Immunohistochemical Marker Expression in Temporal Bone Squamous Cell Carcinoma

Daisuke MAKI[*1], Kenji OKAMI[*1], Koji EBISUMOTO[*1], Akihiro SAKAI[*1], Masashi HAMADA[*1], Go OGURA[*2], Tomoaki MURAKAMI[*1], Mayu YAMAUCHI[*1], Kosuke SAITO[*1], Shoji KANEDA[*1] and Naoya NAKAMURA[*2]

[*1]*Department of Otolaryngology-Head and Neck Surgery, Tokai University School of Medicine*
[*2]*Department of Pathology, Tokai University School of Medicine*

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Background: The correlation between genomic mutations (or the overexpression of abnormal proteins) and prognosis in temporal bone squamous cell carcinoma (TBSCC) is not clear. We investigated the overexpression of EGFR and p53 as a pathological biomarker for predicting the clinical course of patients with TBSCC.
Methods: We performed a retrospective review of 22 TBSCC cases treated in Tokai University Hospital between January 2005 and October 2016. We assessed the overexpression of EGFR and p53 in TBSCC patients through immunohistochemical staining. We also evaluated the association between the overexpression of these proteins and clinicopathological variables, including survival outcomes.
Results: The primary lesion in all patients was the external auditory canal. Nine (40.9%) patients were EGFR positive, and 9 (40.9%) were p53 positive. The 5-year overall survival rate for EGFR-positive patients (55.6%) was significantly lower (p = 0.043) than that of the EGFR-negative patients (92.3%).
Conclusion: EGFR overexpression in TBSCC patients may be a prognostic biomarker.

Key words: temporal bone squamous cell carcinoma, Immunohistochemical marker, EGFR, p53, Cetuximab

INTRODUCTION

Temporal bone cancer is a rare malignancy; it accounts for less than 0.2% of all tumors of the head and neck [1, 2]. Squamous cell carcinoma (SCC) is the most common temporal bone cancer, accounting for 80% of all such lesions [3]. The prognostic factors of temporal bone squamous cell carcinoma (TBSCC) have been reported in previous studies. Histopathologically, the positive surgical margin [2], pT4 disease [2], pathological N+ staging [4], and poorly differentiated SCC [5, 6] have been reported as poor prognostic factors of TBSCC.

On the other hand, several genes, including tumor suppressors such as cyclin-dependent kinase inhibitors, TP53, and oncogenes such as the cyclin family, epidermal growth factor receptor (EGFR), and ras genes have been considered to play a role in the pathogenesis of head and neck cancers [7, 8].

The expression of proteins linked to genetic and epigenetic alterations has been clinically analyzed using immunohistochemical staining, and the extent of expression is expected to correlate with prognosis in patients with various cancers [9]. Thus, the identification of biological markers is important for predicting prognosis and defining individual treatment modalities for patients with TBSCC. However, the correlation between genomic mutations or the overexpression of abnormal proteins and prognosis in TBSCC is not clear. If it becomes clear that overexpression of abnormal proteins in TBSCC is associated with prognosis and therapeutic effect, that information is clinically meaningful for predicting prognosis and selecting a treatment modality. In the present study, we performed a retrospective, single institutional review of 22 patients with TBSCC. We aimed to clarify the association between overexpression of EGFR and p53 in TBSCC patients and clinical data including survival outcomes using immunohistochemical staining.

MATERIALS AND METHODS

Patients and tissue specimens

This study is a retrospective, single-institution review of TBSCC patients who initially underwent curative treatment at the Department of Otolaryngology, Tokai University Hospital, between January 2005 and October 2016. The protocol of this study was approved by the Institutional Review Board of the Tokai University, School of Medicine (ref: 17R-276), on March 28, 2018. Staging was performed using the University of Pittsburgh modified TNM staging system [2]. None of the patients had neck metastasis or distant metastasis before treatment. Patients with no tumor invading the skull base or middle ear underwent lateral temporal bone resection. Patients with tumors invading the carotid canal or jugular foramen underwent radiotherapy or chemoradiotherapy. Patients who refused surgery also underwent radiotherapy or chemora-

Daisuke MAKI, Department of Otolaryngology-Head and Neck Surgery, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan Tel: +81-463-931121 Fax: +81-463-941611 E-mail: d.maki@tokai.ac.jp

diotherapy. No patient underwent subtotal temporal bone resection. All tissue samples were obtained from surgically resected specimens or specimens biopsied before treatment. The relationship between the level of expression of proteins (EGFR, p53) and clinical parameters was assessed.

Immunohistochemistry

For the immunohistochemical analyses, 4-μm thick tissue sections were obtained and stained with an EGFR monoclonal antibody (31G7; NICHIREI BIOSCIENCES INC.) using a BOND-MAX system (Leica Biosystems, Newcastle upon Tyne, United Kingdom) following the manufacturer's protocol. Antigen retrieval was performed by treatment with Bond Epitope Retrieval Solution 2 (Leica) for 30 min. The tissue sections were also stained with a p53 mouse monoclonal antibody (DO-7; Roche Diagnostics Ltd.). Appropriate positive and negative tissue control samples were included in all experiments. The immunohistochemical evaluation was performed by GO and DM who were blinded to clinical information, and discrepancies were discussed using a multi-head microscope until consensus was reached. For EGFR, immunostaining results were divided into four categories: 0 (no or weak staining in < 10% of tumor cells), 1+ (weak staining in part of the membrane in > 10% of the tumor cells), 2+ (complete staining of the membrane with weak or moderate intensity in > 10% of the tumor cells), and 3+ (strong staining in > 10% of the tumor cells) (Fig. 1). The EGFR samples were also categorized into two groups; 0, 1+, and 2+ were classified as negative, while 3+ was positive. For p53, positive immunostaining of proteins was determined from the staining intensity as well as the percentage of immunoreactive cells in the most stained area of each slide based on a previously reported method [10–13]. The intensity score was graded as 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The percentage score was graded as 0 (< 1 % positive tumor cells), 1 (1%–10%), 2 (10%–50%), 3 (50%–90%), and 4 (> 90%) [9]. The sum of the staining intensities and percentages of positive cells was calculated and graded as p53 positive (sum of scores exceeded 3) or p53 negative (sum of scores were ≤ 2) according to previous reports [10, 12].

Statistical analysis

Statistical analyses were conducted using Statmate V (ATMS, GraphPad, Inc., San Diego, CA, USA). Fisher's exact test was used to analyze the relationship between clinicopathological factors and immunostaining results. Overall survival curves were illustrated using the Kaplan–Meier method and analyzed using the log-rank test. A *p-value* of < 0.05 represented statistical significance. The Cox proportional hazard model was used to identify independent prognostic factors if univariate survival analyses revealed statistically significant differences.

RESULTS

Clinicopathological features

Patient characteristics are presented in Table 1. Twenty-two patients with TBSCC were included in this study. The median age of patients was 65 years (range, 33–77 years). Eleven patients were older than 65 years. The patients included 7 men and 15 women. The primary lesion in all patients was the external auditory canal. Based on the T classification, 3 patients were categorized as T1, 9 as T2, 4 as T3, and 6 as T4. No patients had lymph node metastasis or distant metastasis. Sixteen patients were diagnosed with well-differentiated SCC, and 6 patients had moderately or poorly differentiated SCC. The treatment selected for each patient is presented in Table 2. Lateral temporal bone resection without postoperative radiation therapy

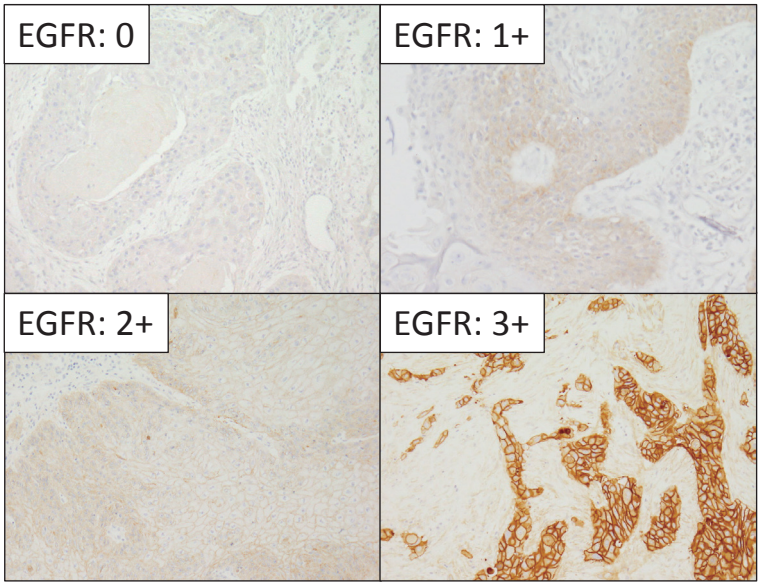

Fig. 1 EGFR immunostaining results were divided into four categories: 0 (no or weak staining in < 10% of tumor cells), 1+ (weak staining in part of the membrane in > 10% of the tumor cells), 2+ (complete staining of the membrane with weak or moderate intensity in > 10% of the tumor cells), and 3+ (strong staining in > 10% of the tumor cells).

Table 1 EGFR and p53 expression and clinicopathological parameters

Total number of patients (n = 22)	EGFR		p value	p53		p value
	positive 9	negative 13		positive 9	negative 13	
Age Median: 65 years						
≤65 years (n = 11)	6	5		5	6	
>65 years (n = 11)	3	8	0.234	4	7	0.694
Sex						
Male (n = 7)	3	4		3	4	
Female (n = 15)	6	9	1	6	9	1
T class						
T1 (n = 3) + T2 (n = 9)	4	8		4	8	
T3 (n = 4) + T4 (n = 6)	5	5	0.666	5	5	0.666
Differentiation						
Well (n = 16)	6	10		6	10	
Moderate or Poor (n = 6)	3	3	1	3	3	1

Table 2 Selected treatment in each cases

T stage	Treatment (case number)
T1 (n = 3)	LTBR (2), RT (1)
T2 (n = 9)	LTBR (3), LTBR + PORT (5), RT (1)
T3 (n = 4)	LTBR + PORT (4)
T4 (n = 6)	LTBR + PORT (1), RT (2), CRT (1), BRT (1)

Table 3 Univariate analyses of the prognostic factors for the overall survival of patients with TBSCC

	Univariable		
	HR	95% CI	*p* value
Age: >65years (vs. ≤65years)	0.237	0.049−1.648	0.161
Sex: female (vs. male)	0.725	0.110−4.635	0.723
T stage: T1 + T2 (vs. T3 + T4)	0.547	0.094−3.190	0.502
Differentiation: Well (vs. others)	1.796	0.250−11.222	0.594
EGFR: positive (vs. negative)	6.924	1.055−40.471	**0.043**
p53: positive (vs. negative)	0.912	0.154−5.415	0.920

was performed in 2 and 3 patients with T1 and T2, respectively. In 5 patients with T2, 4 with T3, and 1 with T4 it was followed by postoperative radiation therapy due to positive or close surgical margins. Definitive radiation therapy (RT) was performed in 1 patient with T1, 1 with T2, and 2 with T4. Definitive chemoradiotherapy or bio-radiotherapy (RT + Cetuximab) was performed in 3 patients with T4. The follow-up period ranged from 5 to 132 months. The median follow-up period was 63 months.

Correlation between IHC expression and clinicopathological parameters

Nine (40.9%) patients were graded as EGFR positive, and 9 (40.9%) were also graded as p53 positive. There was no significant association between immunohistochemical expression and the clinicopathological parameters (Table 1).

Survival analysis

Findings from the univariate analysis are presented in Table 3. A significant association between EGFR overexpression and prognosis was observed. The 5-year overall survival (OS) rate of patients graded as EGFR positive (55.6%) was significantly shorter than that of patients graded as EGFR negative (92.3%) (Fig. 2a). The 5-year OS rate was 77.8% for patients with p53 positivity and 76.9% for those with p53 negative tumors (Fig. 2b). The 5-year OS rate was 83.3% for patients with T1−2 diseases and 70.0% for those with T3−4 diseases (Fig. 2c).

The p53 status and clinicopathologic features, such as age, sex, T-stage, and histopathological differentiation, were not significantly correlated with survival outcomes. Multivariate analysis was not performed.

Cases treated with Cetuximab

Five of the 22 cases were administered with cetuximab as the first- or second-line treatment. Two cases were T2 and 3 cases were T4. In all T2 cases, surgery and postoperative irradiation were performed as the initial treatments, and cervical metastasis occurred. Therefore, the EXTREME regimen comprising cisplatin, 5-FU, and cetuximab was administered for cervical recurrence. One of 2 patients remained had no recurrence for 57 months. In T4 cases, 2 patients were treated with cetuximab as first-line, and 1 was treated with cetuximab as second-line. One of the T4 patients who received irradiation with cetuximab as the initial treatment had no recurrence for 61 months. EGFR overexpression was detected in one of the two cases that had remarkable cetuximab effects. On the other hand, EGFR overexpression was also detected in 2 of 3 cases that had no cetuximab effect. Although there were few cases, there was no correlation between the effect of cetuximab and EGFR expression.

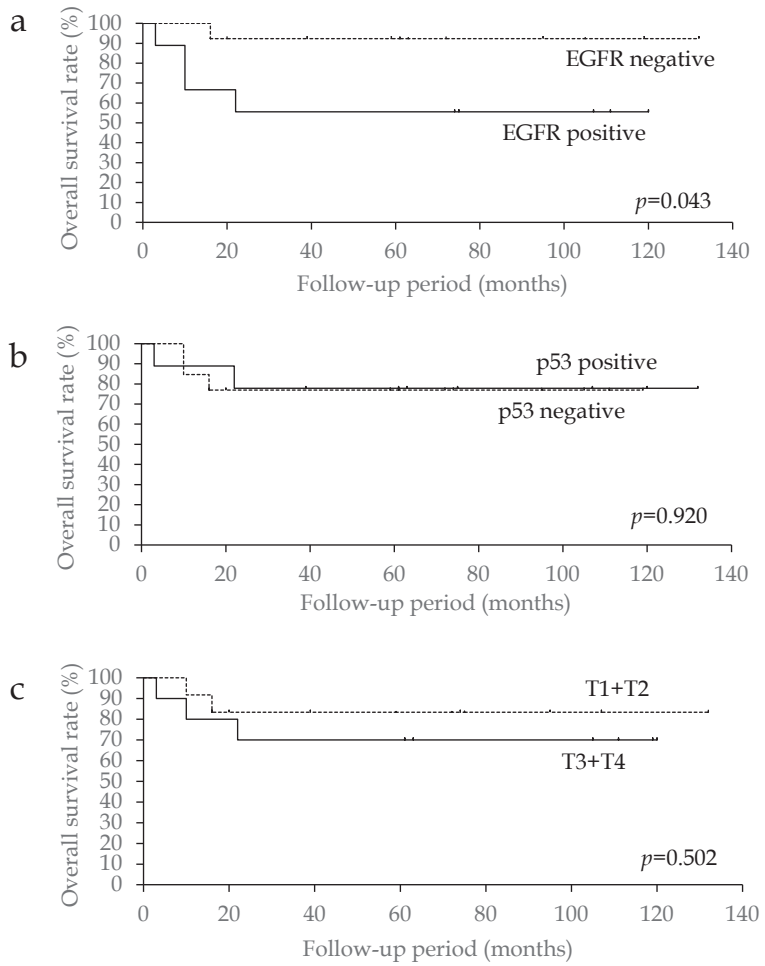

Fig. 2 Overall survival rates of the 22 patients with temporal bone squamous cell carcinoma stratified by the immunohistochemical expression of EGFR (a) and p53 (b). Overall survival rates stratified by T classifications (T1 + T2 vs. T3 + T4) in patients with temporal bone squamous cell carcinoma (c).

DISCUSSION

EGFR is a transmembrane glycoprotein expressed in many epithelial malignancies, and the activation of EGFR leads to the initiation of intracellular signaling pathways that regulate the activation of cell proliferation, invasion, angiogenesis, and metastasis [14]. Morita *et al.* [9] demonstrated that positive EGFR expression in TBSCC tissues was significantly associated with T classification, N classification, and poor survival outcomes. In this study, EGFR overexpression was correlated with a poor prognosis in patients with TBSCC. Advanced T stages (T3 and T4) were associated with poor prognosis with no significant difference. Moreover, the other parameters, including the expression of p53, were not correlated with prognosis. This result may have been influenced by the small sample TBSCC patients, and a large number of TBSCC cases is required.

It was reported that EGFR expression of head and neck squamous cell carcinoma (HNSCC) was a strong independent prognostic factor [15]. It was also reported that there was a correlation between EGFR overexpression and RT resistance [16, 17]. However, it has been reported that EGFR expression levels in HNSCC are not correlated with the effectiveness of cetuximab [18]. Recently, loss of PTEN (Phosphatase and Tensin Homolog Deleted from Chromosome 10) expression has been considered as predictive biomarkers for the effectiveness of cetuximab [19, 20]. However, this is not clear yet.

In this study, 5 cases were treated with cetuximab, and long-term results were obtained in 2 cases. Although there were few cases, there was no correlation between EGFR and cetuximab. To the best of our knowledge, there is only one report of RT with cetuximab as initial therapy for TBSCC by Ebisumoto *et al.* in our hospital [21].

From its origin, TBSCC is considered to be more similar to cutaneous squamous cell carcinoma (cSCC) than HNSCC originated mucosa. Most cutaneous squamous cell carcinomas overexpress EGFR [22], but there are few reports of cetuximab treatment for cSCC. There have been reports that cetuximab has effects on advanced cSCC [23], and RT with cetuximab as postoperative treatment improved the prognosis of cSCC [22]. However, these reports were from retrospective evaluations, and no studies were conducted on EGFR and effect prediction. If biomarkers that predict the effectiveness of cetuximab or RT in cSCC or TBSCC can be evaluated before treatment, TBSCC patients can select treatment that is less invasive than surgery even if they have advanced cancer.

The overexpression of EGFR showed a significant correlation with poor survival outcomes. Cetuximab

had significant effects in 2 cases, but there was no association between EGFR expression and therapeutic effect. If biomarkers that predict the effectiveness of cetuximab are elucidated in TBSCC in the future, it may be possible to propose biotherapy as an alternative to highly invasive temporal bone surgery.

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