Connection between Gab1 Polymorphisms and cholangiocarcinoma patients prognosis

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Abstract

Background We conducted this study to measure the levels of GRB2 associated binding protein 1 (Gab1) mRNA in cholangiocarcinoma (CCA) tissues and the paired normal tissues. And then assess the prognostic significance of Gab1 in CCA patients.

Methods Quantitative real-time (qRT-PCR) was adopted to confirm the expression levels of Gab1 mRNA in both CCA tissues and normal controls. The Chi-square test was applied to estimate the influence of clinical parameters on Gab1 expression. Kaplan-Meier survival analysis with log-rank test were used to determine overall survival rates of patients with different Gab1 expression. Cox regression analysis was carried out to evaluate prognostic value of factors in CCA patients.

Results According to the qRT-PCR result, increased expression of Gab1 mRNA was found in CCA tissues compared with the paired noncancerous controls ($P<0.0001$). The Chi-square test demonstrated that the elevated expression of Gab1 was affected by poor differentiation ($P=0.022$), positive lymph node metastasis ($P=0.008$) and high TNM stage ($P=0.004$). However, age, gender, family history and resection margin had no significant influence on Gab1 levels (all, $P>0.05$). The survival curves suggested that up-regulation of Gab1 was related with poor prognosis of CCA patients. Furthermore, the Cox analysis demonstrated Gab1 was a prognostic biomarker for CCA patients ($P=0.000$, 95%CI=2.576, HR=1.552-4.275).

Conclusion Taken together, Gab1 expression was increased in CCA and may be a useful biomarker to predict the outcomes of CCA patients.

Background

Cholangiocarcinoma (CCA) is a common malignancy all over the world that occurs in biliary tract and derives from the epithelium of bile tract, accounting for about 3% of all gastrointestinal malignancies [1, 2]. It is found that the occurrence of CCA is occult, and the clinical symptoms of CCA often present late. As a result, it is hard to diagnose CCA at early stages, and CCA is a lethal disease with high mortality and morbidity. Moreover, in recent years, the incidence rate and death rate of CCA are increasing year by year [3, 4]. Currently, the only potentially curative management for CCA is surgical resection [5, 6]. However, due to the special anatomical location and the invasion to surrounding tissues, the surgery rate of CCA patients is very low, and prognoses of CCA patients are significantly disappointing [7, 8]. It has been reported that the 5-year survival rate of CCA patients is only 25-30% [9]. Therefore, it is important to find novel prognostic biomarkers for CCA patients to improve their outcomes.

Gab (Grb2-associated binder) proteins are a class of adaptor/docking proteins, which are named after their coupling to the growth factor receptor bound protein 2 (Grb2). The Gab protein family is composed of 5 homologous protein, namely mammalian Gab1, Gab2 and Gab3, Drosophila homolog DOS (daughter of sevenless), and Caenorhabditis elegan homolog SOC-1 (suppressor of clear) [10, 11]. Gab1 protein is encoded by the *Gab1* gene, which is located on human chromosome 4q31.1 [12]. Evidence has revealed

that Gab1 contains a Met-bind domain, an NH2-terminal pleckstrin homology (PH) domain, 16 potential tyrosine phosphorylation sites and 3 proline-rich sequences [13, 14]. *Gab1* has been proved to be implicated in a variety of biological activities, such as signal transduction of various factors, development of different organs, and cell proliferation and differentiation [15-17]. Abnormal behavior of *Gab1* has been found in various cancers, including pancreatic cancer, lung cancer and hepatocellular carcinoma as well as CCA [18-20].

In the present study, we attempted to identify the expression of *Gab1* mRNA in CCA tissues and then investigate the correlations between *Gab1* expression and CCA prognosis.

Materials And Methods

Patients and specimen

A total of 119 CCA patients who were admitted to the PLA Rocket Force Characteristic Medical Center, were recruited in our study, including 64 males and 55 females. They were all subjected to surgical resection with no preoperative chemo- or radio- therapy. All the patients were graded according to the TNM staging system and other clinical parameters of patients, including age, family history, resection margin, lymph node metastasis and differentiation, were listed in **Table 1**. All the 119 pairs of CCA tissues and adjacent noncancerous tissues were immediately put in liquid nitrogen after surgery and then stored at -80℃ for use. A 5-year follow-up was performed on the patients. The information of patients were updated through telephone calls every month during the follow-up. This investigation was performed with the approval from the Ethics Committee of the PLA Rocket Force Characteristic Medical Center and the written informed consents were provided by the patients in advance.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from 119 pairs of CCA tissues and noncancerous controls using the Trizol reagent (Introgen) following the manufacture's protocols. The extracted RNA was then used to synthesize the first strand of cDNA with the reverse transcription kit (Takara, Ostu, Japan). Finally the real-time PCR was carried out using the SYBR Premix Ex Tap Kit (Takara, Ostu, Japan) on a CFX Connect Real-Time PCR system (Bio-Rad, CA, USA) with optimal experimental conditions. Besides, *GAPDH* was used as an internal reference. The relative expression level of *Gab1* was calculated by the $2^{-\Delta\Delta Ct}$ method, and each sample was treated in triplicate.

Statistical analysis

All statistical analyses in the study were analyzed using SPSS 18.0 and Sigmaplot 12.5 softwares (Systat Software Inc.) and the data was presented as mean±SD. The student's t-test was used to compare the

difference of *Gab1* levels between CCA group and the control group. Kaplan-Meier curves were used to determine the survival curves and the differences of overall survival rate were identified using log-rank test. Cox regression analysis was performed to evaluate the prognostic significance of potential biomarkers. *P* values were regarded to be statistical significant when less than 0.05.

Results

Overexpression of Gab1 mRNA in CCA tissues To measure the expression levels of Gab1 mRNA in CCA tissues and corresponding noncancerous tissues, the qRT-PCR assay was performed. As shown in Figure 1, Gab1 mRNA expression in CCA tissues was significantly higher than that in adjacent normal controls (P<0.001). Relationship of Gab1 expression with clinical characteristics of CCA patients To further explore the association between Gab1 expression and clinical features, the patients were divided into high Gab1 group (n=60) and low Gab1 group (n=59) based on the mean level of Gab1 expression (3.43). As listed in Table 1, high Gab1 expression was associated with poor differentiation (P=0.022), advanced TNM stage (P=0.004) and positive lymph node metastasis (P=0.008). However, no significant correlation was identified between Gab1 expression and other clinical characteristics, including gender, age, family history and resection margin (all, P>0.05). Correlation between Gab1 expression with prognosis of CCA patients To determine the relationship between Gab1 expression and outcomes of patients, a 5-year follow-up was adopted. During the follow-up, 71 out of 119 patients (59.7%) died, including 44 cases with high Gab1 expression and 27 cases with low expression. From the Kaplan-Meier curves in Figure 2, the overall survival rate of patients with high Gab1 expression level (26.7%) was significantly lower than that of patients with low Gab1 level (54.2%) (P<0.001). Furthermore, the univariate analysis showed differentiation (P=0.029), lymph node metastasis (P=0.040), TNM stage (P=0.002) and Gab1 expression (P=0.000) were related to CCA prognosis. Besides, the multivariate analysis revealed that lymph node metastasis (P=0.022, HR=1.831, 95%CI=1.090-3.077), TNM stage (P=0.001, HR=2.449, 95%CI=1.468-4.086) and Gab1 level (P=0.000, HR=2.576, 95%CI=1.552-4.275) were candidate markers for predicting the outcomes of CCA patients (Table 2).

Discussion

CCA is a prevalent choledochal malignancy, and the majority of CCA patients are accompanied with adenocarcinoma. It has been reported that CCA is characterized by insidious onset. The radical surgical resection rate of CCA is low because the early diagnosis is difficult and most clinical patients have middle or advanced stage by the time they seek medical intervention [21]. In recent years, the mortality and morbidity are presented with the tendency of increase. Therefore, it is important to investigate the pathogenesis of CCA and seek new effective biomarkers and treatment methods to improve the outcomes of CCA patients. So far, to our knowledge, a large number of biomarkers have been demonstrated to play important roles in the development and progression of CCA. Xu et al. reported that Sig1R overexpression was significantly correlated with progression and prognosis of CCA [22]. In the study of Nakagawa et al., EZH2 was contributed to the progression of CCA cells through modulating cell

cycle and apoptosis [23].

In the current study, we explored the role of Gab1 in the prognosis of CCA patients. Gab1 protein belongs to the Gab protein family and has been found to promote cell growth and differentiation, regulate metabolism and development of organisms, and immunization. Aberrant expression of Gab1 has been observed in various cancers and proved to play crucial roles in the occurrence and development of malignancies. For example, Liu et al. demonstrated that Gab1 expression may represent a promising biomarker for the prognostication of human glioma [24]. Bai et al. revealed that in colorectal cancer cell, Gab1 was modulated by miR-409-3p at both the mRNA and protein levels, and Gab1 was partially responsible for the disease metastasis [25]. These findings suggested that Gab1 was a vital factor in the progression of tumors.

In our study, we firstly investigated the expression levels of Gab1 mRNA in CCA tissues and paired normal controls using qRT-PCR. The findings showed that the expression of Gab1 at mRNA level in CCA tissues was significantly elevated compared with adjacent noncancerous controls, confirming the results in previous studies. In addition, higher Gab1 levels in CCA tissues had close relationships with poor differentiation, positive lymph node metastasis and late TNM stages. All the above results indicated Gab1 might be involved in the development and progression of CCA. Kaplan-Meier curves showed that CCA patients with high Gab1 expression represented poor prognosis and resulted in a significantly low overall survival rate. Both univariate and multivariate analyses from Cox regression demonstrated that Gab1 was a potential biomarker in predicting the outcomes of CCA patients.

Our study is the first investigation to explore the prognostic significance of Gab1 for CCA patients. Though the predictive role of Gab1 in CCA has been identified, its precise mechanism on CCA tumorigenesis, proliferation and migration, which are complex processes with multiple genes and their products, has not been clearly known yet. Studies have proved that Gab proteins play key roles in the pathogenesis and progression of tumors through regulating aberrant tyrosine phosphorylation [26, 27]. Furthermore, Sang et al. showed that down-regulation of Gab1 controlled the proliferation and migration of hilar CCA cells [28]. Besides, Sang et al. also claimed that Gab1 functioned on the proliferation and migration of intrahepatic CCA using the PI3K/Akt pathway [29]. What's more, Gab1 has also been demonstrated to be related with the PTPN11/MAPK pathway [30]. All the above findings and consequences could provide theoretical foundations for our further studies.

Conclusion

In summary, our study confirmed the significantly high expression of Gab1 in CCA tissues and the Gab1 overexpression was influenced by differentiation, lymph node metastasis and TNM stage. Furthermore, the results suggested Gab1 was an independent and efficient biomarker for CCA prognosis.

Abbreviations

GRB2 associated binding protein 1 (*Gab1*)

cholangiocarcinoma (CCA)

Quantitative real-time (qRT-PCR)

growth factor receptor bound protein 2 (Grb2)

pleckstrin homology (PH)

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 Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.**Competing interests**The authors declare that they have no competing interests.**Funding** Not applicable.**Authors' contributions**

N.W. and Y.L. conceived and designed the experiments; Y.Z. conceived and performed the experiments; H.C. and X.W. prepared figures. Z.L. wrote the main manuscript text. All authors reviewed the manuscript.

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Tables

Table 1. Relationship between *Gab1* expression and clinical characteristics of CCA patients

Clinical features	NO. (n=119)	Expression		c^2	*P* value
		High	Low		
Age (years)				3.035	0.081
≦50	53	22	31		
>50	66	38	28		
Gender				3.027	0.082
Female	55	23	32		
Male	64	37	27		
Family history				1.057	0.304
Yes	52	29	23		
No	67	31	36		
Resection margin				2.431	0.119
Positive	59	34	25		
Negative	60	26	34		
Differentiation				5.279	0.022
Well, moderate	62	25	37		
Poor	57	35	22		
Lymph node metastasis				7.064	0.008
Positive	63	39	24		
Negative	56	21	35		
TNM stage				8.094	0.004
I,II	61	23	38		
III,IV	58	37	21		

Table 2. Univariate and multivariate analyses of *Gab1* and clinical factors in CCA patients

Clinical features	Univariate		Multivariate	
	P value	HR(95%CI)	*P* value	HR(95%CI)
Differentiation	0.029	1.685(1.053-2.695)	-	-
Metastasis	0.040	1.650(1.024-2.657)	0.022	1.831(1.090-3.077)
TNM stage	0.002	2.144(1.330-3.457)	0.001	2.449(1.468-4.086)
Gab1 expression	0.000	3.117(1.917-5.067)	0.000	2.576(1.552-4.275)

Figures

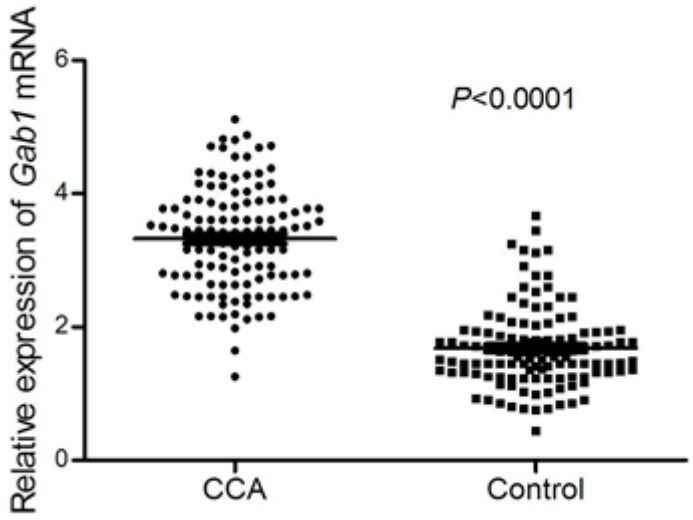

Figure 1

The expression of Gab1 mRNA in CCA tissues and paired noncancerous tissues using qRT-PCR method. It was apparently that Gab1 mRNA expression was significantly up-regulated in CCA tissues compared with normal controls (P<0.0001).

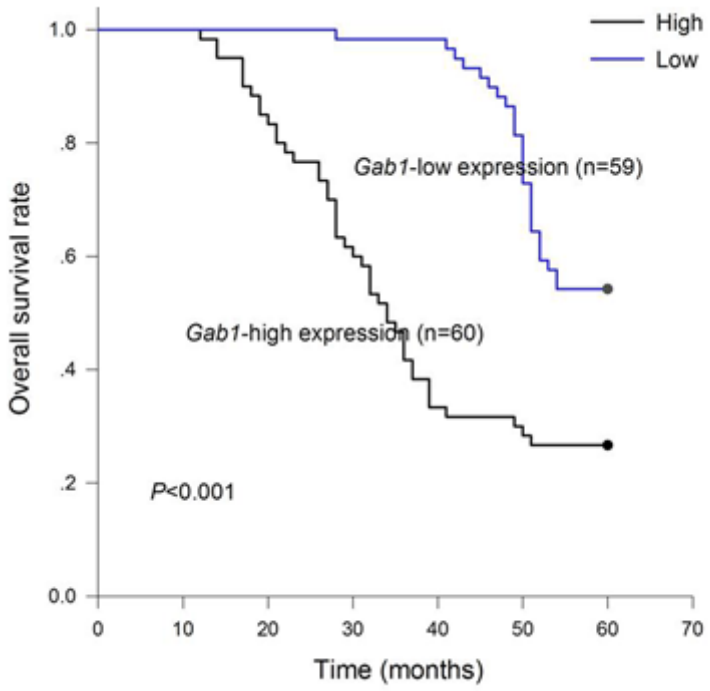

Figure 2

Kaplan-Meier survival curves were plotted according to the expression of Gab1 in CCA tissues. Patients with high Gab1 expression lived shorter than those with low Gab1 expression (P<0.001). P value was calculated by log-rank test.