EEF1A Acts as A Promising Novel Bio-Marker in the Prognosis of Patients With Cholangiocarcinoma

Ning Wang
the PLA Rocket Force Characteristic Medical Center
Yanni Li
the PLA Rocket Force Characteristic Medical Center
Yanfang Zheng
the PLA Rocket Force Characteristic Medical Center
Huoming Chen
the PLA Rocket Force Characteristic Medical Center
Xiaolong Wen
University of Science and Technology Beijing
Zhaoxia Li (zmcfghk@126.com)
the PLA Rocket Force Characteristic Medical Center https://orcid.org/0000-0002-9421-0513

Research article

Keywords: EEF1A2, Prognosis, Cholangiocarcinoma

Posted Date: August 26th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-53241/v1

Abstract

Background: The morbidity and mortality of cholangiocarcinoma (CCA) is increasing in recent years. *EEF1A2* could regulate multiple biological and pathological processes. Our study was designed to investigate the clinical significance of *eEF1A2* for prognosis evaluation in CCA.

Methods: The quantitative analysis for *eEF1A2* in CCA specimens was performed using quantitative real-time polymerase chain reaction (qRT-PCR). The influences of *eEF1A2* expression on disease progression and survival of CCA patients were analyzed by Chi-square test and Kaplan-Meier method, respectively. Additionally, cox regression analyses were adopted to identify the potential predictive biomarker for prognosis of CCA.

Results: *eEF1A2* expression showed increased tendency in CCA tissues (P<0.05). The expression profile of *eEF1A2* was positively correlated with TNM stage (P=0.004), lymph node metastasis (P=0.001) and distant metastasis (P=0.001). In addition, high *eEF1A2* expression predicted dismal survival among CCA cases (P<0.05). *EEF1A2* might be independently correlated with prognosis of CCA (HR=2.724, 95%CI=1.303-5.964, P=0.008).

Conclusions: *eEF1A2* may act as a predictive biomarker for clinical outcomes of CCA.

Background

Cholangiocarcinoma (CCA) is a common malignant tumor originated from the biliary epithelial cells [1, 2]. According to the anatomical sites, CCA was divided into intrahepatic cholangiocarcinoma, hilar cholangiocarcinoma and distal cholangiocarcinoma by the seventh AJCC/UICC [3]. The most pathological types of CCA are differently differentiated adenocarcinoma [4, 5]. CCA is characterized by concealed pathogenesis, rapid lymphatic invasion and distant metastasis, and most cases developed to advanced stages when initial diagnosis, leading to low resection rate and dismal clinical outcomes. In recent years, the incidence of CCA exhibits increasing tendency [6, 7]. Even worse, CCA patients show insensitive to radiotherapy and chemotherapy [8, 9], resulting in poor prognosis. Therefore, the predictive biomarkers are in urgent need to guide the clinical treatments and predictive the clinical outcomes of CCA.

Eukaryotic elongation factor 1 alpha (*eEF1A*) is responsible for protein synthesis. It is a kind of guanine nucleotide-binding regulatory (G protein), and it can regulate the combine of ribosomal A site and amino-acylated tRNAs in order to promote the elongation of ribosomal polypeptide [10, 11]. It is reported that *eEF1A* may take part in multiple cellular processes such as protein translation, protein degradation, apoptosis and cell proliferation [12, 13]. *eEF1A*, contains two isoforms, named *eEF1A1* and *eEF1A2*. *EEF1A2* gene is located in the chromosome 20q13.3 with 12 kb in length, and eEF1A2 is the protein product of *eEF1A2* gene [14]. *eEF1A2* may function as a regulator *for* protein translation [15]. Dysregulation of *eEF1A2* has been reported in several human cancers, and it may play oncogenic roles in carcinogenesis [16]. The clinical investigations confirmed that the increased expression of *eEF1A2*

showed close association with perineural invasion, lymph node metastasis and poor prognosis [17]. However, the predictive value of *eEF1A2* for clinical outcomes of CCA still remained unclear.

In our study, the expression pattern and its influences on clinical parameters of *eEF1A2* were investigated in CCA.Moreover, the survival curves and cox analyses were adopted to measure whether *eEF1A2* could act as a predictive biomarker for CCA patients.

Methods

Study subjects and sample collection

In this study, 121 eligible CCA cases were recruited from the PLA Rocket Force Characteristic Medical Center. Diagnosis of CCA was made based on pathological examinations, and none of the cases had history of anti-tumor treatments, including chemotherapy, radiotherapy, or surgery. The surgical CCA tissues and corresponding non-cancerous tissues were frozen in liquid nitrogen ,and then maintained at -80℃. The CCA patients were followed up every 3 months in the first year and then every 6 months in the subsequent two years, and then annually. The follow-up investigation lasted for five years. The clinicopathological features of CCA patients were listed in Table 1, including gender, age, tumor location, histological grade, TNM stage, lymph node metastasis and distance metastasis.

Table 1
The influences of *eEF1A2* expression on disease progression of CCA patients

Clinical Features	Cases (n = 121)	*eEF1A2* expression		χ^2	*P*
		Low (n = 54)	High (n = 67)		
Age (years)				2.510	0.113
≥ 65	59	22	37		
< 65	62	32	30		
Gender				0.661	0.416
Male	60	29	31		
Female	61	25	36		
Tumor location				0.086	0.958
upper	36	16	20		
midpiece	41	19	22		
hypomere	44	19	25		
Histological grade				1.695	0.428
low	42	22	20		
moderate	39	15	24		
high	40	17	23		
TNM stage				8.260	0.004
I + II	51	15	36		
III + IV	70	39	31		
Lymph node metastasis				11.567	0.001
Negative	52	14	38		
Positive	69	40	29		
Distant metastasis				11.966	0.001
Negative	50	13	37		
Positive	71	41	30		

Table 2
Cox regression analyses performed to indentify the predictive biomarker for prognosis of CCA

Characteristics	HR	95%CI	*P* values
eEF1A2	2.724	1.303–5.694	0.008*
age	1.820	0.981–3.379	0.058
gender	0.979	0.518–1.853	0.948
Tumor location	1.700	0.773–3.737	0.187
Histological grade	0.550	0.228–1.330	0.144
TNM stage	0.909	0.495–1.669	0.758
Lymph node metastasis	1.659	0.894–3.080	0.109
Distant metastasis	1.424	0.777–2.611	0.252

The study permission was obtained from the Ethics Committee of the PLA Rocket Force Characteristic Medical Center. All the participants provided the written informed consents.

Quantitative analysis for *eEF1A2*

RNA extraction was performed by Qiagen RNeasy kits (Qiagen, Crawley, UK), and the experimental procedures were carried out according to the instructions of manufacturer. 1 μg RNA sample with high quality was used for cDNA synthesis, and the Superscript III Reverse Transcriptase (Invitrogen) was adopted. The quantitative analysis for *eEF1A2* was achieved by quantitative real-time polymerase chain reaction (qRT-PCR), and the reaction was carried out by SYBR Green PCR master mix (Applied Biosystems) on the Rotor-Gene 6000 real-time genetic analyzer (Corbett Life Science, USA). *GAPDH* was employed as an internal reference. The specific amplified primers were as follows: *eEF1A2* forward: 5'-CCATGTGTGTGGAGAGCTTCTC-3', reverse: 5'-TCTCCACGTTCTTGATGACGCC-3'; *GAPDH* forward: 5'-GTCTCCTCTGACTTCAACAGCG-3', reverse: 5'-ACCACCCTGTTGCTGTAGCCAA-3' [18]. The relative expression of *eEF1A2* was normalized against *GAPDH*, and analyzed by the method of $2^{-\Delta\Delta Ct}$.

Statistical analysis

The expression level of *eEF1A2* was expressed as mean ± standard deviation (SD), and its comparison between CCA and non-cancerous specimens was achieved by student's t test. The possible relationship between *eEF1A2* expression and the cliincal parameters of CCA cases was confirmed via Chi-square test. The survival curves were plotted using Kaplan-Meier method with log-rank test for CCA cases according to their expression patters of *eEF1A2*. The prognosis analysis was performed by Cox regression analysis to identify the potential predictive biomarker for prognosis evaluation of CCA. $P < 0.05$ indicated the statistical significance of the analysis results.

Results

Upregulation of *eEF1A2* in CCA tissues

The quantitative analysis for *eEF1A2* was performed in 121 pairs of CCA tissues and corresponding normal tissues. As displayed in **Figure 1**, *eEF1A2* expression exhibited significantly increased tendency in CCA tissues compared to the non-cancerous specimens (P<0.05).

Influences of *eEF1A2* expression on disease progression of CCA patients

The included CCA cases were divided into high expression (n=67) and low expression (n=54) groups based on their mean *eEF1A2* expression in CCA tissues. Chi-square test showed that the CCA patients with high expression of *eEF1A2* were more likely to undergo advanced TNM stage (P=0.004), positive lymph node metastasis (P=0.001) and distant metastasis (P=0.001). Meanwhile, the expression level of *eEF1A2* had no obvious association with age, gender, tumor location or histological grade (all P>0.05) (**Table 1**).

Overall survival curves and prognosis analysis

The survival curves plotted for CCA patients according to their *eEF1A2* expression showed that CCA patients with high *eEF1A2* expression had worse overall survival rates than those with lower *eEF1A2* expression (log-rank test P=0.003) (**Figure 2**).

The prognostic analysis was achieved by Cox regression model. Analysis results confirmed that eEF1A2 might be independently correlated with clinical outcomes of (P=0.008). It was shown that increased expression of eEF1A2 had adversely influence to CCA with HR of 2.724 and 95%CI of 1.303-5.694, and it also suggested an increasing risk of dying to CCA patients with up-regulation of eEF1A2.

Discussion

CCA is originated from the biliary epithelium, making up 2–3% of all gastrointestinal tumors [19]. Epidemiological data show that the prevalence of CCA is on rise year by year around the world [20, 21]. Due to the lack of obvious symptoms in early stages, many CCA cases present local infiltration and lymphatic or distal metastasis when initial diagnosis, resulting in dismal outcomes. In China, the 5-year survival rate is only 5% among the patients with advanced CCA [2, 22]. The predictive biomarkers were key for early screening and treatment guidance in CCA. In current study, we explored whether *eEF1A1* could be employed as a biomarker for prognosis evaluation of CCA.

EEF1A is one of the four subunits of eukaryotic elongation factor 1 (*EEF1*), belonging to G protein family [23, 24]. *EEF1A* has two isomerides, *eEF1A1* and *eEF1A2.* Under normal physiological conditions, *eEF1A1* widely expresses in lung and liver, but its expression was rarely detected in cardiac muscle, skeletal muscle and brain. Moreover, *eEF1A2* expression was rarely observed in neither normal lung nor normal liver tissues [25, 26]. Dysregulation of *eEF1A2* may lead to diseases, like tumors. The altered expression of *eEF1A2* was reported to be involved in several human malignancies, such as breast cancer [12],

pancreatic cancer [27], ovarian cancers [28]. In this study, we explored the expression profile of *eEF1A2* in CCA.

In our study, the expression patterns of *eEF1A2* was detected in CCA specimens. The quantitative analysis showed that *eEF1A2* expression level was higher in CCA tissues than that in the matched noncancerous tissues. Moreover, the expression of *eEF1A2* was significantly related to TNM stag, lymph node metastasis and distant metastasis. And the results of Kaplan-Meier survival analysis showed that CCA patients with high *eEF1A2* expression were more likely to undergo poor survival rate compared to those with low expression. In addition, Cox regression analysis suggested that the expression of *eEF1A2* was an independent prognostic factor for the survival of CCA patients. Taken together, high expression of *eEF1A2* predicted aggressive disease progression and poor prognosis of CCA.

In the previous studies, the predictive values of *eEF1A2* for prognosis had been investigated in several cancers. Duanmin et al. have shown that the increased expression of *eEF1A2* in pancreatic ductal adenocarcinoma was closely associated with nodal metastasis, perineural invasion and worse prognosis [17]. In the study of Yang et al., the over-expression of *eEF1A2* was confirmed to be correlated with worse outcomes in gastric cancer patients [29]. Moreover, Kawamura et al. reported that the positive expression of *eEF1A2* in non-small cell lung cancer indicated poor prognosis [30]. However, upregulated *eEF1A2* expression predicted prolonged survival for the cases with ovarian cancer and breast cancer [31, 32], which were not in accordance with our findings. The disparity in prognostic significance of *eEF1A2* implied its multiple biological functions depending on cancer types with distinct genetic background. Therefore, further investigation is necessary to determine the role of *eEF1A2* in the development of CCA.

Conclusions

In conclusion, the up-regulation of *eEF1A2* in CCA predict aggressive disease progression and poor clinical outcomes. *eEF1A2* may be a potential predictive biomarker for prognosis of CCA.

List Of Abbreviations

cholangiocarcinoma (CCA)

Eukaryotic elongation factor 1 alpha (*eEF1A*)

guanine nucleotide-binding regulatory (G protein)

eukaryotic elongation factor 1 (*EEF1*)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of the PLA Rocket Force Characteristic Medical Center, and the experimental procedures were 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

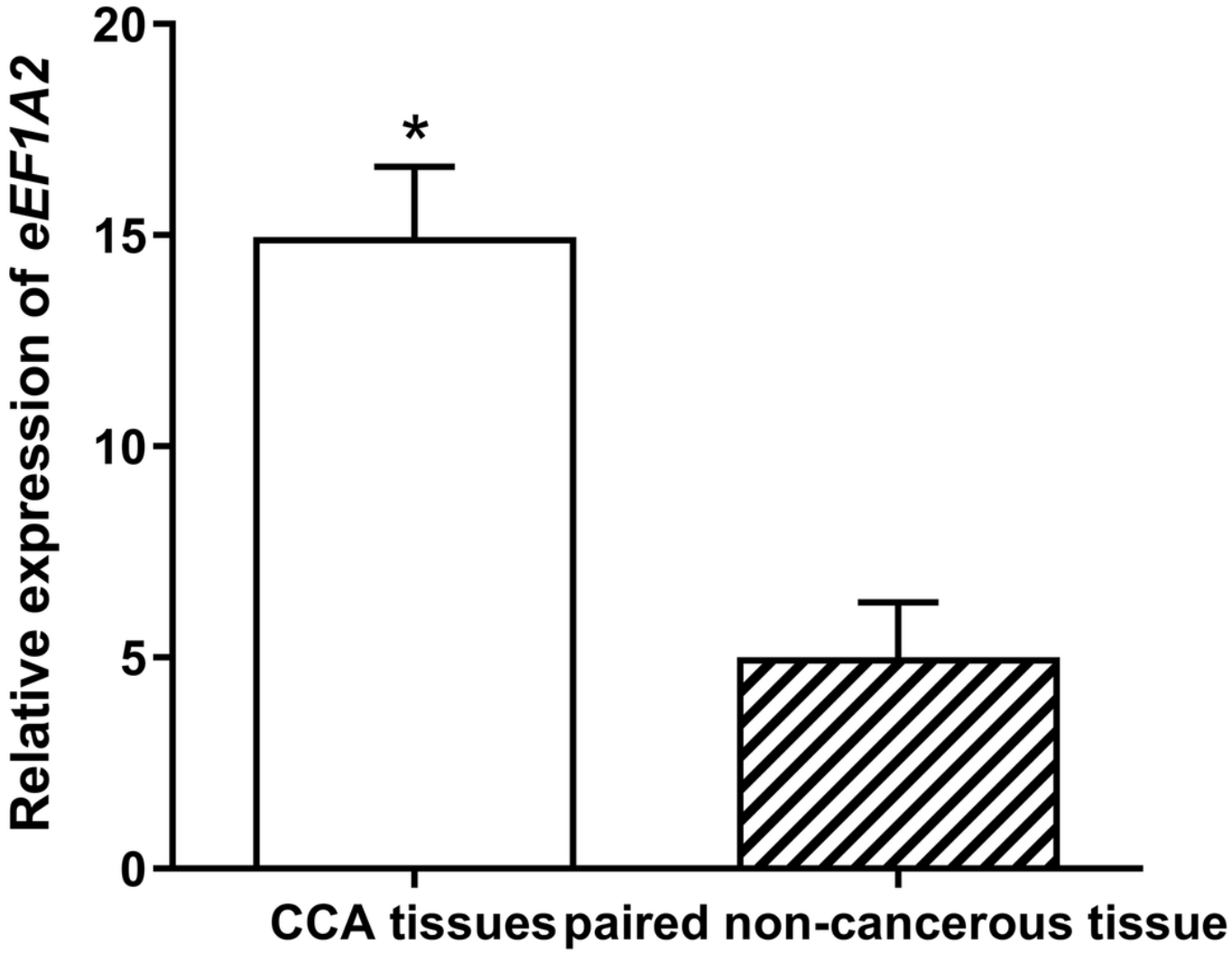

Figure 1

The expression profiles of eEF1A2 expression in CCA tissues and paired adjacent noncancerous tissues (*, P<0.05).

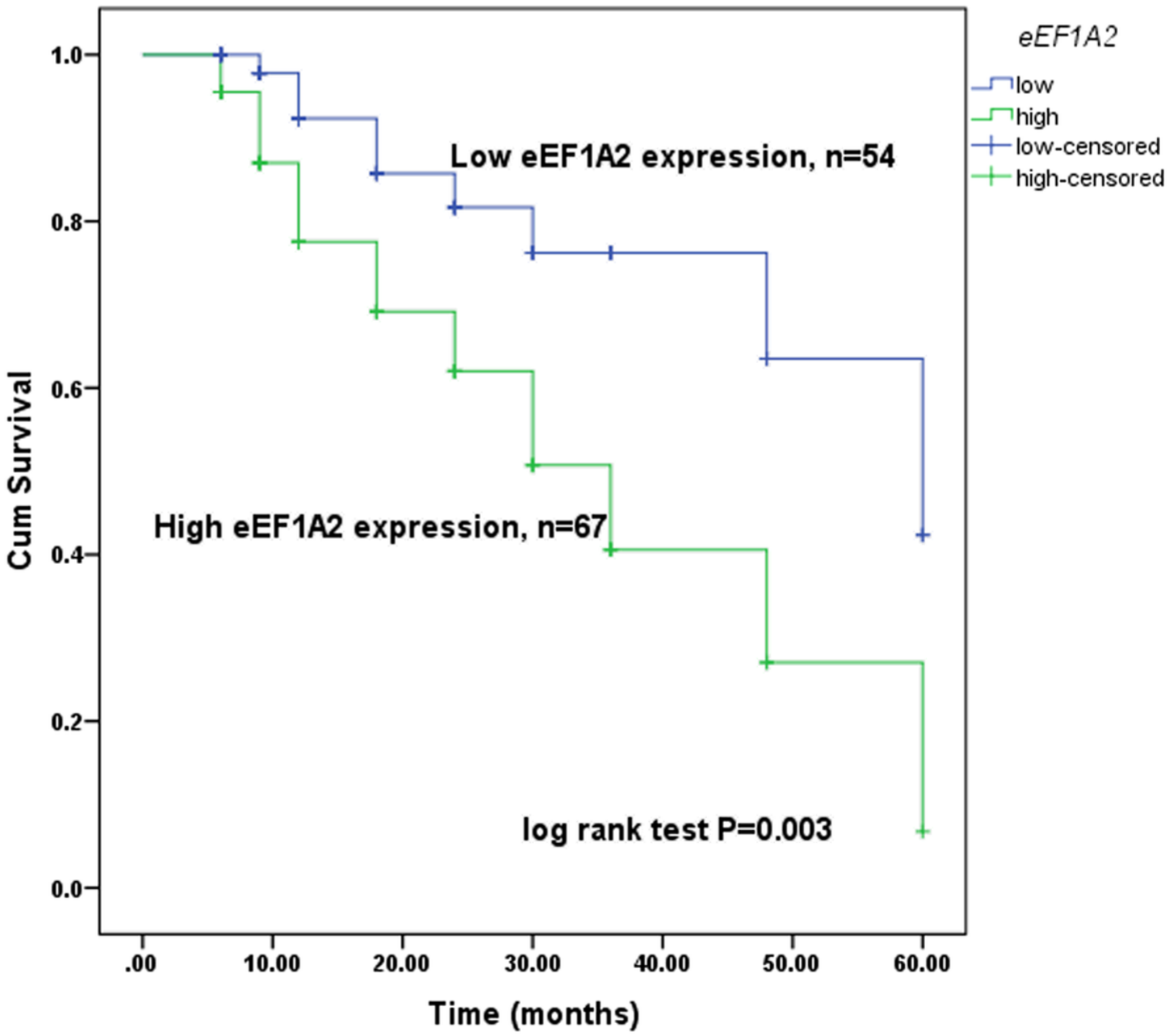

Figure 2

Survival curves were plotted for CCA patients based on their expression of eEF1A2 in CCA tissues.