PLOS ONE

RESEARCH ARTICLE

# Low circadian clock genes expression in cancers: A meta-analysis of its association with clinicopathological features and prognosis

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**Citation:** Zhang J, Lv H, Ji M, Wang Z, Wu W (2020) Low circadian clock genes expression in cancers: A meta-analysis of its association with clinicopathological features and prognosis. PLoS ONE 15(5): e0233508. https://doi.org/10.1371/journal.pone.0233508

**Editor:** Gianfranco D. Alpini, Texas A&M University, UNITED STATES

**Received:** February 21, 2020

**Accepted:** May 6, 2020

**Published:** May 21, 2020



**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Funding:** This review was supported by Shenzhen nanshan district science and technology bureau science development projects (Grant No. 2017035).

**Competing interests:** No authors have competing interests.

## Abstract

### Background

Per1, Per2, Per3, Cry1, Cry2, Bmal1, Npas2 and CLOCK genes are the eight core circadian clock genes. Low expression of these circadian clock genes plays an important role in the progression of cancers. However, its clinicopathological and prognostic value in patients with cancers remains controversial and inconclusive. We performed a meta-analysis of studies assessing the clinicopathological and prognostic significance of low expression of these genes in cancers.

### Methods

Relevant studies were searched from the Cochrane Central Register of Controlled Trials, Embase, EBSCO, Ovid, PubMed, Science Direct, Wiley Online Library database, CNKI and Wan Fang database. The meta-analysis was performed by using STATA version 12 software. A random-effect model was employed to evaluate all pooled hazard ratios (HRs) and odd ratios (ORs).

### Results

A total of 36 studies comprising 7476 cases met the inclusion criteria. Meta-analysis suggested that low expression of Per1 was associated with poor differentiation (Per1: OR=2.30, 95%CI: 1.36 ~ 3.87, *P*=0.002) and deeper invasion depth (Per1: OR=2.12, 95%CI: 1.62 ~ 2.77, <0.001); low Per2 expression was correlated with poor differentiation (Per2: OR=2.41, 95%CI: 1.53 ~ 3.79, <0.001), worse TNM stage (Per2:OR=3.47, 95%CI: 1.88 ~ 6.42, *P*<0.001) and further metastasis (Per2:OR=2.35, 95%CI: 1.35 ~ 4.11, =0.003). Furthermore, the results revealed that low expressions of Per1 and Per2 were also correlated with poor overall survival of cancers (Per1: HR=1.35, 95%CI: 1.06 ~ 1.72, *P*=0.014; Per2: HR=1.43, 95%CI: 1.10 ~ 1.85, *P*=0.007). Subgroup analysis indicated that low Per1 and Per2 expressions were especially associated with poor prognosis of gastrointestinal

caners (Per1: HR=1.33, 95%CI: 1.14 ~ 1.55, <0.001, $^2$=4.2%; Per2: HR=1.62, 95%CI: 1.25 ~ 2.18, *P*<0.001, $I^2$=0.0%).

## Conclusions

Our study suggested that low Per1, Per2 and Npas2 expression played a distinct and crucial role in progression of cancers. Low expressions of Per1 and Per2 could serve as unfavorable indicators for cancers prognosis, especially for gastrointestinal cancers.

## Introduction

Period1 (Per1), period2 (Per2), period3 (Per3), cryptochrome1 (Cry1), cryptochrome2 (Cry2), aryl hydrocarbon receptor nuclear translocator-like protein 1 (Bmal1), neuronal PAS domain protein 2 (Npas2) and circadian locomoter output cycles protein kaput (CLOCK) genes are the eight core circadian clock genes that generate and maintain circadian rhythms in many physiologic processes [1, 2]. Per1, Per2 and Per3 were reported to play an important role in regulating cancer cell growth, proliferation and apoptosis [3, 4, 5]. Cry1 and Cry2 acted as transcriptional regulators and checkpoint proteins for cancer cell proliferation and cell cycle control [6, 7]. Bmal1 and Npas2 could regulate cancer cell proliferation and invasion through suppressing the transcription of c-Myc[8, 9]. CLOCK might interact with HIF-1α/ Bmal1 and activate VEGF to stimulate tumor angiogenesis and metastasis [10]. These eight circadian clock genes take part in the carcinogenesis and development of many cancers. Recent studies demonstrated that disrupted expression of these genes was associated with poor progression and prognosis of cancers. For example, low Per1, Per2 and Per3 expressions in different cancers were found to correlate with worse histological grade and poor prognosis [3, 5, 11–22]; Cry2 was reported downregulated in breast and pancreatic cancer and its low expression was associated with higher tumor grade and shorter survival time [23, 24]; reduced Bmal1 and CLOCK expressions were confirmed to result in poor outcome of colon, pancreatic, kidney, head and neck cancers [15, 25, 26]; low Npas2 expression was also found to be related with worse overall survival (OS) in colorectal and breast cancers [9, 27]. However, several other studies showed that low expression of these genes was not correlated with the prognosis of cancers. For example, low Per1 expression in lung cancer was not related to prognosis [28]; downregulated Per2 and Per3 expression were not correlated with gastric and colorectal cancer (CRC) prognosis [29, 30]; low Cry1 expression was not an independent prognostic factor for ovarian cancer [31];reduced expression of Bmal1 and CLOCK were not associated with lung cancer survival and CRC outcomes[28, 30]; Moreover, some studies even implied that overexpression of some of these genes was associated with unfavorable prognosis in patients with cancers. For example, Cry1 and Cry2 overexpression was associated with poor OS in gastric cancer and CRC [29, 32, 33]; Npas2 was frequently upregulated in hepatocellular carcinoma (HCC) and its overexpression significantly contributed to poor prognosis of HCC patients [34]. The clinicopathological and prognostic value of these circadian clock genes in cancers remains controversial and inconclusive. Therefore, we conducted this meta-analysis by integrating published data and online database to clarify the influence of low expression of these seven circadian clock genes on the clinicopathological features and prognosis of different cancers.

## Materials and methods

### 1. Literature search

We systematically searched through the databases (Cochrane Central Register of Controlled Trials, Embase, EBSCO, Ovid, PubMed, Science Direct and Wiley Online Library, China National Knowledge Infrastructure (CNKI) and Wan Fang database) to obtain relevant articles that were published before 1 January 2020. The following terms and phrases were used as search criteria: 'circadian clock gene' or 'period1 (Per1)' or 'period2 (Per2)', or 'period3 (Per3)' or 'cryptochrome1 (Cry1)' or 'cryptochrome2 (Cry2)' or 'aryl hydrocarbon receptor nuclear translocator-like protein 1 (Bmal1)' or 'neuronal PAS domain protein 2 (Npas2) ' or 'circadian locomoter output cycles protein kaput (CLOCK)' and 'neoplasm' or 'tumor' or 'cancer', 'carcinoma' and 'prognosis' or 'overall survival (OS)' or 'mortality' or 'clinic outcome' or 'clinicopathological feature' or 'odd ratio (OR)' or 'hazard ratio (HR)'. The title and abstract of each study obtained in the search was scanned to exclude any clearly irrelevant ones. The remaining articles were reviewed, analyzed, evaluated to determine whether they contained information on the topic of interest. The reference lists of these articles with information on the topic were also reviewed for additional pertinent studies.

### 2. Inclusion and exclusion criteria

Inclusion criteria were as follows: (1) patients diagnosed with cancers; (2) immunohistochemical (IHC) analysis, quantitative PCR, RNA-Sequence analysis and in situ hybridization detection of circadian clock genes expression in tissues; (3) relationships between abnormal expression of circadian clock genes and clinicopathological features or prognostic indicators that were evaluated; (4) odds ratio (OR), hazard ratio (HR) and 95% confidence intervals (CI) that could be obtained directly or indirectly calculated based on the data provided in the graphics and tables; (5) only the newest studies were retained if the data were repeated in different studies and (6) studies in English or Chinese.

Exclusion criteria were as follows: (1) cell or animal studies, letters, case reports, reviews and meta-analyses; (2) articles with similar content or those with small sample sizes ($\leq$ 10) and (3) articles with language barriers.

### 3. Data extraction

The articles that met the criteria were reviewed by two independent investigators (Zhimo-Wang and HongLv) and extracted data on author, year of publication, nationality, sample size, patient age, detection method, clinical stage and pathological degree. Discrepancies in terms of data extraction were resolved by discussion among all the authors.

### 4. Statistical analysis

The ORs and 95%CI between aberrant circadian clock genes expression and clinicopathological indexes were calculated from the original data in articles using statistical software. The prognostic effects of low circadian clock genes expression were detected by merging the HRs and 95%CI of the included literatures using the forest plot. The HRs and 95% CI values either came from direct extraction of the original text or indirect extraction of survival curve through Engauge Digitizer version 4.1 (https://sourceforge.net/projects/digitizer/) [35].

Heterogeneity was measured by $Q$ statistics as follows: no heterogeneity: $0<I^2<25\%$; low heterogeneity: $25\%\leq I^2<50\%$; moderate heterogeneity: $50\%\leq I^2<75\%$; high heterogeneity: $75\%\leq I^2\leq 100\%$. A random effects model was used to pool HRs and ORs with or without significant heterogeneity. An $I^2<50\%$ was considered acceptable, and a P value>0.10 signified an

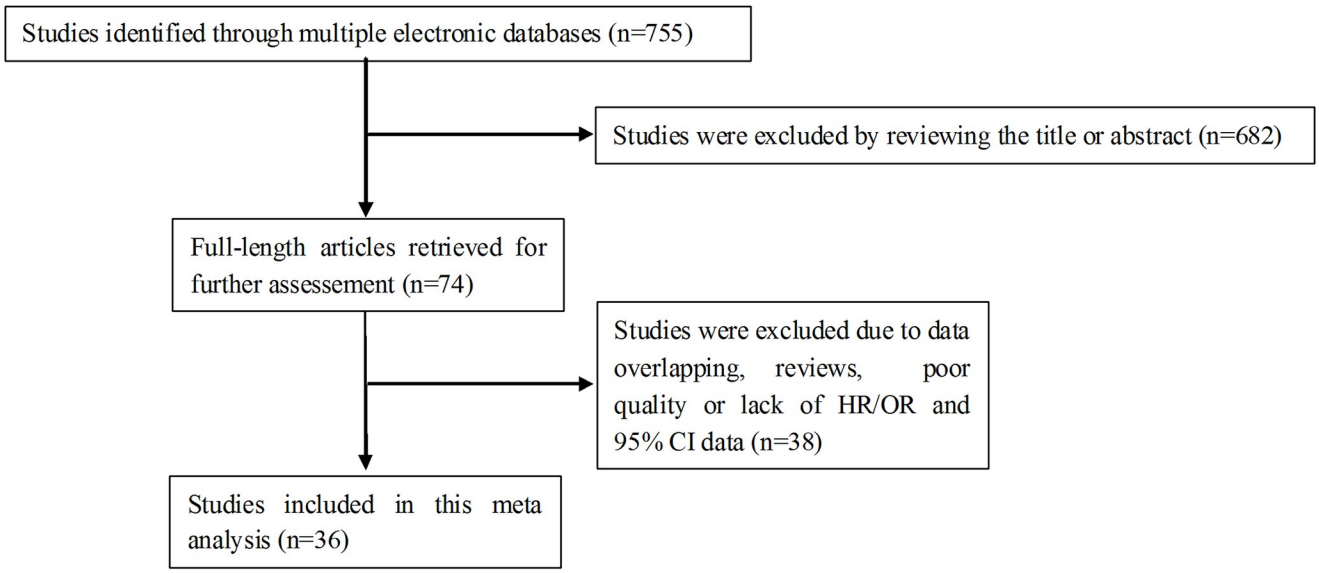


**Fig 1. Flow diagram of the study selection process.**

https://doi.org/10.1371/journal.pone.0233508.g001

acceptable degree of homogeneity. Sensitivity and subgroup analysis for the source of the heterogeneity was performed according to publication year, population, detecting method, pathological types and patient number. Publication bias was detected by Begg's funnel plot and Egger's test. A two-sided *P* value <0.05 was considered to indicate statistical significance. Statistical analyses were carried out with Stata SE 12.0, Engauge, Microsoft Office 2007.

## Results

### 1. Eligible studies

A total of 755 articles were identified from a search of the included databases using the search strategy as described in Fig 1. 682 articles were excluded through reviewing the titles and abstracts. The remaining 74 articles were then fully examined for their fit with the current meta-analysis, and a further 38 articles were excluded because they met one or more of the exclusion criteria. The final 36 studies [3, 6, 9, 11–17, 19, 22, 24, 25, 27–30, 32–34, 36–50] with 7476 cases were included in our meta-analysis (Fig 1). The fundamental features of the included studies were presented in Table 1 and Fig 2. Among the 36 studies, 25 studies [3, 6, 9, 11–17, 19, 22, 28, 30, 33, 36–41, 43, 46, 47, 50] assessed the association between low circadian clock genes expression and clinicopathological features in patients with cancers, and 22 studies [3, 6, 12–15, 19, 22, 24, 27, 28, 29, 30, 32, 33, 34, 41, 42, 47–50] investigated the relationship between low expression of circadian clock genes and OS in multiple cancers.

### 2. Circadian clock genes expression and clinicopathological features of cancers

The correlation between low expression of circadian clock genes and clinicopathological features was exhibited in Table 2 and Figs 3 and 4 as follows. The pooled ORs indicated that the low expressions of Per1, Per2, Per3 and Npas2 were significantly related with poor differentiation (Per1: OR=2.30, 95%CI: 1.36 ~ 3.87,=0.002; Per2: OR=2.41, 95%CI: 1.53 ~ 3.79, *P*<0.001; Per3: OR=2.50, 95%CI: 1.10 ~ 5.66, *P*=0.001 and Npas2: OR=1.89, 95%CI: 1.47 ~ 2.43,

**Table 1. Characteristics of studies included for the meta-analysis.**

| Author | Year | Population | Cancer type | Number of patients | Gender (Male/ Female) | Detection method | Gene (Low expression, high expression) |
|---|---|---|---|---|---|---|---|
| Winter SL, et al.[36] | 2007 | Canadian | Breast cancer | 34 | 0/34 | Quantitative PCR | Per1 (16, 10) |
| Kuo SJ, et al. [37] | 2009 | Chinese | Breast cancer | 53 | 0/53 | Immunohistochemistry | Per1 (26, 27) |
| Climent J, et al.[38] | 2010 | Chinese | Breast cancer | 203 | 0/203 | Quantitative PCR | Per3 (36, 167) |
| Zhang YB, et al. [11] | 2015 | Chinese | Breast cancer | 60 | 0/60 | Immunohistochemistry | Per1 (19, 41), Per2 (13, 47) |
| Mao Y, et al. [24] | 2015 | | Breast cancer | 737 | 0/737 | Microarray | Cry2 |
| Yi C, et al. [27] | 2010 | American | Breast cancer | 287 | 0/287 | Quantitative PCR | Npas2 (94, 193) |
| Zhao N, et al. [40] | 2013 | Chinese | Buccal squamous cell carcinoma | 38 | 16/22 | Immunohistochemistry | Per1 (6, 32) |
| Yang C, et al. [14] | 2018 | Chinese | Cervical squamous cell carcinoma | 239 | 0/239 | IlluminaHiSeq-miRNASeq | Per1 (138, 101) |
| Eisele L, et al. [45] | 2009 | German | Chronic lymphocytic leukemia | 116 | 82/34 | Quantitative PCR | Cry1 (62, 46) |
| Wang X, et al. [22] | 2012 | Chinese | Colon cancer | 203 | 86/117 | Immunohistochemistry | Per3 (36, 167) |
| Wang Y, et al. [41] | 2015 | Chinese | Colon cancer | 203 | 86/117 | Immunohistochemistry | Per1 (21, 182) |
| | | | Colon cancer | 454 | 240/214 | RNA-Seq analysis | CLOCK (258, 196) |
| Oshima T, et al. [30] | 2011 | Japanese | Colorectal cancer | 202 | 110/92 | Quantitative PCR | Per1 (101, 101), Per2 (101, 101), Per3 (101, 101), Cry1 (101, 101), Cry2 (101, 101), Baml1 (101, 101), CLOCK(101, 101) |
| Wu S, et al. [42] | 2016 | Chinese | Colorectal cancer | 214 | | HiSeq platform | Per1 (82, 132) |
| Hasakova K, et al. [44] | 2018 | Slovakian | Colorectal cancer | 61 | 38/23 | Quantitative PCR | Per2 (31, 30), Cry1 (31, 30), Cry2 (31, 30) |
| Yu H, et al. [32] | 2013 | Chinese | Colorectal cancer | 168 | 89/79 | Quantitative PCR | Cry1 (67, 101) |
| Fang L, et al. [33] | 2015 | Chinese | Colorectal cancer | 289 | 147/142 | Immunohistochemistry | Cry2 (165, 124) |
| Xue X, et al. [9] | 2014 | Chinese | Colorectal cancer | 108 | 59/49 | Quantitative PCR | Npas2 (54, 54) |
| Yang SF, et al. [47] | 2016 | Chinese | Colorectal cancer | 120 | 79/41 | Quantitative PCR | Npas2 (97, 23) |
| Zeng Z, et al. [48] | 2014 | Chinese | Colorectal cancer | 82 | | Immunohistochemistry | Baml1 (46, 36) |
| Wang Y, et al. [16] | 2011 | Chinese | Colorectal carcinoma | 38 | 18/20 | Immunohistochemistry | Per2 (24, 14) |
| Momma T, et al. [13] | 2017 | Japanese | Colorectal carcinoma | 51 | 32/19 | In situ hybridization | Per1 (27, 24), Per2 (25, 26), CLOCK(30, 21) |
| Liu HJ, et al. [46] | 2015 | Chinese | Gastrointestinal adenocarcinoma | 63 | 40/23 | Immunohistochemistry | Cry1 (37, 26) |
| Hu ML, et al. [29] | 2014 | Chinese | Gastric cancer | 29 | 20/9 | Quantitative PCR | Per1, Per3 |
| Zhao H, et al. [12] | 2014 | Chinese | Gastric cancer | 246 | 181/65 | Immunohistochemistry | Per1 (143, 103), Per2 (160, 86) |
| Ding HB, et al. [43] | 2018 | Chinese | Gastric cancer | 106 | 68/38 | Immunohistochemistry | Per1 (4, 102), Cry1 (58, 48) |

(*Continued*)

**Table 1.** (Continued)

| Author | Year | Population | Cancer type | Number of patients | Gender (Male/ Female) | Detection method | Gene (Low expression, high expression) |
|---|---|---|---|---|---|---|---|
| Yuan P, et al. [34] | 2017 | Chinese | Hepatocellular carcinoma | 217 | | Quantitative PCR | Npas2 (108, 109) |
| Li B, et al. [50] | 2018 | Chinese | Hepatocellular carcinoma | 158 | 143/15 | Western blot analysis | CLOCK(79, 79) |
| Qiu MJ, et al. [15] | 2019 | Chinese | Kidney cancer | 530 | 344/186 | RNA-Seq analysis | Per1 (338, 192), Per2 (323, 204), Per3 (308, 219), Cry2 (302, 229), Npas2 (320,207), CLOCK (283, 247) |
| | | | Liver cancer | 371 | 250/121 | RNA-Seq analysis | Cry2 (219, 151), Npas2 (230, 141) |
| Qiu MJ, et al. [28] | 2019 | Chinese | Lung adenocarcinoma | 500 | 230/270 | RNA-Seq analysis | Per1 (367, 133), Per2 (333, 167), Per3 (333, 167), Cry1 (298, 202), Cry2 (333, 167), Npas2 (309, 191), Baml1 (310, 190), CLOCK(324, 176) |
| | | | Lung squamous cell carcinoma | 494 | 366/128 | RNA-Seq analysis | Per1 (326, 168), Per2 (318, 167), Per3 (318, 176), Cry1 (270, 224), Cry2 (293, 201), Npas2 (292, 202), Baml1 (291, 203), CLOCK(332,162) |
| De Assis LVM, et al. [49] | 2018 | American | Melanoma | 340 | | RNA-Seq analysis | Baml1 (170, 170) |
| Chi C, et al. [17] | 2013 | Chinese | Non-small cell lung cancer | 60 | 38/22 | Immunohistochemistry | Per2 (17, 43) |
| Liu B, et al. [3] | 2014 | Chinese | Non-small cell lung cancer | 130 | 75/55 | Immunohistochemistry | Per1 (44, 86), Per2 (53, 77), Per3 (48, 82) |
| Chen R, et al. [39] | 2012 | Chinese | Oral squamous cell carcinoma | 41 | 24/17 | Immunohistochemistry | Per1 (7, 34) |
| Xiong H, et al. [19] | 2018 | Chinese | Oral squamous cell carcinoma. | 40 | 25/15 | Quantitative PCR | Per2 (24, 16) |
| Tokunaga H, et al. [31] | 2008 | Japanese | Ovarian cancer | 104 | 0/104 | Quantitative PCR | Cry1 |
| Li W, et al. [25] | 2016 | Chinese | Pancreatic ductal adenocarcinoma | 87 | 51/36 | Immunohistochemistry | Baml1 (61, 26) |

https://doi.org/10.1371/journal.pone.0233508.t001

$P<0.001$), with no heterogeneity to high heterogeneity among studies. Furthermore, we also found that low expression of Per1 was obviously correlated with deeper depth of invasion (OR=2.12, 95%CI: 1.62 ~ 2.77, $P<0.001$; $I^2$=28.8%) and low Per2 expression was significantly associated with more advanced TNM stage (OR=3.47, 95%CI: 1.88 ~ 6.42, $P<0.001$; $I^2$=74.8) and more lymph node metastasis (OR=2.35, 95%CI: 1.35 ~ 4.11, $P$=0.003; $I^2$=79.4). Therefore, although heterogeneity existed, these pooled results suggested that low expressions of Per1, Per2, Per3 and Npas2 might play important roles in the development and progression of cancers.

There was no significant association between low expressions of Cry1, Cry2, CLOCK and clinicopathological parameters. The combined ORs were 0.89 (95%CI: 0.47 ~ 1.68, $P$=0.722) forCry1 and differentiation, 0.86 (95%CI: 0.22 ~ 3.26, $P$=0.825) for Cry1 and invasion depth and 0.55 (95%CI: 0.29 ~ 1.03, $P$=0.062) for Cry1 and lymph node metastasis. The pooled ORs were 1.35 (95%CI: 0.84 ~ 2.15, $P$=0.214) forCry2 and differentiation, 0.85 (95%CI: 0.44 ~ 1.66, $P$=0.636) for Cry2 and invasion depth and 1.10 (95%CI: 0.80 ~ 1.52, $P$=0.543) for Cry2 and lymph node metastasis. The pooled ORs were 0.87 (95%CI: 0.66 ~ 1.16, $P$=0.342) forCLOCK and differentiation, 1.08 (95%CI: (0.59 ~ 2.00, $P$=0.798) forCLOCK and TNM stage.

To explore the heterogeneity among these results, we conducted the subgroup analysis. The results indicated that the correlation between low Per1 expression and differentiation was exhibited in non-IHC group (OR=1.51, 95%CI: 1.10 ~ 2.08, $P$=0.010) and published after 2015

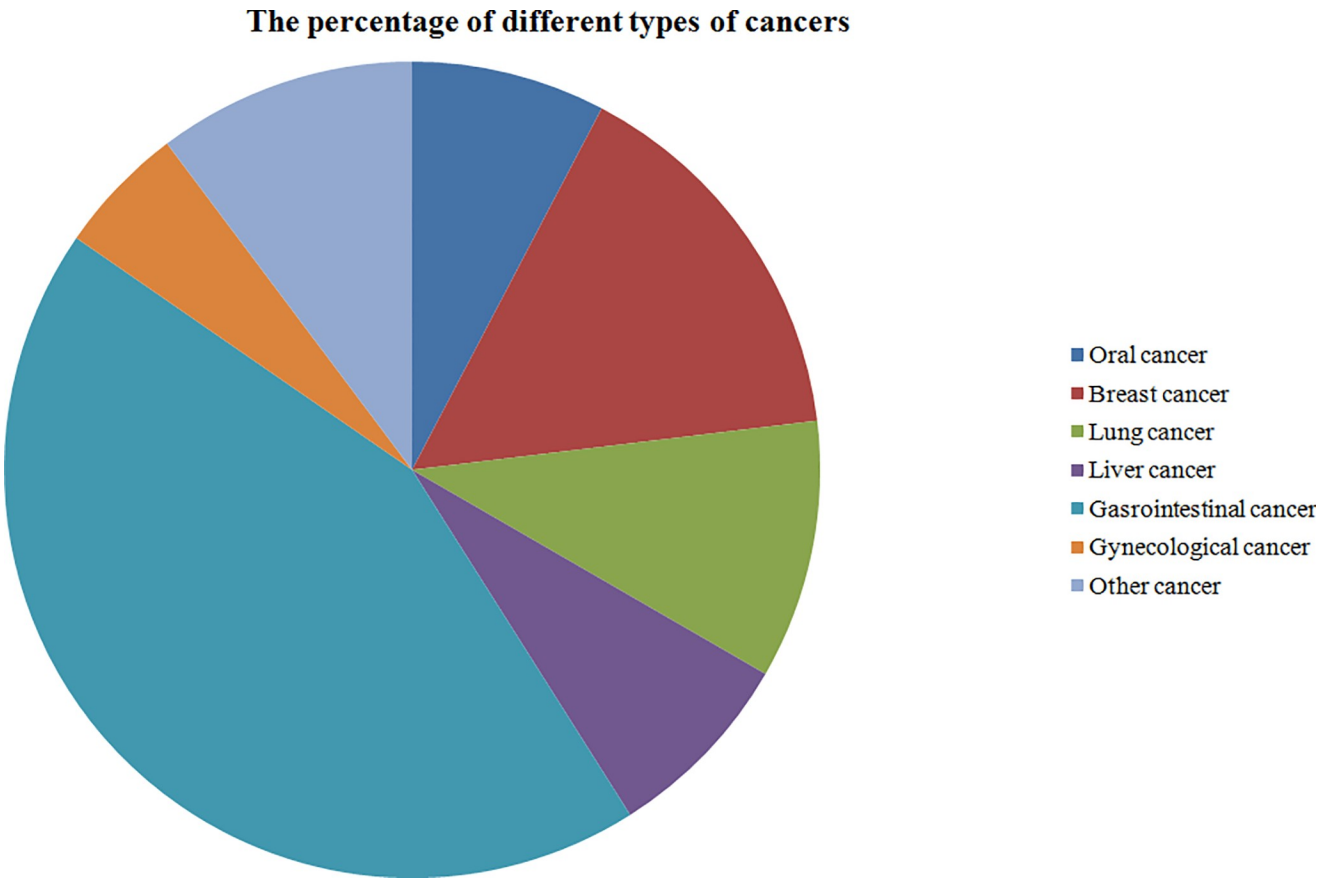


**Fig 2. The percentage of different types of cancer included for the meta-analysis.**

https://doi.org/10.1371/journal.pone.0233508.g002

group (OR=1.62, 95%CI: 1.14 ~ 2.30, *P*=0.007) without heterogeneity ($I^2$=0.0%, *P*=0.680 and $I^2$=0.0%, *P*=0.625, respectively). The relationship between low Per2 expression and differentiation was displayed in non-gastrointestinal cancer group (OR=2.82, 95%CI: 1.91 ~ 4.15, *P*<0.001, $I^2$= 37.2%, *P*=0.173) and published before 2015 group (OR=2.19, 95%CI: 1.66 ~ 2.88, *P*<0.001, $I^2$=0.0%, *P*=0.606). The correlation between low Per2 expression and TNM was also exhibited in IHC group (OR =4.82, 95%CI: 3.27 ~ 7.08, *P*<0.001) and published before 2015 group (OR=4.82, 95%CI: 3.27 ~ 7.08, *P*<0.001) without heterogeneity ($^2$=0.0%, *P*=0.908 and $I^2$= 0.0%, *P*=0.908, respectively). Furthermore, the heterogeneity among studies related to low Per2 expression and lymph node metastasis was obviously decreased in non-gastrointestinal cancer group (OR=3.89, 95%CI: 2.59 ~ 5.84, *P*<0.001, $I^2$=1.4%, *P*=0.363). Additionally, the heterogeneity among studies related to low Per3 expression and differentiation was also decreased in non-gastrointestinal cancer group ($I^2$= 5.6%, *P*=0.276) and IHC group ($I^2$=0.0%, *P*=0.326) (Table 3). These results indicated that the differences in detecting methods, publish years and pathological types might be the source of study heterogeneity.

## 3. Impact of circadian clock genes expression on overall survival of cancers

The association between low expression of circadian clock genes and OS was further explored in this meta-analysis. Low expressions of Per1 and Per2 were related to poor OS in patients with cancers (Per1: HR=1.35, 95%CI: 1.06 ~ 1.72, P=0.014 and Per2: HR =1.43, 95%CI:

**Table 2. Main meta-analysis results of association between low circadian clock genes expression and clinicopathological features in cancers.**

| Circadian clock gene | Clinicopathological parameters | No. of studies | No. of patients | Pooled OR(95% CI) | Zvalue | P-value | Heterogeneity | | Publication bias | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | $I^2$ (%) | P-value | Begg's P value | Egger's P value |
| Per1 | Differentiation (Moderate+Well/ Poor) | 11 | 1588 | 2.30 (1.36, 3.87) | 3.13 | 0.002 | 64.7 | 0.002 | 1.000 | 0.832 |
| | Clinical Stage (I+II/III+IV) | 7 | 692 | 1.85 (0.85, 4.00) | 1.56 | 0.120 | 84.6 | <0.001 | 0.230 | 0.168 |
| | Depth of invasion (T1+T2/T3 +T4) | 7 | 911 | 2.12 (1.62, 2.77) | 5.49 | <0.001 | 28.8 | 0.209 | 0.368 | 0.601 |
| | Lymph node metastasis (Absent/ Present) | 9 | 1051 | 1.98 (0.77, 5.09) | 1.42 | 0.155 | 91.0 | <0.001 | 0.251 | 0.311 |
| | Tumor size (<5CM/≥5CM) | 5 | 639 | 0.91 (0.60, 1.36) | 0.48 | 0.630 | 53.0 | 0.075 | 0.806 | 0.301 |
| Per2 | Differentiation (Moderate+Well/ Poor) | 9 | 1357 | 2.41 (1.53, 3.79) | 3.78 | <0.001 | 68.3 | 0.001 | 0.754 | 0.525 |
| | Clinical Stage (I+II/III+IV) | 4 | 397 | 3.87 (0.40, 37.23) | 1.17 | 0.241 | 96.7 | <0.001 | 0.734 | 0.623 |
| | Depth of invasion (T1+T2/T3 +T4) | 6 | 707 | 1.88 (0.75, 4.74) | 1.35 | 0.178 | 89.5 | <0.001 | 1.000 | 0.847 |
| | TNM (I + II/III + IV) | 4 | 758 | 3.47 (1.88, 6.42) | 3.98 | <0.001 | 74.8 | 0.008 | 1.000 | 0.472 |
| | Lymph node metastasis (Absent/ Present) | 7 | 767 | 2.35 (1.35, 4.11) | 3.00 | 0.003 | 79.4 | <0.001 | 0.548 | 0.391 |
| | Tumor size (<5CM/≥5CM) | 3 | 358 | 0.69 (0.33, 1.42) | 1.01 | 0.314 | 68.6 | 0.041 | 0.296 | 0.150 |
| Per3 | Differentiation (Moderate+Well/ Poor) | 4 | 1065 | 2.50 (1.10, 5.66) | 2.19 | 0.029 | 81.5 | 0.001 | 0.734 | 0.280 |
| | Depth of invasion (T1+T2/T3 +T4) | 3 | 535 | 2.45 (0.78, 7.89) | 1.54 | 0.124 | 86.1 | 0.001 | 0.296 | 0.204 |
| | Lymph node metastasis (Absent/ Present) | 3 | 535 | 1.50 (0.81, 2.79) | 1.29 | 0.197 | 61.2 | 0.076 | 0.296 | 0.173 |
| Cry1 | Differentiation (Moderate+Well/ Poor) | 4 | 539 | 0.89 (0.47, 1.68) | 0.36 | 0.722 | 52.8 | 0.095 | 0.734 | 0.580 |
| | Depth of invasion (T1+T2/T3 +T4) | 4 | 539 | 0.86 (0.22, 3.26) | 0.22 | 0.825 | 85.5 | <0.001 | 0.734 | 0.319 |
| | Lymph node metastasis (Absent/ Present) | 4 | 539 | 0.55 (0.29, 1.03) | 1.87 | 0.062 | 62.9 | 0.044 | 0.734 | 0.453 |
| | Tumor size (<5CM/≥5CM) | 4 | 539 | 1.10 (0.77, 1.56) | 0.52 | 0.603 | 0.0 | 0.464 | 0.734 | 0.326 |
| Cry2 | Differentiation (Moderate+Well/ Poor) | 4 | 1392 | 1.35 (0.84, 2.15) | 1.24 | 0.214 | 71.6 | 0.014 | 0.308 | 0.201 |
| | Depth of invasion (T1+T2/T3 +T4) | 2 | 491 | 0.85 (0.44, 1.66) | 0.47 | 0.636 | 58.6 | 0.120 | 1.000 | |
| | Lymph node metastasis (Absent/ Present) | 2 | 491 | 1.11 (0.80, 1.52) | 0.61 | 0.543 | 0.0 | 0.791 | 1.000 | |
| Npas2 | Differentiation (Moderate+Well/ Poor) | 4 | 1129 | 1.89 (1.47, 2.43) | 4.98 | <0.001 | 0.0 | 0.943 | 0.089 | 0.003 |
| | TNM (I + II/III + IV) | 5 | 2123 | 0.79 (0.40, 1.55) | 0.70 | 0.486 | 86.6 | <0.001 | 0.221 | 0.020 |
| CLOCK | Differentiation (Moderate+Well/ Poor) | 4 | 941 | 0.87(0.66, 1.16) | 0.95 | 0.342 | 0.0 | 0.453 | 1.000 | 0.707 |
| | TNM (I + II/III + IV) | 3 | 1142 | 1.08(0.59, 2.00) | 0.26 | 0.798 | 80.8 | 0.006 | 0.296 | 0.531 |

https://doi.org/10.1371/journal.pone.0233508.t002

1.10 ~ 1.85, P=0.007), with high observed heterogeneity (Per1: $I^2$=77.1%, P<0.001 and Per2: $I^2$=63.1%, P=0.006) (Table 4 and Fig 5). These results demonstrated that low expressions of Per1 and Per2 were significantly associated with worse prognosis in cancers.

No significant correlation was found between low expression of Per3, Cry1, Cry2, Npas2, Baml1, CLOCK and OS (Per3: HR=1.32, 95%CI: 0.99 ~ 1.76; Cry1: HR=0.79, 95%CI:

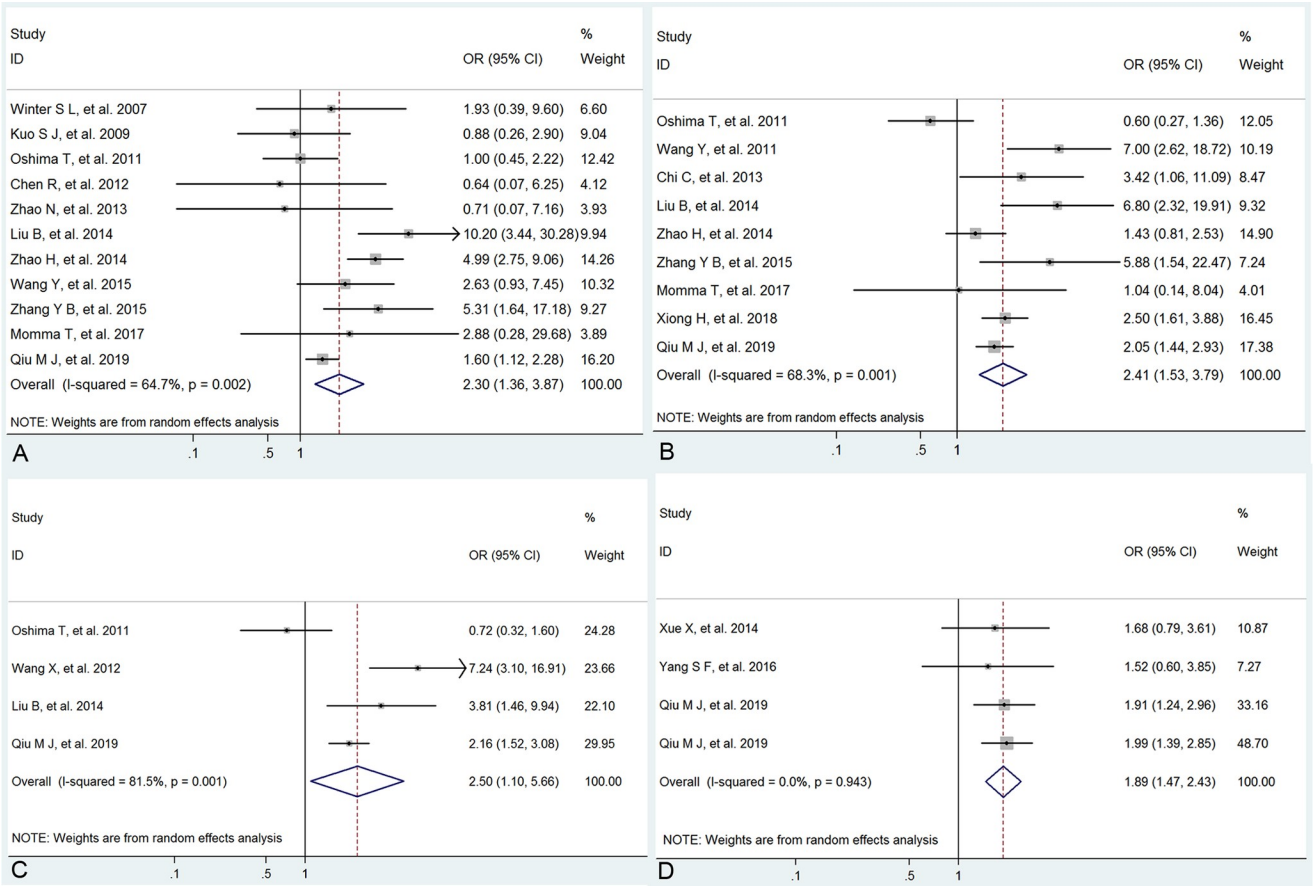


**Fig 3. Forrest plot of odds ratio (OR) for the association of low Per1 (A), Per2 (B), Per3 (C) and Npas2 (D) expression and cancer differentiation.**

https://doi.org/10.1371/journal.pone.0233508.g003

0.54～1.11; Cry2: HR=1.06, 95%CI= 0.82～1.37; Npas2: HR=0.85, 95%CI: 0.61～1.19; Baml1: HR=1.10, 95%CI: 0.82～1.49 and CLOCK: HR=1.05, 95%CI: 0.74～1.48) (Table 4).

To explain the heterogeneity in OS, subgroup analysis was performed, and the results showed that the heterogeneity among studies related to low Per1 expression and OS was obviously decreased in gastrointestinal cancer group (HR=1.33, 95%CI: 1.14～1.55, P<0.001, $I^2$=4.2%, P=0.395) and non-Chinese group (HR=1.52, 95%CI: 1.02～2.08, P=0.041, $I^2$=8.6%, P=0.296) (Table 3). The heterogeneity among studies related to low Per2 expression and OS was significantly decreased in gastrointestinal cancer group (HR=1.62, 95%CI: 1.25～2.18, P<0.001, $I^2$=0.0%, P=0.851), IHC group (HR=1.92, 95%CI: 1.24～2.96, P=0.003, $I^2$=47.1%, P=0.169) and published before 2015 group (HR=1.85, 95%CI: 1.42～2.39, P<0.001, $I^2$=0.0%, P=0.370) (Table 3). The differences in pathological types, populations, detecting methods and publish years might contribute to heterogeneity in these results.

## 4. Sensitivity analysis and publication bias

Sensitivity analysis was performed to check the stability of statistically significant results. As showed in Figs 6, 7 and 8, the pooled ORs or HRs and 95% CIs did not change substantially after removing one study at a time in the comparison between low and high expression of Per1, Per2 and Npas2.However, after removing single study out one by one, the relationship

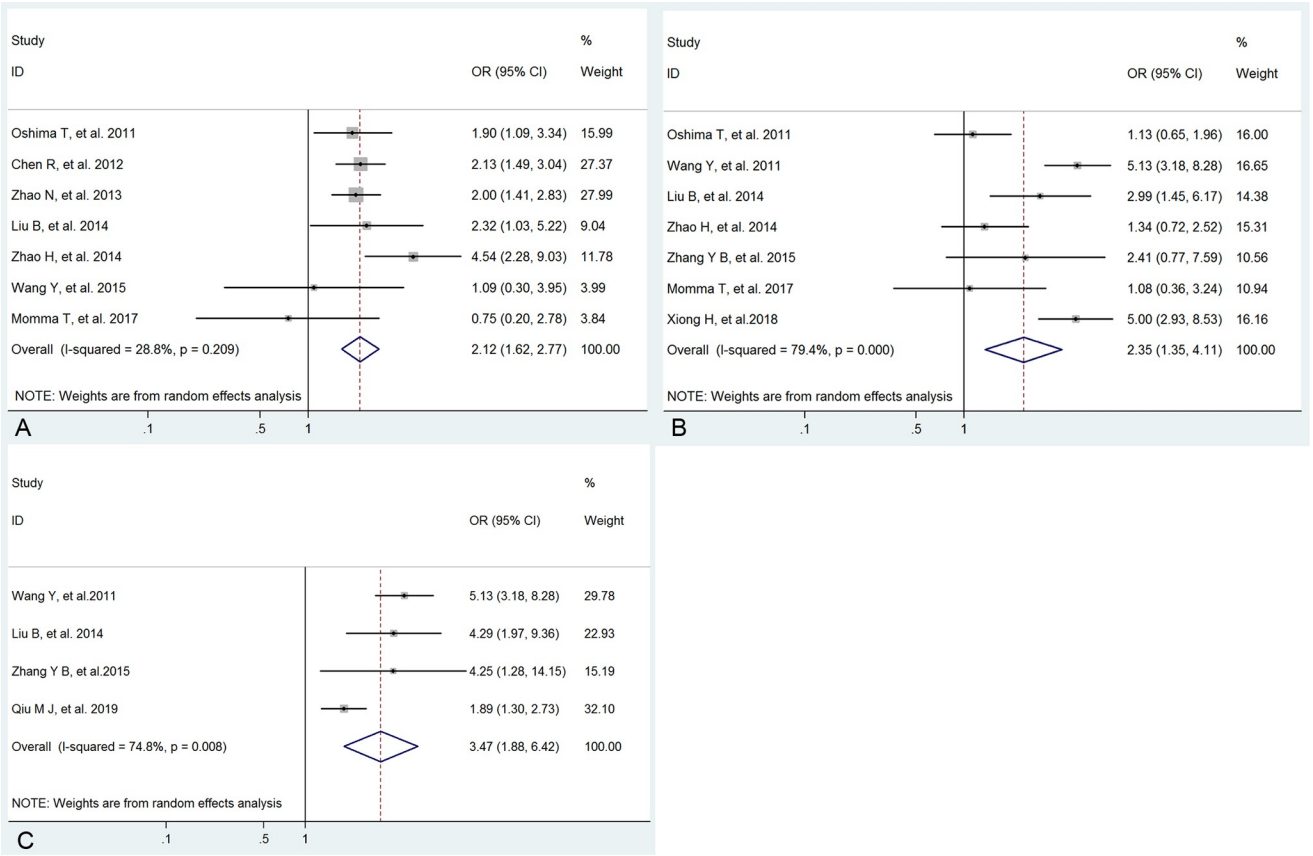


**Fig 4. Forrest plot of odds ratio (OR) for the association of low Per1 expression and depth of invasion (A), low Per2 expression and lymph node metastasis (B) and TNM stage (C).**

https://doi.org/10.1371/journal.pone.0233508.g004

between low expression of Per3 and differentiation become not significant (from OR=2.50, 95%CI: 1.10～5.66to OR=2.68, 95%CI: 0.65～11.07) (Fig 7C). These results suggested that the meta-analysis of Per1, Per2 and Npas2 were reliable and stable. Publication bias was detected by Begg' funnel plot and Egger's test in the current meta-analysis and the shape of the funnel plots seemed symmetrical in Figs 9, 10 and 11. Thus publication bias might not have a substantial influence on the result of this meta-analysis.

## Discussion

Meta-analysis is a quantitative statistical method that summarizes results of different studies with the same theme to reach a general conclusion. This approach has been successfully used for evaluation of clinicopathological and prognostic parameters in patients with cancers. Circadian clock genes and gene products generate overt circadian rhythms. The disruption of circadian clock genes expression leads to loss of circadian oscillations, such as loss of the 24 h rest-activity cycle, serum corticosterone level daily rhythms, lymphocyte count and body temperature rhythm, which has been associated with higher tumorigenesis rates, faster tumor growth in humans and animal models [42, 51]. However, the relationship between circadian clock genes expression and clinicopathological and prognostic features of cancers was controversial. Therefore, it is rather necessary to analyze and combine these data to reach a reasonable conclusion. Recently, many studies have demonstrated that low expression of circadian

**Table 3. Subgroup analysis results of association between low circadian clock genes expression and clinicopathological and prognostic parameters in cancers.**

| Circadian clock gene | Clinicopathological parameters | | No. of studies | No. of patients | Pooled HR or OR (95% CI) | Z value | P-value | Heterogeneity | | Publication bias | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $I^2$ (%) | P-value | Begg's P value | Egger's P value |
| Per1 | Overall survival(Low/High) | Chinese | 10 | 2585 | 1.33 (1.02, 1.74) | 2.09 | 0.037 | 80.0 | <0.001 | 0.592 | 0.183 |
| | | Non-Chinese | 2 | 253 | 1.52 (1.02, 2.28) | 2.05 | 0.041 | 8.6 | 0.296 | 1.000 | |
| | | Gastrointestinal cancer | 7 | 945 | 1.33 (1.14, 1.55) | 3.59 | <0.001 | 4.2 | 0.395 | 1.000 | 0.528 |
| | | Non-gastrointestinal cancer | 5 | 1893 | 1.37 (0.86, 2.17) | 1.32 | 0.188 | 89.2 | <0.001 | 0.086 | 0.054 |
| | | IHC | 3 | 579 | 2.22 (1.29, 3.80) | 2.89 | 0.004 | 64.4 | 0.060 | 1.000 | 0.509 |
| | | Non-IHC | 9 | 2259 | 1.16 (0.92, 1.46) | 1.25 | 0.21 | 69.5 | 0.001 | 0.754 | 0.378 |
| | | Number of patients ≥100 | 9 | 2660 | 1.38 (1.00, 1.89) | 1.98 | 0.048 | 82.1 | <0.001 | 0.348 | 0.069 |
| | | Number of patients <100 | 3 | 178 | 1.30 (0.97, 1.75) | 1.78 | 0.075 | 24.0 | 0.268 | 1.000 | 0.784 |
| | | Published before 2015 | 5 | 162 | 1.66 (1.15, 2.38) | 2.72 | 0.007 | 72.3 | 0.006 | 0.462 | 0.202 |
| | | Published after 2015 | 7 | 2028 | 1.17 (0.85, 1.60) | 0.96 | 0.335 | 75.8 | <0.001 | 0.764 | 0.268 |
| | Differentiation (Moderate+Well/ Poor) | Chinese | 8 | 1301 | 2.61 (1.39, 4.92) | 2.97 | 0.003 | 71.3 | 0.001 | 0.711 | 0.757 |
| | | Non-Chinese | 3 | 287 | 1.23 (0.62, 2.44) | 0.60 | 0.546 | 0.0 | 0.585 | 0.296 | 0.061 |
| | | Gastrointestinal cancer | 4 | 702 | 2.46 (1.01, 6.04) | 1.97 | 0.049 | 70.1 | 0.018 | 1.000 | 0.733 |
| | | Non-gastrointestinal cancer | 7 | 886 | 2.16 (1.04, 4.51) | 2.06 | 0.039 | 62.9 | 0.013 | 1.000 | 0.761 |
| | | IHC | 7 | 771 | 2.95 (1.46, 5.98) | 3.01 | 0.003 | 59.5 | 0.022 | 0.230 | 0.148 |
| | | Non-IHC | 4 | 817 | 1.51 (1.10, 2.08) | 2.58 | 0.01 | 0.0 | 0.680 | 1.000 | 0.914 |
| | | Number of patients ≥100 | 5 | 1311 | 2.77 (1.34, 5.74) | 2.74 | 0.006 | 81.7 | <0.001 | 0.462 | 0.412 |
| | | Number of patients <100 | 6 | 277 | 1.76 (0.83, 3.71) | 1.47 | 0.141 | 19.5 | 0.286 | 1.000 | 0.473 |
| | | Published before 2015 | 9 | 1007 | 2.39 (1.24, 4.59) | 2.60 | 0.009 | 65.7 | 0.003 | 0.466 | 0.302 |
| | | Published after 2015 | 2 | 581 | 1.62 (1.14, 2.30) | 2.70 | 0.007 | 0.0 | 0.625 | 1.000 | |
| Per2 | Overall survival(Low/High) | Chinese | 6 | 1940 | 1.40 (1.04, 1.89) | 2.22 | 0.026 | 74.5 | 0.001 | 0.060 | 0.088 |
| | | Non-Chinese | 3 | 324 | 1.74 (1.00, 3.03) | 1.96 | 0.05 | 0.0 | 0.689 | 0.296 | 0.137 |
| | | Gastrointestinal cancer | 4 | 570 | 1.65 (1.25,2.18) | 3.50 | <0.001 | 0.0 | 0.851 | 0.308 | 0.641 |
| | | Non-gastrointestinal cancer | 5 | 1694 | 1.36 (0.96, 1.93) | 1.75 | 0.08 | 75.7 | 0.002 | 0.221 | 0.114 |
| | | IHC | 2 | 376 | 1.92 (1.24, 2.96) | 2.93 | 0.003 | 47.1 | 0.169 | 1.000 | |
| | | Non-IHC | 7 | 1888 | 1.24 (0.95, 1.60) | 1.59 | 0.111 | 49.3 | 0.066 | 0.548 | 0.215 |
| | | Number of patients ≥100 | 6 | 2102 | 1.39 (1.05, 1.83) | 2.33 | 0.02 | 73.0 | 0.002 | 0.133 | 0.078 |
| | | Number of patients <100 | 3 | 162 | 1.92 (0.84, 4.36) | 1.55 | 0.122 | 5.1 | 0.349 | 0.296 | 0.023 |
| | | Published before 2015 | 3 | 578 | 1.85 (1.42, 2.392) | 4.62 | <0.001 | 0.0 | 0.370 | 1.000 | 0.367 |
| | | Published after 2015 | 6 | 1686 | 1.15 (0.90, 1.47) | 1.10 | 0.27 | 43.1 | 0.118 | 0.707 | 0.379 |
| | Differentiation (Moderate+Well/ Poor) | Chinese | 7 | 1104 | 2.89 (1.92, 4.35) | 5.08 | <0.001 | 57.7 | 0.028 | 0.368 | 0.057 |
| | | Non-Chinese | 2 | 253 | 0.65 (0.31, 1.37) | 1.14 | 0.256 | 0.0 | 0.621 | 1.000 | |
| | | Gastrointestinal cancer | 4 | 537 | 1.62 (0.58, 4.54) | 0.91 | 0.363 | 79.3 | 0.002 | 1.000 | 0.860 |
| | | Non-gastrointestinal cancer | 5 | 820 | 2.82 (1.91, 4.15) | 5.24 | <0.001 | 37.2 | 0.173 | 0.221 | 0.032 |
| | | IHC | 5 | 534 | 3.92 (1.80, 8.54) | 3.45 | 0.001 | 68.2 | 0.014 | 1.000 | 0.054 |
| | | Non-IHC | 4 | 823 | 1.58 (0.89, 2.81) | 1.55 | 0.12 | 69.4 | 0.020 | 0.734 | 0.373 |
| | | Number of patients ≥100 | 4 | 1108 | 1.75 (0.87, 3.50) | 1.58 | 0.114 | 78.7 | 0.003 | 1.000 | 0.963 |
| | | Number of patients <100 | 5 | 249 | 3.40 (2.02, 5.71) | 4.61 | <0.001 | 28.2 | 0.234 | 1.000 | 0.595 |
| | | Published before 2015 | 3 | 621 | 2.19 (1.66, 2.88) | 5.60 | <0.001 | 0.0 | 0.606 | 1.000 | 0.601 |
| | | Published after 2015 | 6 | 736 | 2.85 (1.23, 6.61) | 2.45 | 0.014 | 79.4 | <0.001 | 0.707 | 0.148 |
| | TNM (I + II/III + IV) | IHC | 3 | 228 | 4.82 (3.27, 7.08) | 7.98 | <0.001 | 0.0 | 0.908 | 1.000 | 0.307 |
| | | Published before 2015 | 3 | 228 | 4.82 (3.27, 7.08) | 7.98 | <0.001 | 0.0 | 0.908 | 1.000 | 0.307 |
| | Lymph node metastasis (Absent/ Present) | Chinese | 5 | 514 | 3.14 (1.83, 5.40) | 4.15 | <0.001 | 71.0 | 0.008 | 0.221 | 0.398 |
| | | Non-Chinese | 2 | 253 | 1.12 (0.68, 1.83) | 0.45 | 0.653 | 0.0 | 0.942 | 1.000 | |
| | | Gastrointestinal cancer | 4 | 537 | 1.77 (0.76, 4.16) | 1.31 | 0.189 | 86.1 | <0.001 | 1.000 | 0.456 |
| | | Non-gastrointestinal cancer | 3 | 230 | 3.89 (2.59, 5.84) | 6.54 | <0.001 | 1.4 | 0.363 | 0.296 | 0.294 |
| | | IHC | 4 | 474 | 2.72 (1.39, 5.35) | 2.91 | 0.004 | 73.5 | 0.010 | 0.735 | 0.553 |
| | | Non-IHC | 3 | 293 | 1.90 (0.62, 5.80) | 1.13 | 0.258 | 87.7 | <0.001 | 1.000 | 0.749 |
| | | Number of patients ≥100 | 3 | 578 | 1.59 (0.92, 2.77) | 1.65 | 0.1 | 56.7 | 0.099 | 0.296 | 0.193 |
| | | Number of patients <100 | 4 | 189 | 3.42 (1.89, 6.19) | 4.07 | <0.001 | 61.8 | 0.049 | 0.308 | 0.083 |
| | | Published before 2015 | 2 | 91 | 2.51 (0.56, 11.19) | 1.21 | 0.227 | 83.5 | 0.014 | 1.000 | |
| | | Published after 2015 | 5 | 676 | 2.24 (1.15, 4.34) | 2.38 | 0.017 | 80.5 | <0.001 | 1.000 | 0.743 |

(*Continued*)

**Table 3.** (Continued)

| Circadian clock gene | Clinicopathological parameters | | No. of studies | No. of patients | Pooled HR or OR (95% CI) | Z value | P-value | Heterogeneity | | Publication bias | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $I^2$ (%) | P-value | Begg's P value | Egger's P value |
| Per3 | Differentiation (Moderate+Well/ Poor) | Chinese | 3 | 863 | 3.63 (1.67, 7.87) | 3.26 | 0.001 | 72.3 | 0.027 | 1.000 | 0.312 |
| | | Gastrointestinal cancer | 2 | 405 | 2.27 (0.24, 21.83) | 0.71 | 0.477 | 93.3 | <0.001 | 1.000 | |
| | | Non-gastrointestinal cancer | 2 | 660 | 2.39 (1.56,3.66) | 4.01 | <0.001 | 15.6 | 0.276 | 1.000 | |
| | | IHC | 2 | 333 | 5.46 (2.89, 10.31) | 5.24 | <0.001 | 0.0 | 0.326 | 1.000 | |
| | | Non-IHC | 2 | 732 | 1.33 (0.46, 3.87) | 0.52 | 0.604 | 83.3 | 0.014 | 1.000 | |

https://doi.org/10.1371/journal.pone.0233508.t003

**Table 4. Meta-analysis results of association between low circadian clock genes expression and prognosis in cancers.**

| Circadian clock gene | No. of studies | No. of patients | Pooled HR(95%CI) | Z | P-value | Heterogeneity | | Publication bias | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $I^2$ (%) | P-value | Begg's P value | Egger's P value |
| Per1 | 12 | 2838 | 1.35 (1.06, 1.72) | 2.46 | 0.014 | 77.1 | <0.001 | 0.537 | 0.119 |
| Per2 | 9 | 2264 | 1.43 (1.10, 1.85) | 2.68 | 0.007 | 63.1 | 0.006 | 0.602 | 0.145 |
| Per3 | 7 | 2088 | 1.32 (0.99, 1.76) | 1.91 | 0.056 | 86.1 | <0.001 | 0.230 | 0.033 |
| Cry1 | 8 | 1706 | 0.79 (0.54, 1.11) | 1.37 | 0.170 | 60.8 | 0.013 | 0.711 | 0.849 |
| Cry2 | 9 | 3245 | 1.06 (0.82, 1.37) | 0.47 | 0.635 | 78.1 | <0.001 | 0.754 | 0.190 |
| Npas2 | 7 | 2519 | 0.85 (0.61, 1.19) | 0.93 | 0.352 | 86.0 | <0.001 | 0.548 | 0.142 |
| Baml1 | 7 | 1809 | 1.10 (0.82, 1.49) | 0.64 | 0.519 | 75.2 | <0.001 | 0.764 | 0.438 |
| CLOCK | 7 | 2389 | 1.05(0.74, 1.48) | 0.27 | 0.790 | 82.3 | <0.001 | 0.548 | 0.915 |

https://doi.org/10.1371/journal.pone.0233508.t004

clock genes results in the disruption of the normal circadian rhythm and plays an important role in the development, invasion, and metastasis of many kinds of cancers [3, 26, 29, 39], hence, we focused on meta-analyzing the association of low expression of circadian clock genes and cancers. This meta-analysis was the first comprehensive assessment of the association between low circadian clock genes expression and cancer progression and prognosis. Our results showed that low Per1, Per2, Per3 and Npas2 expression played a distinct and crucial

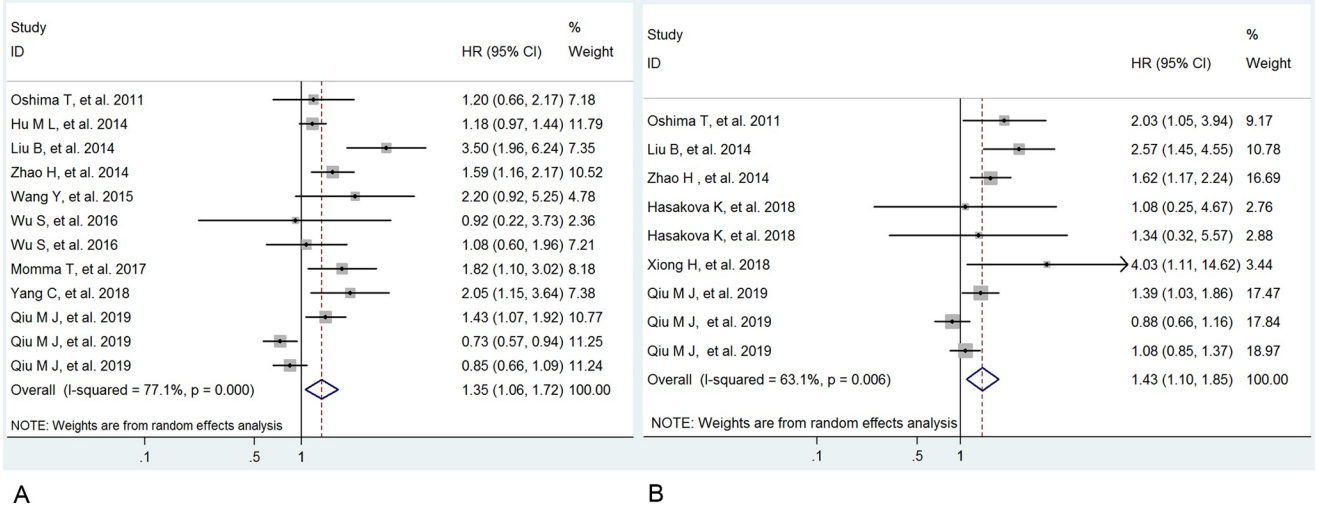


**Fig 5. Forrest plot of hazard ratio (HR) for the association of low Per1 (A) and Per2 (B) expression and overall survival.**

https://doi.org/10.1371/journal.pone.0233508.g005

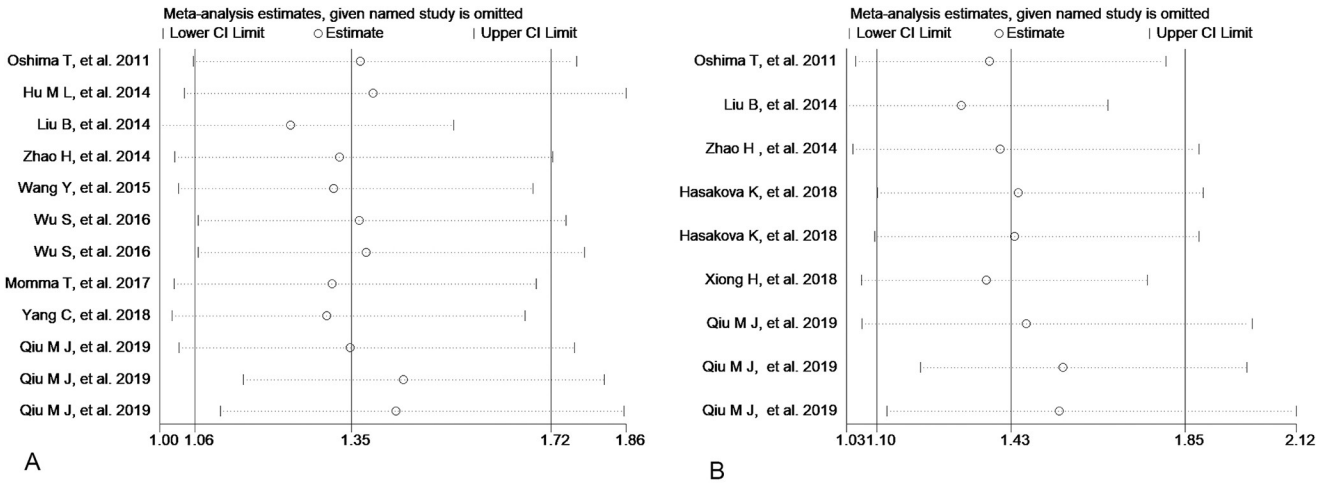


**Fig 6. Sensitive analysis of low Per1 (A), Per2 (B) expression and overall survival.**

https://doi.org/10.1371/journal.pone.0233508.g006

role in progression of cancers. Low expressions of Per1 and Per2 could serve as unfavorable indicators for gastrointestinal cancers prognosis.

Inhibition of endogenous Per1 expressionresulted in the abrogation of the ATM/Checkpoint kinase 2 (Chk2) checkpoint pathway and led to less DNA damage-induced apoptosis of cancer cells [52]. Similar effects were also seen when Per2 was knockdown in human leukemia cells. Knockdown of Per2led to downregulation of p53 and upregulation of Cylin B1 and c-Myc and promoted tumorigenesis [53]. Npas2 had been shown to bind to the c-Mycpromoter and suppress its transcription and lower expression of Npas2 resulted in increased cell growth and cycle progression of tumor cells [4, 9]. These observations concurred with our findings and suggested that low expressions of Per1, Per2 and Npas2 could significantly lead to poor differentiation of cancers through the same underlying mechanism mentioned above. Decreased Perl expression upregulated the expression of matrixmetalloproteinase-2 and increased the cell membrane distribution of laminin receptor 1, thereby enhanced tumor cells invasion [54, 55]. This result might partially account for why low Per1 expression was significantly correlated with deeper invasion depth. The epithelial-to-mesenchymal transition (EMT) is a key step in cancer progression and enables cancer cell metastasis. Low expression of Per2 led to the activation of EMT genes TWIST1 and SLUG and promoted cancer metastasis [56]. Therefore, low expression of Per2 might result in further metastasis as our meta-analysis indicated. Since low expressions of Per1 and Per2 were correlated with poorer tumor cell differentiation, deeper invasion depth and worse metastasis, it was reasonable to suppose that low expression of Per1 and Per2 might result in poorer OS, as studies pointed out [3, 11–19]. As expected, the pooled HR results in our study indicated that patients with low expression of Per1 or Per2 had a shorter OS.

Intestinal cell growth, proliferation, differentiation and gut microbiome had a daily rhythm orchestrated by circadian clock genes [57, 58]. Per1 and Per2 were expressed rhythmically throughout the gastrointestinal tract and had been shown to coordinate gastrointestinal functions such as motility, cell proliferation and migration, and regulate host gut microbiota rhythms [59, 60]. The deregulated expression of Per1 and Per2 was correlated with the host and microbiota circadian rhythms disruption and had been thought to be associated with gastrointestinal cancer progression and prognosis [5, 12, 13, 15, 16, 29, 30]. Circadian rhythms

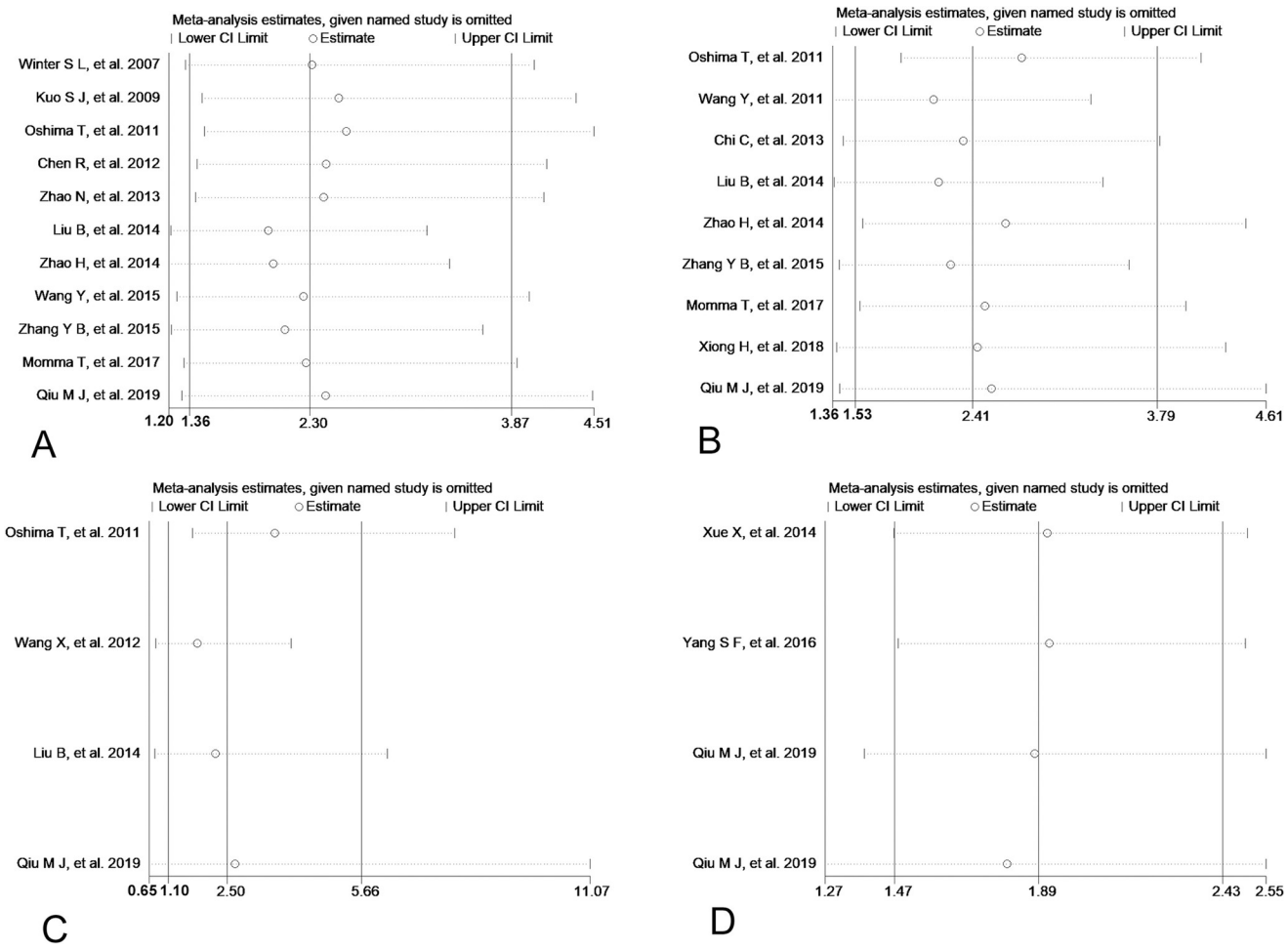


**Fig 7. Sensitive analysis of low Per1 (A), Per2 (B), Per3 (C), Npas2 (D) expression and differentiation.**

https://doi.org/10.1371/journal.pone.0233508.g007

controlled by Per1 and Per2 might have stronger and synergistic influence on the gastrointestinal cancer progression, therefore, the heterogeneity among studies which focused on gastrointestinal cancers prognosis was obviously reduced. Further studies are required to investigate the specific mechanisms involved. The heterogeneity among low Per2 expression and differentiation decreased in non-gastrointestinal cancer group (HR=2.82, 95%CI=1.91 ~ 4.15, P<0.001, $I^2$= 37.2%, P=0.173), whereas the heterogeneity of low Per2 expression and OS disappeared in gastrointestinal cancer group (HR=1.62, 95% CI=1.25 ~ 2.18, P<0.001, $I^2$=0.0%, P=0.851). These two results are inconsistent and the reason for this appeared to be that the ORs varied significantly in gastrointestinal cancers and could not reveal the true state since the time variable was not included in the OR analysis. Further studies are needed to illustrate this inconsistency. No association between the low expression of Cry1, Cry2 and Bmal1 and prognosis of cancers was found. The controversial and inconsistent prognostic results in those studies might be the reason for these negative findings [6, 24, 25, 32, 33, 45, 48, 51], and future large cohorts studies are needed to fully evaluate the relationship between the expression of these clock genes and cancer prognosis.

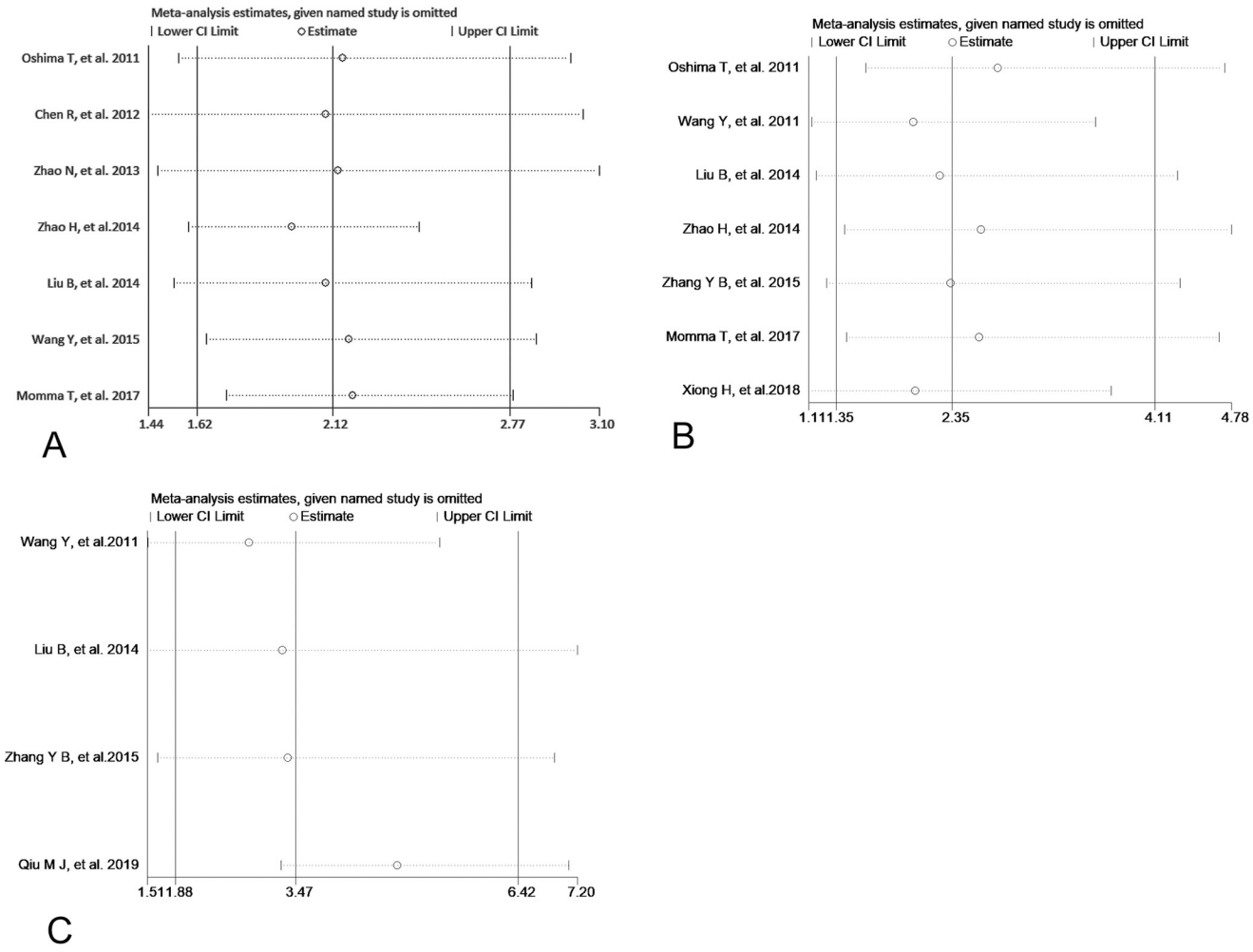


**Fig 8. Sensitive analysis of low Per1 expression and depth of invasion (A), low Per2 expression and lymph node metastasis (B),TNM (C).**

https://doi.org/10.1371/journal.pone.0233508.g008

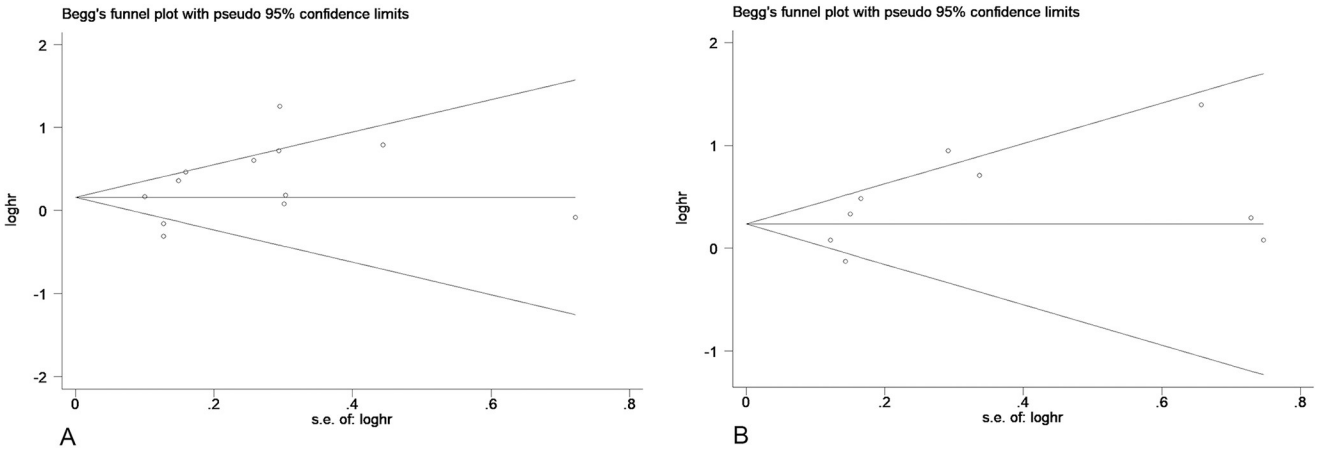


**Fig 9. The Begg's funnel plots assessing the publication bias in analyses of the association of low Per1 (A) and Per2 (B) expression and overall survival.**

https://doi.org/10.1371/journal.pone.0233508.g009

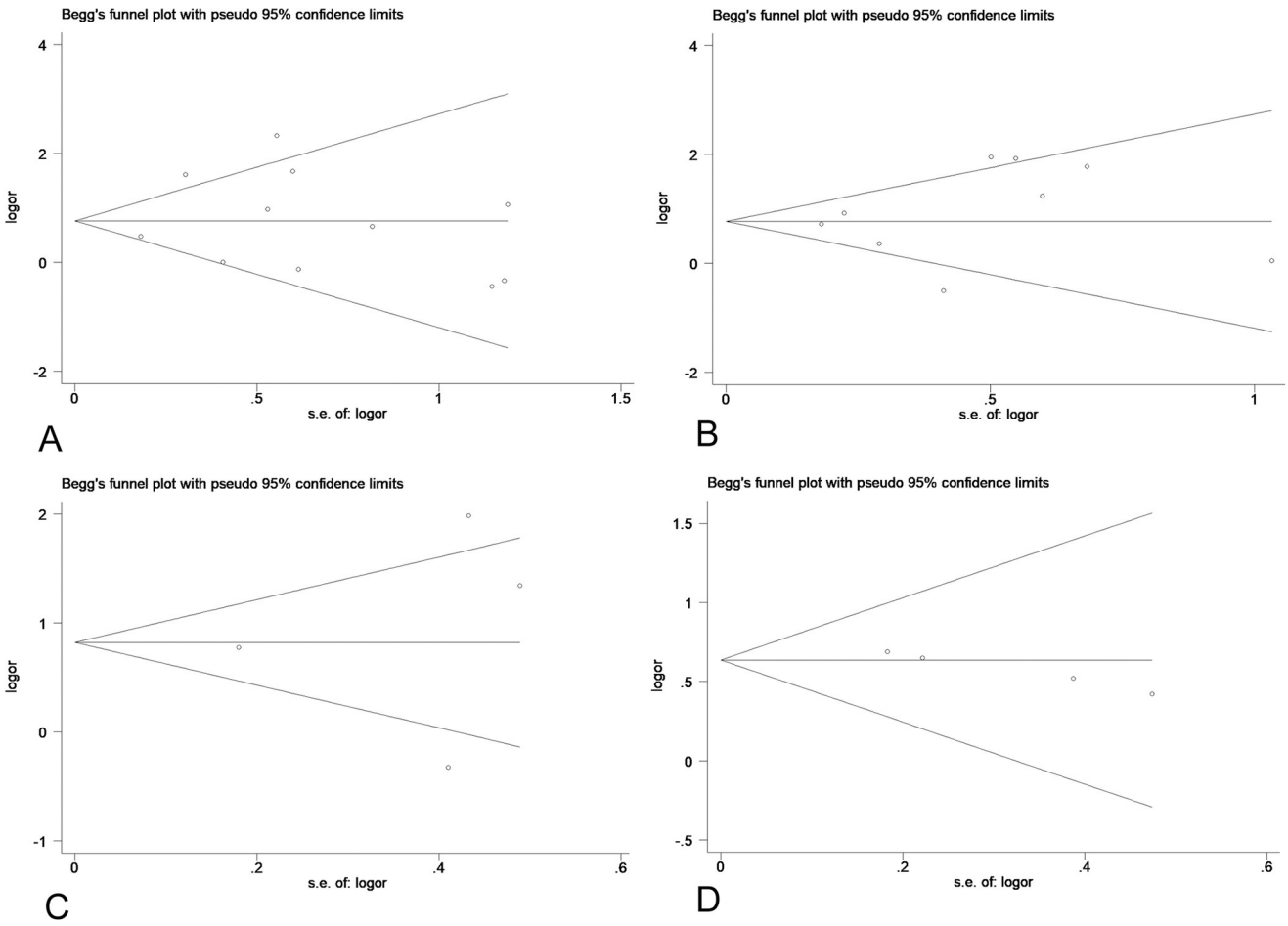


**Fig 10. The Begg's funnel plots assessing the publication bias in analyses of the association of low Per1 (A), Per2 (B), Per3 (C) and Npas2 (D) expression and differentiation.**

https://doi.org/10.1371/journal.pone.0233508.g010

Although some studies focused on other circadian clock genes (such as casein kinase 1ε (CK1ε), receptor subfamily 1 group D member 1/2 (NRD1/2), RAR-related orphan receptor A and B (RORA/B), timeless (Tim) and timeless-interacting protein (Tipin)) and cancers prognosis, these data were not sufficient to meta-analyze HR or OR of these circadian clock genes [13, 23, 29, 49]. The rhythmic expression of clock genes is critical for cancer cell growth, however, only two studies have focused on cosinor analysis of circadian gene expression levels in pancreatic cancer cell lines and tumor bearing mice [40, 61]. Therefore, the rhythmic expression of circadian clock genes was not included in this meta-analysis.

Several limitations do exist in our study. First, potentially relevant unpublished papers and studies published in non-English or Chinese were not included in this meta-analysis, thereby the reliability of our results might be weakened. Second, most of the population in our studies were from Asia, so the conclusion reliability of this meta analysis might also be weakened by this ethic disparity and furtherstudies included more European and American are needed. Third, the sample sizes of the studies ranged from 34 to 737 patients and could be the source of heterogeneity as displayed in Table 4. Fourth, the estimating HRs and their 95% CIs from Kaplan-Meier curves might be less reliable because of the inaccuracy method in extracting survival data. Fifth, we also thought that the difference in published year and detection assays for

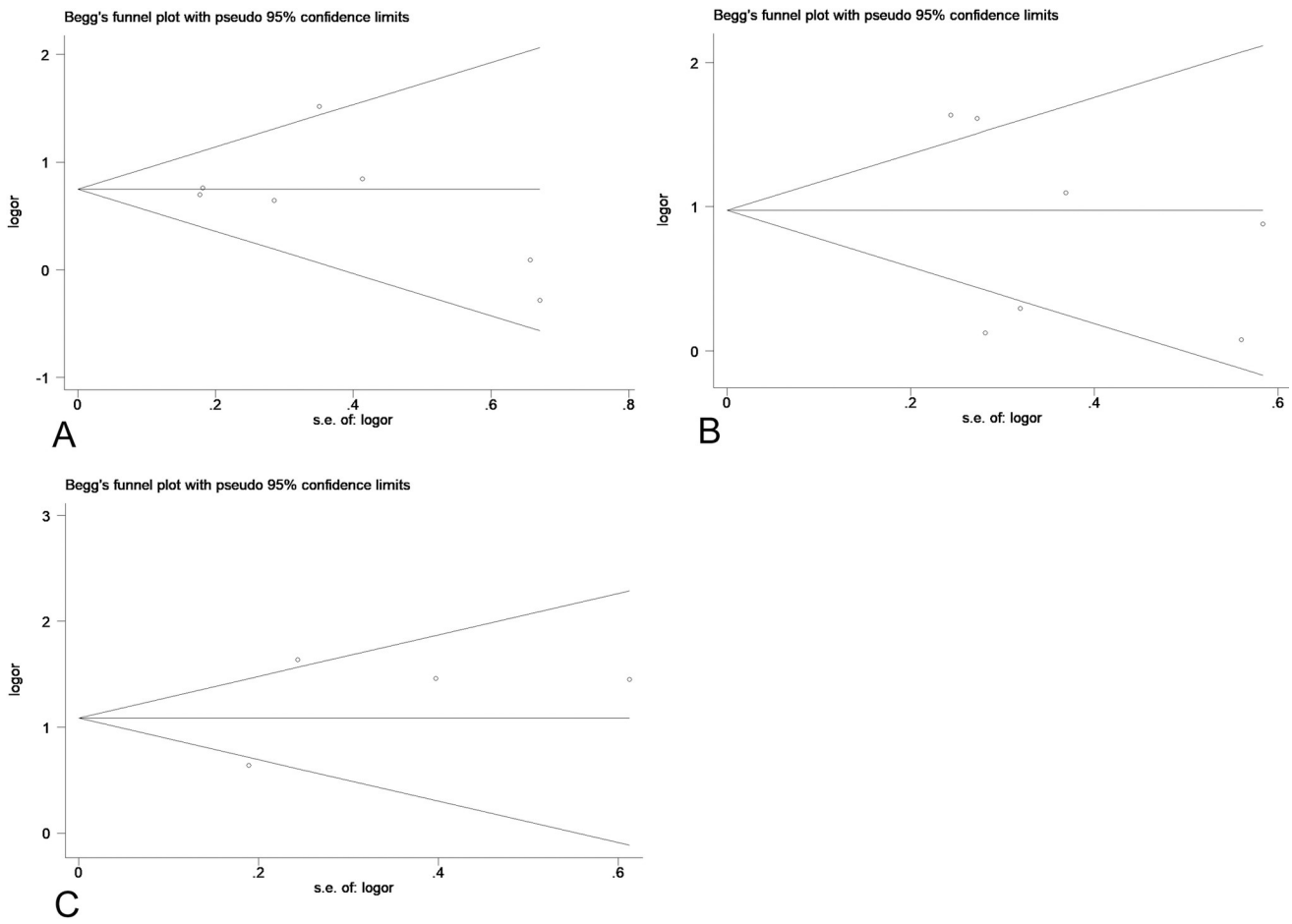


**Fig 11. The Begg's funnel plots assessing the publication bias in analyses of the association of low Per1 expression and depth of invasion (A), low Per2 expression and lymph node metastasis (B), TNM (C).**

https://doi.org/10.1371/journal.pone.0233508.g011

circadian clock genes expression should be taken into consideration. To the best of our knowledge, immunohistochemistry had been widely used for detecting the expression of circadian clock genes, however, recent researchers preferred performing qRT-PCR or microarray to evaluate circadian clock genes expression. These differences might contribute to the methodological heterogeneity.

## Conclusions

In conclusion, our meta-analysis provided evidence that low Per1, Per2and Npas2 expression played a distinct and crucial role in progression of cancers. Low expressions of Per1 and Per2 could serve as unfavorable indicators for cancers prognosis, especially for gastrointestinal cancers. However, well designed, larger-size and higher-quality cohort studies are needed to investigate the precise impact of Per1, Per2and Npas2 on the pathobiological behaviors and prognosis of cancers.

## Supporting information

**S1 Checklist.**
(DOC)

**S1 File.**
(DOC)

## Author Contributions

**Conceptualization:** Jiangguo Zhang, Hong Lv.

**Data curation:** Jiangguo Zhang, Hong Lv, Mingzhu Ji.

**Formal analysis:** Jiangguo Zhang.

**Funding acquisition:** Jiangguo Zhang.

**Investigation:** Jiangguo Zhang.

**Methodology:** Jiangguo Zhang, Hong Lv, Mingzhu Ji.

**Project administration:** Zhimo Wang.

**Resources:** Jiangguo Zhang, Zhimo Wang.

**Software:** Zhimo Wang.

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