Per1 has the capacity to serve as a diagnostic biomarker for cholangiocarcinoma patient

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Research article

Keywords: Cholangiocarcinoma, Per1, Diagnosis

Posted Date: August 19th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-53226/v1

Abstract

Background

Period 1 (*Per1*) had been reported to be involved in the tumorigenesis and progression of human cancers. However, the clinical significance of *Per1* in cholangiocarcinoma (CCA) was unclear. The purpose of this study was to explore the diagnostic value of serum *Per1* in CCA patients.

Methods

Serum levels of *Per1* in CCA patients and healthy individuals were detected by quantitative real-time polymerase chain reaction (qRT-PCR). Chi-square test was used to evaluate the relationship between *Per1* expression and clinical characteristics of patients. The diagnostic value of *Per1* in CCA was estimated by establishing a receiver operating characteristic (ROC) curve.

Results

Serum *Per1* level was significantly down-regulated in CCA patients compared to that in healthy controls ($P < 0.001$). Moreover, the decreased expression of *Per1* was closely associated with poor histological differentiation ($P = 0.040$), advanced TNM stage ($P = 0.035$) and positive lymph node metastasis ($P = 0.007$). ROC curve indicated that the area under the curve (AUC) was 0.863 with a sensitivity of 88.1% and a specificity of 72.1%, revealing the high diagnostic value of serum *Per1* in CCA.

Conclusions

Per1 is down-regulated in CCA and negatively correlated with tumor progression. Serum *Per1* may be a potential biomarker for early screening of CCA.

Background

Cholangiocarcinoma (CCA) is a rare malignant tumor originating from the epithelial cells of bile duct [1] The morbidity and death rates of CCA exhibit increasing trend in the world [2]. CCA is characterized by continually infiltrative growth, high metastasis, poor response to radiotherapy and chemotherapy [3]. Until now, radical surgery remains the only effective strategy for CCA patients. However, due to the silent growth of the cancer and lack of tools for early detection, most CCA patients are diagnosed at late stages, leading to limited therapeutic effects and poor clinical outcomes [4, 5]. Currently, several serum biomarkers are applied for early detection and monitoring of CCA, including CEA, CA19-9, ALP, MUC5AC, and CA-S121. However, their clinical significance is unsatisfactory [6]. Therefore, it is an urgent need to identify novel biomarkers for non-invasive diagnosis of CCA patients.

The circadian rhythm is a basic character of life that can regulate various physiological activities, including cell proliferation and metabolism [7]. In living organisms, circadian system is regulated by many circadian clock genes [8]. To data, several clock genes are confirmed to be implicated with circadian rhythm, such as period 1 (*Per1*), period 2 (*Per2*), period 3 (*Per3*), circadian locomotor output cycles kaput (*Clock*), etc [9]. *Per1* gene is located on human chromosome 17p13.1, belonging to the Per subfamily. Growing evidences have demonstrated that Per subfamily not only plays a crucial role in the modulation of circadian rhythms, but also participates in the development of tumors [10]. The aberrant expression of *Per1* was observed in several tumors, such as colorectal cancer, head and neck squamous cell carcinoma, oral cancer, etc [11–13]. In CCA, it was reported that *Per1* was down-regulated and its over-expression could suppress cell proliferation, cell cycle progression, and induce cell apoptosis [14]. However, little was known about the diagnostic values of serum *Per1* in CCA.

In this study, we sought to detect the serum levels of *Per1* in CCA patients and healthy controls. Chi-square test was applied to evaluate the association of *Per1* expression with clinical characteristics of patients. Then the ROC curve was plotted to estimate the diagnostic value of *Per1* in CCA patients.

Methods

Study subjects and samples

This study was approved by the Ethical Committee of the hospital and the written informed consent was obtained from each participator in advance. In our study, 122 patients who were pathologically diagnosed with CCA at the PLA Rocket Force Characteristic Medical Center were enrolled. None of patients had received any treatments before blood collection. The histopathological diagnoses were confirmed by the experienced pathologists. The detailed clinicopathologic characteristics of the patients were shown in Table 1. Besides, 84 healthy individuals who were matched the cases in age and gender were recruited as healthy controls.

Table 1
Relationship between *Per1* expression and clinical features of CCA patients

Clinical Features	Cases (n = 122)	*Per1* expression		χ^2	*P*
		High (n = 53)	Low (n = 69)		
Age (years)				0.646	0.422
≤ 60	64	30	34		
> 60	58	23	35		
Gender				2.525	0.112
Male	72	27	45		
Female	50	26	24		
Histological differentiation				4.236	0.040
Well/Moderate	63	33	30		
Poor	59	20	39		
TNM stage				4.450	0.035
□-□	65	34	31		
□-□	57	19	38		
Lymph node metastasis				7.324	0.007
Negative	78	41	37		
Positive	44	12	32		
Depth of invasion				3.250	0.071
T1-T2	60	31	29		
T3-T4	62	22	40		

After fasting for one night, 5 mL blood samples were collected from CCA patients and healthy controls, and centrifuged at 3000 rpm for 10 min. Then the supernate was immediately stored at -80°C until for RNA extraction.

RNA extraction and qRT-PCR

Total RNA was isolated from serum samples using TRIzol reagent (Invitrogen, USA). The concentration and purity of RNA were measured via NanoDrop 2000 Spectrophotometer (NanoDrop Technologies, USA). PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, China) was utilized to synthesize the first-strand of cDNA. The relative mRNA expression of *Per1* in sera was detected by SYBR Premix Ex Taq (Takara, China). The sequences of primers for *Per1* and *β-actin* were as follows: *Per1*, forward-5'-ACCCTGATGACCCACTCTTCTC-3', and reverse-5'-CTCCTCCATAG- CCAAGTCCTGA-3'; *β-actin*, forward-5'-CTTCTACAATGAGCTGCGTGTG-3', and reverse-5'-AGAGGCGTACAGGGATAGCACAG-3'. *β-actin* acted as the internal control and the $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression level of *Per1*. Each sample was examined in triplicate.

Statistical analysis

All statistical analyses were performed with SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA). The data were expressed as mean ± standard deviation (SD). Student's t-test was used to evaluate the difference of *Per1* expression between case and control groups. The relationship between *Per1* expression and clinical characteristics of patients was analyzed via chi-square test. A receiver operating characteristic (ROC) curve was plotted to estimate the diagnostic value of *Per1* in CCA patients. If *P* was less than 0.05, the difference was considered statistically significant.

Results

Demographic data of the study subjects

A total of 50 female and 72 male patients with the mean age of 60.39 ± 13.45 years (range, 46–74 years) were recruited in this study. Among these CCA patients, 63 patients had well/moderate histological differentiation and 59 patients had poor differentiation. 65 cases were diagnosed at stage □-□ and 57 cases were at stage □-□. There were 44 patients presenting lymph node metastasis and 60 cases with T1-T2 depth of invasion. The detailed clinical characteristics of patients were shown in Table 1.

Serum *Per1* level was down-regulated in CCA patients

The relative mRNA levels of *Per1* in CCA patients and healthy controls were detected by qRT-PCR. As shown in Fig. 1, serum *Per1* level in CCA patients was significantly lower than that in healthy controls ($P < 0.001$).

Relationship between *Per1* expression and clinical features of CCA patients

According to the mean *Per1* expression, the CCA patients were divided into high expression group (n = 53) and low expression group (n = 69). Then chi-square test was applied to evaluate the relationship between *Per1* expression and clinical characteristics of patients. The result showed that decreased expression of *Per1* was closely associated with poor histological differentiation ($P = 0.040$), advanced TNM stage ($P =$

0.035) and positive lymph node metastasis ($P = 0.007$) (Table 1). However, there was no obvious correlation between *Per1* expression and age, gender or depth of invasion ($P > 0.05$ for all, Table 1).

The diagnostic value of *Per1* in CCA patients

To assess the diagnostic value of *Per1* in CCA, a ROC curve was established. It indicated that serum *Per1* could be a forceful biomarker for discriminating CCA patients from healthy controls. The area under the curve (AUC) was 0.863 (95%CI = 0.816–0.910, $P < 0.001$) with a sensitivity of 88.1% and a specificity of 72.1% (Fig. 2). The cutoff value of *Per1* mRNA for CCA detection was 5.02.

Discussion

Early diagnosis remains a great challenge for CCA patients in clinic. In the present study, we investigated the diagnostic value of serum Per 1 in CCA. The relative mRNA level of serum *Per1* in CCA patients was significantly lower than that in healthy controls. Furthermore, patients with decreased expression of *Per1* were more easily to undergo poor histological differentiation, advanced TNM stage and positive lymph node metastasis. The result of ROC curve analysis indicated that serum *Per1* might be a promising biomarker for the diagnosis of CCA with high sensitivity and specificity.

The Per genes are a subgroup of core clock genes which are involved in various biological processes via regulating circadian rhythm [15]. In human, there are three identified Per family members including, *Per1*, *Per2* and *Per3*. Accumulating evidences have demonstrated that the Per family members were involved in carcinogenesis and development of malignancies. For instances, in lung cancer, down-regulation of *Per2* might lead to aggressive proliferation and migration, as well as inhibition of apoptosis through enhancing the activities of PI3K/AKT/mTOR signaling pathway [16]. In vitro study has shown that the expression of *Per3* was decreased in CRC, moreover, it played a suppressive role in malignant biological behaviors of the cancer cells [17]. In CCA, previous tumor investigations have reported that cicadian clock disruption induced by abnormal expression of clock genes could significantly promote liver carcinogensis [18]. However, the function of Per genes in CCA had been rarely reported.

Per1, a member of the Per subfamily, was proved to play an inhibitory role in several human malignancies via suppressing the biological behaviors of cancer cells [19, 20]. Moreover, high expression of *Per1* might increase the sensitivity of radiotherapy against tumors, contributing to favorable prognosis of tumor patients [21, 22]. In this study, we found that serum level of *Per1* was decreased in CCA patients. and its expression profile showed negative association with malignant clinical parameters. All the data revealed that *Per1* as a tumor suppressor gene was involved in initiation and progression of CCA. In addition to CCA, the anti-tumor action of *Per1* was also reported in other types of human cancer, such as pancreatic cancer, non-small cell lung cancer, and buccal squamous cell carcinoma [23–25]. However, the molecular mechanisms for tumor suppression of *Per1* were poorly known in CCA. Further researches were still required.

Given its function in tumor progression, *Per1* was considered as a candidate biomarker for tumors. A study of Relles et al. had demonstrated that the expression patterns of *Per1* in comparing pancreatic cancer tissues might be a predictive biomarker for survival of the patients [26]. Hsu et al. found that the blood level of *Per1* was closely correlated with postoperative survival of patients with head and neck squamous cell carcinoma, suggesting its potential as a non-invasive biomarker for cancers [22]. In the present study, we estimated the value of serum *Per1* as a non-invasive tool for early screening of CCA. We found that serum *Per1* could be an effective biomarker for CCA patients with high sensitivity and specificity. Circulating *Per1* might be a reliable biomarker for early detection and monitoring of CCA. However, the sample size was relatively small in the current study. The clinical significance of serum *Per1* for CCA diagnosis was needed further verification.

Conclusions

In conclusion, *Per1* is down-regulated in CCA and negatively correlated with the progression of this disease. What's more, serum *Per1* may be a promising biomarker for early diagnosis of CCA.

List Of Abbreviations

Period 1 (*Per1*)

cholangiocarcinoma (CCA)

quantitative real-time polymerase chain reaction (qRT-PCR)

receiver operating characteristic (ROC)

area under the curve (AUC)

period 2 (*Per2*)

period 3 (*Per3*)

standard deviation (SD)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of the PLA Rocket Force Characteristic Medical Center and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

N.W. design of the work; Y.L. the acquisition, analysis, Y.Z. interpretation of data; H.C. the creation of new software used in the work; X.W., Z.L. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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Figures

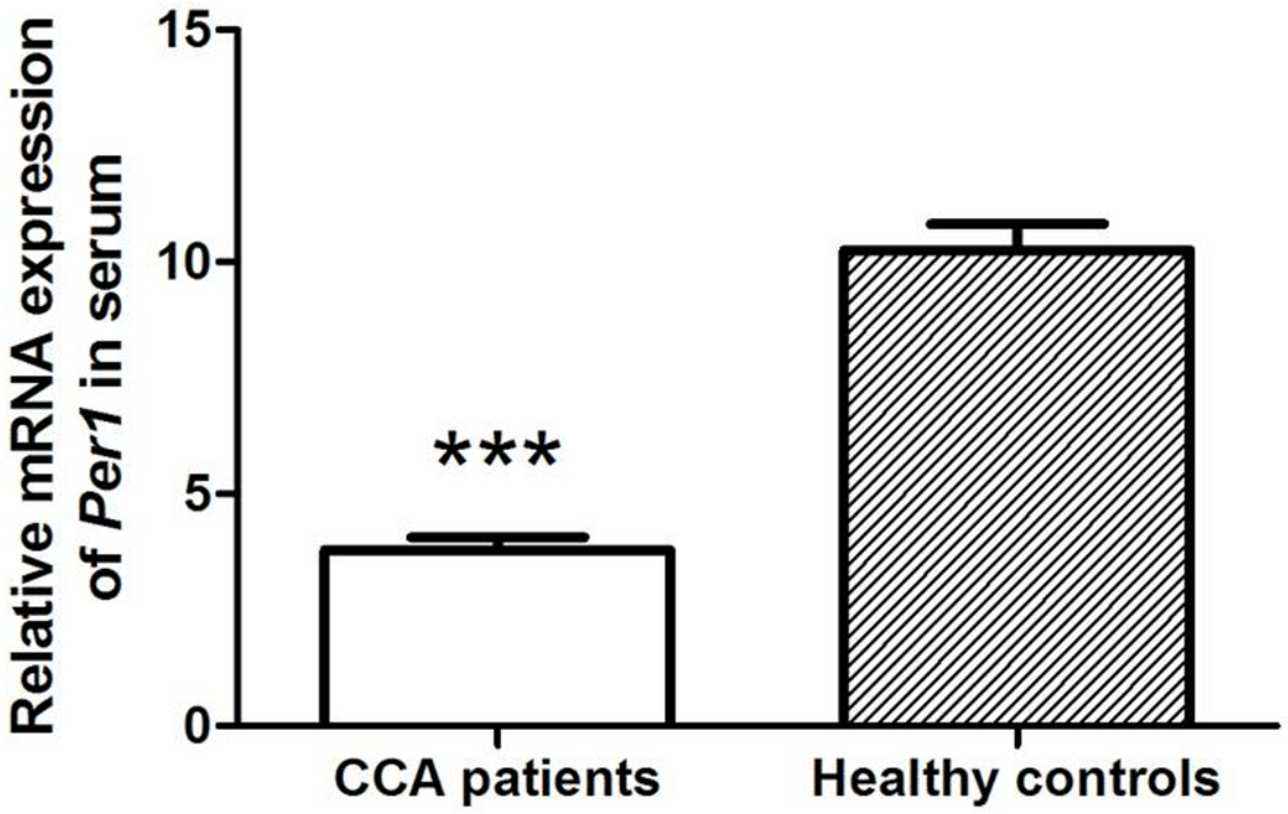

Figure 1

The relative mRNA levels of Per1 in serum of CCA patients and healthy controls. Compared with healthy individuals, serum Per1 level was significantly down-regulated in CCA patients (P<0.001). *** indicated P less than 0.001.

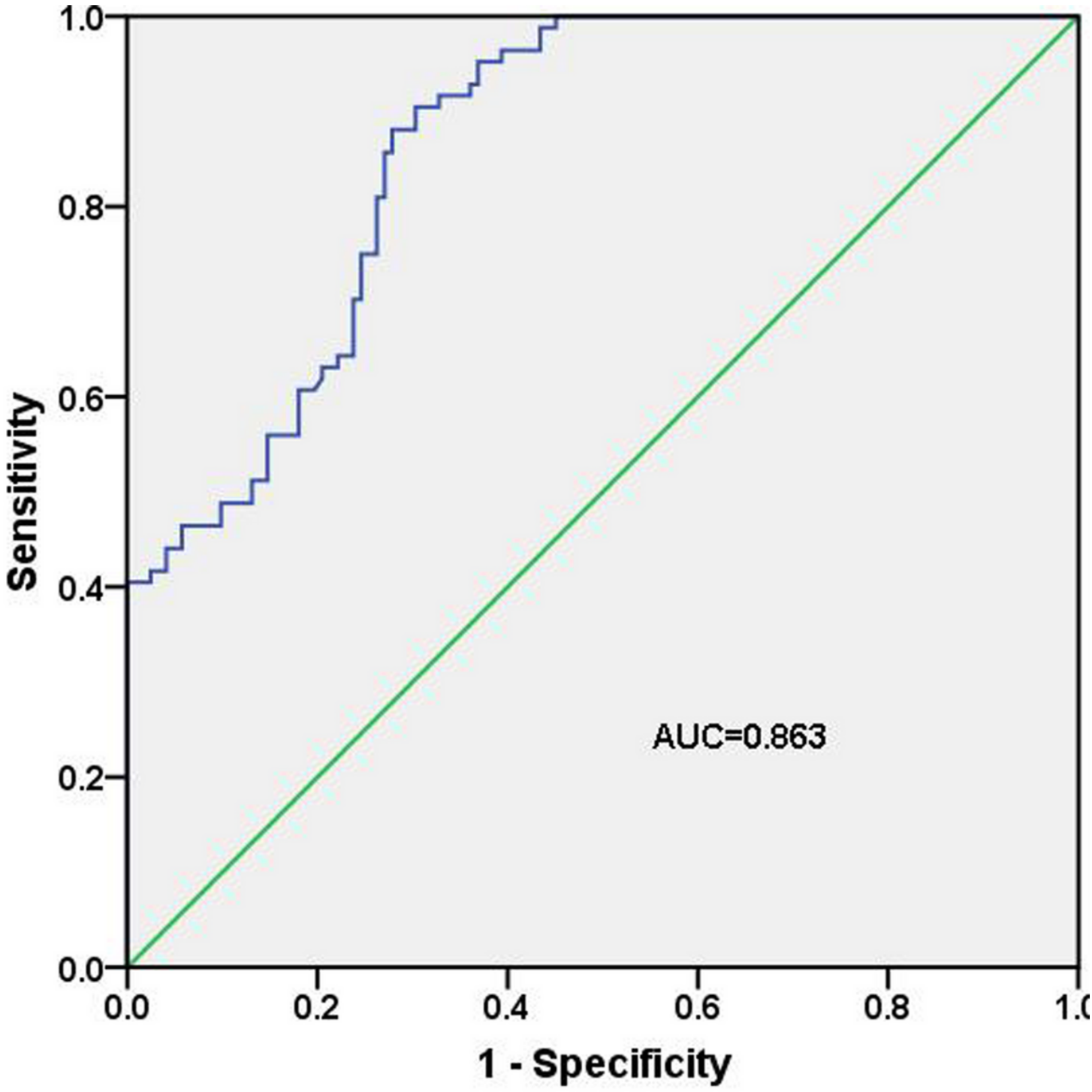

Figure 2

ROC curve analysis was performed to estimate the diagnostic value of Per1 in CCA patients. The AUC was 0.863 with a sensitivity of 88.1% and a specificity of 72.1%, revealing the high diagnostic accuracy of Per1 for CCA.