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Expression of EGFR and p16 in Squamous Cell Carcinoma of External Auditory Canal

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The expression of EGFR and p16 in the external auditory canal squamous cell carcinoma (EACSCC) and their impacts on oncological outcomes were not well studied. Seventeen-one consecutive patients who were treated for EACSCC at Kobe University Hospital from 1995 to 2018 were enrolled in this study. The expression of EGFR, and p16 were evaluated and their impacts on oncological outcomes were statistically analyzed. Positive expression of EGFR was observed in 62 patients (87%). Strong positive expression of p16 were observed in 18 patients (32.4%), and weakly positive expression in 30 patients (42.3%), respectively. While the number of the patients with negative EGFR expression were limited, all the surgically treated patients with negative EGFR expression have been alive without disease. In the patients with T3 & T4a EACSCC, prognosis of the patients with positive p16 expression EACSCC tended to be better than those with negative p16 expression. These results suggest the clinical significance of EGFR and p16 expressions in the patients with advanced EACSCC to predict oncological outcomes.

INTRODUCTION

Cancer of the external auditory canal is a rare malignant neoplasm that occurs in 1–6 per million people and accounts for about 0.2% of all head and neck cancers (1). Histopathological types include squamous cell carcinoma (SCC), adenoid cystic carcinoma (AdCC), adenocarcinoma, basal cell carcinoma and malignant melanoma, with SCC being the most frequent type (2). Chronic mechanical irritation, such as habitual ear cleaning, and chronic inflammation due to chronic otitis external have been considered as risk factors for the development of SCC of EAC (EACSCC) (3). The modified Pittsburgh classification (4) has been widely used for staging and predicting prognosis. T classification, facial nerve palsy, positive surgical margin, N classification, dural invasion have been reported as factors affecting oncological outcomes (5–7). Surgical resection followed by postoperative radiotherapy (RT) have been the mainstay of the treatment for EACSCC and provided satisfactory oncological outcomes in the patients with T1 and T2 disease (3, 8). On the other hand, the reported oncological outcomes of the patients with advanced disease treated by surgical resection followed by RT were unfavorable with sever postoperative sequelae, such as hearing loss, balance disorder, facial deformity, malocclusion, and difficulty in swallowing due to lower cranial palsies. To address these issues, recently, concurrent chemoradiotherapy with the combination of Docetaxel, Cisplatin, and Fluorouracil (TPF), has been developed in Japan and becoming the standard of care for advanced EACSCC. However, 5-year survival rate still remains ranging around 50% (9, 10).

Recently, mutations and abnormal expression of genes such as EGFR, cyclin D1, p53, and p16 have been reported in the head and neck SCC (10), suggesting the possible roles as prognostic factors for the patients with head and neck SCC including EACSCC (11). Encouraged with these previous reports, in this study, we examined the expression of EGFR, and p16 by immunohistological staining and statistically analyzed their impacts on oncological outcomes using Kaplan-Meier's method.

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PATIENTS AND METHODS

Patients

Between January 1995 and March 2018, 119 patients were treated for malignant tumors of the external auditory canal at the Department of Otolaryngology-Head and Neck Surgery, Kobe University Hospital. In all patients, pathological diagnoses were determined using biopsy or surgical specimen. Among them, 71 patients with EACSCC who were followed up at least for 5 years or until they died and whose paraffin embedded formalin fixed specimen either at the time of biopsy or surgical resection were available were included in this study. Clinical data were retrospectively obtained from medical records. Primary lesion was staged according to the modified Pittsburg classification (4). In addition, T4 was subclassified to T4a (resectable T4) and T4b (unresectable T4) according to the recently proposed KOBE classification (12). Regional lymph node metastasis and distant metastasis were staged according to the AJCC 8th edition staging of head and neck cancer (13).

Methods

Immunohistological staining was performed for EGFR, and p16 as described in elsewhere. Briefly, mouse monoclonal antibody against EGFR (31G7, Invitrogen 28-0005; Thermo Fisher Scientific, USA), and mouse monoclonal antibody against p16(clone E6H4, #518-1009912; Roche Diagnostics, Austria) were used according to the manufacture's protocols. The expression of immunostaining was determined according to the previous reports as follows. EGFR was determined as "positive" when a positive signal was detected on the tumor cell membrane, and "negative" when no positive signal was detected at all. p16 was considered as "strongly positive" when positive staining in the nucleus and cytoplasm was observed in more than 70% of the tumor cells, as "weakly positive" when those showed weaker expression than the internal control group, and as "negative" when positive staining was observed in less than 70% of the tumor cells. Assessments for EGFR and p16 expression were performed twice by a single experienced pathologist, blind to patient treatment and prognosis. Statistical analyses were processed using BellCurve for Excel (Social Survey Research Information Co., Ltd.), and overall survival according to the T classifications at the time of initial treatment and expression status of EGFR or p16 were analyzed using Kaplan-Meier's method. This retrospective study was approved by the institutional review board of Kobe University Hospital (C180010).

RESULTS

Characteristics of the Patients

Of the 71 patients with EACSCC, 27 patients were male, and 44 patients were female, with a median age of 64 years old, ranging from 38 to 89 years. The right ear was affected in 38 patients and left ear was affected in 33 patients. Distant metastasis was observed in no patients at the time of initial diagnosis. As an initial treatment, lateral temporal bone resection (LTBR) was performed in 37 cases and subtotal temporal bone resection in 13 cases. Postoperative radiotherapy (and chemotherapy) was performed in 2 patients with T1, and 2 patients with T2, and 5 for T3. Lateral temporal bone resection (LTBR) was performed in 37 patients and subtotal temporal bone resection (STBR) in 13 patients. Ines with T3 and T4a, 11 of 15 patients with T3 were surgically treated. Four of them underwent LTBR, 7 of them underwent STBR, and 5 of them received postoperative radiotherapy. Five of 12 patients with T4a were surgically treated. One of them underwent LTBR, 4 of them underwent STBR, and no T4a patients did not receive postoperative irradiation. Seven of the 51 surgically treated patients were positive for surgical margin, 37 were negative for surgical margin, and 7 were not documented. 12 patients underwent radiotherapy (RT), and cisplatin-based chemoradiotherapy (CRT) was performed in 9 patients (Table I).

Table I. Clinical characteristics of 71 patients with EACSCC

	No. of patients		No. of patients		No. of patients
Sex		T4a	12	**Treatment**	
Male	27	T4b	11	RT	12
Female	44	**N classification**		CRT	9
T classification		N0	65	LTBR + (C)RT	5
T1	12	N1	3	LTBR − (C)RT	32
T2	21	N2	3	STBR + (C)RT	4
T3	15	**M classification**		STBR − (C)RT	9
T4	23	M0	71		
		M1	0		

RT, radiotherapy; CRT, chemoradiotherapy; ( ), followed by (C)RT, LTBR, lateral temporal bone resection; STBR, subtotal temporal bone resection.

Expression of EGFR and p16

Immunohistochemically, EGFR was positive in 62 cases (87.3%), negative in 9 cases (12.7%). p16 was strongly positive in 23/71 (32.4%), weakly positive in 30/71 (42.3%), and negative in 18/71 (25.4%) (Fig. 1). Expressions of EGFR, and p16 according to the T classification were summarized in Table II. There were no specific tendencies in the expression of EGFR and p16 among T1, T2, T3 and T4.

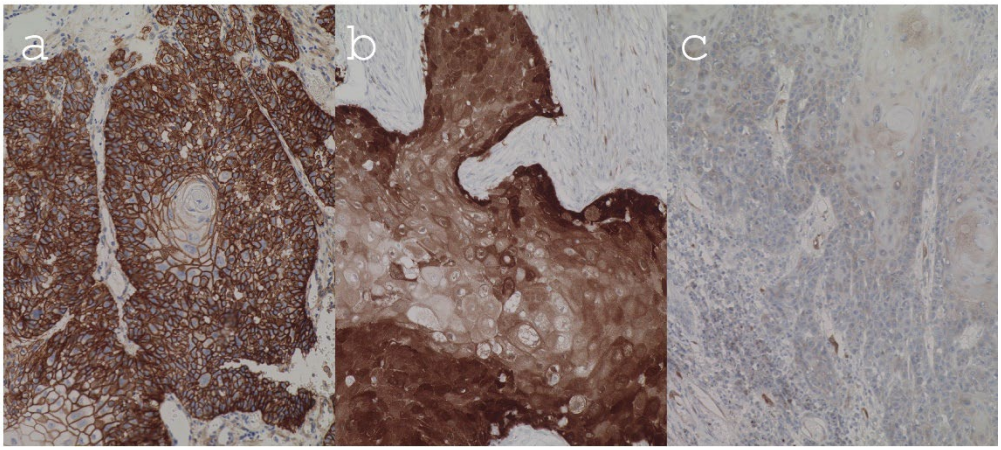

Fig. 1. Expression of EGFR and p16
a. positive expression of EGFR
b. strongly positive expression of p16
c. weakly positive expression of p16

Table II. Expression of EGFR and p16 according to T classification

	EGFR		P16		
	positive	negative	positive	weakly positive	negative
T1	11	1	4	3	5
T2	17	4	5	10	6
T3	14	1	2	9	4
T4a	10	2	4	5	3
T4b	10	1	3	3	5

Oncological Outcomes

Follow-up period ranged from 2–218 months (median 55.5 months). 3-year and 5-year overall survival rates of all the 71 patients were 57.7%, 54.1%, respectively. 5-year overall survival rates of the patients with T1, T2, T3, and T4 EACSCC were 100%, 83.1%, 75.0%, and 19.6%, respectively (Fig. 2). In the surgically treated patients (n = 50), the 5-year overall survival rates of the patients with T1, T2, T3, and T4a EACSCC were 100%, 82.1%, 80.0%, and 42.9%, respectively (Fig. 3). In the patients with T3, T4a and T4b EACSCC treated by CRT, the 5-year overall survival rate of the patients with T3 or T4a disease tended to be better significantly than those with T4b (66.7% and 0.0%).

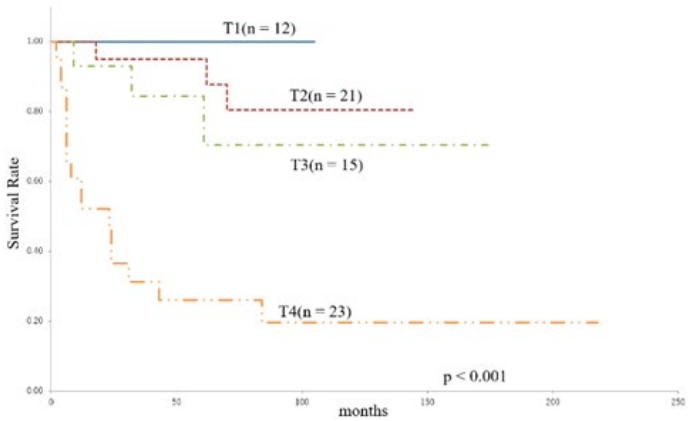

Fig. 2. Overall survival of all the patients according to T classification

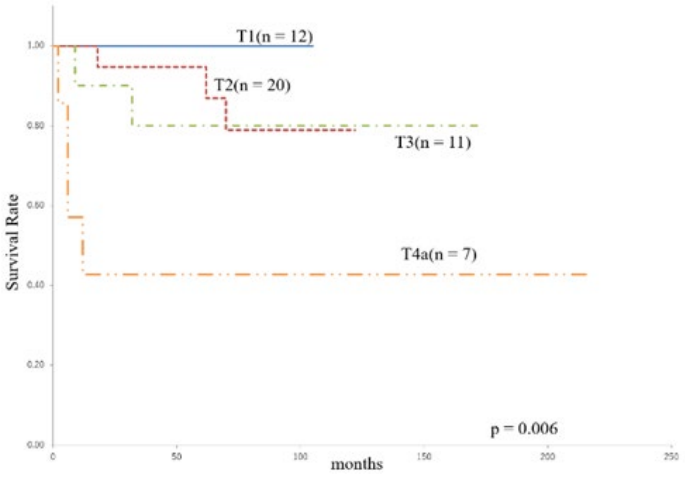

Fig. 3. Overall survival of surgically treated patients according to T classification

Among the surgically treated patients, 5-year survival rate of the patients with EGFR-negative disease (n = 6) was favorable compared to those with EGFR-positive disease (n = 44) (100% vs. 82.9%, p = 0.9; Fig. 4). As for p16, the survival rate of the patients with strongly or weakly p16-positive disease (n = 34), tended to be better compared with that of p16-negative EACSCC (n = 16) (82.7% vs. 73.4%, p = 0.21; Fig. 5).

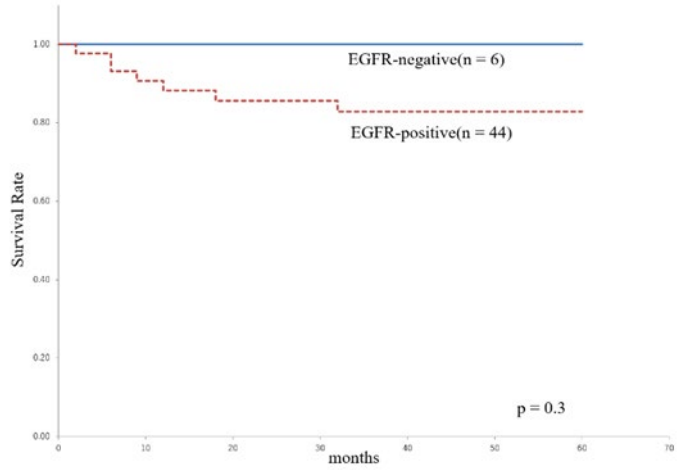

Fig. 4. Overall survival of surgically treated patients according to EGFR expression

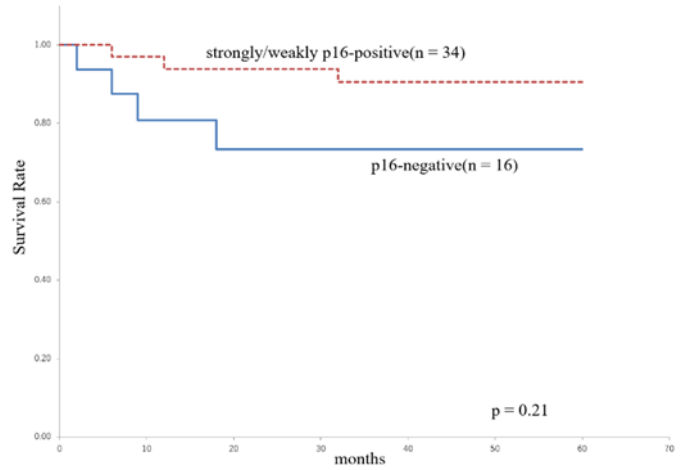

Fig. 5. Overall survival of surgically treated patients according to p16 expression

Since the prognoses of the patients with T1 and T2 EACSCC were quite favorable and prognosis of the patients with T4b EACSCC was extremely unfavorable, next, we excluded the patients with T1, T2 and T4b EACSCC,

and examined the impact of p16 expression on the prognosis of the patients with T3 and T4a EACSCC (n = 27). Among the patients with T3 and T4a EACSCC, the 5-year survival rates of the patients with strongly positive, weakly positive and negative were 85.7%, 62.3%, and 38.1% (p = 0.16; Fig. 6a), respectively. Survival rate of the patients with strongly or weakly p16-positive disease (n = 20) tended to be better than those with p16-negative disease (n = 7) (72.5% vs. 38.1%, p = 0.07; Fig. 6b).

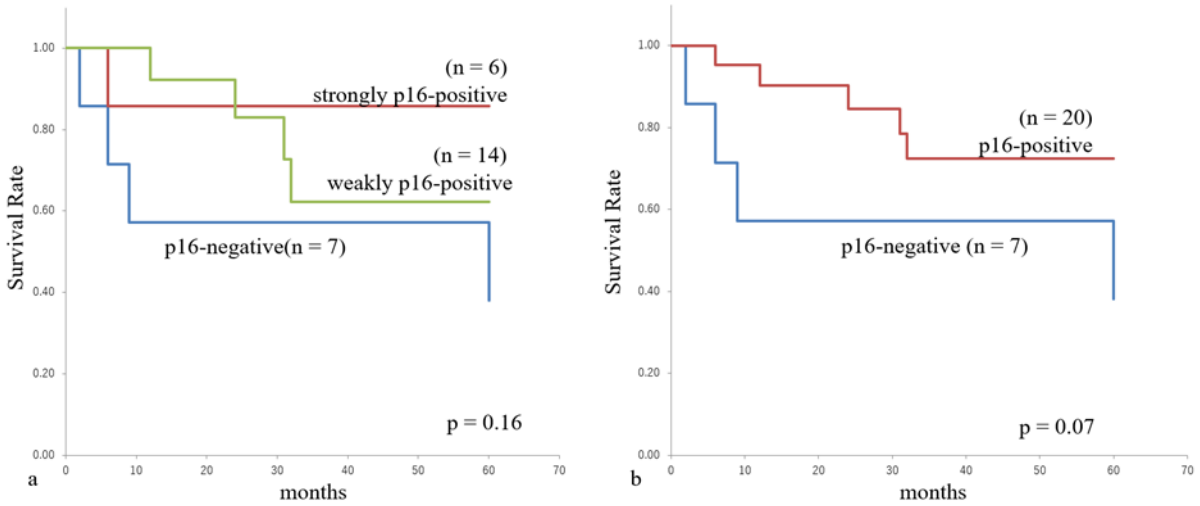

Fig. 6. Overall survival of all the patients with T3/T4a EACSCC
a: strongly positive vs. weakly positive vs. negative
b: positive (strongly + weakly) vs. negative

DISCUSSION

Staging System for EACSCC

Since malignant neoplasms of EAC is extremely rare as described above, current UICC TMN classification does not provide specific staging system for EAC, while it provides staging system for skin cancer of the head and neck. Alternatively, the modified Pittsburgh classification proposed by Moody et al. (4) has been used for T classification. However, during the last two decades, advances in the surgical treatment such as surgical navigation system, and microvascular free flap reconstruction provided better oncological results in the patients with advanced EACSCC in compared with the previous reports. In addition, concurrent chemoradiotherapy with multiple agents provided better oncological and functional outcomes even in the patients with T4 disease (13).

Reflecting these advances, recently we have subdivided T4 to T4a (resectable T4) and T4b (unresectable T4) and proposed KOBE classification (12). As shown in this study, KOBE classification well predicted the prognosis of the surgically treated patients as well as the patients treated by CRT. While satisfactory oncological outcomes were obtained by surgical treatment in the patients with early stages, the survival rates of the patients with T3 and T4a are still unfavorable and those with T4b remained poor. Thus, development of prognostic factors to predict the survival of the patients with advanced EACSCC in considering additional treatment strategies for these patients with advanced EACSCC. Above these backgrounds, in order to better understand the molecular mechanism of EACSCC, develop the personalized treatment with molecular-targeted medicines and explore the molecular prognostic factors for the patients with EACSCC, in this study, we examined the expression of EGFR and p16 in EACSCC by immunohistological staining.

Expression of EGFR

EGFR is a tyrosine kinase involved in the regulation of cell differentiation and proliferation. Binding of ATP to EGFR ligands activates cell differentiation and proliferation (14). EGFR is expressed in various organs including skin and mucous membrane in upper aerodigestive tract. Under normal conditions, the EGFR gene is responsible for directing cell proliferation and inhibiting cell death. However, mutations in this gene activate the EGFR protein, which in turn causes abnormal cell proliferation, leading to cancer. A novel molecular mechanism has been elucidated in which genetic abnormalities of epidermal growth factor receptor (EGFR) in head and neck cancer and lung cancer regulate the Hippo pathway, a signaling pathway via tyrosine phosphorylation, to activate the downstream oncogene YAP/TAZ, resulting in increased cancer cell growth and resistance to EGFR inhibitors (15). Mutations and overexpression of EGFR gene have been reported to occur in head and neck, breast, lung

cancer and several other organs. EGFR mutations are detected at high rates in non-small cell lung cancer, and multivariate analysis has reported that patients with positive EGFR mutations have a better prognosis than those with negative mutations (16). On the other hand, positive EGFR mutation has been reported as a poor prognostic factor in rectal cancer, ovarian cancer, bladder cancer (17), and in the head and neck region, overexpression of EGFR has been suggested to be a poor prognostic factor in oral cancer (18). In recent years, EGFR inhibitors, have become widely used in cancer therapy and have been shown to prolong survival (14, 17). For metastatic or recurrent squamous cell carcinoma of the head and neck, there are reports of improved survival with the addition of cetuximab, antibody against EGFR, to CDDP+5-FU therapy, which had been the standard of care (19). Similarly, the addition of another antibody against EGFR, Panitumumab, to CDDP+5-FU, was found to prolong the running rate and progression-free survival (20). And at present, cetuximab, antibody against EGFR, has been approved and used for head and neck cancers in various countries (21, 22).

As we expected, distinct expression of EGFR is observed in the majority of EACSCC. However, EGFR expression is absent in several cases. These results suggest that expression of EGFR should be evaluated in the consideration of applying cetuximab. Of note, while statistical analysis did not reach significance, prognosis of EGFR-negative group in consistent with the previous report (11), suggesting that the lack of EGFR expression may be a favorable prognostic factor for EACSCC.

Expression of p16

p16 as well as p53 have been known as tumor suppressor genes that regulate the cell cycle, arrest the cell cycle when DNA damage occurs, and suppress tumor growth by inducing DNA repair and apoptosis. p16 is compensatory overexpressed by inactivation of p53 by human papilloma virus. To take advantage of this mechanism, p16 has been used as a surrogate marker to indirectly evaluate the involvement of HPV in the tumor cells. Currently, in UICC and AJCC TNM classification, the diagnosis of HPV-associated oropharyngeal cancer (OPC) requires the positive immunohistological staining of p16 (13). Now, it is well known that the prognosis of the patients with p16-positive HPV related OPC is significantly better than those with p16-negative HPV-unrelated OPC regardless of the treatment modalities (upfront surgery or CRT).

In the present study, strong positive staining of p16 was observed in approximately one-third of EACSCC, which was lower than in OPC but was much higher than the other head and neck SCC (23, 24). As with p16-positive OPC, prognosis of the patients with p16-positive disease were better than those with p16-negative disease. While the implications of positive staining of p16 in EACSCC will need to be explored in the future, these results suggest the possible role of p16 staining as a prognostic marker for patients with EACSCC.

Limitations

One of the limitations in this study is the limited number of the patients. While the number of the patients in this study is one of the largest ever reported at a single institution, the number of the patients in each T classification is relatively small. In addition, due to the nature of retrospective analysis in a single institute over a long-period, treatment strategy for advanced EACSCC has been shifting from radical surgery to CRT. Thus, the number of cases in each T classification for respective treatment strategy is limited. A multi-institutional study should be conducted to accumulate a large number of the patients and draw more definitive conclusions to the findings of the present study.

Another limitation is the lack of testing the presence of HPV using PCR, or RT-PCR. In p16-positive OPC, good concordance between p16 positivity and HPV attributability with HPV attributability definition using PCR and/or RT-PCR[21]. However, there is no standard definition of techniques to define HPV attributability in non-OPC. Moreover, it is not always clear whether p16 immunoreactivity indicates oncogenic HPV infection or risk of progression (25). Thus, the meaning of positive p16 expression in this study remains unclear. To address this issue, now, we are preparing to test the HPV attributability in the p16-positive EACSCC using RT-PCR and RT-PCR technique.

CONCLUSIONS

The present study demonstrated that expression of EGFR is not observed in a part of EACSCC and p16 is expressed in one-third of EACSCC. Expression of EGFR and p16 might have roles as prognostic factors for advanced EACSCC.

REFERENCES

1. Marlon RP, Stell PM, Derrick PP. Epidemiology of cancer of the middle ear cleft. Cancer. 1984;53(7):1612–7.
2. Tammaro A, Adebanjo GAR, Chello C, Parisella FR, Cantisani C, Farnetani F, et al. Malignant lesions of the

ear. Arch Dermatol Res. 2022;314(9):839–845.
3. Yin M, Ishikawa K, Honda K, Arakawa T, Harabuchi Y, Nagabashi T, et al. Analysis of 95 cases of squamous cell carcinoma of the external and middle ear. Auris Nasus Larynx. 2006;33(3):251–257.
4. Moody SA, Hirsch BE, Myers EN. Squamous cell carcinoma of the external auditory canal: an evaluation of a staging system. Am J Otol. 2000;21(4):582–588.
5. Chi FL, Gu FM, Dai CF, Chen B, Li HW. Survival outcomes in surgical treatment of 72 cases of squamous cell carcinoma of the temporal bone. Otol Neurotol. 2011;32(4):665–669.
6. Higgins TS, Antonio SA. The role of facial palsy in staging squamous cell carcinoma of the temporal bone and external auditory canal: a comparative survival analysis. Otol Neurotol. 2010;31(9):1473–1479.
7. Masterson L, Rouhani M, Donnelly NP, Tysome JR, Patel P, Jefferies SJ, et al. Squamous cell carcinoma of the temporal bone: clinical outcomes from radical surgery and postoperative radiotherapy. Otol Neurotol. 2014;35(3):501–508.
8. Morita S, Homma A, Nakamaru Y, Sakashita T, Hatakeyama H, Kano S, et al. The outcomes of surgery and chemoradiotherapy for temporal bone cancer. Otol Neurotol. 2016;37(8):1174–1182.
9. Takenaka Y, Cho H, Nakahara S, Yamamoto Y, Yasui T, Inohara H. Chemoradiation therapy for squamous cell carcinoma of the external auditory canal: a meta-analysis. Head Neck. 2015;37(7):1073–1080.
10. Mountzios G, Rampias T, Psyrri A. The mutational spectrum of squamous-cell carcinoma of the head and neck: targetable genetic events and clinical impact. Ann Oncol. 2014;25(10):1889–1900.
11. Morita S, Nakamaru Y, Homma A, Yasukawa S, Hatakeyama H, Sakashita T, et al. Expression of p53, p16, cyclin D1, epidermal growth factor receptor and Notch1 in patients with temporal bone squamous cell carcinoma. Int J Clin Oncol. 2017;22(1):181–189.
12. Shinomiya H, Uehara N, Fujita T, Yoshida K, Imamura Y, Teshima M, et al. New proposal to revise the classification for squamous cell carcinoma of the external auditory canal and middle ear. J Laryngol Otol. 2021;135(4):297–303.
13. Amin MB, Edge SB, Greene FL, editors. AJCC Cancer Staging Manual. 8th ed. New York: Springer; 2017. p. 55–181.
14. Huang SM, Harari PM. Epidermal growth factor receptor inhibition in cancer therapy: biology, rationale and preliminary clinical results. Invest New Drugs. 1999;17(3):259–269.
15. Ando T, Arang N, Wang Z, Costea DE, Feng X, Goto Y, et al. EGFR Regulates the Hippo pathway by promoting the tyrosine phosphorylation of MOB1. Commun Biol. 2021;4(1):1237.
16. Suda K, Mitsudomi T, Shintani Y, Okami J, Ito H, Ohtsuka T, et al. Clinical impacts of EGFR mutation status: analysis of 5780 surgically resected lung cancer cases. Ann Thorac Surg. 2021;111(1):269–276.
17. Nicholson RI, Gee JMW, Harper ME. EGFR and cancer prognosis. Eur J Cancer. 2001;37 Suppl 4:S9–15.
18. Awawdeh MA, Sasikumar R, Aboalela AA, Siddeeqh S, Gopinathan PA, Sawair F, et al. Evaluation of Prognostic Significance of the Expression of p53, Cyclin D1, EGFR in Advanced Oral Squamous Cell Carcinoma after Chemoradiation—A Systematic Review. Appl Sci. 2023;13(9):5292.
19. Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med. 2008;359(11):1116–1127.
20. Vermorken JB, Stöhlmacher WJ, Davidenko I, Licitra L, Winquist E, Villanueva C, et al. Cisplatin and fluorouracil with or without panitumumab in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck (SPECTRUM): an open-label phase 3 randomised trial. Lancet Oncol. 2013;14(8):697–710.
21. Ang KK, Berkey BA, Tu X, Zhang HZ, Katz R, Hammond EH, et al. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. Cancer Res. 2002;62(24):7350–7356.
22. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med. 2006;354(6):567–578.
23. Ndiaye C, Mena M, Alemany L, Arbyn M, Castellsague X, Laporte L, et al. HPV DNA, E6/E7 mRNA, and p16INK4a detection in head and neck cancers: a systematic review and meta-analysis. Lancet Oncol. 2014; 15(12):1319–1331.
24. Mena M, Wang X, Tous S, Quiros B, Clavero O, Alejo M, et al. Concordance of p16(INK4a) and E6*I mRNA among HPV-DNA-Positive Oropharyngeal, Laryngeal, and Oral Cavity Carcinomas from the ICO International Study. Cancers (Basel). 2022;14(15):3787.
25. Liu Y, Alqatari M, Sultan K, Ye F, Geo D, Sigel K, et al. Using p16 immunohistochemistry to classify morphologic cervical intraepithelial neoplasia 2: correlation of ambiguous staining patterns with HPV subtypes and clinical outcome. Hum Pathol. 2017;66:144–151.