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Original Article

Decreased expression of *ECRG4* in serum predicts poor prognosis for patients with nasopharyngeal carcinoma

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Abstract: Purpose: The present study tended to explore the expression of esophageal carcinoma related gene 4 (*ECRG4*) in patients with nasopharyngeal carcinoma (NPC). Besides, the correlation between *ECRG4* expression and prognosis of NPC patients was also evaluated. Methods: The relative expression level of *ECRG4* mRNA in serum was detected by Quantitative real-time PCR (qRT-PCR). Chi-square test was performed to analyze the relationship between *ECRG4* expression and clinical characteristics of NPC patients. Kaplan-Meier method was used to analyze the association of *ECRG4* expression and overall survival of NPC patients. Cox regression analysis was conducted to study the role of *ECRG4* in the prognosis of NPC patients. Results: *ECRG4* was weakly expressed in serum of NPC patients compared to the controls ($P < 0.001$). There was significant relationship between *ECRG4* expression and such clinical characteristics as high TNM stage, metastasis and N classification ($P < 0.05$). Survival curves illustrated that the survival rate of patients with low *ECRG4* was significantly lower than those with high *ECRG4* expression ($P = 0.003$). Cox regression analysis demonstrated that *ECRG4* could act as a prognostic factor for NPC patients ($P = 0.036$, HR = 2.930, 95% CI = 1.072-8.009). Conclusion: *ECRG4* expression was tightly related with the prognosis of NPC patients.

Keywords: *ECRG4*, nasopharyngeal carcinoma, prognosis

Introduction

Nasopharyngeal carcinoma (NPC) is a type of fast-growing malignant tumor which occurs in the nasopharyngeal region [1-3]. The incidence and mortality of NPC were different in ethnic groups and geographic regions, becoming frequent in Southeast Asia and Southern China [4-6]. NPC is frequently characterized with high proliferation, adjacent region invasion and neck lymph nodes metastasis [7, 8]. Owing to the biological and anatomical specificity of NPC, the standard treatments for NPC are mainly radiation therapy and chemoradiotherapy, which are often followed by adjuvant chemotherapy [9, 10]. However, the carotid artery is easily damaged by ionizing radiation, which may result in carotid atherosclerosis [11, 12]. Besides, the five-year survival rate after the combined treatments is still relatively low and various [13]. Therefore, an innovate and promising biomarker for therapy and prognosis of NPC patients is urgently needed.

The esophageal carcinoma related gene 4 (*ECRG4*), also known as *C2ORF40*, locates at chromosome 2q12.2 and encodes a secretory protein that is produced in such endocrine tissues as adrenal gland, pituitary gland and choroid plexus [14-16]. *ECRG4* is first identified and cloned in the Key Laboratory of Molecular Oncology in Peking Union Medical College from human esophageal epithelia [17, 18]. *ECRG4* plays important roles on cell migration, cell cycle progression and cell differentiation [19, 20]. *ECRG4* has been reported to be downregulated in several tumors or cancers, including colerectal carcinoma and glioma, squamous cell carcinoma of the head and neck, gastric cancer, prostatic carcinoma and esophageal squamous cell carcinoma, therefore be regarded as a tumor suppressor [16, 21-23]. Previous reports have demonstrated that *ECRG4* was an independent prognostic biomarker for ESCC and low expression of *ECRG4* in patients with ESCC was correlated with poor prognosis [24].

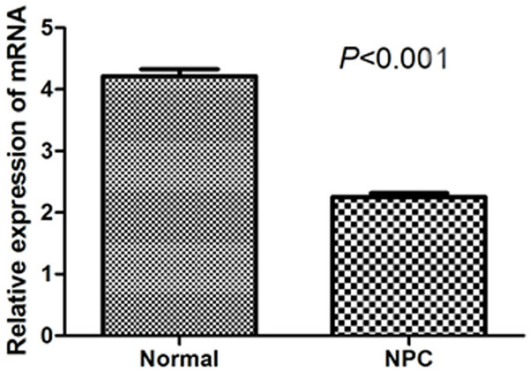

Figure 1. The expression of *ECRG4* in serum of NPC patients and the controls was measured using qRT-PCR. The relative expression of *ECRG4* was normalized to *GAPDH* and presented as mean ± SEM. The expression of *ECRG4* was significantly lower in serum of NPC patients than the controls ($P < 0.001$).

Table 1. Relationship between *ECRG4* expression and clinicopathologic characteristics

Characteristics	Case NO.	Expression		χ^2	*P* value
		Low	High		
Gender				0.054	0.817
Male	37	29	8		
Female	42	32	10		
Age				0.498	0.481
≤ 20	47	35	12		
> 20	32	26	6		
Smoking				1.286	0.257
Never	39	28	11		
Ever	40	33	7		
TNM stage				4.183	0.041
I + II	36	24	12		
III + IV	43	37	6		
Metastasis				4.347	0.037
Yes	39	34	5		
No	40	27	13		
N classification				6.034	0.014
N0 + N1	37	24	13		
N2 + N3	42	37	5		

In this study, we attempted to examine the expression of *ECRG4* in NPC patients and explored the correlation between *ECRG4* expression and prognosis of NPC patients.

Material and methods

Patients and specimens

A total of 79 serum specimens, which were immediately extracted from the peripheral blood, were obtained from patients (37 males and 42 females with a median age of 46 years) diagnosed as NPC clinically and radiologically in The First Affiliated Hospital of Zhengzhou University. All the patients were treated with the same therapeutic strategies. In addition, twenty two normal serum samples from healthy donors were provided by the Blood Center of The First Affiliated Hospital of Zhengzhou University as controls. All serum specimens were immediately stored at -80°C until use. The study began upon approval of the Ethics Committee and written informed consents were provided by all the patients.

Quantitative real-time PCR (qRT-PCR)

The total RNA in serum of NPC patients and the controls was extracted and purified by a QIAamp blood mini kit (Qiagen, Hilden, Germany) based on the manufacturer's instructions. Then the reverse transcription was conducted to synthesize the first chain of cDNA with PrimeScript® RT reagent Kit (TaKaRa Biotechnology Co., Ltd) and qRT-PCR was applied to detect the expression of *ECRG4* mRNA normalized with *GAPDH* as internal standard, using the SYBR Premix Ex Taq™ II (TaKaRa Biotechnology Co., Ltd). The reaction was performed with an Applied Biosystems 7500 (Applied Biosystems, USA), $2^{-\Delta\Delta CT}$ method was used for relative quantification. qRT-RCR was conducted in triplicate.

Statistical analysis

All data were carried out by SPSS 18.0 software (SPSS Inc, IL, USA). The relationship between *ECRG4* expression and clinical characteristics was evaluated by Chi-square test. The survival curves were plotted to describe the overall survival rate of NPC patients, which was described by Kaplan-Meier method. The relevance between *ECRG4* expression and prognosis of NPC patients was analyzed by Cox regression. It was considered significant when *P* was less than 0.05.

Results

Decreased expression of ECRG4 in NPC patients

The expression of *ECRG4* in NPC serum and the controls were detected by qRT-PCR. The relative expression level of *ECRG4* mRNA normalized to *GAPDH* in NPC serum was 2.25 ± 0.06

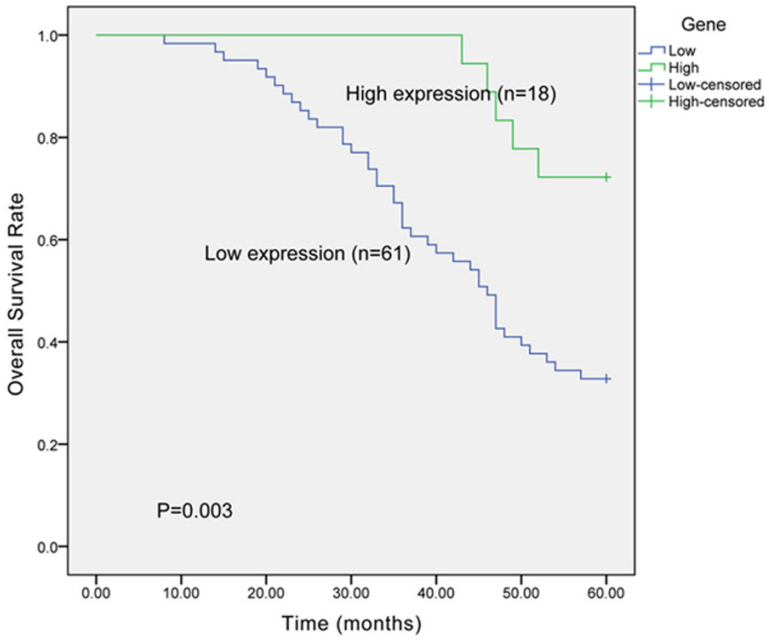

Figure 2. Survival curves of NPC patients were plotted by Kaplan-Meier method. Patients with low expression of ECRG4 was more likely to die than those with high expression of ECRG4 (P = 0.003). *P* value was calculated by log-rank test.

Table 2. The multivariate analysis of prognostic factors in NPC

Characteristics	*P* value	HR	95% CI
Gender	0.375	0.743	0.386-1.432
Age	0.699	0.879	0.458-1.686
TNM stage	0.381	1.350	0.690-2.643
Metastasis	0.203	1.543	0.791-3.008
N classification	0.549	2.930	0.628-2.396
ECRG4 expression	0.036	2.930	1.072-8.009

(mean ± SEM), while that in the controls was 4.22 ± 0.11. The result revealed that the expression of *ECRG4* in NPC serum was significantly lower than that in the control (**Figure 1**, *P* < 0.001).

Correlation between ECRG4 expression and clinicopathologic characteristics of NPC patients

The relationship between *ECRG4* expression and clinical characteristics was evaluated by Chi-square test. We manually grouped the 79 specimens into two groups according to the relative expression of *ECRG4* mRNA. Relative expression of *ECRG4* mRNA more than belonged to the *ECRG4* high expression group, and the rest were to the *ECRG4* low expression group. The result indicated that there was a significant correlation between *ECRG4* expression and high TNM stage (*P* = 0.041), metastasis (*P* = 0.037) and N classification (*P* = 0.014). However, *ECRG4* expression shared no statistical association with gender, age and smoking (all *P* > 0.05) (**Table 1**).

Low expression of ECRG4 was correlated with poor prognosis of NPC patients

The survival rate of NPC patients was evaluated by Kaplan-Meier survival analysis. The mean follow-up in this study was 46.44 months. During the follow-up, 41 out of 61 (67.2 %) patients with low expression of *ECRG4* died, while only 5 out of 18 (27.8 %) died among those with high *ECRG4* expression. As displayed in **Figure 2**, the NPC patients with low expression of *ECRG4* had lower survival rate than those with high *ECRG4* expression (*P* = 0.003). In addition, Cox regression analysis demonstrated that there was significant correlation between *ECRG4* expression and prognosis of NPC patients (**Table 2**, *P* = 0.036, HR = 2.930, 95 % CI = 1.072-8.009), indicating that *ECRG4* might be a prognostic biomarker for NPC patients.

Discussion

NPC is one of the most frequent malignancies on head and neck in southern China with high prevalence. Recently, researchers have sought to investigate the roles of various oncogenes on the prognosis of NPC. Zhuo et al. [25] claimed that over expression of *TWIST* was related to distant and lymphatic metastasis and *TWIST* might act as an unfavorable prognostic marker for NPC. Xia et al. [26] explained that *HMGA2* was related to epithelial-mesenchymal and predicted poor prognosis in NPC. In this study, we engaged in discovering more molecular biomarkers to better predict the prognosis of NPC patients.

ECRG4 is a recently identified tumor suppressor and it might be involved in the development of multi-tumors [27]. Our investigations displayed that down-regulation of *ECRG4* was frequently noticed in NPC serum specimens. It indicated that *ECRG4* might act as a tumor suppressor in NPC, the result was similar to previous study [19]. Then we explored the correlation of the *ECRG4* expression and clinicopathologic characteristics of NPC patients. We found *ECRG4* expression was significantly associated with high TNM stage, metastasis and N classification of NPC. This might reveal that *ECRG4* participates in the development of NPC. Down-regulation of *ECRG4* has been identified to associate with poor prognosis of patients with ESCC [25]. So we hypothesized that *ECRG4* expression might associate with prognosis of NPC patients. In this study, survival analysis revealed that the survival rate of patients with low expression of *ECRG4* was lower than those with high expression of *ECRG4*. Additionally, Cox analysis declared that low expression of *ECRG4* was correlated with poor prognosis of NPC patients, indicating that *ECRG4* could be an independently prognostic factor.

Taken together, low expression of *ECRG4* was observed in NPC and shared tight relationship with high TNM stage, metastasis and N classification. Our study also demonstrated that patients with low *ECRG4* expression had a lower survival rate than those with high *ECRG4* expression. We suggest that *ECRG4* was an independent biomarker for the prognosis of NPC patients.

Disclosure of conflict of interest

None.

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