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EGFR gene copy number alteration is a better prognostic indicator than protein overexpression in oral tongue squamous cell carcinomas

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ABSTRACT

Although Epidermal growth factor receptor (EGFR) is particularly important in the pathogenesis of head and neck squamous cell carcinomas (HNSCCs), conflicting data have been reported on the correlation between *EGFR* copy number and survival and the association between *EGFR* copy number and protein expression. Anatomical site of the tumour in HNSCCs may likely contribute to the discordance of the above points as EGFR expression may differ between the sub-sites of HNSCCs. Thus, in this study, we focused on oral tongue squamous cell carcinomas (OTSCCs). To investigate the association between *EGFR* copy number alteration and overexpression and to determine which is the more reliable prognostic indicator, Fluorescence in situ hybridisation (FISH) and immunohistochemical staining (IHC) were performed at a single institution on samples from 89 patients with OTSCCs undergoing surgery as the primary treatment modality. Thirty-two (36%) of 89 cases demonstrated an *EGFR* copy number alteration. EGFR protein expression was found in all 89 cases, of which 82.0% showed overexpression. No significant correlation was found between gene copy number and protein overexpression. Gene copy number alteration was significantly associated with reduced disease-free survival ($P = 0.048$) and overall survival ($P = 0.001$). Multivariate Cox proportional hazards analysis demonstrated that *EGFR* copy number increase was significantly correlated with overall survival ($P = 0.001$). *EGFR* copy number status is a more reliable indicator than protein overexpression of the survival rate in OTSCCs. FISH analysis of the *EGFR* status is useful in predicting poor prognosis in OTSCCs.

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1. Introduction

The epidermal growth factor receptor (*EGFR*) located at the region of p12 on chromosome 7 is a member of the ErbB family of tyrosine kinase receptors that regulates cell growth. Binding of its specific ligands, such as epidermal growth factor (EGF) and transforming growth factor-α (TGF-α), promotes homo- or hetero-dimerisation with other family members (ERBB2, ERBB3 and ERBB4) and subsequent autophosphorylation and initiates a number of signalling pathways. Upregulated EGFR signalling in tumours has been correlated with cell proliferation, invasion, angiogenesis, metastasis, migration and inhibition of apoptosis.[1] EGFR is overexpressed in many epithelial malignancies, including approximately 80% of head and neck squamous cell carcinomas (HNSCCs),[2] and overexpression occurs early during the course of malignant development.[3] Thus, EGFR is important in the pathogenesis of HNSCCs, and is an interesting target for therapy.

Recently, several clinical trials of EGFR inhibitors in HNSCC treatment have demonstrated a clear benefit of these drugs in a small subset of patients. Phase II studies of patients with recurrent or metastatic HNSCCs found encouraging clinical activity of several EGFR inhibitors.[4,5] Moreover, a large randomised phase III trial showed that a combination of EGFR inhibitors and radiation therapy in locally advanced HNSCCs significantly prolongs overall survival.[6] Therefore, subsets of HNSCCs appear to respond to EGFR inhibitors, and it is critical that we are able to select those patients who will best respond to such treatment. The identification of predictive markers for treatment response is also a task of high priority. In non-small cell lung cancer (NSCLC), increased *EGFR* copy number, as assessed by fluorescence in situ hybridisation (FISH), is significantly correlated with improved clinical outcome in EGFR inhibitor-treated patients,[7,8] suggesting that gene copy number may be a useful predictor. However, this association has not yet been clearly demonstrated for HNSCCs.

In addition, there have been several conflicting reports on the association between EGFR protein expression by immunohistochemistry (IHC) and survival in HNSCCs. Many studies have indicated that EGFR protein expression is significantly correlated with survival in several human malignancies, including NSCLC[9] and HNSCC,[10,11] whilst others found no association between EGFR protein expression and HNSCC prognosis.[12,13] Furthermore, conflicting data have been reported on the correlation between *EGFR* copy number and HNSCC survival and the association between *EGFR* copy number and protein expression.[14–18] These discrepancies may result from differences in tumour site and histology, patient numbers, case heterogeneity and the methods used to assess copy number. In particular, variations in case heterogeneity and treatment strategies may contribute to conflicting evaluations of EGFR as a prognostic indictor for HNSCCs.

HNSCC is itself a heterogeneous disease, and there might be differences in signalling depending on the pathobiology of the tumour. EGFR expression may also differ between the site of HNSCC; for example, it was reported to be low in laryngeal tumours compared with those from the pharynx and oral cavity.[19] Additionally, the presence of human papilloma virus (HPV), more common in the base of the tongue than in the oral cavity, is associated with favourable prognosis in oropharyngeal cancer.[20–22] Therefore, to evaluate the prognostic significance of EGFR protein expression and copy number status, it is essential to evaluate at separate sub-sites.

Thus, in the present study, we focused on oral tongue SCCs (OTSCCs) in 89 patients with OTSCCs undergoing surgery as the primary treatment modality at a single institution. We examined the association between *EGFR* copy number alteration and protein expression to determine which is more reliable as a prognostic indicator in this malignancy. Using this approach, we could correctly determine prognostic significance of this gene alteration without affection of other factors such as site-specific factors and treatment modalities.

2. Materials and methods

2.1. Patients

Tissue samples were obtained from 89 patients with OTSCC who had undergone primary surgical excision with curative intent at the Department of Maxillofacial Surgery, Graduate School, Tokyo Medical and Dental University (Tokyo, Japan) between 1999 and 2009. None of these patients received preoperative treatment. All protocols of this study were reviewed and approved by the Research Ethics Committee of Tokyo Medical and Dental University. Informed consent was obtained from all patients in accordance with our Institutional guidelines. Clinical staging was defined according to the International Union against Cancer TNM classification system.[23] Tumours were classified histopathologically as well, moderately or poorly differentiated according to their cellular differentiation as defined by the World Health Organisation criteria.[24] The mode of tumour invasion at the tumour–host borderline was classified according to the modified Jacobsson criteria.[25,26] Disease-free survival (DFS) was calculated from the time of initial examination to the time of local, regional or distant recurrence of the disease or the time of last follow-up. Overall survival (OS) was calculated from the time of initial examination to the time of death or last follow-up.

2.2. FISH analysis

Samples were taken from 89 tumours by fine-needle aspiration (FNA) technique and FISH analysis were performed as described previously using the EGFR SpectrumOrange/CEP 7 SpectrumGreen probe (Vysus/Abbott Molecular, Des Plaines, IL).[27] *EGFR* FISH patterns were classified as follows: balanced disomy, chromosome/nucleus ratio (C/N) $\leqslant$2.5; balanced trisomy, C/N 2.6–3.0; balanced polysomy, C/N >3.0 (where balanced patterns had an average ratio gene/chromosome copy number per nucleus (G/C) of 0.9–2.0); and amplification, G/C >2.0 and gene/nucleus ratio >3.0.[18,27–30] Tumours showing disomy were regarded as unchanged in gene number, with all other tumours being considered to have gene numerical alterations.