EYA4 could be an indicator signifying colorectal cancer sufferers' prognoses

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Abstract

Background

Eyes absent 4 (*EYA4*) is involved in various biological processes. The aim of this study was to investigate the expression of *EYA4* and its prognostic value in colorectal cancer (CRC).

Methods

The mRNA level of *EYA4* in diseased tissues and adjacent normal tissues of CRC patients were detected by quantitative real-time polymerase chain reaction (qRT-PCR). The association between *EYA4* expression and clinicopathological characteristics was analyzed by χ^2 test. Kaplan-Meier analysis with log rank test was performed to evaluate the effects of *EYA4* expression on overall survival of CRC patients. Cox regression model was applied for prognosis analysis in CRC.

Results

The mRNA level of *EYA4* was significantly decreased in CRC tissues compared with that in the adjacent normal tissue ($P < 0.01$). And its expression was affected by DUKE stage ($P = 0.034$), differentiation ($P = 0.027$) and vascular invasion ($P = 0.037$). Survival curve showed that patients with low expression of *EYA4* had a significantly shorter overall survival than those with high expression (log rank test, $P = 0.008$). Low expression of *EYA4* (HR = 1.989, 95%CI = 1.090-3.62902, $P = 0.025$) was an independent biomarker for poor prognosis in CRC patients.

Conclusion

EYA4 expression is decreased in CRC patients and negatively correlated with aggressive tumor progression. *EYA4* may be a potential prognostic biomarker for CRC.

Background

Colorectal cancer (CRC) is one of the most common malignancies in the world, being responsible for many cancer-related deaths in adult [1]. The inflammatory bowel disease (IBD), especially chronic ulcerative colitis (UC) and Crohn's colitis, can enhance the risk of CRC [2, 3]. Despite of the great improvements in surgical resections as well as the integration of chemoradiotherapy and targeted therapy, the long term survival of patients with CRC is still very poor [4, 5]. Many CRC cases will develop local recurrence or distant metastasis within 5 years after operation, resulting in therapeutic failures and high mortality [6]. Until now, there is no widely accepted parameter to provide reliable information for tumor progression and prognosis in CRC [7]. For this reason, it is urgently needed to identify efficient cancer-related genetic biomarkers for improving the poor prognosis of CRC.

The Eyes Absent 4 gene (*EYA4*), is a member of *EYA* gene family which includes four members, *EYA1*-*EYA4*, in mammals [8]. The *EYA* family was initially identified in *Drosophila*, and played pivotal roles in eye development [9]. Growing evidences have reported that *EYA4* is implicated in the formation and development of mammalian organs, regulation of cellular behaviors, apoptosis, innate immunity, DNA damage repair, angiogenesis, as well as tumorigenesis [10]. Recently the aberrant hypermethylation of *EYA4* promoter has been observed in a number of cancers, such as melanomas, lung cancer, pancreatic cancer, and hepatocellular carcinoma [11–14]. It was reported that hypermethylation mediated *EYA4* silence might contribute to tumorigenesis, revealing the inhibitory roles of *EYA4* in carcinogenesis [14]. The anti-tumor action of *EYA4* was also studied in CRC. Sung-Jin Kim et al, demonstrated that over-expression of *EYA4* could lead to inhibition of cell proliferation via inducing up-regulation of *DKK1* and inhibiting the Wnt signaling pathway, indicating its capacity as a tumor suppressor gene in CRC [15]. However, the clinical significance of *EYA4* in prognosis of CRC was rarely reported.

In this study, we mainly investigated the expression pattern of *EYA4* in CRC tissues samples, and its association with clinicopathological characteristics of the patients. Additionally, we further estimated the prognostic value of *EYA4* in CRC.

Materials And Methods

Patients and specimens

The study population was consisted of 155 patients pathologically diagnosed with CRC, including 71 females and 84 males. The tumor tissues and adjacent normal tissues specimens were collected from the patients through surgical resection at Chinese PLA General Hospital. None of these patients had received chemotherapy or radiation therapy prior to surgery. The specimens were immediately put into liquid nitrogen after surgery and then stored at -80°C until RNA extraction. The detail clinical information of the patients was listed in Table 1. A 5-years follow-up was conducted for the patients. In our study, we excluded the patients who were died from unexpected events or other diseases.

Table 1
The relationship between *EYA4* expression and clinicopathological features in CRC patients

Characteristics	Cases (n = 155)	<i>EYA4</i> expression		χ^2	<i>P</i>
		Low (n = 56)	High (n = 99)		
Age (years)				1.278	0.258
< 60	73	23	50		
≥ 60	82	33	49		
Gender				0.792	0.374
Male	84	33	51		
Female	71	23	48		
Lymph node metastasis				3.653	0.056
Negative	93	28	65		
Positive	62	28	34		
DUKES clinical stage				4.511	0.034
A + B	92	27	65		
C + D	63	29	34		
Differentiation				4.875	0.027
median + well	90	26	64		
poor	65	30	35		
Tumor location				0.581	0.446
Colon	92	31	61		
Rectum	63	25	38		
Vascular invasion				4.332	0.037
Negative	89	26	63		
Positive	66	30	36		

This study was approved by the Research Ethics Committee of Chinese PLA General Hospital. All the patients gave written informed consent prior to surgery.

Rna Extraction And Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from tissues specimens using Trizol reagent (Invitrogen, Carlsbad, CA) and reversely transcribed into cDNA by the RevertAid First Strand cDNA Synthesis Kits (Fermentas, Lithuania) according to the manufacturer's protocol. The relative expression value of *EYA4* was evaluated by qRT-PCR reaction and cDNA samples served as amplification template. The reaction was carried out using SYBR Premix Ex Taq™ kit (TaKaRa, Japan) in an Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). The relative expression of *EYA4* mRNA was evaluated via the comparative cycle threshold (CT) method. *GAPDH* was used as an endogenous control to normalize the relative expression of *EYA4*. The primer sequences were as follows: *EYA4* forward-5'-AGATGGAGCGCTTGACACTT-3' and reverse-5'-TCCCCGAATACTGAGTCTGG-3'. *GAPDH* forward primer 5'-CGGAGTCAACGGATTTGGTCGTAT-3', reverse primer 5'-AGCCTTCTCCATGGTGGTGAAGAC-3'. Each test was performed in triplicate.

Statistical analysis

Statistical analyses were performed using the software of SPSS 21.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5 (GraphPad, San Diego, CA, USA). Continuous data were presented as mean $\pm$ SD. The different levels of *EYA4* mRNA between CRC tissues and adjacent normal tissues were analyzed with student's t test. χ^2 test was used to assess the relationship between *EYA4* expression and the clinicopathological characteristics. Kaplan–Meier analysis with log rank test was used to evaluate the effects of *EYA4* expression level on overall survival of CRC patients. Cox regression analysis was conducted to evaluate the prognostic value of *EYA4* and clinical parameters in CRC. The difference was considered to be statistically significant when *P* value less than 0.05.

Results

The expression of EYA4 was decreased in CRC tissues

To study the expression pattern of *EYA4* in CRC, qRT-PCR method was applied in this study. As shown in Fig. 1, the mRNA expression level of *EYA4* was remarkably downregulated in CRC tissues compared with adjacent cancer tissues ($P < 0.01$).

Relationship between EYA4 expression and clinicopathological characteristics of patients with CRC

To investigate whether *EYA4* was involved in the development of CRC, the patients were divided into high expression group ($n = 99$) and low expression group ($n = 56$) according to the mean expression of *EYA4*. The expression of *EYA4* was significantly associated with DUKE stage ($P = 0.034$), differentiation ($P = 0.027$) and vascular invasion ($P = 0.037$). However, no correlation was found between *EYA4* expression and age, gender, lymph node metastasis or tumor location ($P > 0.05$ for all) (Table 1).

Association of EYA4 expression with prognosis of CRC patients

To evaluate the prognostic potential of *EYA4* in CRC, we analyzed the association between *EYA4* expression and the overall survival using Kaplan–Meier analysis with the log-rank test. The survival curves showed that patients with low expression of *EYA4* had a shorter overall survival than those with high expression (Fig. 2, log rank test, $P=0.008$). The univariate analysis revealed that lymph node metastasis (HR = 2.037, 95%CI = 1.121–3.702, $P=0.020$), DUKES clinical stage (HR = 1.819, 95%CI = 1.006–3.289, $P=0.048$), and *EYA4* expression (HR = 2.179, 95%CI = 1.202–3.950, $P=0.010$) showed significant association with outcomes of patients with CRC. Multivariate analysis demonstrated that lymph node metastasis (HR = 1.840, 95%CI = 1.005–3.366, $P=0.048$) and *EYA4* expression (HR = 1.989, 95%CI = 1.090-3.62902, $P=0.025$) were independent prognostic biomarkers for CRC patients (Table 2).

Table 2
Cox regression model for prognosis analysis in CRC patients

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95%CI	<i>P</i>	HR	955CI	<i>P</i>
Age	1.510	0.823–2.773	0.183	–	-	-
Gender	1.061	0.578–1.946	0.849	-	-	-
Lymph node metastasis	2.037	1.121–3.702	0.020	1.840	1.005–3.366	0.048
DUKES Clinical stage	1.819	1.006–3.289	0.048	-	-	-
Differentiation	1.293	0.699–2.392	0.412	-	-	-
Tumor location	0.992	0.540–1.823	0.978	-	-	-
Vascular invasion	1.512	0.832–2.746	0.175	-	-	-
<i>EYA4</i> expression (low vs high)	2.179	1.202–3.950	0.010	1.989	1.090–3.629	0.025
Note: HR: hazard radio, 95%CI: 95% confidence interval, -: indicated no related data, $P<0.05$ was considered to be statistically significant.						

Discussion

CRC is a frequently diagnosed malignant tumor worldwide, with high morbidity and mortality [16]. In China, the incidence of CRC shows increasing tread, posing a great threat to human health [17]. Despite of the various available treatments for CRC, the long term outcomes of CRC patients still remain unsatisfactory, which may be attributed to high occurrence of postoperative recurrence and metastasis [18]. Tumor progression prediction represents a challenge for CRC patients in clinic, due to the lack of credible biomarkers [19]. Therefore, identification of effective prognostic biomarkers for CRC may be a promising approach to improve management and prognosis of patients with CRC.

The development and progression of CRC is a complex process, with the involvement of a variety of molecules and signaling pathways, such as oncogene activation, suppressor gene inactivation, and epigenetic modifications [20]. Up to now, the molecular mechanisms underlying CRC is still unclear. In order to improve the management and prognosis of CRC, various researches were devoted to explore the genetic biomarkers for prognosis prediction in CRC. For instance, Zhu et al., found that *Twist1* could enhance proliferation and chemoresistance of CRC cells which might be a potential prognostic marker and a molecular therapeutic target for the cancer [21]. Zhang et al. indicated that up-regulation of nemo-like kinase (*NLK*) was positively correlated with recurrence and distant metastasis of CRC patients, predicting poor prognosis for patients with the disease [22]. The clinical significance of *MTA2* expression for prognosis of CRC was investigated by Ding et al. They found the expression profile of *MTA2* exhibited high in CRC tissues specimens, and significantly correlated with overall survival of the patients, suggesting its possible as a prognostic biomarker for CRC [23]. These cancer-related molecules might provide reliable information for tumor progression of CRC. In the current study, we investigated the prognostic value of *EYA4* for CRC.

EYA4, a member of *EYA* family, has recently been identified as a tumor suppressor gene in many kinds of cancers, including hepatocellular carcinoma, acute myeloid leukemia, oral cancer, etc [14, 24, 25]. In the current study, we investigated the functional roles of *EYA4* in CRC. We detected the expression of *EYA4* at mRNA level in CRC tumor tissues and the adjacent normal tissues. Our results showed that the level of *EYA4* was significantly down-regulated in tissues compared with adjacent normal tissues. Then we further investigated the effects of *EYA4* expression level on the development of CRC. The results indicated that *EYA4* was negatively associated with DUKE stage, differentiation and vascular invasion. All the data revealed the anti-tumor action of *EYA4* in CRC, and its reduced expression could contribute to malignant progression of the disease.

Given its function in tumorigenesis, *EYA4* was identified as a biomarker for several cancers. In pancreatic ductal adenocarcinoma, down-regulation of *EYA4* predicted short overall survival for the patients [26]. The expression pattern of *EYA4* also held referenced significance for prognosis of patients with intrahepatic cholangiocarcinoma [27]. In CRC, Liu et al. reported that the serum methylation levels of *EYA4* were significantly different between stage I CRC patients and healthy individuals that might be an useful biomarker for early detection of CRC [28]. However, the prognostic value of *EYA4* in CRC was still unclear. In this study, Kaplan-Meier analysis showed that patients with low expression of *EYA4* had a shorter overall survival than those with high expression. According to cox regression analysis, *EYA4* might be employed as an independent prognostic factor for CRC. Low expression of *EYA4* predicted poor prognosis for the patients.

Conclusions

In conclusion, *EYA4* is decreased in CRC patients and its expression is significantly correlated with tumor differentiation, metastasis and clinical stage. *EYA4* may be a potential prognostic biomarker for CRC.

Abbreviations

Eyes absent 4 (*EYA4*)

colorectal cancer (CRC)

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

inflammatory bowel disease (IBD)

ulcerative colitis (UC)

cycle threshold (CT)

nemo-like kinase (*NLK*)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Chinese PLA General Hospital 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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Not applicable.

Authors' contributions

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

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Figures

Figure 1

The relative expression level of EYA4 mRNA in CRC tissues and adjacent normal tissue. The expression level of EYA4 in CRC tissues was lower than that in the normal specimens (**: suggested $P < 0.01$).

Figure 2

Kaplan-Meier analysis for overall survival of patients with CRC. Patients with low EYA4 expression had a shorter overall survival than those high expression (log rank test, $P=0.008$).