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# Clinical significance of integrin-linked kinase in laryngeal squamous cell carcinoma

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ABSTRACT

*Objective:* To investigate the expression of integrin-linked kinase (ILK) and its relationship with clinicopathological parameters in laryngeal squamous cell carcinoma (LSCC).

*Methods:* 116 patients who had previously undergone complete resection of tumor for LSCC were studied retrospectively. The level of ILK expression in tumor tissues and adjacent nontumor tissues were determined by immunohistochemistry.

*Results:* Increased expression of ILK was found in 65.5% of cases. The expression of ILK protein was significantly associated with tumor grade ($p = 0.046$), lymph node metastasis ($p = 0.020$), and pTNM stage ($p = 0.019$). Kaplan–Meier survival estimates showed a significant correlation between ILK expression and patient survival rate (log-rank $p < 0.05$). The multivariate survival analysis revealed that N status was statistically significant prognostic factor ($p < 0.001$). Other parameters, such as ILK expression, cannot predict disease prognosis separately.

*Conclusion:* Increased expression of integrin-linked kinase is associated with lymph node metastases and patient survival rate in laryngeal squamous cell carcinoma. However, it does not appear to be an independent prognostic predictor in LSCC.



## 1. Introduction

Laryngeal carcinoma, classified as squamous cell carcinoma (LSCC) in 90% of the cases, is a common cause of morbidity and mortality worldwide, accounting for 5% of all human malignancies [1]. Despite tremendous development in surgery, radiotherapy, and chemotherapy, the long-term prognosis for patients with LSCC remains unsatisfactory [2,3]. Locoregional recurrence, cervical lymph nodes metastases, and distant metastases are the factors that significantly affect the prognosis in LSCC patients [4]. Recognition and identification of tumor marker associated with recurrence and/or metastasis have great significance in the prediction of tumor biological behavior and direction of therapeutic strategy.

Integrin-linked kinase (ILK) was discovered in 1996 as an β1-integrin subunit cytoplasmic domain interactor [5], is implicated in the regulation of anchorage-dependent cell growth and survival, cell cycle progression, epithelial–mesenchymal transition (EMT), invasion and migration, cell motility and contraction, and vascular development [6–8]. Increased ILK expression has also been reported in many types of tumors, such as melanoma, colon, prostate, ovarian cancer, non-small cell lung cancer, and head and neck squamous cell carcinomas [6,9–11]. Overexpression of ILK

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participates in a panel of processes involved in tumor progression, including lymphangiogenesis [6], invasion and migration [9,10], indicating the significance of ILK as a potential target for cancer therapy. Small-molecule inhibitors of ILK activity have been identified and shown to inhibit tumor growth, invasion, and angiogenesis [12,13]. However, there were few published reports evaluating the role of ILK protein expression in LSCC. In order to gain better insight into the clinical relevance of ILK in LSCC, the present study was carried out to investigate ILK expression in a larger number of LSCC tissue samples and to further assess whether ILK expression was correlated with clinicopathological parameters and prognosis in patients with LSCC.

## 2. Methods

### 2.1. Patient characteristics

A total of 116 patients who were diagnosed as LSCC at the Department of Otolaryngology, Head and Neck Surgery, the Second Xiangya Hospital of Central South University between March 2002 and January 2007 were studied retrospectively. Patients involved in this study signed the informed consent. The entire study was approved by the local Ethics Committee. Specimens of primary tumor and the corresponding histological nontumor tissues from the same patients were separated by two experienced pathologists. In this study, there were 110 male and 6 female patients with an average age of 63.4 years (age range: 47–75 years). All patients had no history of previous malignancies, no history of radiotherapy or chemotherapy. Patients with distant metastasis were excluded from this study. Tumors staging was performed according to TNM staging system of the International Union Against Cancer (UICC, 2002). Histological types of LSCC were determined according to the system of World Health Organization. Patients included in our study underwent different types of surgeries according to the size of primary site. In 116 laryngeal carcinoma patients, 23 patients underwent laser resection, 44 patients underwent partial laryngectomy, and 49 patients underwent total laryngectomy. Patients with lymph nodes metastases confirmed by preoperative evaluations or with supraglottic tumor underwent neck dissection. In the postoperative histological examination, 63 patients with locally advanced tumors, cervical lymph node metastasis with extranodal spread or positive surgical excision margins received post-surgical radiotherapy and chemotherapy.

### 2.2. Immunohistochemistry

The specimens of formalin-fixed, paraffin-embedded tissues were cut into 4 μm sections by a microtome mounted on poly-L-lysine coated glass slides and air-dried overnight at 37 °C. Paraffin tissue sections were deparaffinized in xylene and rehydrated in descending ethanol series and water according to standard protocols. Endogenous peroxidase activity was blocked by immersion for 20 min in 3% hydrogen peroxidase, after which the slides were rinsed in phosphate-buffered saline (PBS, pH 7.2–7.4). Then the sections were boiled for 10 min in a 0.01 M citrate buffer (pH 6.0) for antigen retrieval. After preincubation with 1% bovine serum albumin (Sigma Chemical Co., St. Louis, MO) for 30 min, tissue sections were incubated overnight at room temperature with rabbit polyclonal IgG of ILK (1:500, Santa Cruz Biotechnology, USA). After incubation, the sections were rinsed in PBS and incubated with the secondary biotinylated goat-anti-rabbit IgG (1:200, Santa Cruz Biotechnology, USA) for 30 min at room temperature. After washing in PBS, the slides were incubated with streptavidin conjugated with horse radish peroxidase (Sigma). The peroxidase reaction was developed with 3,3′-diaminobenzidine (DAB, Sigma). Finally, the sections were counterstained with hematoxylin, dehydrated, cleared in xylene and finally embedded in Entellan. Negative control sections were treated using PBS in the primary antibody. All the immunostained tissue sections were reviewed and scored under a microscope for expression and localization of ILK protein by two pathologists independently and blindly. The scores of each section were compared and if there was a discrepancy, the two pathologists reviewed them again and reached a consensus. Briefly, five high-powered fields under the microscope were randomly chosen and 100 cells in each field were counted. The staining scores (IHS) were calculated by combining an estimate of the percentage of immunoreactive cells (quantity score) with an estimate of the staining intensity (staining intensity score). For the percentage of staining, score 0 indicated no staining; score 1, 1–10% of cells stained; score 2, 11–50%; score 3, 51–80%; score 4, 81–100%. Staining intensity scores were as follows: score 0, no staining; score 1, light yellow (weak); score 2, yellow (moderate); and score 3, deep yellow (strong). We defined the IHC staining score as the multiply of the intensity and percentage scores. Patients were classified into two groups according to the staining score of tumor tissues as follows: samples with scores 0–6 of tumor stained as negative staining and samples with scores 7–12 of tumor stained were considered as positive [14,15].

### 2.3. Follow-up

After the completion of treatment, patients underwent routine surveillance every 1–3 months. Recurrence and metastasis were diagnosed by physical examination, imaging evaluation, operation, and postoperative pathological examinations. Follow-up rate was 100% (116/116). Overall survival (OS) and disease-free survival (DFS) were calculated from the day of surgery to the date of death or that of tumor relapse. Deaths from other causes were treated as censored cases. The follow-up time ranged from 23 to 60 months, with a median follow-up time of 44 months (SD = 11.466).

### 2.4. Statistical analysis

The association between ILK expression and clinicopathological parameters was statistically evaluated by using Fisher's exact test or the chi-square test. Survival curves were calculated by the Kaplan–Meier method and were compared using the log-rank test. The correlation of variables with survival was analyzed by multivariate analysis using a Cox proportional

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