ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2023 June 21; 29(23): 3574-3747

Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

Contents

Weekly Volume 29 Number 23 June 21, 2023

EDITORIAL

MINIREVIEWS

ORIGINAL ARTICLE

Basic Study

Retrospective Study

Observational Study

World Journal of Gastroenterology

Weekly Volume 29 Number 23 June 21, 2023

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Gastroenterology*, Kamran Rostami, FRACP, MD, PhD, Department of Gastroenterology, MidCentral District Health Board Palmerston Hospital, Palmerston North 4472, New Zealand. kamran.rostami@midcentraldhb.govt.nz

AIMS AND SCOPE

The primary aim of *World Journal of Gastroenterology* (*WJG*, *World J Gastroenterol*) is to provide scholars and readers from various fields of gastroenterology and hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJG* mainly publishes articles reporting research results and findings obtained in the field of gastroenterology and hepatology and covering a wide range of topics including gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, gastrointestinal oncology, and pediatric gastroenterology.

INDEXING/ABSTRACTING

The *WJG* is now abstracted and indexed in Science Citation Index Expanded (SCIE, also known as SciSearch®), Current Contents/Clinical Medicine, Journal Citation Reports, Index Medicus, MEDLINE, PubMed, PubMed Central, Scopus, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 edition of Journal Citation Reports® cites the 2022 impact factor (IF) for *WJG* as 4.3; IF without journal self cites: 4.1; 5-year IF: 5.3; Journal Citation Indicator: 0.82; Ranking: 32 among 93 journals in gastroenterology and hepatology; and Quartile category: Q2. The *WJG*'s CiteScore for 2021 is 8.3 and Scopus CiteScore rank 2022: Gastroenterology is 22/149.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Yi-Xuan Cai*; **Production Department Director:** *Xiang Li*; **Editorial Office Director:** *Jia-Ru Fan*.

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORS-IN-CHIEF
Andrzej S Tarnawski

EDITORIAL BOARD MEMBERS
http://www.wjgnet.com/1007-9327/editorialboard.htm

PUBLICATION DATE
June 21, 2023

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Gastroenterology

Submit a Manuscript: https://www.f6publishing.com

World J Gastroenterol 2023 June 21; 29(23): 3645-3657

DOI: 10.3748/wjg.v29.i23.3645

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

High expression of the circadian clock gene NPAS2 is associated with progression and poor prognosis of gastric cancer: A single-center study

Xiao-Meng Cao, Wen-Di Kang, Tian-Hong Xia, Shao-Bin Yuan, Chang-An Guo, Wen-Jie Wang, Hong-Bin Liu

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C, C, C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Benna C, Italy; Papadia C, United Kingdom; Qin SS, China

Received: February 9, 2023
Peer-review started: February 9, 2023
First decision: March 9, 2023
Revised: March 16, 2023
Accepted: May 4, 2023
Article in press: May 4, 2023
Published online: June 21, 2023

Xiao-Meng Cao, Department of General Surgery, Gansu Provincial Hospital of TCM, Lanzhou 730050, Gansu Province, China

Xiao-Meng Cao, Shao-Bin Yuan, The First Clinical Medical College, Gansu University of Chinese Medicine, Lanzhou 730030, Gansu Province, China

Wen-Di Kang, Department of Interventional Therapy, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

Tian-Hong Xia, Clinical Medicine College, Ningxia Medical University, Clinical Medicine college, Ningxia Medical University, Yinchuan 750004, Ningxia Hui Autonomous Region, China

Chang-An Guo, Department of Emergency, Lanzhou University Second Hospital, Lanzhou 730030, Gansu Province, China

Wen-Jie Wang, Department of General Surgery, Lanzhou University Second Hospital, Lanzhou 730030, Gansu Province, China

Hong-Bin Liu, Department of General Surgery, The 940th Hospital of Joint Logistics Support Force of Chinese People's Liberation Army, Lanzhou 730050, Gansu Province, China. 1554153823@qq.com

Corresponding author: Hong-Bin Liu, MD, Professor, Department of General Surgery, The 940th Hospital of Joint Logistics Support Force of Chinese People's Liberation Army, No. 333 Nanbinhe Road, Qilihe District, Lanzhou 730050, Gansu Province, China. 1554153823@qq.com

Abstract

BACKGROUND

The prognostic assessment of patients after surgical resection of gastric cancer (GC) patients is critical. However, the role of the circadian clock gene NPAS2 expression in GC remains unknown.

AIM

To explore the relationship between NPAS2 and the survival prognosis of GC

patients and clarify its role in evaluating GC prognosis.

METHODS

The tumor tissues and clinical data of 101 patients with GC were collected retrospectively. Immunohistochemical staining (IHC) was used to detect the expression of NPAS2 protein in GC and adjacent tissues. Univariate and multivariate Cox regression analysis was used to determine the independent prognostic factors of GC, and a nomogram prediction model was established. The receiver operating characteristic (ROC) curve, the ROC area under the curve, the calibration curve, and C-index were used to evaluate the predictive effectiveness of the model. Kaplan Meier analysis was used to compare the risk stratification of subgroups according to the median score in the nomogram model of each patient.

RESULTS

Microarray IHC analysis showed that the positive rate of NPAS2 protein expression in GC tissues was 65.35%, which was significantly higher than 30.69% in adjacent tissues. The high expression of NPAS2 was correlated with tumor-node-metastasis (TNM) stage ($P < 0.05$), pN stage ($P < 0.05$), metastasis ($P < 0.05$), venous invasion ($P < 0.05$), lymphatic invasion ($P < 0.05$), and lymph node positive ($P < 0.05$) of GC. Kaplan Meier survival analysis showed that the 3-year overall survival (OS) of patients with high NPAS2 expression was significantly shortened ($P < 0.0001$). Univariate and multivariate COX regression analysis showed that TNM stage ($P = 0.009$), metastasis ($P = 0.009$), and NPAS2 expression ($P = 0.020$) were independent prognostic factors of OS in GC patients for 3 years. The nomogram prediction model based on independent prognostic factors has a C-Index of 0.740 (95%CI: 0.713-0.767). Furthermore, subgroup analysis showed that the 3-year OS time of the high-risk group was significantly lower than that of the low-risk group ($P < 0.0001$).

CONCLUSION

NPAS2 is highly expressed in GC tissues and is closely related to worse OS in patients. Therefore, the evaluation of NPAS2 expression may be a potential marker for GC prognosis evaluation. Notably, the nomogram model based on NPAS2 can improve the accuracy of GC prognosis prediction and assist clinicians in postoperative patient management and decision-making.

Key Words: NPAS2; Gastric cancer; Tissue microarray; Survival analysis; Prediction model; Nomogram

Core Tip: The prognostic assessment of patients after surgical resection of gastric cancer (GC) is critical. However, the role of NPAS2 expression in GC remains unknown. Our study identified for the first time that high NPAS2 protein expression was associated with poor overall survival prognosis in GC patients and was an independent risk factor for patients after radical resection of GC. We constructed a nomogram prediction model by combining NPAS2 and other clinically independent risk factors, thus improving the predictive accuracy of GC prognosis.

Citation: Cao XM, Kang WD, Xia TH, Yuan SB, Guo CA, Wang WJ, Liu HB. High expression of the circadian clock gene NPAS2 is associated with progression and poor prognosis of gastric cancer: A single-center study. *World J Gastroenterol* 2023; 29(23): 3645-3657

URL: https://www.wjgnet.com/1007-9327/full/v29/i23/3645.htm

DOI: https://dx.doi.org/10.3748/wjg.v29.i23.3645

INTRODUCTION

Gastric cancer (GC) ranks fifth and fourth in the incidence and mortality of global cancer, respectively, and is more likely to occur in men[1,2]. The etiology of GC remains unclear, involving multiple cell types and complex molecular mechanisms[3]. Despite recent improvements in the diagnosis and treatment of GC, the overall treatment outcome remains poor[4]. Notably, radical gastrectomy is the primary treatment method for GC. However, postoperative recurrence rates and mortality remain high, and their mortality rates are closely associated with peritoneal, hematologic, and lymph node metastases in GC[5]. Therefore, there is an urgent need to identify new markers to predict the malignant biological behavior and prognosis of GC to guide the treatment strategy and improve the clinical outcome of GC.

Biological rhythm is a regulatory system in almost all living things. It produces 24-h periodic rhythm changes in many essential behaviors and physiological processes[6], including the sleep-wake cycle, body temperature cycle, hormone secretion, heart rate, blood pressure, excretion, *etc.*[7,8]. The destruction of normal biological rhythm adversely affects mammalian physiology[9]. The role and mechanism of biological rhythm-related genes in tumors, and the design of tumor treatment methods targeting biological rhythm genes, are one of the hot spots in the current cancer research field[10]. NPAS2, also known as MOP4, is the largest core circadian gene found so far, with a length of about 176.68Kb, located on human chromosome 2 (2q11.2), encoding proteins belonging to the basic helix loop helix PAS domain of transcription factors[11]. NPAS2 is mainly present in the forebrain of mammals and is also expressed in some peripheral organs such as the liver and skin[12]. Additionally, it is an important part of the positive circadian rhythm feedback circuit[8]. Furthermore, Studies have confirmed that NPAS2 is associated with various rhythm-related diseases, such as cancer, diabetes, depression, sleep disorders, obesity, *etc.*[13].

Recent studies have shown that low expression of NPAS2 accelerates cell growth and tumor cell cycle progression and plays a unique and critical role in cancer progression[14]. The low expression of NPAS2 promotes the proliferation and invasion of colorectal cancer cells. Additionally, it increases the wound-healing ability of colorectal cancer cells, indicating that NPAS2 has a key tumor inhibitory effect. Additionally, its high expression is positively correlated with overall survival (OS)[15]. However, some studies have found that NPAS2, as an oncogene, promotes the proliferation of hepatocellular carcinoma (HCC) cells and participates in the critical process of hepatocellular carcinogenesis. Its high expression is related to the invasive clinical characteristics and low OS of HCC patients[16]. It promotes the survival of HCC cells by up-regulating the expression of mitotic cycle 25A and inhibiting mitochondrial-dependent intrinsic apoptosis[16]. These results have also been observed in acute myeloid leukemia [17]. In contrast, there are few studies on the expression and role of NPAS2 in GC at home and abroad, and there is a significant lack of experimental research. Therefore, the expression level of NPAS2 in GC and its role in the occurrence and development of GC in different signal pathways are controversial and need to be further studied.

This study intends to detect the expression level of NPAS2 in GC and paraneoplastic tissues by using tissue microarray immunohistochemical staining to establish a nomogram prediction model based on NPAS2 and other clinicopathologically independent risk factors. Furthermore, we aim to investigate the effect of NPAS2 on the prognosis of GC and its value in the prognosis prediction of GC patients.

MATERIALS AND METHODS

This study was approved by the Medical Ethics Committee of the Second Hospital of Lanzhou University. Informed consent was obtained from all patients, and all specimens were pseudonymous. The tumor tissues, precancerous tissues, and clinical data of 101 patients with primary GC resected by surgery from July to October 2019 in the second Hospital of Lanzhou University were collected and followed up by standard outpatient clinic and telephone. The inclusion criteria of the study were as follows: (1) Histopathological diagnosis of gastric adenocarcinoma; (2) age ≥ 18 years old; (3) patients with primary GC underwent surgical resection; (4) complete pathological, treatment, surgical and follow-up data; and (5) the patient gave written informed consent. Exclusion criteria: (1) Death before discharge; (2) preoperative radiotherapy and chemotherapy or neoadjuvant chemotherapy; (3) multiple cancers; and (4) gastric stump cancer. 10% neutral formalin fixation and routine paraffin-embedded GC and paraffin-embedded tissues were confirmed by pathology, and all pathology was independently reviewed by two pathologists. The patients were followed up every 3 mo in the first 2 years and every 6 mo in the third year. Follow-up included laboratory examination, chest X-ray examination, and abdominal computed tomography (CT) examination. The follow-up period was 36 mo, and OS was defined as from the date of the first operation to the end of follow-up or the time of death.

Construction of tissue microarray and immunohistochemical staining

One hundred one paraffin-embedded primary GC tissues and adjacent tissues were fixed with formalin and then serially sectioned on a microtome with a thickness of 4 μm. After drying, hematoxylin and eosin staining (HE) were performed. A professional pathologist selected and marked the representative positions of HE-stained pathological sections and corresponding wax blocks according to the view under the light microscope. The tissue chip wax blocks were made by a drilling mechanism and sliced with a thickness of 4μm. After being dewaxed in xylene and washed in gradient ethanol, the slices were immersed in a pressure tank containing 800 mL of 0.1% sodium citrate buffer solution (pH 6.0) and boiled for 3 min, followed by cooling. Following the manufacturer's instructions, we then conducted a streptavidin-Biotin Complex assay (Fuzhou Maxin Biotechnology Development Company). The slides were then incubated overnight with the primary antibody against NPAS2 at 4 ℃ (diluted, 1:200, rabbit anti-human NPAS2 polyclonal antibody, Biossantibodies, China). Color development, hematoxylin re-staining, differentiation, blueing, and dehydration were performed prior to sealing.

Evaluation of immunostaining

The complete immunohistochemical sections of 101 cases were observed under the microscope. NPAS2 was mainly expressed in the cytoplasm and nucleus, and the positive cells were yellow and brown granular. Two professional pathologists scored the section staining and did not know the clinicopathological factors and clinical outcome of the patient. The positive results were judged by the semi-quantitative integration method. Five high-power visual fields (400 ×) were selected for each chip on each slide under the microscope, and cells' positive rate and staining intensity were observed. (1) The score of cell positive rate: 0 indicates that the proportion of positive cells is less than 5%. The score of 5%-25% is 1, 26%-50% is 2, 51%-75% is 3, and more than 75% is 4; and (2) Staining intensity score: 0 = no coloring, 1 = light yellow, 2 = brown, 3 = brown. The immunostaining score was obtained by multiplying the positive rate of cells with the result of staining intensity. 0 was negative, 1 to 4 was weakly positive, 5 to 8 was moderately positive, and 9 to 12 was strongly positive. In this study, negative and weak positive was defined as low expression of NPAS2, while medium positive and strong positive was defined as high expression.

Statistical analysis

All data were analyzed using R software (Version 4.1.1, http://www.r-project.org) and SPSS (Version 26). Classification variables are represented by percentage (%), chi-square test, and continuity correction. Pearson's chi-squared test was used to evaluate the correlation between NPAS2 expression and clinicopathological factors. Survival analysis was performed by Kaplan-Meier curve and log-rank test. Univariate and multivariate Cox analysis determined the independent prognostic factors of OS and established the Cox proportional hazards model, and the hazard ratios (HRs) and 95% confidence intervals (CIs) were subsequently calculated. "Survival" and "surminer" R package were used for survival and Cox analysis. The "rms" package drew a nomogram model based on independent risk factors to predict OS. Furthermore, the receiver operating characteristic (ROC) curve, aera under the curve (AUC), calibration curve, and C-index were used to evaluate its prediction performance. In this study, $P < 0.05$ was considered to be statistically significant.

RESULTS

High expression of NPAS2 in human GC tissues

To explore the prognostic value of NPAS2 in GC, we performed immunohistochemical staining on 101 GC tissues collected. Strong or moderate positive immunostaining of NPAS2 protein was observed in the nucleus and cytoplasm of tumor cells in GC tissues. In contrast, weak positive or negative immunostaining was mainly detected in adjacent tissues. Representative positive staining results of NPAS2 showed that the positive rate of NPAS2 expression in GC tissues was 65.35% (66/101) (Figure 1), while the positive rate in corresponding adjacent tissues was 30.69% (31/101).

High expression of NPAS2 correlates with clinicopathological factors in GC patients

Of the 101 patients with GC, 65 (64.36%) died during follow-up. Table 1 summarizes the correlation between clinicopathological characteristics and NPAS2, showing that high expression of NPAS2 is associated with clinical progression in GC patients. The high expression of NPAS2 was significantly correlated with tumor-node-metastasis (TNM) stage ($P = 0.001$), pN stage ($P = 0.009$), metastasis ($P = 0.032$), venous invasion ($P = 0.012$), lymphatic invasion ($P = 0.001$) and lymph node positivity ($P = 0.001$).

The high expression of NPAS2 is associated with the prognosis of GC patients

The prognostic value of NPAS2 expression in GC was further investigated by Kaplan-Meier analysis and log-rank test. The results demonstrated that GC patients with high NPAS2 expression were significantly associated with shorter OS ($P < 0.001$) (Figure 2). Univariate Cox regression analysis showed that the TNM stage of GC patients (HR = 7.07, 95%CI: 3.47-14.41, $P < 0.001$), pN stage (HR = 1.72, 95%CI: 1.38-2.15, $P < 0.001$), metastasis (HR = 4.85, 95%CI: 2.24-10.46, $P < 0.001$), venous invasion (HR = 5.01, 95%CI: 1.57-15.98, $P = 0.007$), neural invasion (HR = 1.73, 95%CI: 1.03-2.9, $P = 0.037$), lymphatic invasion (HR = 3.53, 95%CI: 1.88-6.64, $P < 0.001$), lymph node positivity (HR = 3.35, 95%CI: 1.82-6.18, $P < 0.001$), and high expression of NPAS2 (HR = 5.13, 95%CI: 2.6-10.13, $P < 0.001$) were significantly correlated with OS. Multivariate Cox regression analysis showed that the TNM stage (HR = 3.61, 95%CI: 1.37-9.49, $P = 0.009$), metastasis (HR = 3.07, 95%CI: 1.33-7.1, $P = 0.009$), and high expression of NPAS2 (HR = 2.43, 95%CI: 1.15-5.13, $P = 0.020$) were independent risk factors for predicting OS (Table 2). Therefore, these collective data suggest that the expression level of NPAS2 has a good predictive value in the prognosis of GC patients.

Construction and evaluation of the prediction model

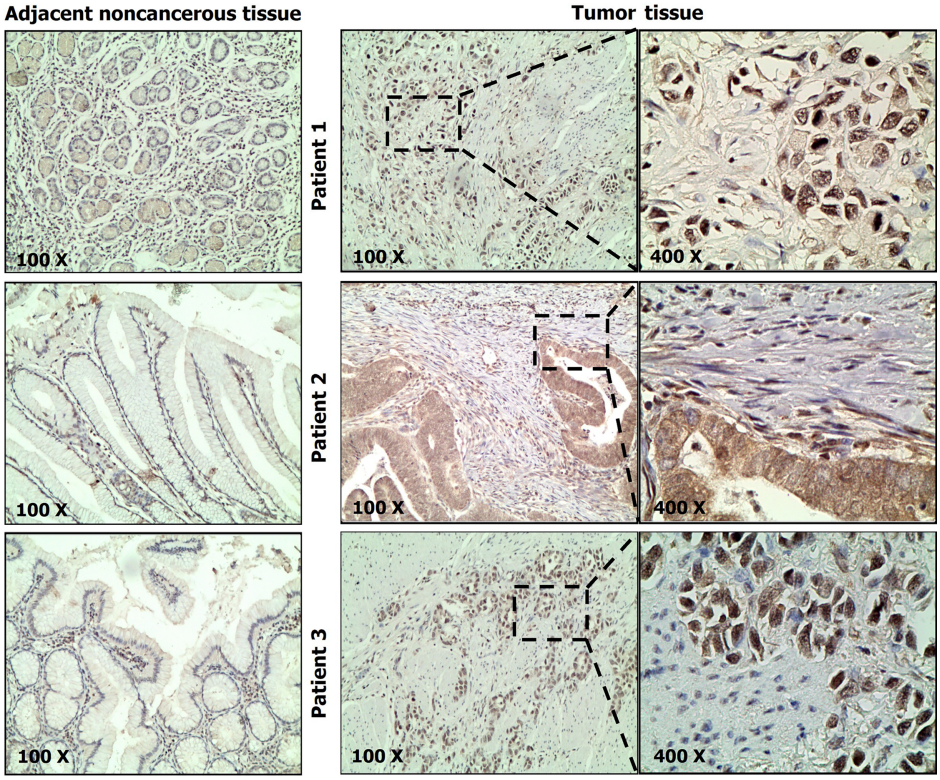
To obtain the best prediction model, TNM stage and metastasis were determined as independent

Table 1 Correlation between clinicopathological characteristic and NPAS2 in gastric cancer

Characteristic	Low expression of NPAS2	High expression of NPAS2	*P* value
Number	35	66	
Age, *n* (%)			0.599
< 60	21 (60.0)	36 (54.5)	
≥ 60	14 (40.0)	30 (45.5)	
Sex, *n* (%)			0.626
Female	7 (20.0)	16 (24.2)	
Male	28 (80.0)	51 (75.8)	
TNM stage, *n* (%)			0.001
I-II	25 (71.4)	11 (16.7)	
III-IV	10 (28.6)	55 (83.3)	
pT stage, *n* (%)			0.351
T1-T2	6 (17.1)	29 (82.9)	
T3-T4	7 (10.6)	59 (89.4)	
pN stage, *n* (%)			0.009
N0	16 (45.7)	12 (18.2)	
N1-N3	19 (54.3)	54 (81.8)	
Metastasis, *n* (%)			0.032
M0	35 (100.0)	58 (87.9)	
M1	0 (0.0)	8 (12.1)	
Tumor size, *n* (%)			0.626
< 5	15 (42.9)	25 (37.9)	
≥ 5	20 (57.1)	41 (62.1)	
Tumor location, *n* (%)			0.611
Antrum	14 (40.0)	33 (50.0)	
Body	9 (25.7)	13 (19.7)	
Cardia	12 (34.3)	20 (30.3)	
Venous invasion, *n* (%)			0.012
Negative	9 (25.7)	5 (7.6)	
Positive	26 (74.3)	61 (92.4)	
Neural invasion, *n* (%)			0.736
Negative	15 (42.9)	26 (39.4)	
Positive	20 (57.1)	40 (60.6)	
Lymphatic invasion, *n* (%)			0.001
Negative	22 (62.9)	12 (18.2)	
Positive	13 (37.1)	54 (81.8)	
Positive lymph node, *n* (%)			0.001
Negative	22 (62.9)	14 (21.2)	
Positive	13 (37.1)	52 (78.8)	
Differentiated degree, *n* (%)			0.16
Poorly	27 (77.1)	58 (87.9)	
Moderately and highly	8 (22.9)	8 (12.1)	

Lauren's Classification, *n* (%)			0.569
Intestinal	23 (65.7)	47 (71.2)	
Diffuse	12 (34.3)	19 (28.8)	

pT stage: Pathological assessment of primary tumor; pN stage: Pathological assessment of regional lymph nodes; TNM: Tumor-node-metastasis.



DOI: 10.3748/wjg.v29.i23.3645

Figure 1 Expression of NPAS2 in gastric cancer. The expression of NPAS2 in tumor tissues of gastric cancer was detected by immunohistochemical staining and compared with adjacent normal tissues. High expression of NPAS2 in gastric cancer and low expression in adjacent non-cancerous tissue.

prognostic factors for GC according to multivariate Cox regression analysis and combined with NAPS2 high expression. Together, these were used as a combined prediction model for predicting OS at 1, 2, and 3 years after GC resection was constructed. The prediction model was visualized by nomogram (Figure 3). The 1-year, 2-year, and 3-year ROC curves of the Nomogram prediction model and other independent factors (Figure 4A) and the Time-dependent ROC curve were used to show the model's prediction performance (Figure 4C). The AUC of the nomogram prediction model for 1, 2, and 3 years is 0.763, 0.800, and 0.848, respectively (Figure 4B). Additionally, the C-index of each model shows the prediction performance of different models (Figure 4D), and the nomogram has the best prediction performance with a C-index of 0.740 (95%CI: 0.713-0.767). Finally, the prediction accuracy of the nomogram was verified by calibration curves, and the results showed that the combined nomogram model's 1-year, 2-year, and 3-year survival rates based on NPAS2 were in good agreement with the actual survival probability (Figure 5).

Risk stratification of OS by the nomogram model

To evaluate the positive effect of the nomogram prediction model in different subgroups, we divided all patients into a low-risk subgroup (score < 158) and a high-risk subgroup (score ≥ 158) according to the median nomogram score. Our results showed that patients in the high-risk group had significantly lower OS than those in the low-risk group ($P < 0.0001$) (Figure 6).

DISCUSSION

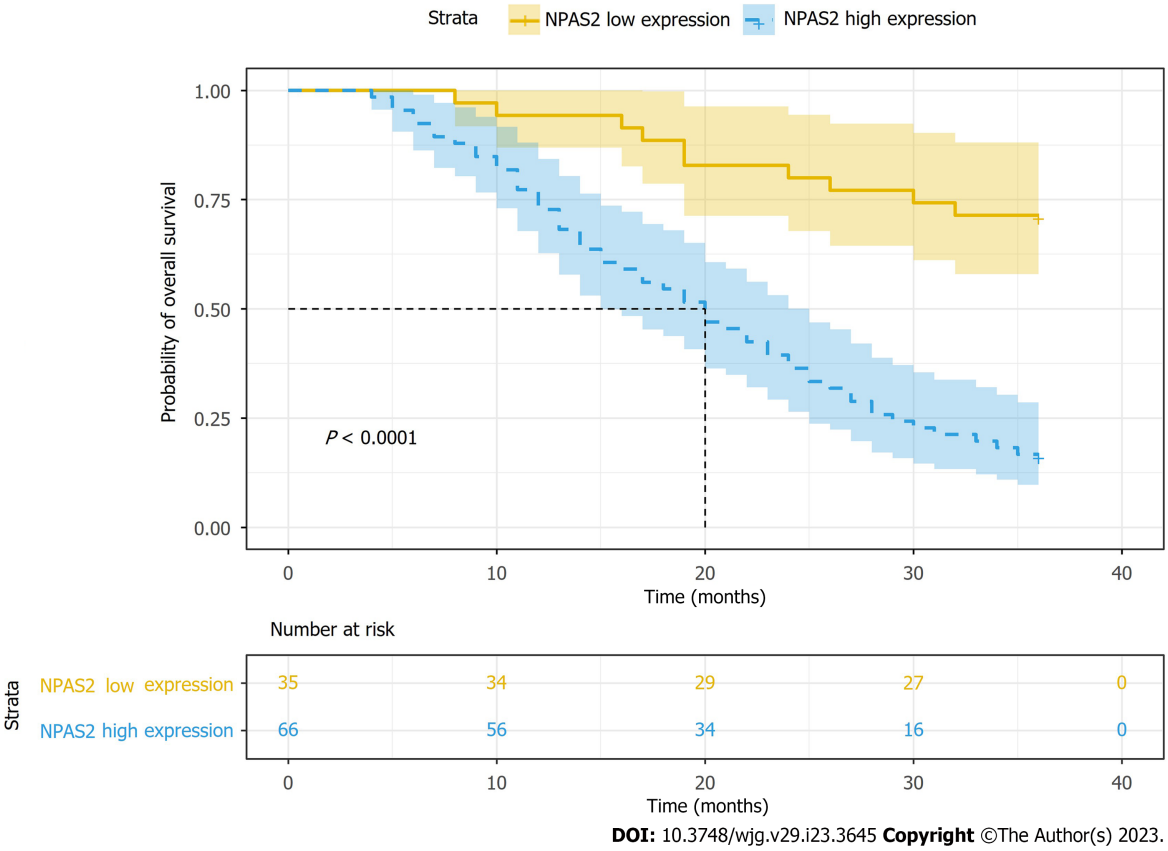
Epidemiological studies have shown that the disruption of normal circadian rhythm may increase the

Table 2 Univariate and multivariate Cox analysis of clinicopathological factors associated with the overall survival of gastric cancers

Characteristics	Univariate analysis		Multivariate analysis	
	Hazard ratio (95%CI)	*P* value	Hazard ratio (95%CI)	*P* value
Age				
< 60	1			
≥ 60	1.33 (0.82-2.17)	0.25		
Gender				
Female	1			
Male	1.13 (0.63-2.05)	0.679		
TNM stage				
I-II	1			
III-IV	7.07 (3.47-14.41)	< 0.001	3.61 (1.37-9.49)	0.009
pT stage				
T1-T2	1			
T3-T4	1.87 (0.81-4.35)	0.143		
pN stage				
N0	1			
N1-N3	1.72 (1.38-2.15)	< 0.001	1.66 (0.68-4.07)	0.265
Metastasis				
M0	1			
M1	4.85 (2.24-10.46)	< 0.001	3.07 (1.33-7.1)	0.009
Tumor size				
< 5	1			
≥ 5	1.63 (0.97-2.72)	0.063		
Tumor location				
Antrum	1			
Body	0.92 (0.53-1.60)	0.76		
Cardia	1.13 (0.58-2.23)	0.72		
Venous invasion				
Negative	1			
Positive	5.01 (1.57-15.98)	0.007	1.69 (0.49-5.84)	0.409
Neural invasion				
Negative	1			
Positive	1.73 (1.03-2.9)	0.037	1.71 (0.98-3)	0.06
Lymphatic invasion				
Negative	1			
Positive	3.53 (1.88-6.64)	< 0.001	0.39 (0.09-1.77)	0.225
Positive lymph node				
Negative				
Positive	3.35 (1.82-6.18)	< 0.001	2.23 (0.58-8.49)	0.241
Differentiated degree				
Moderately and highly	1			
Poorly	0.62 (0.29-1.30)	0.203		

Lauren's Classification				
Diffuse type	1			
Intestinal type	0.97 (0.57-1.64)	0.908		
	0.97 (0.57-1.64)			
NPAS2 expression				
Low	1			
High	5.13 (2.6-10.13)	< 0.001	2.43 (1.15-5.13)	0.020

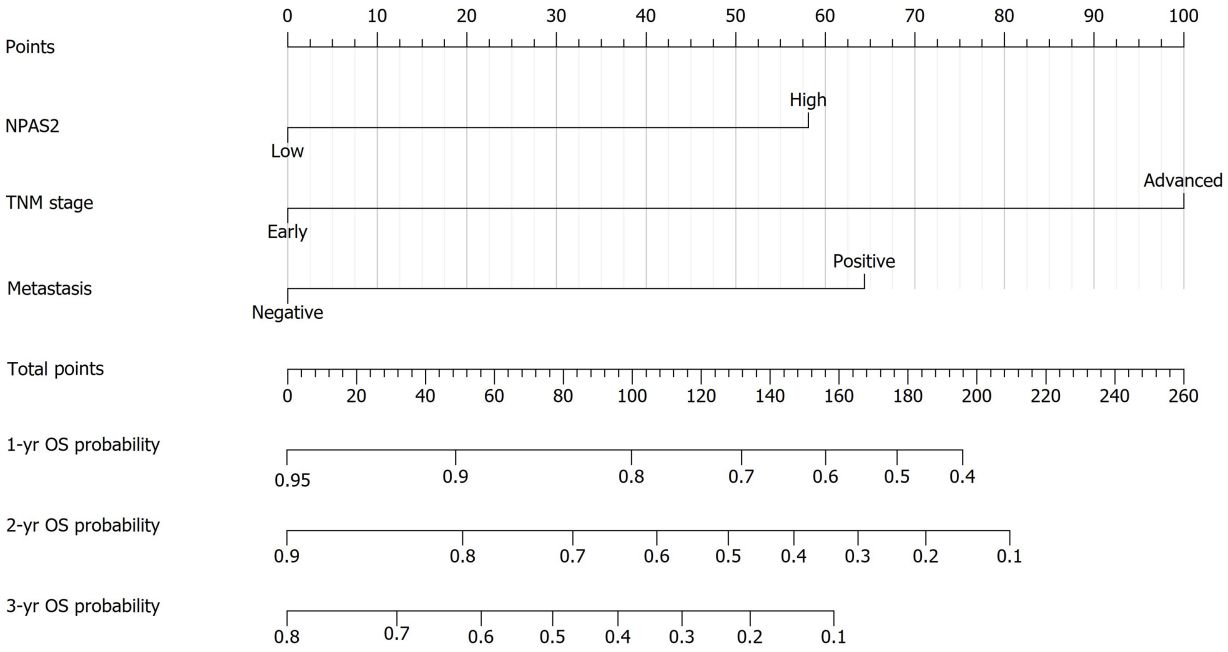
pT stage: Pathological assessment of primary tumor; pN stage: Pathological assessment of regional lymph nodes; TNM: Tumor-node-metastasis.



DOI: 10.3748/wjg.v29.i23.3645

Figure 2 Kaplan–Meier curves for NPAS2 expression in gastric cancer. Gastric cancer patients with high NPAS2 expression were significantly associated with shorter overall survival.

risk of a variety of cancers[18], such as breast cancer[19], lung cancer[20], bladder cancer[21], prostate cancer[22], and liver cancer[23]. Notably, women on night shifts have a higher risk of breast cancer[19]. Likewise, breast cancer patients with irregular circadian rhythms have a worse prognosis because of changes in the expression of NPAS2 in breast cancer tissues[19]. Additionally, the disruption of biorhythm can promote the occurrence of lung cancer, which is a promising diagnostic marker for patients with lung adenocarcinoma and an independent prognostic marker for non-small cell lung cancer[24]. Notably, NPAS2 regulates the expression of several genes as markers of bladder cancer and further reduces the migration ability of bladder cancer cells[21]. The development of prostate cancer depends on androgen levels, and NPAS2 may interact with dihydrotestosterone, thus affecting the androgen receptor-dependent signaling pathway leading to prostate cancer[25]. Importantly, NPAS2 plays a role in reprogramming glucose metabolism and the progression of liver cancer, suggesting that NPAS2 may be an important therapeutic target to normalize the abnormal glucose metabolism that leads to the advancement of HCC[23]. Furthermore, NPAS2 plays different roles in different tumors, which can be understood that the expression level and function of NPAS2 are tumor-specific. The exact molecular mechanism of the change of NPAS2 expression in GC remains unclear. Moreso, there are no reports on the correlation between NPAS2 expression and clinicopathological data of GC patients.



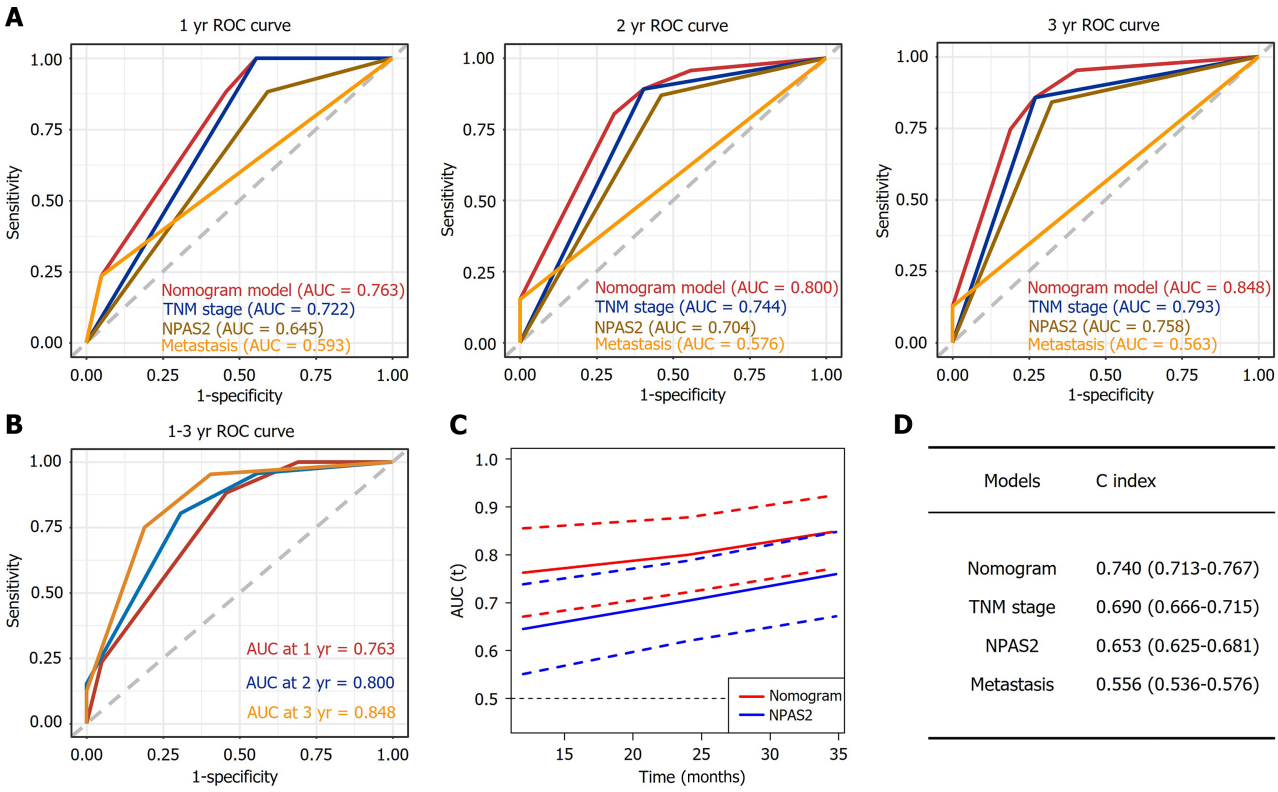
DOI: 10.3748/wjg.v29.i23.3645

Figure 3 Nomogram prediction of 1-, 2-and 3-year overall survival rate in patients with gastric cancer. The total points is calculated by adding up the points of each factor. The total points corresponds to the 1-year, 2-year and 3-year survival probability of the patients. OS: Overall survival; TNM: Tumor-node-metastasis.

The tissue microarray results in this study showed that NPAS2 was expressed in GC and precancerous tissues and was primarily located in the nucleus and cytoplasm. The results showed that the expression was high in 65.35% of GC tissues and low in 34.65% of GC tissues. To the best of our knowledge, our experimental data demonstrate the role of NPAS2 in the diagnosis and prognosis of GC for the first time. The survival analysis showed that the high expression of NPAS2 in GC was significantly correlated with the shorter OS ($P < 0.001$). Univariate and multivariate Cox analysis also showed that the high expression of NPAS2 was an independent risk factor for GC. Furthermore, the ROC curve shows that NPAS2 is valuable in predicting the prognosis of GC. Therefore, the expression level of NPAS2 in GC tissue directly affects the OS of GC patients and may be used as a tumor-promoting factor to affect the occurrence and development of GC, which is a new prognostic biomarker. Various animal and *in vitro* studies have found that NPAS2 can regulate the expression of oncogenes (such as c-myc), tumor suppressor genes (such as P53 and P21), and transcription factors[26], thereby regulating cell cycle, apoptosis, DNA damage and repair systems, invasion, metastasis, carcinogen metabolism, and detoxification[27]. Notably, DNA repair maintains genetic stability and protects DNA from internal and external stimuli. Once human biorhythm genes are mutated (due to spontaneous mutations, prolonged late nights, or other environmental factors), it may lead to irreversible disorders and loss of DNA repair capacity[27].

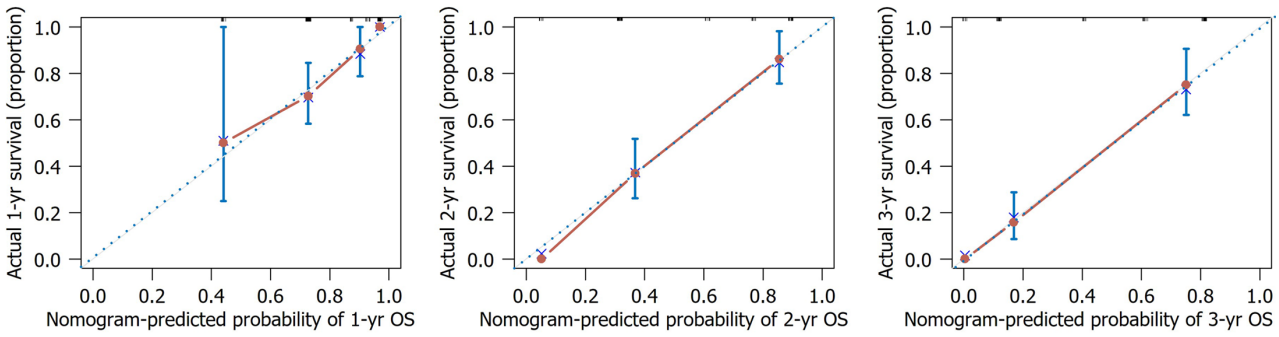
The American Joint Commission on the Cancer TNM staging system is widely used to monitor the prognosis of patients with GC[28]. Our study analyzed the clinicopathological factors affecting OS in patients with GC, and our results showed that TNM stage and metastasis were independent risk factors for poor survival outcomes. Although the TNM stage and metastasis of GC patients have been widely used in the formulation of the treatment plan and the evaluation of prognosis, with the increasing number of GC patients, more studies and related factors are needed to evaluate the survival and prognosis of these patients more accurately and individually. Our study combined three independent risk factors of NPAS2 and TNM stage and metastasis to establish a Cox regression model for predicting 3-year OS in patients with GC. Compared with the TNM stage alone, it has more predictive power and can be used as a more convenient and accurate tool to predict the prognosis of patients with GC following gastrectomy. Therefore, the accurate prognosis prediction by nomogram score is of great significance for postoperative management and diagnosis and treatment of patients.

However, we can not solve the causal relationship in this study. Therefore, further basic and clinical research is needed to explore these aspects. Our advantage is that this is the first study to show that the increased expression of NPAS2 may be related to the malignant biological behavior and poor prognosis of patients with GC. Of course, our research has the following noted limitations. First, the sample size of our study is small, and there may be selection bias. Secondly, this study is a single-center retrospective study, so it is necessary to conduct a multicenter prospective study to verify it further. Finally, we used immunohistochemical staining to detect the expression of NPAS2 in the center and periphery of each



DOI: 10.3748/wjg.v29.i23.3645 **Copyright** ©The Author(s) 2023.

Figure 4 Construction of prognostic nomogram in gastric cancer. A: NPAS2, tumor-node-metastasis (TNM) stage, metastasis, and nomogram predict the receiver operating characteristic (ROC) curves of 1-year, 2-year and 3-year overall survival (OS) in gastric cancer patients; B: Nomogram predict the ROC curve of 1-year, 2-year and 3-year OS in gastric cancer patients; C: Comparison of time-dependent ROC curves between nomogram and NPAS2; D: C-index for nomogram, NPAS2, TNM stage, metastasis. ROC: Receiver operating characteristic; OS: Overall survival; TNM: Tumor-node-metastasis; AUC: Area under the curve.



DOI: 10.3748/wjg.v29.i23.3645 **Copyright** ©The Author(s) 2023.

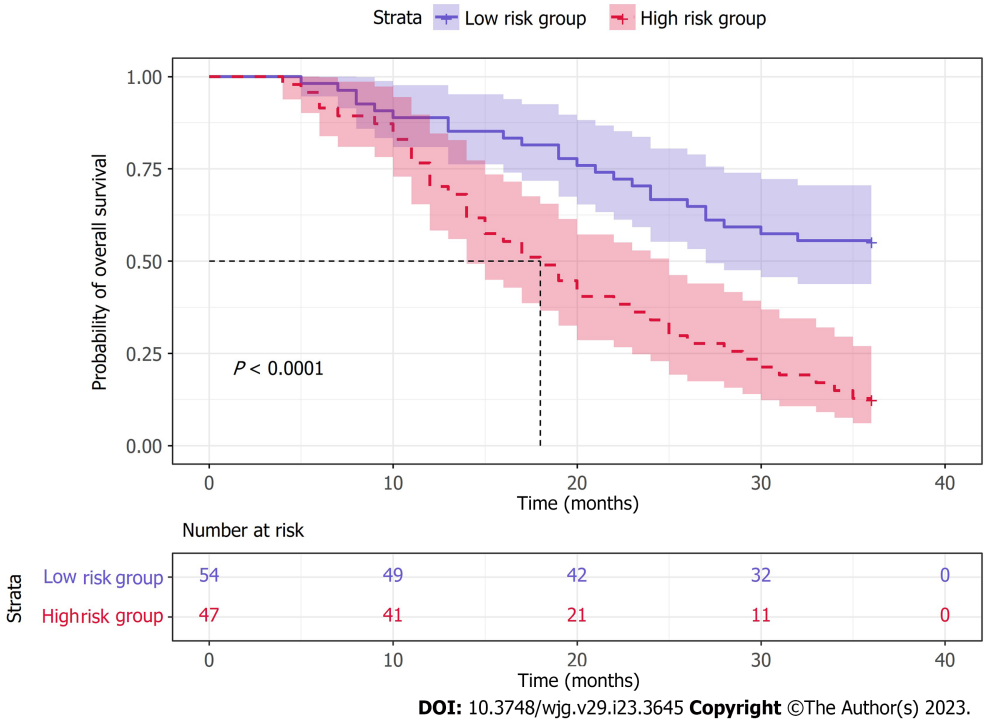
Figure 5 Nomogram calibration plots to predict 1-, 2-, and 3-year overall survival in gastric cancer patients. OS: Overall survival.

GC tissue. However, considering the heterogeneity, the expression level of NPAS2 in the sampling site may not represent whole tumor regions.

Based on these investigations, further exploration of the molecular mechanism and influence of NPAS2 in the occurrence and development of GC will help to promote its clinical application. The established nomogram prediction model provides a potential objective clinical prediction tool to assist clinicians in predicting the prognosis of GC patients and making postoperative management and clinical decisions.

CONCLUSION

In conclusion, our study found that high expression of NAPS2 is associated with poor prognosis of OS in patients with GC and is an independent risk factor for patients after radical resection of GC. We



DOI: 10.3748/wjg.v29.i23.3645

Figure 6 Overall survival Kaplan-Meier curves for patients in the low- and high-risk groups.

further constructed a nomogram model by combining NAPS2 and other independent risk factors, thus improving the accuracy of predicting the prognosis of GC.

ARTICLE HIGHLIGHTS

Research background

The circadian clock gene NPAS2 expression is associated with multiple tumor prognosis, its role in gastric cancer (GC) is unknown.

Research motivation

NPAS2 can improve the accuracy of GC prognosis prediction and assist clinicians in postoperative patient management and decision-making.

Research objectives

This study aimed to explore the relationship between the circadian clock gene NPAS2 and the survival prognosis of GC patients and clarify its role in evaluating GC prognosis.

Research methods

Immunohistochemical staining was used to detect the expression of NPAS2 protein in GC and adjacent tissues. Univariate and multivariate Cox regression analysis was used to determine the independent prognostic factors of GC, and a nomogram prediction model was established. The receiver operating characteristic curve and area under the curve, the calibration curve, and C-index were used to evaluate the predictive effectiveness of the model.

Research results

The NPAS2 expression were independent prognostic factors of overall survival in GC patients for 3 years.

Research conclusions

NPAS2 is highly expressed in GC and is closely related to worse overall survival in patients. Therefore, the evaluation of NPAS2 expression may be a potential marker for GC prognosis evaluation.

Research perspectives

Based on NPAS2 expression, clinicians can predict patient prognosis and guide clinical decision making and follow up.

FOOTNOTES

Author contributions: Cao XM and Kang WD contributed equally to this work and share first authorship; Cao XM, Kang WD, and Liu HB designed the project and reviewed and edited the manuscript; Cao XM and Kang WD analyzed the data and wrote the main manuscripts; Xia TH and Yuan SB carried out research selection and data collection; Guo CA, Wang WJ and Liu HB participated in the discussion of classification criteria.

Institutional review board statement: The studies involving human participants were reviewed and approved by Medical Ethics Committee of the Second Hospital of Lanzhou University, approval No. 2021A-561. The patients/participants provided written informed consent to participate in this study.

Informed consent statement: The Medical Ethics Committee of the Second Hospital of Lanzhou University waived the need for informed consent due to the retrospective nature of the study.

Conflict-of-interest statement: The authors declare no conflict of interest.

Country/Territory of origin: China

ORCID number: Hong-Bin Liu 0000-0003-3668-6166.

S-Editor: Yan JP
L-Editor: A
P-Editor: Chen YX

REFERENCES

1 **Ferlay J**, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, Bray F. Cancer statistics for the year 2020: An overview. *Int J Cancer* 2021 [PMID: 33818764 DOI: 10.1002/ijc.33588]
2 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]
3 **Lei ZN**, Teng QX, Tian Q, Chen W, Xie Y, Wu K, Zeng Q, Zeng L, Pan Y, Chen ZS, He Y. Signaling pathways and therapeutic interventions in gastric cancer. *Signal Transduct Target Ther* 2022; **7**: 358 [PMID: 36209270 DOI: 10.1038/s41392-022-01190-w]
4 **Ugai T**, Sasamoto N, Lee HY, Ando M, Song M, Tamimi RM, Kawachi I, Campbell PT, Giovannucci EL, Weiderpass E, Rebbeck TR, Ogino S. Is early-onset cancer an emerging global epidemic? Current evidence and future implications. *Nat Rev Clin Oncol* 2022; **19**: 656-673 [PMID: 36068272 DOI: 10.1038/s41571-022-00672-8]
5 **Gwee YX**, Chia DKA, So J, Ceelen W, Yong WP, Tan P, Ong CJ, Sundar R. Integration of Genomic Biology Into Therapeutic Strategies of Gastric Cancer Peritoneal Metastasis. *J Clin Oncol* 2022; **40**: 2830 [PMID: 35649219 DOI: 10.1200/JCO.21.02745]
6 **Shostak A**. Circadian Clock, Cell Division, and Cancer: From Molecules to Organism. *Int J Mol Sci* 2017; **18** [PMID: 28425940 DOI: 10.3390/ijms18040873]
7 **Milev NB**, Reddy AB. Circadian redox oscillations and metabolism. *Trends Endocrinol Metab* 2015; **26**: 430-437 [PMID: 26113283 DOI: 10.1016/j.tem.2015.05.012]
8 **Franzoni A**, Markova-Car E, Dević-Pavlić S, Jurišić D, Puppin C, Mio C, De Luca M, Petruz G, Damante G, Pavelić SK. A polymorphic GGC repeat in the NPAS2 gene and its association with melanoma. *Exp Biol Med (Maywood)* 2017; **242**: 1553-1558 [PMID: 28799406 DOI: 10.1177/1535370217724093]
9 **Liu H**, Gao Y, Hu S, Fan Z, Wang X, Li S. Bioinformatics Analysis of Differentially Expressed Rhythm Genes in Liver Hepatocellular Carcinoma. *Front Genet* 2021; **12**: 680528 [PMID: 34149816 DOI: 10.3389/fgene.2021.680528]
10 **Sancar A**, Van Gelder RN. Clocks, cancer, and chronochemotherapy. *Science* 2021; **371** [PMID: 33384351 DOI: 10.1126/science.abb0738]
11 **Chen W**, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; **66**: 115-132 [PMID: 26808342 DOI: 10.3322/caac.21338]
12 **Renthlei Z**, Gurumayum T, Borah BK, Trivedi AK. Daily expression of clock genes in central and peripheral tissues of tree sparrow (Passer montanus). *Chronobiol Int* 2019; **36**: 110-121 [PMID: 30365349 DOI: 10.1080/07420528.2018.1523185]
13 **Roenneberg T**, Merrow M. The Circadian Clock and Human Health. *Curr Biol* 2016; **26**: R432-R443 [PMID: 27218855 DOI: 10.1016/j.cub.2016.04.011]
14 **Zhang J**, Lv H, Ji M, Wang Z, Wu W. Low circadian clock genes expression in cancers: A meta-analysis of its association with clinicopathological features and prognosis. *PLoS One* 2020; **15**: e0233508 [PMID: 32437452 DOI: 10.1371/journal.pone.0233508]

15 **Qiu MJ**, Liu LP, Jin S, Fang XF, He XX, Xiong ZF, Yang SL. Research on circadian clock genes in common abdominal malignant tumors. *Chronobiol Int* 2019; **36**: 906-918 [PMID: 31014126 DOI: 10.1080/07420528.2018.1477792]

16 **Yuan P**, Li J, Zhou F, Huang Q, Zhang J, Guo X, Lyu Z, Zhang H, Xing J. NPAS2 promotes cell survival of hepatocellular carcinoma by transactivating CDC25A. *Cell Death Dis* 2017; **8**: e2704 [PMID: 28333141 DOI: 10.1038/cddis.2017.131]

17 **Song B**, Chen Y, Liu Y, Wan C, Zhang L, Zhang W. NPAS2 regulates proliferation of acute myeloid leukemia cells *via* CDC25A-mediated cell cycle progression and apoptosis. *J Cell Biochem* 2019; **120**: 8731-8741 [PMID: 30536616 DOI: 10.1002/jcb.28160]

18 **Kiessling S**, Beaulieu-Laroche L, Blum ID, Landgraf D, Welsh DK, Storch KF, Labrecque N, Cermakian N. Enhancing circadian clock function in cancer cells inhibits tumor growth. *BMC Biol* 2017; **15**: 13 [PMID: 28196531 DOI: 10.1186/s12915-017-0349-7]

19 **Nagata C**, Tamura T, Wada K, Konishi K, Goto Y, Nagao Y, Ishihara K, Yamamoto S. Sleep duration, nightshift work, and the timing of meals and urinary levels of 8-isoprostane and 6-sulfatoxymelatonin in Japanese women. *Chronobiol Int* 2017; **34**: 1187-1196 [PMID: 28933565 DOI: 10.1080/07420528.2017.1355313]

20 **Gao LW**, Wang GL. Comprehensive bioinformatics analysis identifies several potential diagnostic markers and potential roles of cyclin family members in lung adenocarcinoma. *Onco Targets Ther* 2018; **11**: 7407-7415 [PMID: 30425528 DOI: 10.2147/OTT.S171705]

21 **Iyyanki T**, Zhang B, Wang Q, Hou Y, Jin Q, Xu J, Yang H, Liu T, Wang X, Song F, Luan Y, Yamashita H, Chien R, Lyu H, Zhang L, Wang L, Warrick J, Raman JD, Meeks JJ, DeGraff DJ, Yue F. Subtype-associated epigenomic landscape and 3D genome structure in bladder cancer. *Genome Biol* 2021; **22**: 105 [PMID: 33858483 DOI: 10.1186/s13059-021-02325-y]

22 **Yu CC**, Chen LC, Chiou CY, Chang YJ, Lin VC, Huang CY, Lin IL, Chang TY, Lu TL, Lee CH, Huang SP, Bao BY. Genetic variants in the circadian rhythm pathway as indicators of prostate cancer progression. *Cancer Cell Int* 2019; **19**: 87 [PMID: 30996687 DOI: 10.1186/s12935-019-0811-4]

23 **Yuan P**, Yang T, Mu J, Zhao J, Yang Y, Yan Z, Hou Y, Chen C, Xing J, Zhang H, Li J. Circadian clock gene NPAS2 promotes reprogramming of glucose metabolism in hepatocellular carcinoma cells. *Cancer Lett* 2020; **469**: 498-509 [PMID: 31765736 DOI: 10.1016/j.canlet.2019.11.024]

24 **He Y**, Wang G, Wang Q, Zhao Z, Gan L, Yang S, Wang Y, Guo S, An J, Zhang J, Zhang Z, Zhou F. Genetic variants in NPAS2 gene and clinical outcomes of resectable non-small-cell lung cancer. *Future Oncol* 2021; **17**: 795-805 [PMID: 33541123 DOI: 10.2217/fon-2020-0211]

25 **Mukhopadhyay NK**, Ferdinand AS, Mukhopadhyay L, Cinar B, Lutchman M, Richie JP, Freeman MR, Liu BC. Unraveling androgen receptor interactomes by an array-based method: discovery of proto-oncoprotein c-Rel as a negative regulator of androgen receptor. *Exp Cell Res* 2006; **312**: 3782-3795 [PMID: 17011549 DOI: 10.1016/j.yexcr.2006.07.017]

26 **Yang SL**, Ren QG, Wen L, Hu JL, Wang HY. Research progress on circadian clock genes in common abdominal malignant tumors. *Oncol Lett* 2017; **14**: 5091-5098 [PMID: 29113149 DOI: 10.3892/ol.2017.6856]

27 **LeVan TD**, Xiao P, Kumar G, Kupzyk K, Qiu F, Klinkebiel D, Eudy J, Cowan K, Berger AM. Genetic Variants in Circadian Rhythm Genes and Self-Reported Sleep Quality in Women with Breast Cancer. *J Circadian Rhythms* 2019; **17**: 6 [PMID: 31303884 DOI: 10.5334/jcr.184]

28 **Lu J**, Zheng ZF, Xie JW, Wang JB, Lin JX, Chen QY, Cao LL, Lin M, Tu RH, Huang CM, Zheng CH, Li P. Is the 8th Edition of the AJCC TNM Staging System Sufficiently Reasonable for All Patients with Noncardia Gastric Cancer? A 12,549-Patient International Database Study. *Ann Surg Oncol* 2018; **25**: 2002-2011 [PMID: 29725896 DOI: 10.1245/s10434-018-6447-0]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com