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

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Received April 30, 2015; Accepted June 22, 2015; Epub February 1, 2016; Published February 15, 2016

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 colorectal 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].
ECRG4, a novel predictor for prognosis of NPC patients

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-ΔΔCT method was used for relative quantification. qRT-PCR 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
ECRG4, a novel predictor for prognosis 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 %) patients 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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References


