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The prognostic role of Eg5 expression in laryngeal squamous cell carcinoma

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Summary

Eg5 is identified as a microtubule-dependent motor protein and has been suggested to play important role in cancer development. The aim of this study was to detect the expression of Eg5 and its clinicopathological characteristics in patients with laryngeal squamous cell carcinoma. One-step quantitative reverse transcription-polymerase chain reaction (qPCR) test with 20 fresh-frozen LSCC samples and immunohistochemistry (IHC) analysis in 137 LSCC cases were performed to investigate the relationship between Eg5 expression and the clinicopathological characteristics of LSCC. The Eg5 mRNA and protein levels were significantly higher in LSCC tissues compared with corresponding non-cancerous tissues ($p < 0.05$). Eg5 protein expression was significantly correlated to lymph node metastasis ($p = 0.021$) and TNM stage ($p = 0.030$). Kaplan–Meier survival and multivariate analysis with Cox regression model indicated that high Eg5 expression ($p = 0.031$) and TNM stage ($p = 0.011$) were independent factors to predict unfavourable prognosis for patients with LSCC. The data suggested that Eg5 may be identified as a novel prognostic biomarker and targeting Eg5 seems to be a novel strategy for LSCC treatment.

Key words: Eg5; LSCC; qPCR; IHC.

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INTRODUCTION

Laryngeal squamous cell carcinoma (LSCC) is a common type of malignant tumour of the head and neck and is the second most common malignancy in the respiratory tract.[1] The majority of LSCCs have developed in the glottis (>60%) and supraglottis, with the subglottis representing the minority of LSCC cases (<5%).[2] Carcinogenic elements in daily life such as smoking and alcohol consumption are the major risk factors for LSCC development and the more commonly affected group is males aged over 40 years.[3] Surgery and radiation therapy with or without chemotherapy have widely been utilised as the main treatments for LSCC, while several other progressive treatment strategies have also been expanded and evolved, including molecular targeted therapy, gene therapy and immunotherapy.[4] However, the increased local recurrence rate and frustrating decreased 5-year overall survival (OS) rates from 48% to 54% have been witnessed over the past decades,[5–7] underlining the significance and urgency to identify sensitive and specific biomarkers in LSCC and develop novel therapeutic strategies.

The mitotic kinesin Eg5, encoded by the KIF-11 gene located at chromosome 10q24.1, is a part of the kinesin-5 molecule (a member of the kinesin superfamily) and exerts critical function in the assembly and maintenance of the bipolar spindle.[8] Eg5 takes part in the movements of the spindle and chromosomes in dividing cells and affects spindle function by both its cellular localisation and mutation effectiveness.[9] Normally, Eg5 expresses in proliferating human tissues, such as tonsils, testis and bone marrow, while it is lacking in post-mitotic human cells, such as central nervous system neurons. These special functions suggest that Eg5 could be a candidate target for antimitotic therapies.[10] Recently, cumulative evidence stated that Eg5 expression crucially correlated with several kinds of human cancers. Differential expression of Eg5 has been detected in pancreatic cancer,[8] lung cancer,[11] and prostate cancer.[12] Activation of Eg5 expression contributes to the development of leukemia;[13] ectopic expression of Eg5 promotes pancreatic tumorigenesis,[14] while inhibition of Eg5 expression leads to impaired angiogenesis and tumour development.[15] Several Eg5 inhibitors also exhibit anti-tumour activity.[12,16,17] Thus, Eg5 also represents an attractive target for novel anticancer therapy in certain types of cancers. However, the characteristics of Eg5 in LSCC are rarely reported. What function does Eg5 play in LSCC development and what is the relationship between Eg5 expression and clinical features of LSCC? We conducted this research.

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In this retrospective study, the mRNA and protein expression of Eg5 was evaluated using one-step quantitative reverse transcription-polymerase chain reaction (qPCR) analysis and immunohistochemistry (IHC) analysis respectively. Moreover, the association between Eg5 expression and significant clinicopathological items of LSCC, especially the prognostic status, were further examined.

MATERIALS AND METHODS

LSCC sample collection

Twenty samples of fresh LSCC tissues and matched non-cancerous tissues (confirmed by two pathologists independently) were collected from the archives of the Department of Pathology, the Affiliated Hospital of Nantong University. At the same time, a total of 137 paraffin-embedded LSCC tissues and corresponding non-cancerous tissues were also collected from the Department of Pathology at the Affiliated Hospital of Nantong University, between January 2000 and December 2010. Histological diagnosis of LSCC was performed according to the latest World Health Organization (WHO) criteria.[18,19] Original clinic data, including age, tobacco and alcohol consumption, histopathological grade, lymph node metastasis and TNM stage, were retrospectively collected from medical records. All patients received radical surgery. None of the patients received radiotherapy, chemotherapy or immunotherapy before surgery. Ethical approval of this present research was acquired from the Human Research Ethics Committee of the local hospital and written informed consent was obtained from each patients enrolled in this study.

One-step qPCR test

Twenty samples of fresh LSCC and matched non-cancerous tissues were collected. Total RNA was extracted using Trizol reagent (Invitrogen, USA) and then reversely transcribed into cDNA using Moloney murine leukaemia virus retrotranscriptase (Promega, USA). The primers were designed with the assistance of Beacon Designer 7.7 software (Premier Biosoft, USA) and are as follows: Eg5 forward: 5′-GAA CAA TCA TTA GCA GCA GAA-3′; Eg5 reverse: 5′-TCA GTA TAG ACA CCA CAG TTG-3′; β-actin forward: 5′-TAA TCT TCG CCT TAA TAC TT-3′; β-actin reverse: 5′-AGC CTT CAT ACA TCT CAA-3′. qPCR test was performed using SYBR green dye and a Bio-Rad iQ50 Real-time PCR system in accordance with the manufacturer's instructions. Total RNA extraction, amplification conditions and one-step qPCR procedure were described in our previous publication.[19] Expression data were normalised to the geometric mean of the β-actin housekeeping gene and analysed using the 2-△△Ct method as previously described.[20]

Tissue microarray (TMA) construction and IHC analysis

Formalin fixed, paraffin embedded LSCC samples and non-cancerous samples were collected and TMAs were produced by Xinchao Biotech (China). The TMA was cut into 4-μm sections and placed on Superfrost charged glass microscope slides.

TMA microarray sections were incubated with anti-Eg5 antibody (1:100; Abcam, USA) overnight at 4°C, followed by incubation with biotinylated secondary antibody at 37°C for 30 min. Sections were then incubated with a streptavidin-horseradish peroxidase complex, colourised with 3,3-diaminobenzidine (DAB) chromogen solution and counterstained with haematoxylin. Results were analysed as previously described.[21,22] Briefly, the percentage of Eg5 positive cells was scored as follow: 0 for 0%, 1 for 1–33%, 2 for 34–66% and 3 for 67–100%. The intensity of Eg5 staining was also scored as follows: 0 for negative staining, 1 for yellow colour staining, 2 for light brown colour staining and 3 for brown colour staining. Samples with a sum score <2 were considered to exhibit low Eg5 expression, and those with a sum score of 2–6 were considered to exhibit high Eg5 expression.

Statistical analysis

Statistical analysis was executed using STATA 12.0 software (Stata Corporation, USA). Comparison of Eg5 mRNA expression in fresh-frozen LSCC tissues as well as non-cancerous tissues was analysed with the Wilcoxon signed rank non-parametric test. The association between Eg5 protein expression and clinicopathological parameters was evaluated by chi-square test. Univariate and multivariate analysis was performed using Cox's proportional hazard regression model. Survival rate was estimated by Kaplan–Meier method and log-rank test. For all tests, a two-tailed $p < 0.05$ was considered statistically significant.

RESULTS

Analysis of Eg5 mRNA expression in LSCC by qPCR test

To investigate the expression of Eg5 mRNA in LSCC, we performed qPCR test. When normalised to β-actin, we observed a significant increase in Eg5 mRNA in LSCC tissues compared with non-cancerous tissues (0.875 ± 0.1207 versus 0.361 ± 0.0425, respectively, $t = 4.016$, $p = 0.0003$). The average level of Eg5 mRNA was 2.42-fold higher in LSCC tissues compared with that in non-cancerous tissues (Fig. 1).

Detection of Eg5 protein expression in LSCC by IHC analysis

We next investigated the expression of Eg5 protein in LSCC by IHC analysis. As is demonstrated in Fig. 2, Eg5 positive staining was predominantly localised in the cytoplasm of cancer cells. In comparison, the staining of Eg5 in normal epithelium was extremely low. Expression of Eg5 was significantly higher in LSCC tissues compared with non-cancerous tissues ($p < 0.001$). Specifically, high expression of Eg5 was detected in 76 of 137 (55.5%) LSCC tissues, while only 49 of 137 (35.8%) non-cancerous tissues exhibited high Eg5 expression.

Relationship between Eg5 expression and clinicopathological parameters

The relationship between Eg5 protein expression and clinicopathological parameters of 137 LSCC patients is shown in Table 1. High Eg5 expression in cancer cells was significantly correlated to lymph node metastasis ($p = 0.021$) and TNM stage ($p = 0.030$), while no statistical correlation was observed with other clinical attributes, including age, tobacco and alcohol consumption, or histopathological grade.

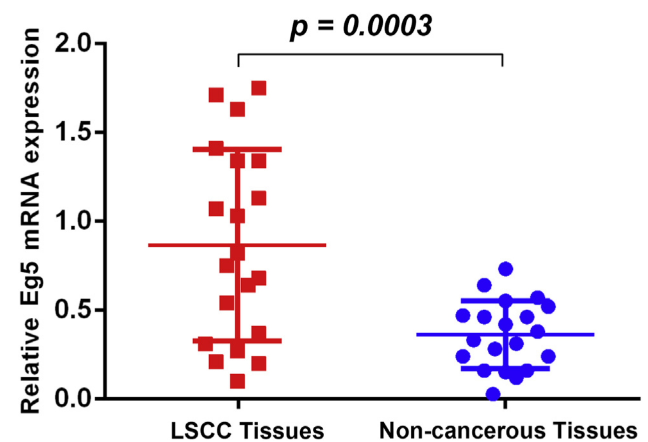

Fig. 1 One-step quantitative polymerase chain reaction (qPCR) was employed to detect Eg5 mRNA expression levels in LSCC compared with non-cancerous tissues. When using β-actin as internal control, the Eg5 mRNA level in LSCC tissue (0.875 ± 0.1207) is statistically higher than that in corresponding non-cancerous tissue (0.361 ± 0.0425).

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