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The value of insulin-like growth factor-1 receptor for predicting early glottic carcinoma response to radiotherapy

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ABSTRACT

Objective: The optimal management of laryngeal carcinoma requires tumor treatment and preservation of laryngeal functions, such as swallowing and voice quality. Radiotherapy (RT) can fulfill both criteria, although it remains important to identify predictors of radioresistance and reduce unnecessary irradiation. Insulin-like growth factor-1 receptor (IGF-1R) is a transmembrane receptor that plays a key role in cancer development, although its prognostic value after RT remains unknown. We evaluated the predictive value of IGF-1R expression for RT response in patients with early glottic squamous cell carcinoma.

Methods: We retrospectively reviewed 43 patients with T1N0 and T2N0 glottic squamous cell carcinoma who were treated with RT alone. Biopsy specimens were stained using an anti-IGF-1R antibody, and we evaluated the relationships between IGF-1R expression and T classification or tumor recurrence. We also evaluated the loco-regional control (LRC) rate and the prognostic value of various clinical factors.

Results: All cases achieved complete response after the initial RT, and 10 (23.3%) patients experienced local tumor recurrence. Twenty-five patients (58.1%) exhibited high IGF-1R expression, although the level of IGF-1R expression was not correlated with T classification. Local recurrence was observed in 36% (9/25) of patients with high IGF-1R expression and in only 5% (1/18) of patients with low IGF-1R expression ($p < 0.05$). The 2-year LRC rate was 94.1% for the low IGF-1R expression group, compared to 49.8% for the high IGF-1R expression group ($p = 0.04$). Anterior commissure involvement and IGF-1R expression were independent adverse factors for LRC.

Conclusion: High IGF-1R expression was more common among patients with recurrent early glottic carcinoma, which suggests that there is a biological relationship between IGF-1R expression and RT response. Thus, IGF-1R may be a useful screening parameter for RT response in laryngeal carcinoma.

1. Introduction

Laryngeal cancer represents the largest subgroup of head and neck cancers, with current treatment options including radiotherapy (RT), surgery, chemotherapy, or a combination of these modalities. The optimal management of laryngeal carcinoma requires tumor treatment and preservation of laryngeal function (such as swallowing and voice quality), and RT is a generally accepted treatment modality that meets both of these criteria. Furthermore, because primary RT provides good preservation of laryngeal function, it is the standard therapy for early glottic carcinoma. Although the appropriate treatment strategy for advanced laryngeal carcinoma remains controversial, concurrent chemoradiotherapy is widely used as the initial treatment modality. Unfortunately, RT

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failures do occur, and TNM classification cannot predict an individual tumor's response to RT, despite this classification being widely used to guide cancer management [1]. Therefore, it has become increasingly important to identify the molecular mechanisms that underlie radioresistance, and several molecular markers have been analyzed as predictors of clinical outcomes. In this context, epidermal growth factor receptor [2,3] and p53 [4,5] are related to local RT failure in laryngeal cancer, although different studies have reported conflicting outcomes.

Insulin-like growth factor 1 receptor (IGF-1R) is a transmembrane receptor with tyrosine kinase activity that is widely distributed in human tissues [6], and plays a key role in the development of cancer [7–10]. In this context, the IGF-1R signaling system affects cancer cell proliferation, adhesion, migration, and cell death; these functions are critical for cancer cell survival and metastasis [11]. Beyond its role in promoting tumorigenesis, IGF-1R can influence clinical outcomes via altered radioresistance and by providing mechanisms for escape from conventional cancer therapies [12]. Furthermore, previous studies have demonstrated that increased IGF-1R expression and signaling significantly enhance the proliferation, motility, and tumorigenicity of head and neck cancer cell lines [13–15]. However, there are currently no data regarding the value of IGF-1R for predicting RT response in patients with head and neck squamous cell carcinoma (HNSCC), and the role of IGF-1R expression in regulating RT response remains unclear. Therefore, the present study aimed to assess the expression of IGF-1R in patients with early glottic squamous cell carcinoma who underwent RT, and to determine its role in predicting clinical outcomes.

2. Methods

2.1. Patients

We retrospectively reviewed the medical records of 43 patients with previously untreated T1N0 and T2N0 glottic larynx squamous cell carcinoma; these patients were treated with RT alone at the University of Juntendo between January 2007 and October 2014. Thirty-nine patients were men, and the average patient age was 67 years (range, 41–88 years). The median follow-up duration was 33 months, and follow-ups ranged from 2 months to 94 months. Representative pathological paraffin-embedded specimens of the patients' untreated glottic cancer had been obtained at their treatment, and these specimens were used to evaluate IGF-1R expression for the present study. The study protocol was approved by our institutional review board, and the investigators obtained written informed consent from each patient.

2.2. Treatment

The planning target volume was calculated by adding a 10-mm margin to the clinical target volume and an additional 15-mm margin from the skin. No prophylactic neck lymph node area irradiation was performed. In the present study, the field size was approximately 6 cm × 6 cm in the lateral view, and all sites were irradiated with 4-MV photon beams (Clinac 21EX; Varian) in parallel opposed fields. The daily dose ranged from 2 Gy to 2.5 Gy per fraction (one fraction per day), with a maximum total dose of 63.0–70 Gy. At our institution, the standard fraction size was 2.0 Gy before 2010, although this was changed to 2.25 Gy per fraction after 2010. Fraction size and total dose were modified in each case based on the tumor's volume, anterior commissure invasion, and age.

2.3. Immunohistochemical analysis

Formalin-fixed and paraffin-embedded specimens were obtained from biopsies that were representative of the characteristics of the tumors. These samples were stained with an anti-IGF-1R antibody (#3027, Cell Signaling Technology Inc., Beverly, MA) to evaluate IGF-1R expression. In brief, the tissue sections (4 μm thick) were deparaffinized, rehydrated with xylene and ethanol, blocked with methanol and 3% hydrogen peroxide, and subsequently incubated with the primary antibodies (1:500 dilution in phosphate-buffered saline [PBS] for anti-IGF-1R, and 1:300 in PBS for anti-PY1316) for 2 h at room temperature. The sections were then stained with Histofine Simple Stain MAX-PO (MULTI) and Simple Stain DAB Solution (Nichirei Bioscience, Tokyo, Japan), according to manufacturer's instructions, and counterstained with hematoxylin. Immunostaining was assessed under a light microscope using representative fields (200× magnification), and the expression was graded semi-quantitatively, based on the number of immunopositive tumor cells: 0, no staining; 1, ≤10% of the tumor cells stained; 2, 11–25% of the tumor cells stained; 3, 25–50% of the tumor cells stained; 4, >50% of the tumor cells stained. The IGF-1R staining was evaluated by an author (SO) who was blinded to the patient's clinical characteristics and outcome. Final evaluations for ambiguous cases were made using a multi-headed microscope in collaboration with the other authors. A score of ≥2 was defined as high IGF-1R expression.

2.4. Statistical analysis

We evaluated the relationships between IGF-1R expression and T classification or tumor recurrence using Fisher's exact test. We also evaluated the loco-regional control (LRC) rate using the Kaplan–Meier method. For this analysis, LRC was defined as tumor control within the radiation field. Furthermore, we evaluated the prognostic value of various clinical factors (age, sex, T classification, anterior commissure involvement, total dose, fraction size, overall treatment time, and IGF-1R expression) for LRC using univariate (log-rank test and Cox's univariate hazards model) and multivariate (Cox's proportional hazards model) analyses. A p-value of <0.05 was considered statistically significant, and all statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL).

3. Results

The clinical characteristics of the 43 included patients are listed in Table 1. All cases achieved complete response after the

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