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Original Article

Prognostic relevance of Period1 (Per1) and Period2 (Per2) expression in human gastric cancer

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Abstract: Period1 (Per1) and Period2 (Per2) are members of the circadian genes. Mounting evidence suggests that the deregulation of the circadian clock plays an important role in the development of mammalian cancer. However, the expression and clinical significance of Per1 and Per2 in gastric cancer is still unexplored. Here, we evaluated the expression pattern of Per1 and Per2 in 246 gastric cancer specimens and their adjacent, non-tumorous tissues using immunohistochemical assays. Per1 expression was significantly associated with clinical stage ($p < 0.001$), depth invasion ($p < 0.001$), lymph node metastasis ($p < 0.001$) and pathologic differentiation ($p < 0.001$). On the other hand, Per2 was associated with clinical stage ($p = 0.021$) and depth invasion ($p = 0.007$). Per1 expression was positively correlated with Per2 expression in the 246 gastric cancer patients ($r = 0.378$, $p < 0.001$), and the expression levels of Per1 and Per2 were down-regulated in gastric cancer tissues when compared with adjacent, non-tumorous tissues in 45 gastric cancer samples ($p < 0.001$, $p = 0.003$). Patients with lower Per1 and Per2 tumor expression had a shorter survival time than those with higher expression. Univariate and Multivariate analyses indicated that Per2 expression is an independent prognostic factor ($p = 0.023$). Our results demonstrate that Per1 and Per2 may play important roles in tumor development, invasion and prognosis, and Per2 may serve as a novel prognostic biomarker of human gastric cancer.

Keywords: Circadian clock gene, Per1, Per2, gastric cancer, expression, prognostic

Introduction

Gastric cancer is the fourth-most commonly diagnosed malignancy and is the second-most frequent cause of cancer deaths [1, 2]. Most gastric cancer patients who undergo surgery are already at an advanced stage. Despite some advances in the chemotherapy and surgical techniques for treating gastric cancer, the overall survival of patients is still low. Therefore, identification of new prognostic and predictive markers to determine the risk of poor prognosis is important for early molecular diagnosis, risk analysis and the development of new therapies [3].

Different types of living organisms are driven by the daily light-dark cycles of the earth. Such circadian rhythm is one of the basic characteristics of an organism's life activities and is controlled by the circadian system, which is composed of a series of circadian clock genes [4]. The circadian timing system that is responsible for the generation of these rhythmic variations is composed of master and peripheral oscillators. Recently, several studies have reported that the circadian system has a master-and-slave structure, i.e., the central pacemaker and master oscillator, which is located in the suprachiasmatic nuclei (SCN) of the brain, is entrained to the environmental light-dark cycle by photic inputs that are conveyed by the retinohypothalamic tract and synchronizes slave oscillators in peripheral tissues [5-7]. The human circadian rhythm is controlled by several core circadian genes, including positive activators such as the three transcription factors CLOCK, neuronal PAS domain protein2 (NPAS2)

and BMAL1, negative effectors, such as two cryptochromes (CRY1 and CRY2) and three period (PER1, PER2 and PER3) genes [8].

Circadian rhythms influence many physiologic processes and pathologic conditions. Disruption of the circadian clock may deregulate normal cellular biological functions and have significant effects on human health, e.g., causing conditions such as sleep disorders, gastrointestinal and cardiovascular illnesses and depression. Growing evidence shows that alterations in circadian rhythm can be a risk factor for the development of cancers in animals and humans [9-11]. Epidemiologic studies have shown that disruption of the normal circadian rhythm may increase the risk of developing various types of cancer such as breast, prostate, colorectal, liver and endometrial cancers [12, 13]. Of all the known clock genes, Per1 and Per2 have been shown to play a major role in cancer development. Overexpression of Per1 or Per2 in cancer cells inhibits their neoplastic growth and increases their apoptotic rate [14]. Importantly, the involvement of Per1 and Per2 in ataxia telangiectasia mutated (ATM)-checkpoint kinase DNA damage response pathways implicate the participation of the circadian system in tumor suppression. Thus, the Per genes may act as tumor suppressors [15].

It has not been reported that Per1 and Per2 expression is associated with clinicopathological features and outcomes of gastric cancer. Therefore, we used immunohistochemistry to investigate the expression of Per1 and Per2 in 246 gastric cancer samples and evaluated their prognostic significance by correlating Per1 and Per2 protein expression with clinicopathological parameters.

Materials and methods

Patient information and tumor tissue samples

The use of clinical materials for this research was approved by the Ethics Committee of Sun Yat-Sen University Cancer Center, and written informed consent was obtained from all patients before surgery.

Two hundred and forty-six paraffin-embedded, archived samples from patients with various clinical stages of gastric cancer and who were treated at the Cancer Center of Sun Yat-Sun University between January 2000 and July 2010 were included in the study. Pathological parameters, such as tumor invasive depth, differentiation grade and histological pattern were collected from pathological reports and verified by pathologists. The clinical and clinicopathological classification and stage of each tumor were classified according to the 7th Union International Cancer Control (UICC) TNM staging system. The cancer tissues were surgically obtained at the following time points: 53 cases were between 10:00 and 12:00, 46 cases were between 12:00 and 14:00, 134 cases were between 14:00 and 16:00, 8 cases were between 16:00 and 18:00, and 13 cases were between 18:00 and 20:00.

The patients were followed-up once every 3 months for the first 2 years, once every 6 months during the third to fifth years and annually for an additional 5 years or until postoperative patient death. All patients were contacted by phone to determine their health status, and the last follow-up date was July 1, 2013. The overall survival (OS), which was defined as the time from the operation to patient death or the last follow-up, was used as a measure of prognosis.

Immunohistochemistry (IHC)

We used previously described IHC standard methods [16]. Briefly, paraffin-embedded tissues were sectioned continuously at a thickness of 4 μm and heated for 1 h at 60°C, the sections were then deparaffinized using xylene at 37°C for 20 min and rehydrated with a series of graded alcohol and distilled water. The tissue slides were then treated with 3% hydrogen peroxide in methanol for 20 min at 37°C to block endogenous peroxidase activity. The sections were subsequently immersed in 10 mM citrate buffer (pH 6.0), microwaved for antigenic retrieval and allowed to cool to room temperature. This treatment was followed by incubation overnight with a primary antibody, either rabbit polyclonal anti-Per1 (Abcam, #ab3443, HK, dilution 1:250) or mouse monoclonal anti-Per2 (Abnova, M01, Taiwan dilution 1:150), in a humidified container at 4°C. The tissue slides were washed three times with PBS, incubated with the corresponding secondary anti-bodies, either an anti-rabbit (D13-110, GBI labs Co.,

Table 1. Clinical characteristics and Per1 and Per2 expression of the 246 patient samples of gastric cancer

	Number of cases (%)
Gender	
Male	181 (73.6)
female	65 (26.4)
Age (years)	
≤ 60	131 (53.3)
> 60	115 (46.7)
Location	
Upper	108 (43.9)
Middle/lower	138 (56.1)
Size (cm)	
≤ 5	154 (62.6)
> 5	92 (37.4)
Clinical stage	
I-II	63 (25.6)
III-IV	183 (74.4)
Depth of invasion	
T1-2	48 (19.5)
T3-4	198 (80.5)
Lymph node metastasis	
N0	52 (21.1)
N1-3	194 (78.9)
Histological types	
Well differentiation adenocarcinoma	25 (10.2)
Moderate differentiation adenocarcinoma	46 (18.7)
poor differentiation adenocarcinoma	175 (71.1)
Vital status (at follow-up)	
Alive	63 (25.6)
Death (All gastric cancer -related)	183 (74.4)
Expression of PER1	
Low expression	143 (58.1)
High expression	103 (41.9)
Expression of PER2	
Low expression	160 (65)
High expression	86 (35)

USA) or anti-mouse (PV-6002, Zhongshan Goldenbridge Biotechnology Co., China), at 37°C for 30 minutes then thoroughly washed three times with PBS. The sections were developed with diaminobenzidine tetrahydrochloride (DAB) and counterstained with hematoxylin.

Evaluation of staining

Two independent observers who were blinded to the patient data and specialize in gastric cancer evaluated the IHC results. The extent of the staining and the proportion of stained cells were used as the criteria of evaluation. The total Per1 and Per2 immunostaining scores were estimated using the percentage of positively stained tumor cells and the staining intensity. For each sample, the proportion of Per1 and Per2 expressing cells varied from 0% to 100%, and the intensity of staining varied from weak to strong. One score was given according to the percent of positive cells as follows: ≤ 10% = 0, > 10% to ≤ 25% = 1, > 25% to ≤ 50% = 2, > 50% to ≤ 75% = 3, and > 75% = 4. Another score was given according to the intensity of staining as negative = 0, weak = 1, moderate = 2, or strong = 3. The two scales were multiplied, and the cells with a value greater than or equal to 4.0 were counted as having high expression. Otherwise, the tumor was considered to have low expression.

Statistical analysis

All statistical analyses were carried out using the SPSS software (version 16.0; Chicago, IL, USA). The relationship between Per1 and Per2 expression and the clinicopathologic characteristics were calculated using the chi-square test, and were displayed in cross-tables. Survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test. Cox proportional-hazard analysis was used for Univariate and Multivariate analyses to explore the effect of clinicopathological variables and Per1 and Per2 expression on survival. The association between Per1 and Per2 were assessed using Spearman's correlation coefficient analysis. All *p* values were two-sided, and $p < 0.05$ was determined to be statistically significant.

Results

Characteristics of patients and tumors

The characteristics of the 246 patients are summarized in **Table 1**. The age distribution

Table 2. Correlation between Per1 and Per2 expression and clinicopathological variables of the 246 gastric cancer cases

Characteristics	Per1		*P*-value	Per2		*P*-value[a]
	Low No. cases (%)	High No. cases (%)		Low No. cases (%)	High No. cases (%)	
Gender			0.144			1.000
Female	43 (30.1)	22 (21.4)		42 (26.2)	23 (26.7)	
Male	100 (69.9)	81 (78.6)		118 (73.8)	63 (73.3)	
Size (cm)			0.789			0.784
≤ 5.0	91 (63.6)	63 (61.2)		99 (68.9)	55 (64.0)	
> 5.0	52 (36.4)	40 (38.8)		61 (38.1)	31 (36.0)	
Age (years)			0.897			0.789
≤ 60	77 (53.8)	54 (52.4)		84 (52.5)	47 (54.7)	
> 60	66 (46.2)	49 (47.6)		76 (47.5)	39 (45.3)	
Location			1.000			0.687
Upper	63 (40.1)	45 (43.7)		72 (45.0)	36 (41.9)	
Middle/lower	80 (59.9)	58 (56.3)		88 (55.0)	50 (58.1)	
Clinical Stage			< 0.001			0.021
I + II	19 (13.3)	44 (42.7)		33 (20.6)	30 (34.9)	
III + IV	124 (86.7)	59 (57.3)		127 (79.4)	56 (65.1)	
Depth of invasion			< 0.001			0.007
T1 + T2	14 (9.8)	34 (33.0)		23 (14.4)	25 (29.1)	
T3 + T4	129 (90.2)	69 (67.0)		137 (85.6)	61 (70.9)	
Lymph node metastasis			< 0.001			0.413
Negative	19 (13.3)	33 (32.0)		31 (19.4)	21 (24.4)	
Positive	124 (86.7)	70 (68.0)		129 (80.6)	65 (75.6)	
Pathologic differentiation			< 0.001			0.239
Well + Moderate differentiation adenocarcinoma	22 (15.4)	49 (47.6)		42 (26.2)	29 (33.7)	
Poor differentiation adenocarcinoma	121 (84.6)	54 (52.4)		118 (73.3)	57 (66.3)	

[a]*p* value < 0.05.

ranged from 23 to 79 years, and the mean patient age was 57.1 years (SD, 11.6). The median follow-up duration was 25 months (range, 1 to 161), and 48 of the 246 tumors (19.5%) were T1 or T2. The samples included 29 cases of clinical stage I (11.8%), 34 cases of stage II (13.8%), 149 cases of stage III (60.6%) and 34 cases of stage IV (13.8%) gastric cancer. Per1 and Per2 protein expression levels were used for IHC analysis. Per1 and Per2 were both localized in the cytoplasm and nuclear regions of the cells, but were mainly found in the cytoplasm.

Per1 and Per2 expression

The association of Per1 or Per2 expression and clinicopathological variables is described in **Table 2**. According to the scoring system, high Per1 and Per2 protein expression was detected in 103 (41.9%) and 86 (35%) of the tumor samples, respectively, while low staining was observed in 143 (58.1%) and 160 (65%) of the tumor samples, respectively.

Our statistical analyses showed that Per1 expression was significantly correlated with the stage of disease ($p < 0.001$), pathologic differentiation ($p < 0.001$), depth invasion ($p < 0.001$) and presence of lymph node metastasis ($p < 0.001$). The expression of Per2 was significantly correlated with the stage of the disease ($p = 0.015$) and depth invasion ($p = 0.006$). As shown in **Figure 1**, higher staging was associated with lower Per1 and Per2 expression. Consistently, Per1 expression was negatively correlated with pathologic differentiation (**Figure 2**).

Forty-five gastric cancer and matched adjacent non-tumorous tissues samples were present in the 246 paraffin-embedded gastric cancer tissues. Our data revealed that Per1 and Per2 expression levels were lower in gastric cancer samples than in matched, adjacent, non-tumor-

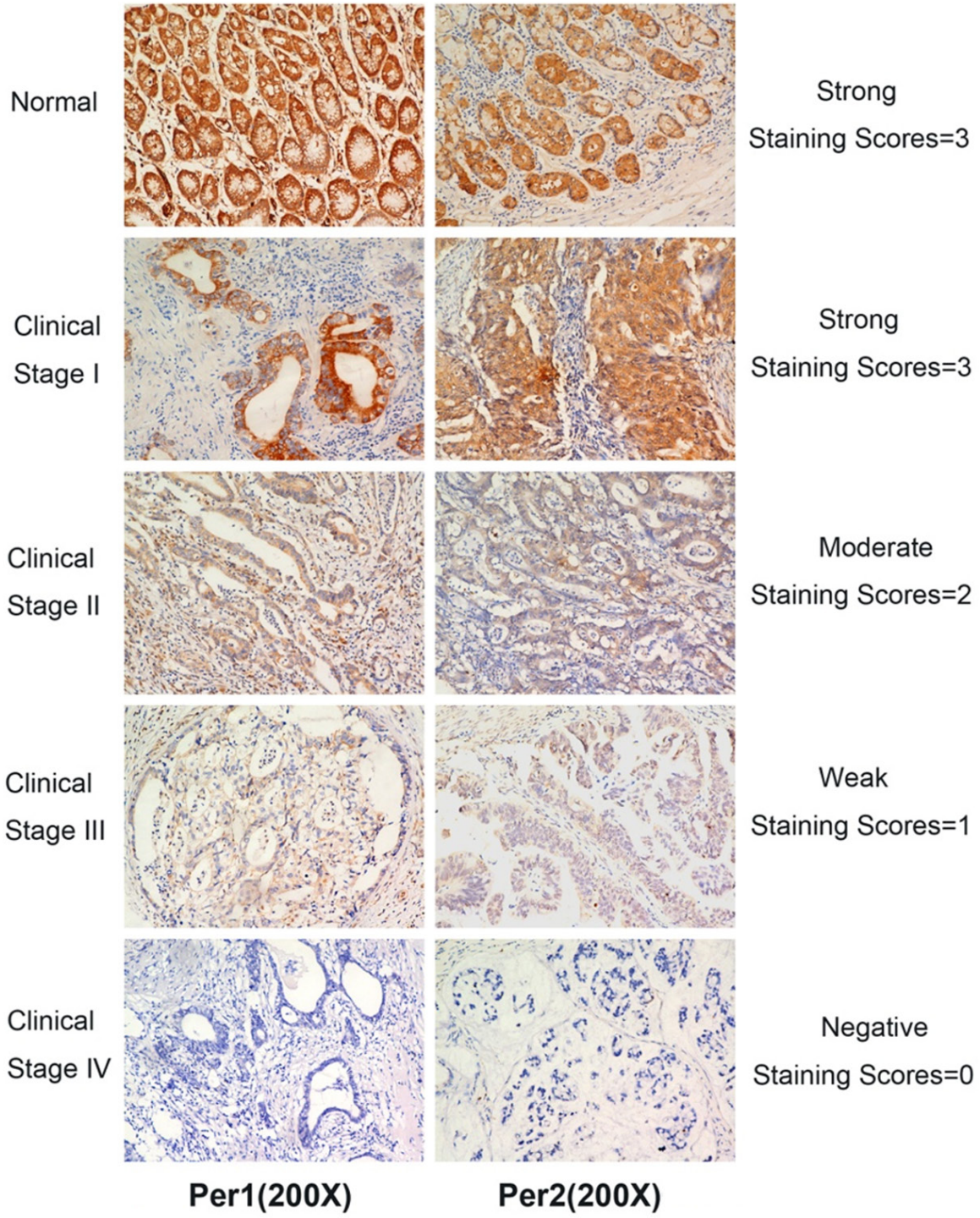

Figure 1. Decreased expression of Per1 and Per2 in advanced gastric cancer. Representative IHC analyses of Per1 and Per2 expression in normal tissues and gastric cancer specimens at different clinical stages. Examples of the scoring system for different scores are also shown (right column).

ous tissues ($p < 0.001$, $p = 0.003$, respectively). Six pairs of representative slides are shown in **Figure 3A** and **3B**. We detected decreased expression of Per1 and Per2 in 24/45 (53.73%) and 30/45 (66.7%) of the gastric cancer tissue samples, respectively, compared with only 6/45 (13.3%) and 15/45 (33.3%) in adjacent, non-tumorous tissues.

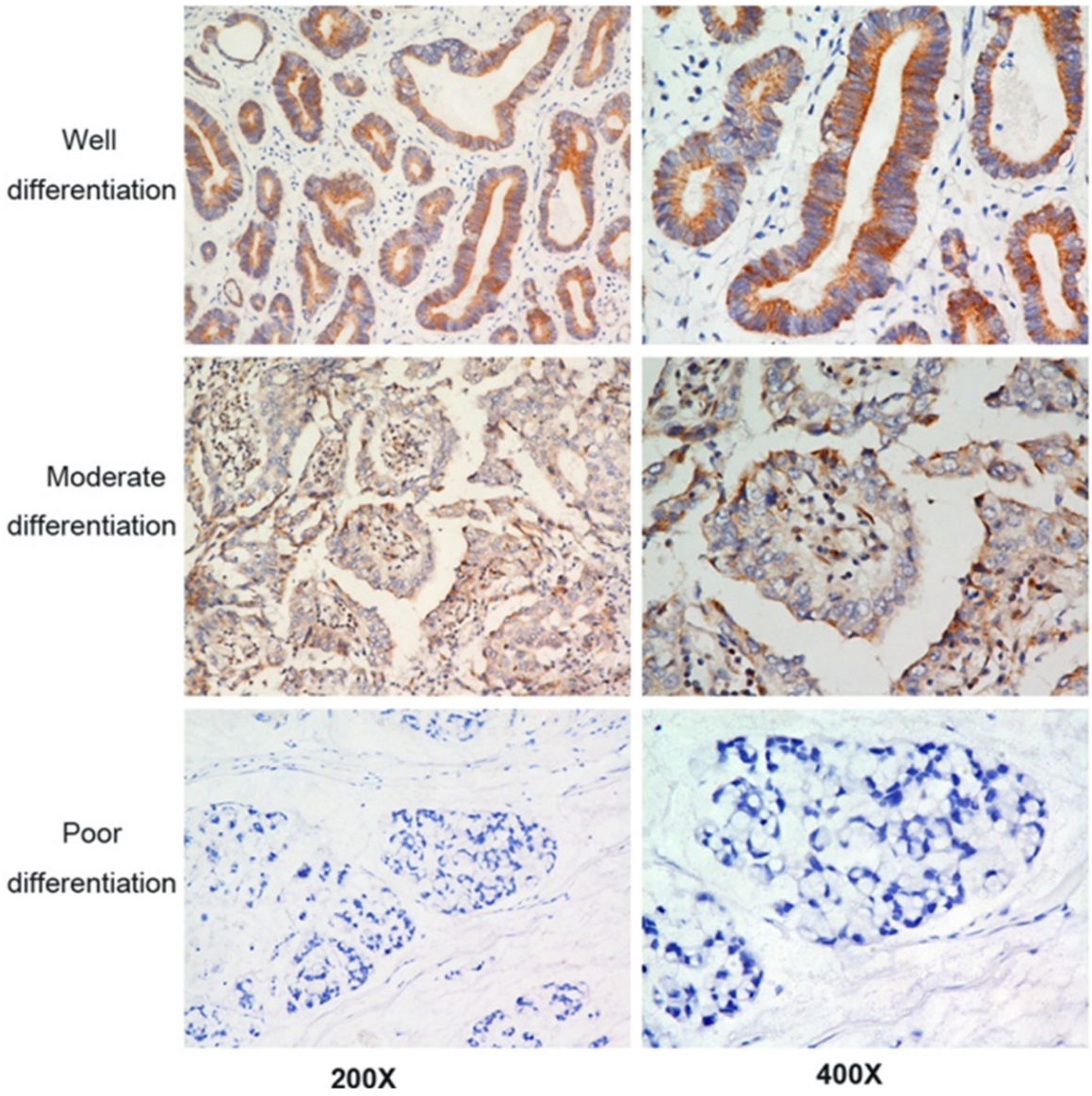

Figure 2. Per1 expression in gastric cancer tissues at different differentiation states.

Correlation between Per1 and Per2 expression

For these 246 cases, the Per1 and Per2 protein expression in the gastric cancer tissues exhibited four different patterns: type I (51 cases): Per1 and Per2 both exhibited high expression; type II (52 cases): the Per1 showed high expression, while the Per2 showed low expression; type III (35 cases): Per1 showed low expression, while Per2 showed high expression; and type IV (108 cases): Per1 and Per2 both showed low expression.

We used the staining final score to analyze the relation between Per1 and Per2 in the 246 gastric cancer specimens (**Figure 3C**). The results indicated that the expression levels of Per1 were positively correlated with the expression levels of Per2, the r coefficient was 0.378, and $p < 0.001$.

Prognostic implications of Per1 and Per2 expression in gastric cancer

Kaplan-Meier analysis and the log-rank test were used to calculate the effects of the clinicopathological characteristics and Per1 and Per2 expression on survival. The gastric cancer patients with low Per1 and Per2 expression had significantly shorter overall survival time

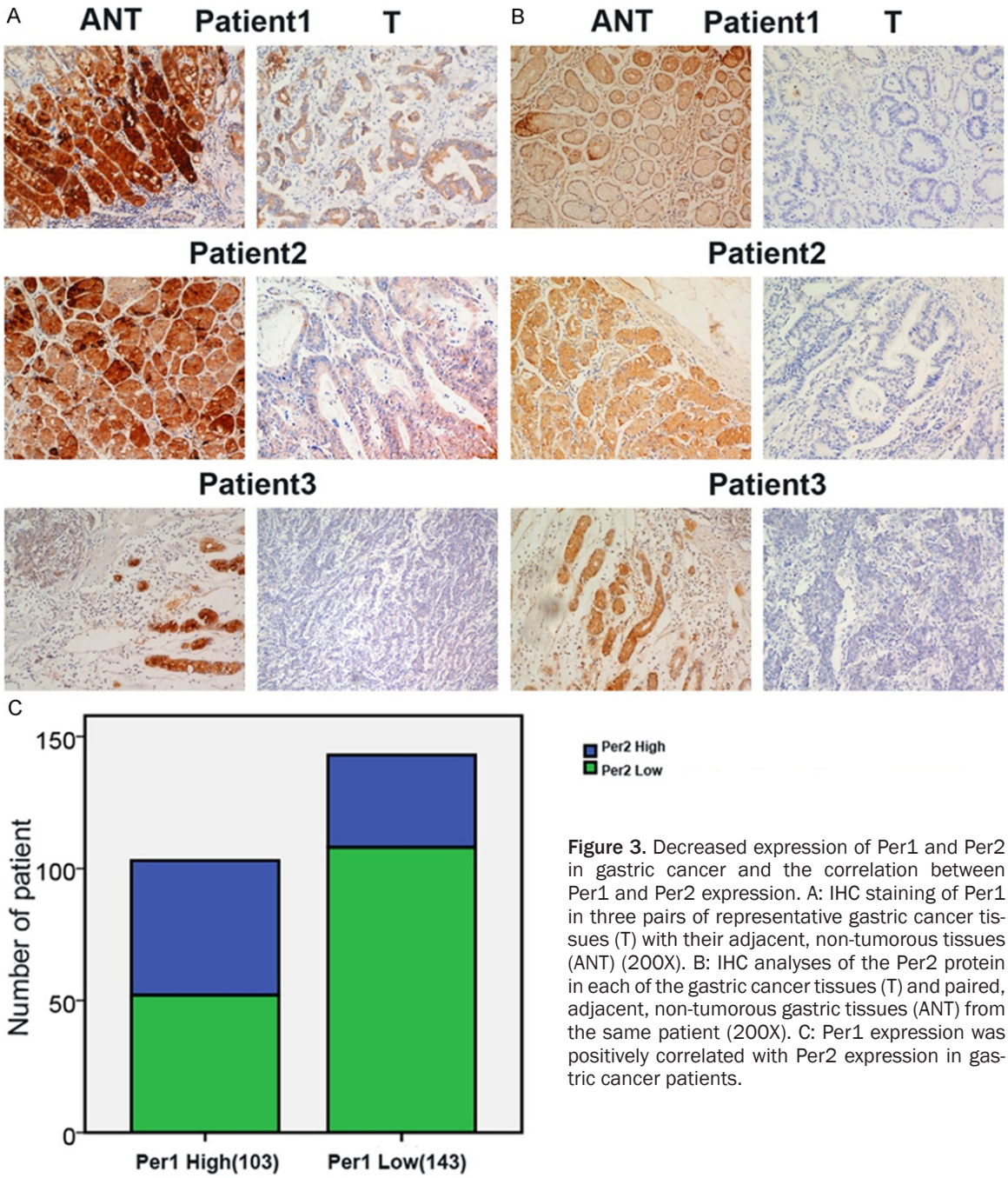

Figure 3. Decreased expression of Per1 and Per2 in gastric cancer and the correlation between Per1 and Per2 expression. A: IHC staining of Per1 in three pairs of representative gastric cancer tissues (T) with their adjacent, non-tumorous tissues (ANT) (200X). B: IHC analyses of the Per2 protein in each of the gastric cancer tissues (T) and paired, adjacent, non-tumorous gastric tissues (ANT) from the same patient (200X). C: Per1 expression was positively correlated with Per2 expression in gastric cancer patients.

than those with high Per1 and Per2 expression (**Figure 4A** and **4B**, $p < 0.001$, $p = 0.002$). The postoperative median OS of patients with high staining of Per1 was 40 months, while that of patients with low staining of Per1 was 21 months. The overall three-year, and five-year accumulative survival rates were 38.6%, and 32.1%, respectively. The 3-year and 5-year cumulative survival rates of patients with Per1 negative expression were 30.1%, and 23.1%, compared with 50.5% and 44.7%, of patients with Per1 positive expression, respectively, and the postoperative median OS of patients with high staining of Per2 was 43 months, while that of patients with low Per2 expression was 21 months. The 3- and 5-year OS rates in patients exhibiting elevated Per2 were significantly higher than in those exhibiting reduced Per2 levels (52.3% and 45.3% vs. 31.3% and 25.0%, respectively). When the survival of patients with a high Per1/high Per2 was compared with that of those with low Per1/low Per2, Kaplan-

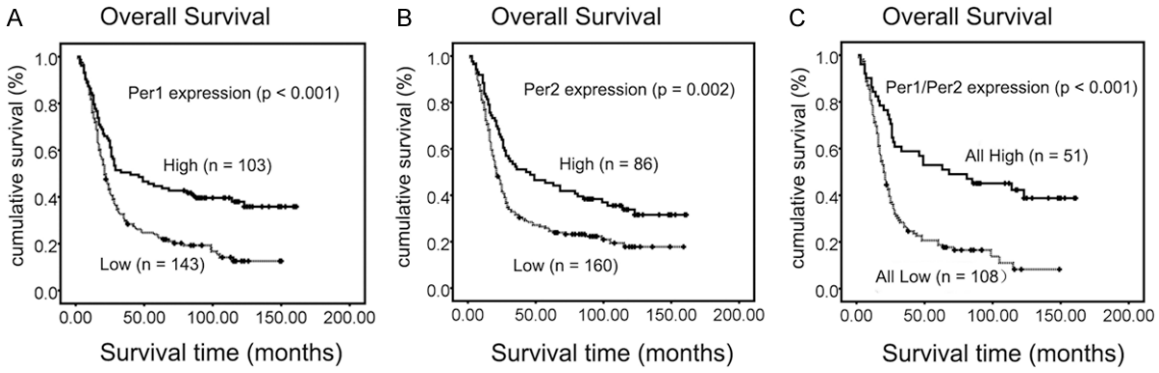

Figure 4. Kaplan-Meier survival curves for gastric cancer patients with low Per1 and Per2 expression (dotted line) versus high Per1 and Per2 expression (solid line). A: The overall survival of patients (clinical stages I-IV) with low/high Per1 expression. B: The overall survival of patients (clinical stages I-IV) with low/high Per2 expression. C: High Per1/high Per2 expression revealed a significant difference in overall survival when compared with low Per1/low Per2.

Meier analysis revealed a significant difference on overall survival ($p < 0.001$, **Figure 4C**).

Univariate analysis demonstrated that Per1 expression ($p < 0.001$), Per2 expression ($p = 0.002$), larger tumor size ($p = 0.001$), depth of invasion ($p < 0.001$), lymph node metastasis ($p < 0.001$), clinical stage ($p < 0.001$), location ($p < 0.001$) and pathologic differentiation ($p = 0.029$) were significantly associated with the overall survival of gastric cancer patients (**Table 3**).

Furthermore, by Multivariate Cox Regression analysis, Per2 expression ($p = 0.023$), the depth of invasion ($p = 0.013$) and the lymph node metastasis ($p = 0.037$) were independent prognostic factors, which suggested that Per2 may be a prognostic factor for the survival of gastric cancer patients (**Table 3**).

Discussion

Per1 and Per2 are two important core clock genes that, regulate the proliferations of various cells in the human body. Aberrant Per1 and Per2 expression can lead to abnormal cell proliferation, which is similar to the basic characteristic of cancer, uncontrolled and disordered cell proliferation [17-19]. In recent years many studies have indicated that deregulation of Per1 and Per2 expression was highly linked to the carcinogenesis and development of malignant tumors, including breast cancer, prostate cancer, colorectal carcinoma and gliomas [8, 12, 20-22], However, the expression and prognostic value of the Per1 and Per2 genes in gastric patients have not been reported. Aiming to investigate this question directly, we studied the relations between the expression levels of the Per1 and Per2 genes and outcomes and clinicopathological features. We also examined the expression levels of those genes in gastric cancer and in adjacent non-tumorous tissues.

Several previous studies explored the expression and clinical significance of Per1 and Per2 in other types of cancer. One study found that the expression of the Per1 gene in colorectal cancer tissue was significantly lower compared with that in adjacent, normal mucosa, and high expression of the Per2 gene was associated with better outcomes. Additionally, Per2 expression functions as an independent prognostic factor [23]. Another study found that the expressions of Per1 and Per2 in sporadic and familial primary tumors were significantly lower than those in normal breast tissues [22]. In human oral squamous cell carcinoma (OSCC), the expression of the Per1 mRNA and protein were significantly reduced compared with that in adjacent noncancerous tissue. Per1 expression was significantly correlated with stage of disease, depth invasion and the presence of lymph node metastasis [24]. In our findings, the same results were found in gastric cancer.

In this study, we found that low levels of Per1and Per2 expression were more frequently observed in gastric cancer patients with depth of invasion or those at advanced stages (**Figure 1**). We also determined the relationship between Per1 expression with lymph node

Table 3. Univariate and Multivariate analyses of the overall survival of the 246 gastric cancer patients

Variables	Univariate analysis		Multivariate analysis		
	No.	*P* value[a]	Hazard Ratio	95% CI	*P* value[a]
Per1		< 0.001	1.245	0.887-1.747	0.205
Low expression	143				
High expression	103				
Per2		0.002	1.462	1.053-2.031	0.023
Low expression	160				
High expression	86				
Gender		0.177			
Female	65				
Male	181				
Size (cm)		0.001	1.318	0.971-1.789	0.076
≤ 5.0	154				
> 5.0	92				
Age (years)		0.598			
≤ 60	131				
> 60	115				
Depth of invasion		< 0.001	2.581	1.219-5.464	0.013
T1 + T2	48				
T3 + T4	198				
Lymph node metastasis		< 0.001	2.419	1.054-5.552	0.037
Negative	52				
Positive	194				
Clinical Stage		< 0.001	1.078	0.433-2.685	0.871
I + II	63				
III + IV	183				
Location		< 0.001	0.762	0.563-1.032	0.079
Upper	108				
Middle/lower	138				
pathologic differentiation		0.029	0.983	0.688-1.406	0.925
Well + Moderate differentiation adenocarcinoma	71				
Poor differentiation adenocarcinoma	175				

CI, confidence interval. [a]*p* value < 0.05.

metastasis and pathologic differentiation in gastric cancer patients (**Figure 2**). The results showed the first evidence that Per1 and Per2 might play a potential role in suppressing the progression and metastasis of gastric cancer.

In addition, Kaplan-Meier analysis showed that, in general, patients with high Per1 and Per2 expression experienced a better survival outcome, while patients with low Per1 and Per2 expression experienced a poorer survival outcome (**Figure 4A-C**). Moreover, Per1 and Per2 expression was down-regulated at the protein level in gastric cancer tissues compared with matched, adjacent, non-tumorous tissues. We found that Per1 and Per2 expression was low in most gastric cancer tissue samples (24/45, 30/45), when compared with only 6/45 and 15/45 in the adjacent, non-tumorous tissues, respectively (**Figure 3A** and **3B**). However, we only observed a small number of adjacent, non-tumorous tissues; therefore we could not determine whether Per1 and Per2 expression was significantly decreased in tumor tissue samples. The inclusion of a greater number of adjacent non-tumorous tissues samples should resolve this problem.

Furthermore, Multivariate Cox model analysis indicated that the Per2 expression status was an independent prognostic factor, which suggested that Per2 could be a potential prognos-

tic factor of gastric cancer. These results suggested that decreased Per2 expression might help identify gastric cancer patients with a poor prognosis and could potentially be a novel prognostic marker for gastric cancer. However, we failed to prove that the Per1 expression status was an independent prognostic factor, and this might due to the limited number of cases. Furthermore, the Per2 gene appears to be a more functional component of the mammalian circadian clock than the Per1 gene, because functional disruption of the Per2 gene resulted in the complete loss of circadian locomotor activity in mice [25], whereas rhythmicity remained in Per1 knockout mice [26, 27]. In addition, in contrast to the phase-shifting effect of surgical stress on Per1 expression in peripheral blood mononuclear cells, the effect on Per2 expression was more prominent and different between esophageal and early gastric cancer [28]. These could explain why Per1 expression was not an independent prognostic factor.

Animals with mutations in Per1 and Per2 have a much more dramatic and immediate loss of rhythmicity in constant darkness, which suggests that Per1 and Per2 can compensate for one another to some extent to help maintain circadian rhythms. In contrast, mice with a mutation in Per3 have surprisingly few disruptions in circadian locomotor activity. Indeed, mice with double mutations in Per3 and Per1 or Per2 do not exhibit an increase in circadian rhythm disruption over the single Per1 or Per2 mutations, which suggests that Per3 has a minimal role in core circadian clock function, although it displays rhythmic expression levels in the SCN [29, 30]. The loss of Per1 results in an enhanced level of Per2 in the mutant, which suggests that Per1 normally represses Per2 levels in vivo. Because Per1 and Per2 have been shown to interact in vitro and in vivo, the posttranscriptional regulation of Per2 by Per1 may be mediated through a direct protein-protein interaction [31]. Interestingly, we observed a significant association between Per1 and Per2 in the 246 gastric cancer patients (**Figure 3C**).

As a cluster of core circadian genes, the Per genes function in maintaining the circadian rhythm of cells and in sustaining the normal cell cycle. It has been reported that 2-10% of all mammalian genes are clock-controlled, and recent studies reported that approximately 7% of clock-controlled genes that were identified in rodents regulated cell proliferation or apoptosis [32]. It has been shown that CpG methylation of promoter sequences, which is an epigenetic alteration, can inactivate promoter functions and lead to down-regulation and inhibition of gene expression [33]. Per gene deregulation is not caused by genetic mutations, but most likely occurs by methylation of the Per1 or Per2 promoters. Because the circadian clock controls the expression of cell cycle-related genes, we suggest that disturbances in Per gene expression may disrupt the control of the normal circadian clock, thus promoting the survival of cancer cells and promoting carcinogenesis [20]. Based on the series of experiments that were mentioned above, the loss of clock gene expression could be due to DNA methylation of the promoters rather than by mutations of the clock genes. Recently, Remco et al. identified the miR-192 / 194 cluster as a potent inhibitor of the entire Period gene family using a forward genetic screen, and they unveiled a new mechanism for the down-regulation of the circadian clock genes at the post-transcriptional level [34]. Over expression of Per1 and Per2 sensitized human cancer cells to DNA damage-induced apoptosis; in contrast, inhibition of Per1 and Per2 in similarly treated cells blunted apoptosis. The apoptotic phenotype was correlated with altered expression of key cell cycle regulators; therefore, we hypothesize that this could be the mechanism by which Per1 and Per2 expression correlates with the clinical features of gastric cancer patients. However, the mechanisms leading to the reduced expression of Per1 and Per2 and the manner by which Per1 and Per2 suppress tumorigenesis remain unclear. Thus, the potential role of Per1 and Per2 in gastric cancer development and their underlying mechanisms must be investigated further.

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