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Clinicopathological and prognostic significance of preoperative serum epidermal growth factor levels in patients with oral squamous cell carcinoma

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Abstract. Epidermal growth factor (EGF) promotes tumourigenesis and tissue repair of epithelial and mesenchymal cells and has a role in chemotaxis, mitogenesis, cell motility, and cytoprotection. It also enhances the growth of cancers. EGF may therefore have a role in the initiation or promotion of oral carcinogenesis. The cases of 152 patients with oral squamous cell carcinoma whose preoperative serum EGF level was determined by enzyme-linked immunosorbent assay were analyzed retrospectively, along with those of 40 age- and sex-matched controls. Patients with higher levels of EGF were more likely to have neck lymph node metastasis ($P = 0.026$), advanced stage cancer ($P = 0.04$), and a worse survival status ($P = 0.0019$). Multivariate analysis using the Cox proportional hazards model indicated that the EGF level was an independent predictor of poor survival (hazard ratio 1.99, $P = 0.018$). Patients with higher preoperative serum EGF levels had significantly poorer cancer-specific survival by Kaplan–Meier analysis ($P = 0.032$). This study indicates that a higher preoperative serum EGF level is associated with neck lymph node metastasis, more advanced stage, and poor survival. EGF should be considered as a potential prognostic biomarker and a therapeutic target for patients with oral cancer.

Key words: epidermal growth factor; oral squamous cell carcinoma; serum; survival.

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Oral squamous cell carcinoma (OSCC) is found worldwide. It is one of the leading human malignancies in most Asian countries, including Taiwan. OSCC patients generally have a poor prognosis, and the 5-year survival rate has not improved significantly over the past 20 years[1]. Many molecular factors contribute to the pathogenesis of OSCC, and it is likely that additional factors will be identified in the future[2–4]. However, the increased identification of such factors does not provide information about how they contribute to the tumour progression process[5,6].

Epidermal growth factor (EGF) is a 53-amino acid polypeptide that was originally isolated from mouse salivary glands. Initial research showed that EGF stimulated epithelial growth and differentiation upon injection into newborn mice[7,8]. Recent research has indicated that EGF has roles in the activation of pathways that promote cell proliferation, survival, migration, and differentiation in epithelial tissues, fibroblasts, and endothelial cells[9]. EGF also promotes several cellular events involved in the repair of epithelial and mesenchymal cells, and has roles in chemotaxis, mitogenesis, cytoprotection, development, and tissue homeostasis[10]. In contrast, EGF also promotes tumourigenesis and metastasis in a variety of cancer types. In particular, EGF promotes the growth of bladder, lung, breast, and colon cancers[11–14]. Thus, it has been suggested that EGF also has a role in the initiation or promotion of oral carcinogenesis[15].

The biological activities of EGF depend upon its binding to the EGF receptor (EGFR). More than 95% of head and neck squamous cell carcinomas (HNSCC) have elevated levels of EGFR due to increased copy numbers[8,9]. A previous study reported that increased EGFR expression in HNSCC correlates with reduced survival and an increased incidence of lymph node metastasis[10].

A previous study has investigated the use of salivary proteins and serum as potential diagnostic markers for OSCC, and it appears that EGF may play a role in the development of OSCC[16].

This long-term retrospective follow-up study of patients with OSCC investigated the effect of preoperative serum EGF levels and clinicopathological features upon the patient prognosis.

Patients and methods

Study protocol

The study protocol was approved by the Institutional Review Board of Mackay Memorial Hospital, Taipei, Taiwan. All enrolled patients provided written informed consent. The diagnosis and clinical staging of OSCC were based on histopathology, computed tomography (CT) scans, whole-body bone scans, chest radiograms, and whole-abdomen echograms. All patients underwent the same surgical procedure: wide tumour excision with modified radical neck dissection. Samples of the primary tumours and neck lymph nodes were collected for histopathological verification. None of the enrolled patients had any other condition that could potentially have affected their immune responses. Patients who had experienced a previous malignancy, a recent inflammatory state, or had any acute infection were excluded. Clinical staging was performed according to the American Joint Committee on Cancer/Tumour Node Metastasis (AJCC/TNM) system, and tumour type and malignancy grade were determined by histopathological analysis.

Patient characteristics

A total of 152 patients with OSCC were diagnosed during the study period (January 2005 to November 2010). Forty blood samples were collected from age- and sex-matched control subjects. Matched controls were subjects who had attended the department for a routine dental check-up. All of these patients were checked to ensure that they had no diseases of the oral mucosa. Control subjects with a history of cancer, any systemic disease, or infectious diseases were excluded. Preoperative serum sampling was performed 1 day prior to surgery. All blood samples were drawn after overnight fasting, and none of the patients received any drug therapy or blood transfusion before blood collection. Blood was obtained by venous puncture, and serum was immediately separated by centrifugation at 1000 *g* (4 °C) and then stored at −80 °C until subsequent analysis. All patients were followed in the department postoperatively. The follow-up period was at least 48 months.

Enzyme-linked immunosorbent assay for serum EGF

Serum EGF levels were measured using a commercially available human enzyme-linked immunosorbent assay (ELISA) kit (Quantine Human EGF, catalogue number DEG00; R&D Systems Inc., Minneapolis, MN, USA). This assay employs a quantitative sandwich enzyme immunoassay technique. Each serum sample was analyzed in triplicate. Post-reaction optical density was measured in a spectrophotometer (Thermo Fisher Scientific, Pittsburgh, PA, USA). Linear calibration curves were drawn using the standard EGF solutions provided with the kit.

Statistical analysis

All analyses were performed using GraphPad Prism 5 statistical software (GraphPad Software Inc., San Diego, CA, US) and SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Mann–Whitney and Wilcoxon matched-pairs tests were used to compare differences among the various clinical variables. Binary logistic regression analysis was used to determine adjusted odds ratios and their 95% confidence intervals (CI). A P-value of <0.05 was considered significant. The extent to which the level obtained could be used to efficiently separate different clinical subsets was determined using receiver operating characteristics (ROC) curve analysis; the area under the curve (AUC) was used to test discriminative ability. A Kaplan–Meier analysis was used to assess influence on survival. The association of non-reactive variables with disease-specific survival was assessed using a multivariate Cox proportional hazards model. Differences were considered significant given any of the following conditions: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Cross-comparisons showing no significance were not marked.

Results

Patient characteristics

In total, 152 patients with OSCC and 40 matched healthy controls were enrolled in this study. The clinical characteristics of the patients with OSCC are listed in Table 1.

Serum EGF as a potential diagnostic marker

The enrolled OSCC patients had a higher mean preoperative serum level of EGF than the controls (1137 ± 559 pg/ml vs. 512.1 ± 272 pg/ml, $P = 0.0001$) (Fig. 1A). When an EGF level of 847.7 pg/ml was used as the cut-off, this marker yielded an AUC of 0.84 and an accuracy of 0.74, as defined by leave-one-out cross-validation (LOOCV) (Fig. 1B, Table 2). Multivariate logistic regression analysis indicated an adjusted odds ratio of 26.33 (95% CI 5.82–119.07; $P < 0.001$) (Table 2).

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