hTERT Represents an Innovative Bio-marker in Cholangiocarcinoma Detection

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Abstract

Background: The study was aimed to examine the diagnostic value of serum human telomerase reverse transcriptase (*hTERT*) in patients with cholangiocarcinoma (CCA).

Methods: Serum *hTERT* in CCA patients and healthy controls was detected by quantitative real-time PCR (qRT-PCR) and compared by student's t-test. Relationships of serum *hTERT* and clinical parameters were explained by Chi-square test. The receiver operating characteristics (ROC) analysis was conducted to identify the diagnostic value of serum *hTERT* in CCA.

Results: Serum *hTERT* was up-regulated in CCA patients compared with healthy controls ($P<0.05$). Furthermore, serum *hTERT* was closely related with differentiation ($P=0.005$), distant metastasis ($P=0.015$) and TNM stage ($P=0.009$). The AUC value of ROC curve was 0.901, demonstrating that serum *hTERT* could discriminate between CCA and healthy individuals, with the cutoff point of 1.88. Besides, the diagnostic specificity and sensitivity of serum *hTERT* in CCA were 89.7% and 76.0%, respectively.

Conclusions: *hTERT* may be a promising biomarker for diagnosis of CCA.

Background

Cholangiocarcinoma (CCA) is a rare malignancy in the intrahepatic or extrahepatic biliary tree, originating from the epithelial cells of bile ducts [1, 2]. According to the anatomical and clinical criteria, CCA is classified into three subtypes: intrahepatic CCA, extrahepatic CCA and hilar CCA [3, 4]. Surgical resection strategy remains the only potential curative treatment for CCA patients [5, 6]. However, the major CCA cases are usually diagnosed with disseminated or advanced stage that miss the optimal time for operation, leading to poor outcomes [7]. Although the symptoms of advanced CCA include biliary strictures, abdominal pain, jaundice and fever, even anorexia, vomiting and nausea, the cancer is asymptomatic at early stage, increasing the difficulty for early diagnosis [8, 9]. So far, several biomarkers are identified for CCA screening, such as CEA, CA19-9, ALP, MUC5AC, and CA-S121. However, their clinical value is limited by low diagnosis accuracy [10]. Therefore, it is urgently needed to explore novel biomarker for CCA diagnosis.

Telomerase, a type of ribonucleoprotein, locates at the ends to the chromosomes and consists of two subunits: telomerase RNA template (hTR) and human telomerase reverse transcriptase (*hTERT*) [11]. Telomerase plays a pivotal role in cell division via catalyzing the synthesis of telomeric repeat DNA [12, 13]. *hTERT*, a catalytic subunit of telomerase, can regulate the activity of telomerase [14, 15]. *hTERT* is undetectable in normal physiological conditions, however, its over-expression has been observed in various human cancers, including gastric cancer [12], hepatocellular carcinoma [15], and colorectal cancer [16]. Furthermore, its up-regulation shows significant association with aggressive potential of cancer cells, suggesting is promoting function in tumor development and progression [17]. For example, Liang et al. reported that patients with endometrial cancer showed significantly higher blood *hTERT* level than the healthy individuals, moreover, its expression pattern was positively associated with tumor stage,

revealing its predictive function for tumor initiation [18]. The elevated expression of *hTERT* was also reported in CCA, however, its diagnostic value for the disease remained unidentified [19].

In the present study, we attempted to detect the serum level of *hTERT* in patients with CCA and estimate its diagnostic value for this disease.

Methods

Patients and specimens

A total of 107 CCA patients at the PLA Rocket Force Characteristic Medical Center were enrolled in this study. All the recruited patients were pathologically diagnosed by two physicians. None of them had received chemo- or radio- therapy 3 weeks before investigation. The clinical information of patients, such as age, gender, differentiation and TNM stage, was listed in **Table 1**. In addition, 75 healthy donors in the same hospital were recruited as healthy controls. Serum samples were isolated from peripheral blood of both CCA patients and healthy controls via centrifugation. This study was approved by the Ethics Committee of the PLA Rocket Force Characteristic Medical Center, and all patients had provided the informed contents in advance.

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

Total RNA was isolated from serum samples using Trizol RNA extraction kit (Invitrogen) according to the manufacture's instructions. Then total RNA was used to synthesize the first strand of cDNA using the iScript cDNA synthesis Kit (Bio-Rad, USA). The relative mRNA level of *hTERT* was evaluated by qRT-PCR. The reaction was conducted by SYBR Premix Ex TaqTM (TaKaRa, Japan) in Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA) under optimal conditions. *GAPDH* was used as an internal standard. The primer sequences were as follows: *hTERT* forward: 5'-CGGAAGAGTGTCTGGAGCAA-3', reverse: 5'-GGATGAAGCGGAGTCTGGA-3'; *GAPDH* forward: 5'-GTCAACGGATTTGGTCTGTATT-3', reverse: 5'-AGTCTTCTGGGTGGCAGTGAT-3'. The relative expression value of *hTERT* mRNA was calculated by $2^{-\Delta\Delta C_t}$ method.

Statistical analysis

All data were analyzed in SPSS18.0 software (SPSS, Inc., Chicago, IL, USA). Student's t-test was used to compare the expression difference of serum *hTERT* between groups. The association of *hTERT* expression and clinical parameters was validated by χ^2 test. Receiver operating characteristics (ROC) curve was plotted to assess the diagnostic value of serum *hTERT* in CCA. The results were considered to be significant when *P* value was less than 0.05.

Results

Up-regulation of serum *hTERT* mRNA in CCA patients

Serum *hTERT* mRNA levels were determined in 107 CCA patients and 75 healthy controls using qRT-PCR. The expression of serum *hTERT* mRNA in CCA patients was significantly higher than that in healthy controls was ($P<0.001$) (**Figure 1**).

Association between *hTERT* expression and clinical parameters

The patients were divided into two groups according to the median level of serum *hTERT* mRNA: high expression group and low expression group. Chi-square test demonstrated that the expression profile of *hTERT* showed close link with differentiation ($P=0.005$), distant metastasis ($P=0.015$) and TNM stage ($P=0.009$) (**Table 1**). However, no obvious correlation was detected between serum *hTERT* expression and age, CA19-9 level, or gender ($P>0.05$ for all).

Diagnostic performance of serum *hTERT* in CCA

The diagnostic accuracy of serum *hTERT* in CCA was determined by ROC analysis. As shown in **Figure 2**, the AUC value of the curve was 0.901, suggesting that serum *hTERT* could discriminate between CCA patients and healthy individuals. The diagnostic sensitivity and specificity of serum *hTERT* in CCA were 89.7% and 76.0%, respectively, with the optimal cutoff value of 1.88.

Table 1. Relationship of *hTERT* expression and clinical characteristics

Clinical characteristics	Case NO.	Expression		χ^2	P value
		High (n=53)	Low (n=54)		
Age (years)				3.549	0.060
<60	63	36	27		
≥ 60	44	17	27		
Gender				2.697	0.101
Male	48	28	20		
Female	59	25	34		
CA19-9 (U/mL)				1.149	0.284
<69	50	22	28		
≥ 69	57	31	26		
Differentiation				7.941	0.005
Well, moderate	61	23	38		
Poor	46	30	16		
Distant metastasis				5.923	0.015
Present	58	35	23		
Absent	49	18	31		
TNM stage				6.805	0.009
I,II	56	21	35		
III,IV	51	32	19		

Discussion

CCA is a fatal disease of bile duct epithelial cells, with dismal prognosis [20]. Due to the delay in early diagnosis, the major of CCA patients are diagnosed with metastatic disease, leading to limited therapeutic effects and poor outcomes [21]. Thus, identification of novel biomarkers for early diagnosis of CCA may be a promising way to improve the prognosis of CCA patients. Like other kinds of tumors, the pathogenesis of CCA is a complex process, with the involvement of various molecules. In the previous studies, number of molecular biomarkers were identified for CCA. In the study of Khoontawad et al., *EXT1* was proved to be overexpressed in CCA and showed close correlation with CCA genesis, suggesting its potential as a biomarker for the disease [22]. Tang et al. revealed that up-regulation of *EXH2* predicted aggressive clinical characteristics and poor prognosis for patients with CCA, suggesting its prognostic significance for the disease [23]. Boonjaraspinyo et al. reported that the expression of *PDGGA* was

positively correlated with aggressive progression of CCA that might be a candidate biomarker for diagnosis and treatment strategies of the disease [24]. To explore the molecular biomarkers for CCA early detection and prognosis evaluation may be an effective approach to improve the outcomes of the patients. In the current study, we investigated the prognostic significance of serum *hTERT* for CCA.

The molecular abnormalities in tumorigenesis are mainly involved in independent growth, unlimited replication, aging avoidance, block of growth inhibitory signal and escape of apoptosis. Telomeres play an important role in maintaining chromosome stability and cell activity in different animal cells. In normal cells, telomeres are usually presented with procedural shortening, resulting in the apoptosis and senescence of cells. However, in the immortalized and tumor cells, the telomere lengths are maintained by the activated telomerase, resulting the infinite proliferation and canceration [25]. *hTERT*, a subunit of telomerase, plays a crucial role in telomerase activity mainly [26, 27]. Overexpression of *hTERT* has been found in a large number of cancers, as well as cholangiocarcinoma [19, 28]. Furthermore, its expression patterns showed close association with development and progression of cancer. Based on this, we deduced that *hTERT* might hold the potential to serve as a biomarker for CCA.

In the present study, we investigated the expression of serum *hTERT* mRNA in CCA patients and healthy controls using qRT-PCR. The results showed that serum *hTERT* was increased in CCA patients compared with healthy controls. Furthermore, the elevated expression of *hTERT* was positively associated with poor tumor differentiation, positive distant metastasis and advanced TNM stages. All the data revealed that *hTERT* played a carcinogenic role in development and progression of CCA. The conclusion was consistent with the previous study [19]. It was reported that *hTERT* might regulate tumor metastasis via miR-29a-ITGB1 pathway [29]. A related research also reported that *hTERT* promoted cancer invasion and metastasis through cooperating with c-Myc to upregulate the expression of heparanase [12]. However, the specific oncogenic mechanisms for *hTERT* in CCA remained unidentified. Further researches were still needed.

In addition, ROC analysis was established to assess the diagnostic performance of serum *hTERT* in CCA. The result demonstrated that serum *hTERT* could discriminate between CCA patients and healthy individuals with high diagnostic sensitivity and specificity. Additional to CCA, the predictive function of *hTERT* was also determined in other malignancies. In gastric cancer, the expression profile of *hTERT* showed close link with clinical factors and disease-free survival of the patients that might be a potential prognostic biomarker for the cancer [30]. The plasma *hTERT* mRNA level was significantly different between healthy individuals and prostate cancer cases, and ROC analysis demonstrated that *hTERT* was an useful non-invasive diagnostic biomarker for the disease [31]. Taken together, *hTERT* might be a potential molecular biomarker for human cancer that might be widely used for early detection, targeted treatment, and prognosis evaluation in tumor.

Conclusion

In conclusion, serum *hTERT* expression is significantly increased in CCA patients, and positively related with aggressive clinical characteristics. Serum *hTERT* may be a potential biomarker for CCA diagnosis.

Abbreviations

human telomerase reverse transcriptase (*hTERT*)

cholangiocarcinoma (CCA)

quantitative real-time PCR (qRT-PCR)

receiver operating characteristics (ROC)

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.

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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.

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Figures

Figure 1

Relative expression value of serum hTERT mRNA in CCA patients and healthy individuals. The level of serum hTERT mRNA in CCA showed significantly higher than that in healthy controls (***: indicated $P < 0.001$).

Figure 2

ROC analysis for diagnostic value of serum hTERT in CCA. From the curve, we found that serum hTERT could act as a diagnostic marker in CCA with the AUC value of 0.901, combining with the sensitivity of 89.7% and the specificity of 76.0%. The cut-off value of serum hTERT for CCA diagnosis was 1.875.