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Original contribution

Epidermal growth factor receptor: a novel biomarker for aggressive head and neck cutaneous squamous cell carcinoma☆

Sydney Ch'ng MBBS[a,b], Irene Low MBChB, FRCPA[c], Daniel Ng BSc[d], Helen Brasch MBChB, FRCPA[e], Michael Sullivan MBChB, PhD, FRACP[f], Paul Davis PhD[a], Swee T. Tan MBBS, FRACS, PhD[b,*]

[a]*Department of Medicine, Wellington School of Medicine & Health Sciences, Wellington, 6021 New Zealand*
[b]*Wellington Regional Plastic, Maxillofacial, and Burns Unit, Hutt Hospital, Wellington, 5011 New Zealand*
[c]*Department of Anatomical Pathology, Lab Plus, Auckland City Hospital, Auckland, 1010 New Zealand*
[d]*Innovative Genetic Diagnosis, Auckland, 1010 New Zealand*
[e]*Department of Pathology, Hutt Hospital, Wellington, 5011 New Zealand*
[f]*Children's Cancer Research Group, Christchurch School of Medicine & Health Sciences, Christchurch, 8011 New Zealand*

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Summary There is currently no prognostic tool that reliably predicts the risk of metastasis in cutaneous squamous cell carcinoma, most of which occur in the head and neck region. Epidermal growth factor receptor has received much interest in recent years with the advent of epidermal growth factor receptor–targeted molecular therapy in clinical oncology. We investigate the role of epidermal growth factor receptor as a biomarker for head and neck cutaneous squamous cell carcinoma. Using immunohistochemistry and fluorescence in situ hybridization, we assessed the epidermal growth factor receptor protein expression and gene copy in 3 groups of head and neck cutaneous squamous cell carcinoma: primary lesions not associated with metastasis (P), primary lesions associated with subsequent metastasis (PM), and metastatic nodal disease (M). Epidermal growth factor receptor overexpression was detected in 36% and 79% of P and PM cases, respectively. Epidermal growth factor receptor overexpression was significantly associated with PM ($P = .03$) and was found to be an independent prognostic factor for metastasis on multivariate analysis ($P = .05$). However, epidermal growth factor receptor overexpression was only maintained in 47% of cases in the M group. None of the 27 cases that overexpressed the epidermal growth factor receptor protein showed gene amplification: the results were uninterpretable in 2, and polysomy and balanced disomy were detected in 5 and 20 cases, respectively. These observations may have important prognostic and therapeutic implications for head and neck cutaneous squamous cell carcinoma.

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* Corresponding author.
E-mail address: sweetan@plastsurg.co.nz (S. T. Tan).

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1. Background

Squamous cell carcinoma (SCC) is the second commonest cutaneous malignancy, with 60% occurring in the head and neck region. Most primary SCCs are curable. However, there is a 14% incidence of metastasis for cutaneous SCC in the head and neck, which carries a mortality of up to 40% [1-3]. Histologic parameters such as tumor depth, degree of differentiation, perineural spread, and vascular and lymphatic invasion in the primary SCC are known to be associated with worse prognosis [3]. However, none of these is consistently reliable. Better understanding of the molecular basis of the disease is essential for the development of prognostic tools that will reliably predict the risk of metastasis.

In a number of malignancies, including mucosal SCC of the aerodigestive tract, tumors with overexpression of epidermal growth factor receptor (EGFR) have higher tumor stage, increased lymph node metastasis, and shorter relapse-free and overall survivals [4-8]. These findings, to the best of our knowledge, have not been demonstrated in cutaneous SCC. EGFR plays a major role in the normal cellular processes of proliferation, differentiation, and development of the skin [9].

The EGFR gene is located on chromosomal region 7p12 and encodes a 170-kDa plasma membrane glycoprotein. EGFR is a tyrosine kinase receptor whose main ligands are epidermal growth factor and transforming growth factor-α [10]. With the advent of targeted molecular therapy such as monoclonal antibody (eg, cetuximab) and small molecule tyrosine kinase inhibitor (eg, erlotinib), EGFR has generated much interest recently. EGFR contributes to tumorigenesis through autocrine stimulation of cell proliferation, cell migration, and angiogenesis.

The aims of our study were to (1) determine if there was a correlation between EGFR expression and the metastatic potential of head and neck cutaneous SCC and (2) investigate the role of gene amplification in cases of EGFR protein overexpression.

2. Materials and methods

2.1. Patient selection

Patients with head and neck cutaneous SCCs treated at the Wellington Regional Plastic, Maxillofacial, and Burns Unit over the past decade were identified from our prospective head and neck database. Fifty-four cases of SCC treated between 1998 and 2005 were retrieved from the archives at the Department of Pathology, Hutt Hospital. There were 14 cases of primary SCC with concurrent or subsequent metastasis (PM group), 15 cases of metastatic SCCs (M group), and 25 primary SCCs without metastasis (P group) making up the control group. Five patients provided 2 specimens each to the PM and M groups, respectively. Inclusion/exclusion criteria for the PM group are listed in Table 1. The control (P group) was age- and sex-matched for the PM group and have all been followed up for a minimum of 3 years. This study was approved by the Wellington Regional Ethics Committee.

Table 1 Inclusion/Exclusion criteria for the PM group

The index lesion must be at least 0.5 cm in its greatest dimension
The index lesion must be located within the lymphatic drainage area of the nodal basin involved with metastatic disease
There cannot be other SCC within the lymphatic drainage field
The interval between diagnosis of the index lesion and metastasis must be less than 5 years
The histology of the index lesion and metastasis must have similar features

2.2. Immunohistochemistry

Four-micrometer formalin-fixed paraffin-embedded sections were mounted on silane-coated slides, deparaffinized in xylene, rehydrated in graded alcohol, and brought to distilled water. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 minutes. The sections were then subjected to 5 minutes of proteolytic epitope retrieval with proteinase K (Dako Cytomation, Glostrup, Denmark) and incubated at room temperature with monoclonal mouse antihuman EGFR, clone E30 (Dako Cytomation), at 1:100 dilution for 30 minutes. Positive control (tonsil) and negative control (Dako Cytomation mouse IgG1, clone DAK G01, 1:100 dilution) were run simultaneously with the SCC specimens. Visualization was performed with the LSAB2/HRP kit (Dako Cytomation) with diaminobenzidine as the chromogen. All slides were counterstained with hematoxylin.

All slides were interpreted independently by 2 of the authors (S.C. and I.L.) based on the US Food and Drug Administration-approved Dako EGFR pharmDx guidelines. The clinical information was unknown to either observer at the time of slide interpretation. In cases of disagreement, a consensus was achieved after joint review. Positive staining was defined as membranous staining above background level in greater than 1% of tumor cells. Staining intensity was graded on a scale of 1+ (weak), 2+ (moderate), and 3+ (strong). Protein overexpression was defined by cases that demonstrate 3+ staining.

Correlation between EGFR expression and metastatic potential was evaluated using the χ^2 test, and a P value of .05 was considered statistically significant.

2.3. Fluorescence in situ hybridization

Four-micrometer sections were deparaffinized and brought to distilled water. The slides were pretreated with Digestion Enzyme Solution 00-8401 (Zymed, Invitrogen, Carlsbad, CA) for 60 minutes at 37°C, dehydrated, codenatured at 85°C with 1:1 EGFR/CEP7 probe (Zymed) for 20 minutes, then incubated overnight at 37°C. After overnight hybridization, the slides were washed in 0.4 SSC/0.3%