tumor environments and organisms, showing the complexity of the circadian transcriptional loops. For example, TIMELESS was associated with poorer survival in patients with ovarian cancer but better survival in cervical squamous cell carcinoma, while others, such as PER2 and PER3, were associated with better survival in patients with kidney renal clear cell carcinoma. TIMELESS is known to be a positive regulator of DNA replication and TIMELESS-TIPIN functions as a replication fork stabilizer. (Leman et al., 2010) TIMELESS depletion increases γH2AX and causes G2 / M arrest, restricting cell proliferation. Activation of ERK can lead to the over-expression of TIMELESS in cancer, which stimulates the proliferation of cancer cells. (Neilsen et al., 2019) Differently, P53 is an important anti-cancer gene, and previous studies have shown that reducing the expression of PER2 can directly depress the activation of P53 protein, thus accelerating the development of tumor. (Fu et al., 2002) However, the exact molecular
mechanisms behind the complex clock mechanism are still largely unknown. Further confirmation of the complex function of the biological circadian rhythm in cancer is needed in the future.

The circadian genetic factors identified included both gene expression and SNPs. SNPs can cause disruption to circadian rhythms and alterations in gene expression, but a single SNP may provide moderate or undetectable effects. Besides, the analysis of SNPs needs reference alleles. When genotype or frequency data are available, it may only be known which allele is dominant and which allele is minor in a particular population. As a result, interpretation of polymorphism varies, depending on the type of cancer or the population in which it is being evaluated. Relatively, gene expression is related to the function of genes, and its biological significance is easier to be interpreted. However, the examination of SNPs is relatively convenient. Venous blood is the most commonly sampled specimen and can be easily obtained by venipuncture, while the examination of gene expression usually needs tissue samples. It is suggested to test the association between SNP and disease more effectively by jointly modeling the relationship between SNP, gene expression and disease. (Huang et al., 2014)

Overall, these results need to be interpreted with caution and further validated in more studies.

However, there existed some inconsistent results between the review and the public databases. Zhang et al. revealed that an increase in the expression of \textit{TIMELESS} was linked to poorer overall survival and disease-free survival in early-stage cervical carcinoma. (Zhang et al., 2016) Our results based on Kaplan-Meier
plotter database suggested that higher expression of \textit{TIMELESS} was related to significantly better overall survival in patients with cervical squamous cell carcinoma. The discrepancy of the results may be partly explained by different tumor subtypes or stages. Several \textit{CLOCK} genes were differentially expressed between tumor stages and showed significant differences in different patient subtypes within cancer types, suggesting that \textit{CLOCK} genes may be altered during tumor progression. (Ye et al., 2018) Different tumor subtypes or stages may have different genomic patterns and influence the relationship between circadian genes and prognosis. The studies used different study population. In Zhang et al.’s study, tumor specimens were collected from 189 patients with cervical cancer who underwent surgical treatment in the Cancer Center of Sun Yat-sen University from 2006 to 2010. (Zhang et al., 2016) GENT2 used gene expression profiles from more than 34,000 samples of different human cancer and normal tissues generated by the Affymetrix U133A or U133Plus2 microarray platform. (Park et al., 2019) Besides, the study population may have different treatment of cancer after surgery, which can also become a confounder.

What’s more, in the review, all the associations between circadian factors and cancer survival were significant. However, in the study using GENT2 and Kaplan-Meier plotter, quite a number of the results between circadian factors and cancer survival were not significant. It may be due to the publish bias that authors publish only results that show a significant finding disturbs the balance of findings.

This study had several limitations. Most of the significant associations were not replicated more than once. Besides, the study did not stratify tumors according to
stages and molecular subtypes. There are still huge gaps in the understanding of the association between circadian genes and different types of cancer, different subtypes of cancer, and different stages of cancer. What’s more, the timing of the acquisition of biological samples from cancer patients is unknown, which may result in significant differences in the omics data. (Ye et al., 2018) Because the results lack complete consistency, these potential biomarkers should not be used to make clinical decisions related to cancer treatment at this time. However, these findings warrant the need for further research and make the area a focus of research. Future research fields of the clinical implications of circadian genetic factors may include indicating survival, serving as potential treatment targets and supporting cancer chrono therapy.

With the development of genomic technology, molecular diagnosis has been widely used in cancer diagnosis, and more and more biomarkers have been developed. Mutations in genes such as BRCA1 and BRCA2 may increase the risk of breast, ovarian and other cancers, which have been used as biomarkers for cancer risk in clinical practice. (Chen and Parmigiani, 2007) The findings from this study suggest that dysfunctions or functional clocks may become the candidate of indicators of cancer survival and therapeutic response outcomes. Biomarker testing can help personalize the management of cancer and help cancer patients make better treatment decisions, thereby reducing cancer mortality. The clinical applicability of biomarkers is one of the most challenging parts of converting research results into practical applications. In order to apply these results to maximize patient benefits, the effectiveness of markers should be evaluated using the disciplined application of well-
designed clinical trials.

Uncovering the function of genes linked to cancer and finding less harmful ways to improve cancer outcomes has long been the focus of cancer researchers around the world. Currently, targeted therapy is the focal point of anticancer drug development. Effective identification of potential targets that play essential roles in the growth and survival of cancer cell is required by the realization of targeted therapies. In this study, the significant associations between circadian genetic factors and cancer survival may help find potential therapeutic targets for cancer. If activators of positive regulator circadian genes or inhibitors of negative regulator circadian genes can be developed, it may be possible to use them as treatments and effectively improve patients’ survival.

Understanding the role and relationship of clock genes in cancer provides an important basis for the development of timed therapy for cancer. The influence of circadian rhythm biology on the system level has been recognized in the field of chronotherapy. In essence, the field seeks to understand how time of day affects the efficacy, metabolism, toxicity, and off-target effects of therapeutic agents. (Levi and Schibler, 2007) The concept of circadian-rhythm-based cancer treatment has also developed rapidly, whose basic objective is to improve the effectiveness of chemotherapy drugs as well as minimize adverse reactions through appropriate timing of administration. Giacchetti et al. conducted a meta-analysis on the effects of circadian chemotherapy on patients’ survival with metastatic colorectal cancer, finding that overall survival was significantly improved in males on chrono
modulated chemotherapy (median values of 20.8 months) rather than on conventional chemotherapy (median values of 17.5 months). (Giacchetti et al., 2012) Giving drugs at a time with relative high expression level of positive regulator genes and low expression level of negative regulator genes may facilitate the development of effective new anticancer drugs or improved drug delivery plans and ultimately improve patients’ health. By modeling, simulation of complex systems and clinical trials, it may help determine the optimal model structure and parameters, as well as the proper time. Currently, circadian rhythm studies are not yet a routine part of drug safety and efficacy trials, and the effects of the duration on most drugs on the market has not been widely explored. In the future, timed therapy focusing on circadian gene expression may become a possible direction for improving cancer treatment in clinical.

Conclusions

This study reviewed and demonstrated the significant associations between circadian genetic factors and cancer survival across different cancer types. Although results have been achieved in this area of research, clinical trials important to exploit them have not yet been carried out. More research is warranted before circadian genetic factors can be used as biomarkers of cancer survival outcomes.
Table 1. Sample size for each cancer type in GENT2 and KM Plotter.

<table>
<thead>
<tr>
<th>Database</th>
<th>Cancer Type</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENT2</td>
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<tr>
<td></td>
<td>blood cancer</td>
<td>554</td>
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<tr>
<td></td>
<td>brain cancer</td>
<td>50</td>
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<tr>
<td></td>
<td>colon cancer</td>
<td>1003</td>
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<tr>
<td></td>
<td>lung cancer</td>
<td>445</td>
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<tr>
<td></td>
<td>ovary cancer</td>
<td>166</td>
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<tr>
<td></td>
<td>stomach cancer</td>
<td>300</td>
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<tr>
<td>KM Plotter</td>
<td>Bladder Carcinoma</td>
<td>405</td>
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<tr>
<td></td>
<td>Breast cancer</td>
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<td></td>
<td>Cervical squamous cell carcinoma</td>
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<td></td>
<td>Esophageal Adenocarcinoma</td>
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<td></td>
<td>Esophageal Squamous Cell Carcinoma</td>
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<td></td>
<td>Head-neck squamous cell carcinoma</td>
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<td></td>
<td>Kidney renal clear cell carcinoma</td>
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<tr>
<td></td>
<td>Kidney renal papillary cell carcinoma</td>
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<td></td>
<td>Liver hepatocellular carcinoma</td>
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<td></td>
<td>Lung adenocarcinoma</td>
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<td></td>
<td>Lung squamous cell carcinoma</td>
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<td>Ovarian cancer</td>
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<td>Pancreatic ductal adenocarcinoma</td>
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<td>Pheochromocytoma and Paraganglioma</td>
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<td>Rectum adenocarcinoma</td>
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<td>Testicular Germ Cell Tumor</td>
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<td></td>
<td>Thymoma</td>
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<td></td>
<td>Thyroid carcinoma</td>
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<td></td>
<td>Uterine corpus endometrial carcinoma</td>
<td>543</td>
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Table 2. Summary of significant associations between circadian genetic factors and cancer survival.
<table>
<thead>
<tr>
<th>Cancer</th>
<th>Gene</th>
<th>Related Health Outcomes</th>
<th>P-value</th>
<th>HR (95% CI)</th>
<th>Tissue type</th>
<th>Sample size</th>
<th>FDR Authors</th>
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<td>Breast cancer</td>
<td>noggin-like expression of PER2 in Brca1-/- mice and heterogeneous expression of CLOCK</td>
<td>poor prognosis</td>
<td>P &lt; 0.05</td>
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<td>tissue samples</td>
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<td>0.039</td>
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<td>P &lt; 0.01</td>
<td>HR = 1.44, 95% CI: 1.05-1.91</td>
<td>tissue samples</td>
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<td>P &lt; 0.03</td>
<td>HR = 0.62, 95% CI: 0.40-0.95</td>
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<td>expression of TIMELESS</td>
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<td>HR = 0.80, 95% CI: 0.70-0.92</td>
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**Figure 1. A. The number of circadian factors related with cancer survival by**
cancer type. B. The number of papers indicating associations between circadian factors and cancer survival by cancer type.

Figure 2. The number of cancer types related to genetic circadian factors.
Figure 3. The Kaplan Meier survival curves for cancer patients with high and low circadian
gene expression using GENT2 database. It includes fifteen genes: *PER1, PER2, PER3, CRY1, CRY2, BMAL1, NPAS2, RORA, RORC, NR1D2, DEC1, TIPIN, CLOCK, NPAS3* and *TIMELESS*.
Figure 4. The Kaplan Meier survival curves for cancer patients with high and low circadian gene expression using Kaplan-Meier plotter database. It includes six genes: PER2, PER3, RORA, RORC, DEC1 and TIMELESS.
Supplemental Figures

Figure 5. The Kaplan Meier survival curves for cancer patients with high and low circadian gene expression using Kaplan-Meier plotter database.

**PER2**

**Kidney renal clear cell carcinoma**

HR = 0.58 (0.43 - 0.79)  
logrank P = 0.00042

**Pancreatic ductal adenocarcinoma**

HR = 0.65 (0.43 - 0.98)  
logrank P = 0.038

**PER3**

**Cervical squamous cell carcinoma**

HR = 0.56 (0.35 - 0.91)  
logrank P = 0.016

**Kidney renal clear cell carcinoma**

HR = 0.55 (0.4 - 0.75)  
logrank P = 0.00012
References


Mao Y, Fu A, Hoffman AE, et al. The circadian gene CRY2 is associated with breast


Xiong H, Yang Y, Yang K, Zhao D, Tang H, Ran X. Loss of the clock gene PER2 is associated with cancer development and altered expression of important tumor-