Auris Nasus Larynx 42 (2015) 472–477

Contents lists available at ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

Prognostic factors and outcome analysis of salivary duct carcinoma

Myung Woul Han [a], Jong-Lyel Roh [b], Seung-Ho Choi [b], Soon Yuhl Nam [b], Hee Jin Lee [c], Kyung-Ja Cho [c], Sang-Wook Lee [d], Sang Yoon Kim [b,e,*]

[a] Department of Otolaryngology, Ulsan University Hospital, Ulsan, Republic of Korea
[b] Department of Otolaryngology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea
[c] Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea
[d] Department of Radiation Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea
[e] Biomedical Research Institute, Korea Institute of Science and Technology, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 20 May 2014
Accepted 27 April 2015
Available online 28 May 2015

Keywords:
Salivary duct carcinoma
Radiotherapy
Distant metastasis
c-erbB-2
EGFR

ABSTRACT

Objective: Salivary duct carcinoma (SDC) is highly aggressive, with high rates of recurrence and nodal and distant metastases. The aim of the current study was to evaluate the clinical implication of EGFR and HER2 expression for predicting prognosis and to identify the factors associated with outcome.
Methods: The medical records of 28 patients with SDC underwent surgery and adjuvant RT. Expression of *c-erbB-2* and EGFR was determined immunohistochemically on the 25 SDC specimens. Disease-free survival (DFS), overall survival (OS) and distant metastasis-free survival (DMFS) were analyzed.
Results: Three-year DFS, OS and DMFS rates were 38.3%, 78.1% and 45.7%, respectively. Expression of *c-erbB-2* and EGFR was seen in 64% and 40%. *c-erbB-2* and EGFR expression did not correlate with recurrence or metastasis. Advanced N classification and perineural invasion (PNI) were significant predictors of DFS and DMFS.
Conclusion: *c-erbB-2* and EGFR expression did not correlate with recurrence or metastasis. Despite aggressive surgery and RT, approximately 50% of SDCs failed systemically. More effective therapy to inhibit distant metastases in patients with advanced N classification and PNI should be considered.

1. Introduction

Salivary duct carcinoma (SDC) is an uncommon and highly malignant tumor of the salivary gland. Clinically, SDC is characterized by very aggressive behavior, and high rates of recurrence, nodal and distant metastases and tumor-related death [1–4]. The clinical aspects and histopathological patterns of SDC have been well described. This tumor type is most often seen in elderly men, occurring predominantly in the parotid gland and, occasionally, in the submandibular gland (SMG). Pain, facial palsy, and presence of calcification on CT (Computed Tomography) scans are suggestive features of SDC. The adjuvant chemoradiotherapy (CRT) with a platinum-based regimen resulted in excellent local control in a subgroup of patients with adverse prognostic factors [5–8].

Histologically, SDC bears a striking resemblance to breast carcinoma of the ductal type, with both intraductal and invasive components [3,9,10] and frequently overexpresses EGFR and HER2 [9,11–19], but, it is not yet clear whether HER2 and EGFR expression is associated with poor prognosis in SDC [15,19–21]. Although a small-scale study showed that cetuximab in 30 patients with recurrent and/or metastatic salivary gland carcinomas was effective [22], the role of molecular targeted therapy including EGFR inhibition in the treatment of salivary gland malignancies has not been well defined and there are no convincing data to support the routine use of other cytotoxic, hormonal, or targeted agents in salivary gland malignancies [21,23,24]. HER2 gene amplification status has improved the identification of possible responders to targeted therapy with trastuzumab, but the role of targeted therapy has been reported only in cases of disseminated disease in SDC [15,25–27].

Although radical excision and adjuvant radiation therapy (RT) appear appropriate for local and regional controls, distant metastasis is the most common cause of treatment failure in SDC, and its early detection and treatment continue to pose a major challenge [1,4]. To design effective therapeutic options for SDC, it is essential to identify the factors that are associated with distant metastasis. Since little is known about the predicting factors that determine survival outcome and distant metastasis and about the

* Corresponding author at: Asan Medical Center, 88 Olymhic-Ro 43-Gil, Songpa-Gu, Seoul 138-736, Republic of Korea. Tel.: +82 2 3010 3710; fax: +82 2 489 2773.
E-mail address: sykim2@amc.seoul.kr (S.Y. Kim).

http://dx.doi.org/10.1016/j.anl.2015.04.005
0385-8146/

outcome in patients underwent postoperative RT or CRT in SDC, the present study was performed. To identify effectiveness of postoperative RT and necessity of new targeted chemotherapy, hear, possible risk factors for recurrence and distant metastasis in only SDC patients treated by surgery and postoperative RT, including overexpression of *c-erbB-2* (*HER2/neu* protein) and EGFR were evaluated.

2. Patients and methods

2.1. Patients

The medical records of all consecutive patients treated for salivary gland carcinoma at our institution between January 1997 and December 2009 were reviewed. Eligibility criteria included the following: (1) histologically proven cases of SDC, (2) patients who underwent radical surgery with or without neck dissection as the initial treatment, and (3) patients who received postoperative RT as adjuvant therapy at our institution. Patients were excluded if they had concurrent second primary malignancy, distant metastasis at diagnosis, or a history of prior radiotherapy to analyze disease-free survival (DFS), overall survival (OS), and distant metastasis-free survival (DMFS) after surgery and adjuvant radiotherapy. In total, 28 patients (26 men, two women; mean age, 61.3 years; range, 39–75 years) were enrolled. All were preoperatively evaluated by biopsy (fine-needle aspiration) and CT or magnetic resonance imaging. The patients were staged according to the American Joint Committee on Cancer Staging guidelines. The patient data were reviewed retrospectively after receiving approval from the Institutional Review Board of Asan Medical Center (AMC IRB).

2.2. Immunohistochemical analysis

In total, 25 SDC formalin-fixed, paraffin-embedded specimens were available for the immunohistochemical analysis of *c-erbB-2* and EGFR. Spots of each primary tumor were chosen under a microscope. All immunostains were performed in a Benchmark automatic immunostaining device (Ventana Medical System, Tucson, AR, USA). Sections (4-μm thick) were obtained with a microtome, transferred onto adhesive slides, and dried at 62 °C for 30 min. After incubation with primary antibodies against EGFR (1:100 dilution; Zymed, South San Francisco, CA, USA) and *c-erbB-2* (1:500 dilution; Dako), immunodetection was performed by using a labeled streptavidin biotin kit. Thus, the sections were incubated with biotinylated anti-mouse immunoglobulin, then peroxidase-labeled streptavidin, and finally the 3,3′-diaminobenzidine chromogen substrate. The primary antibody incubation step was omitted in the negative control slides. The slides were counterstained with Harris hematoxylin. *c-erbB-2* and EGFR immunoreactivity were evaluated semi-quantitatively by two pathologists according to established criteria for grading HER-2 expression in mammary carcinoma cells (DAKO), as follows (Fig. 1): a score of 0 (negative staining) was given if there was membranous staining in $<10\%$ of the tumor cells, while positive scores of 1+, 2+, and 3+ were given if there was partial, weak complete, or strong complete membranous staining in $\geq 10\%$ of the tumor cells, respectively.

2.3. *Fluorescence* in situ *hybridization (FISH)*

Tissue microarrays were generated from eight and seven formalin-fixed and paraffin-embedded specimens that had positive immunohistochemical expression for the *c-erbB-2* and EGFR proteins, FISH was performed by using the commercially available multi-target FISH assay (LAVysion; Vysis; Downers Grove, IL, USA) according to the manufacturer's instruction with minor modifications The kit includes directly labeled DNA FISH probes for EGFR (7p12, spectrum Red) and HER-2/CEP17 (chromosome 17 centromere, Spectrum Orange). After staining, 4′,6-diamidino-2-phenylinole (DAPI I) (Abbot/Vysis; Downers Grove, IL, USA) was applied to the target areas and the slides were analyzed under a fluorescence microscope using a single-band-pass filter. HER-2/neu and EGFR amplification was evaluated by calculating the signal ratio. If the ratio of probes to controls exceeded 2.0, the HER-2/neu or EGFR gene was considered to have been amplified.

2.4. Statistical analysis

Disease-free survival (DFS), overall survival (OS), and distant metastasis-free survival (DMFS) were estimated from survival curves as a function of follow-up time by using the log-rank test. The Cox proportional hazards model was used for multivariate analysis using the variables shown to be significant ($p < 0.05$) in univariate analysis. All statistical comparisons were performed with SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL). $p < 0.05$ was regarded as being statistically significant.

3. Results

3.1. Clinicopathological findings

The patient demographics and TNM staging are listed in Table 1. The most common primary site was the parotid gland (20 patients, 71.4%). Nineteen patients (67.9%) had locally advanced primary tumors (T3, T4), 23 (82.1%), and had stage III or IV disease, besides 18 (64.3%) with pathologically positive necks (N+). SDC could be diagnosed on the basis of fine-needle aspiration biopsy (FNAB) in only five cases (17.9%). In 22 cases (78.6%), the

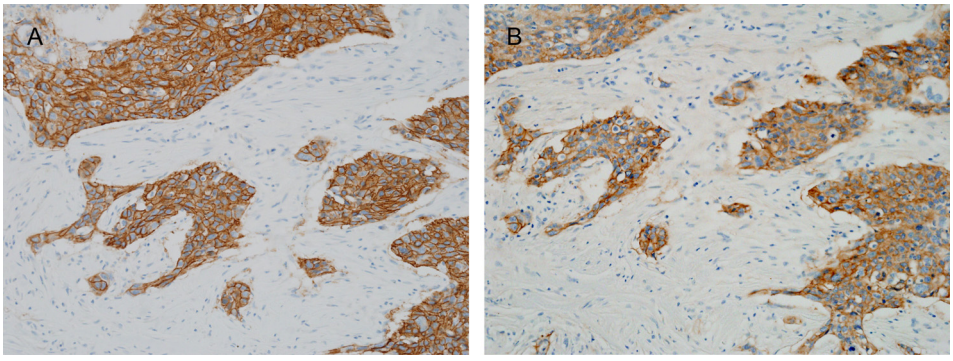

Fig. 1. Immunohistochemical analysis of c-erbB-2 and EGFR expression by salivary duct carcinoma specimens. (A) Anti-c-erbB-2 antibodies and (B) anti-EGFR antibodies stained the tumor cells diffusely and strongly with a membranous pattern (3+ grade, 200×).